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18 - 21 October 2009, Milan, Italy

ABSTRACT BOOK

Official Organ of the European Hematology Association

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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 hematology journal
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ABSTRACT BOOK

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Milano, Italy, October 18-21, 2009

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BEST ABSTRACTS

BEST-01

ABSENCE OF RESIDUAL VEIN THROMBOSIS AFTER AN EPISODE OF IDIOPATHIC DEEP VEIN THROMBOSIS: SHORT-TERM ANTICOAGULATION IS SAFE. THE "EXTENDED DACUS STUDY"

Siragusa S., Malato A., Saccullo G., Caramazza D., Pizzo G., Lo Coco L., Pinto A., Mariani G.

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Background. The optimal duration of Oral Anticoagulant Therapy (OAT) for Deep Vein Thrombosis (DVT) can be tailored by Residual Vein Thrombosis (RVT) (Siragusa S *et al.* Blood 2003;102(11):OC183), a marker able to assess the individual risk for recurrent thrombosis. However, in patients with idiopathic DVT the safety of early interruption of OAT, because of absence of RVT, is still debated. Objective of the study. In the present study, we evaluated the safety of withholding OAT, in patients with idiopathic DVT and without RVT, three months after the index thrombotic episode. *Study design.* Prospective controlled study with two groups: patients without RVT stopped OAT after 3 months while those with RVT continued for additional 3 months. Materials and *Methods.* Consecutive patients with a first episode of idiopathic DVT of the lower limbs. Patients with cancer or known thrombophilia were excluded. At the third months of OAT, RVT was assessed as previously described; briefly, RVT was considered absent when a clot occupying less than 40% of the vein lumen was detected by compression ultrasonography. Events, classified as recurrent DVT and/or Pulmonary Embolism and/or major and minor bleeding were evaluated; all patients were followed-up for at least 12 months after OAT discontinuation.

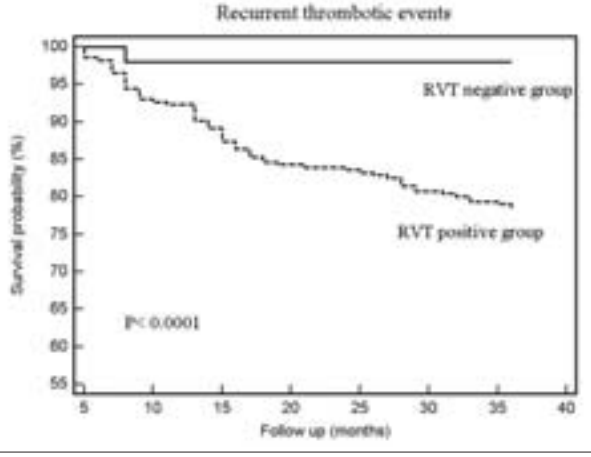

Figure.

Results. During the period 1999-2006, 518 patients were included in the study. In 206 (39.7%) RVT was considered absent (RVT negative group) and they stopped OAT; the remaining 312 patients continued anticoagulants for additional 3 months (RVT positive group). Total duration of follow-up (FU) was 184.7 years for RVT negative group (with a mean FU of 3.0+0.83 years) and 191.3 years for RVT positive group (with a mean FU of 3.1+0.89 years). The recurrences rates were 0.9% in RVT negative group and 20.2% (p<0.005) in RVT+ group; the number/100 person-year were 1.0 and 30.9 in RVT-negative and RVT+ group (p<0.0005). No differences between groups were found regarding incidence of major bleeding. *Conclusions.* This investigation shows that in patients without RVT, three months of OAT are safe even after an episode of idiopathic DVT. This hold for at least 30% of the entire DVT population and has an important clinical impact; in fact, it is possible to select a group of patients with a very low risk for recurrency over a period of 3 years. This approach carries also a negligible risk for bleeding.

BEST-02

SINGLE TIMEPOINT (WEEK 10) MINIMAL RESIDUAL DISEASE (MRD) EVALUATION IS HIGHLY PREDICTIVE OF LONG-TERM OUTCOME IN UNSELECTED ADULT PATIENTS WITH B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

Peruta B.,[1] Spinelli O.,[1] Tosi M.,[1] Salmoiraghi S.[1], Zanghì P.,[1] Bussini A.,[1] Giupponi D.,[1] Salvi A.,[1] Intermesoli T.,[1] Oldani E.,[1] Borlenghi E.,[2] Terruzzi E.,[3] Cassibba V.,[4] Cortelezzi A.,[5] Gianfaldoni G.,[6] Bernardi F.M.,[7] Mattei D.,[8] Di Bona E.,[9] Romani C.,[10] Scattolin A. M.,[11] Bassan R.,[1] Rambaldi A.[1]

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We recently demonstrated the importance of MRD to predict relapse in adult patients with ALL in a Phase II clinical study of the Northern Italy Leukemia Group. MRD was evaluated at fixed timepoints during induction and consolidation therapy (week 10, 16 and 22), the results confirming the value of a treatment strategy based on MRD risk classification evaluated at weeks 16 and 22. MRD evaluation at week 10 was not a decision-making point in the prospective trial and is herein considered to assess comparatively the predictive value of the earlier timepoint. Two hundreds and eighty patients were enrolled and 253 had sufficient diagnostic material to perform TCR, Ig and translocation analysis in order to identify a sensitive MRD probe.

Table.

Patients (no.)	*TP1-MRD (no.)*	*5-yr OS*	*(P)*	*5-yr DFS*	*(P)*	*% CIR*	*(P)*
All (130)	Neg (55)	0.66	0.0000	0.63	0.0000	34	0.0006
	Pos (75)	0.26		0.18		75	
B-lineage (97)	Neg (36)	0.79	0.0001	0.73	0.0001	22	0.0006
	Pos (61)	0.32		0.21		71	
T-lineage (33)	Neg (19)	0.41	0.0011	0.44	0.0039	56	0.0039
	Pos (14)	0.0		0.0		86	

A total of 308 specific probes were obtained for 223 patients, 30 cases lacked a suitable marker; a single probe was available in 61.8% and two probes in 38.2%; probe sensitivity was > or = 10^{-4} in 89.3%. A MRD-based risk definition was obtained in 112/142 patients who completed consolidation; of these, based on MRD analysis at w16 and 22, 58 were classified MRD^- and 54 MRD^+, this correlating well with long-term outcome. Beside the study model, we analyzed the effects of week

10 MRD in predicting long-term outcome (OS, DFS) and relapse risk (CIR, cumulative incidence of relapse): the analysis was available in 130 patients and correlated strongly with all outcome parameters (Table). When considered separately for B-precursor ALL and T-ALL, these figures remained highly significant in the former subset whereas the correlation with the relapse risk was somewhat weaker in T-ALL. When compared to prospective study model (MRD weeks 16 and 22), week 10 MRD results correlated very well using MRD levels $>10^{-4}$ to define MRD positivity and undetectable MRD for negativity (p=0.001). In conclusion we demonstrated the usefulness of MRD analysis at week 10 as prognostic factor in unselected patients with ALL. This time point may be critical as it defines the response of ALL to a substantial amount of early chemotherapy, indicating the risk of subsequent relapse. For clinical studies, it is important to consider the different CIR rates between T and B lineage MRD-neg ALL: the findings in T-ALL may suggest a different kinetics of relapse and reliability of MRD studies with sensitivity levels of $10^{-4}/10^{-5}$.

BEST-03

RESPONSE TO IMATINIB AND LONG-TERM OUTCOME IN EARLY CHRONIC PHASE CML: THE GIMEMA CML WORKING PARTY EXPERIENCE ON 559 PATIENTS

Castagnetti F.,[1] Gugliotta G.,[1] Breccia M.,[2] Sartor D.,[3] Cavazzini F.,[4] Rupoli S.,[5] Pregno P.,[6] Bruno M.[7] Ferrero D.,[8] Annunziata M.[9] Capucci A.,[10] Palandri F.,[1] Amabile M.,[1] Testoni N.,[1] Marzocchi G.,[1] Poerio A.,[1] Iacobucci I.,[1] Alimena G.,[2] Martinelli G.,[1] Saglio G.,[11] Pane F.,[12] Baccarani M.,[1] Rosti G.[1]

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Background. Imatinib (IM) 400 mg is the standard treatment for Chronic Myeloid Leukemia (CML) in early Chronic Phase (ECP): the results of the IRIS trial have shown for the IM arm a Complete Cytogenetic Response (CCgR) 12-months probability of 69%, with different CCgR rates according to Sokal score (76%, 67% and 49% in low, intermediate and high risk, respectively). At 84 months, overall survival (OS), event-free survival (EFS) and progression-free survival (PFS) were 86%, 81% and 93%, respectively. The outcome was significantly influenced by the Sokal score, but, once the CCgR was obtained, the relative risk did not influenced the probability of subsequent progression. *Aims.* To investigate the response to imatinib and the long-term outcome, overall and by Sokal Risk, of Ph^+ CML patients in ECP.

Table.

	Total	*Low Risk*	*Int. Risk*	*High Risk*	*p*
Patients, n (%)	559	219 (39)	216 (39)	124 (22)	/
Age, median (range)	52 (18-84)	44 (18-69)	61 (18-84)	52 (21-79)	/
CCgR 12M, %	79	85	83	65	< 0.01
MMR 12M, %	54	62	58	44	< 0.01
CCgR overall, %	88	95	94	79	0.11
CCgR overall, %	88	95		79	0.04
MMR overall, %	83	92	89	72	< 0.01
EFS, %	71	79	74	50	< 0.01
FFS, %	81	89	83	56	< 0.01
PFS, %	90	95	89	85	0.02
OS, %	91	96	91	79	0.01

Methods. Between January 2004 and April 2007, 559 patients were enrolled in 3 investigator-initiated, multicentric trials of the GIMEMA CML WP: CML/021 (Clin Trials Gov. NCT00514488), phase II, IM 800 mg in intermediate Sokal risk; CML/022 (Clin Trials Gov. NCT00510926), phase III, IM 400 vs 800 mg in high Sokal risk; CML/023, observational, IM 400 mg. Response monitoring was based on conventional cytogenetic examination of bone marrow cells every 6 months and Q-PCR evaluations (peripheral blood) after 3, 6, 12 months, and every 6 months thereafter. *Definitions:* Major Molecular Response (MMR): BCR-ABL/ABL ratio < 0,1% I.S.. Failures: no CHR at 6 months, no CgR at 6 months, no PCgR at 1 year, no CCgR at 18 months, loss CHR or CCgR, progression and death. Events: failures, treatment discontinuation, refusal and lost to follow-up. All the analysis has been made according to the intention-to-treat. RESULTS: 559 patients were treated with 400 mg (76%) or 800 mg (24%) IM daily. The median follow-up is currently 42 (1-64) months. The CCgR rate was 68%, 79% and 79% at 6, 12 and 18 months respectively. Responses and outcome were significantly different by Sokal score (Table). High risk CCgR patients have a significantly greater probability to fail treatment (ELN criteria) with respect to low and intermediate risk CCgR ones. *Conclusions.* These data confirms IM results in a large nationwide multicentric experience. Sokal risk significantly influences the response and the outcome. Further therapeutic strategies are needed to overcome the adverse prognosis of high risk patients. *Acknowledgements.* European LeukemiaNet, COFIN, University of Bologna and BolognAIL.

BEST-04

PAX5 GENE IS FREQUENTLY THE TARGET OF GENETIC ALTERATIONS IN BCR-ABL1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

Iacobucci I.,[1] Lonetti A.,[1] Ferrari A.,[1] Cilloni D.,[3] Messa F.,[3] Guadagnolo V.,[1] Storlazzi C.T.,[2] Chiaretti S.,[5] Papayannidis C.,[1] Messina M.,[5] Vitale A.,[5] Ottaviani E.,[1] Soverini S.,[1] Arruga F.,[3] Piccaluga P.P.,[1] Paolini S.,[1] Saglio G.,[3] Pane F.,[6] Foà R.,[5] Baccarani M.,[1] and Martinelli G.[1]

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Background. Recently, using single nucleotide polymorphism (SNP) microarrays, a high frequency of genetic alterations of regulators of B-lymphoid development (Mullighan *et al.*, Nature 2008) was described in paediatric B- ALL, but a comprehensive analysis in adults is still lacking. *Aim.* To characterize, by high resolution SNP arrays, the rearrangements on 9p involving the PAX5 locus. *Patients and Methods.* Affymetrix Human Mapping 250K NspI and SNP6.0 arrays and FISH assay were used to profile the genome of 98 adult BCR-ABL1-positive ALL patients. *Results.* The most frequent somatic copy number alterations affecting B-lymphoid development were deletions on 7p12 involving the IKZF1 gene (76/98, 78%), which encodes the transcription factor Ikaros, and mono-allelic copy number changes on 9p chromosome involving the PAX5 gene (31/98, 32%). Overall mono-allelic loss of PAX5 was identified in 28 patients (29%), whereas internal amplification in only 3 cases (3%). Four major PAX5 losses occurred: 1) focal deletions involving only the PAX5 gene (3%) with the minimal overlapping region of 101 kb; 2) deletions involving only a portion of PAX5 and flanking genes (8%) with a median size of 364 kb; 3) broader deletions involving PAX5 and a variable number of flanking genes (11%) with a median size of 947 kb; 4) deletion of all chromosome 9 or 9p (6%). In 23 patients (23%) we identified the deletions of both IKZF1 and PAX5; in 51 patients (52%) only the deletion of IKZF1 was found, while the presence of deletion or rearrangements of only PAX5 gene was found in 5 patients (5%). In two cases we identified the loss of IKZF1 and the gain of an internal region of PAX5. According to the type of deletion we could have PAX5 haploinsufficiency or PAX5 mutants with impaired DNA-binding or transactivating activity. FISH analysis with three overlapping BAC probes encompassing the whole PAX5 gene confirmed what obtained by SNP-array analysis. To investigate the consequences of genomic PAX5 alteration, quantitative PCR (q-PCR) was used, demonstrating that genomic alterations on 9p13.2 lead to a significant down-modulation at the transcript level of the Pax5. *Conclusions.* PAX5 rearrangements occurred at an incidence of about 30% in adult BCR-ABL1 ALL and its impairment may be associated with the development of this poor prognosis subtype of leukemia.

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BEST-05

SUPERIORITY OF RIC ALLOGENEIC TRANSPLANTATION OVER CONVENTIONAL TREATMENT FOR HODGKIN LYMPHOMA PATIENTS RELAPSING AFTER AUTOLOGOUS TRANSPLANTATION: A GITMO RETROSPECTIVE STUDY BASED ON TIME OF HLA-TYPING AND DONOR AVAILABILITY

Sarina B.,[1] Castagna L.,[1] Farina L.,[2] Ciceri F.,[3] Crocchiolo R.,[3] Benedetti F.,[4] Carella AM.,[5] Falda M.,[6] Rigacci L.,[7] Patriarca F.,[8] Bonini A.,[9] Tamiazzo S.,[10] Ferrari S.,[11] Malagola M.,[11] Morello E.,[12] Milone G.,[13] Sorasio R.,[6] Mordini N.,[14] Viviani S.,[15] Giordano L.,[1] Santoro A. Corradini P.[2]

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Hodgkin Lymphoma (HL) patients (pts) relapsing after autologous transplantation (auto-SCT) have a very poor outcome with no chemotherapy options able to obtain a long term disease control.Allogeneic stem cell transplantation (allo-SCT) employing reduced intensity conditioning (RIC) is increasingly used in lymphomas, but no clear data exist on the clinical role of allo-RIC as an effective salvage option. *Aims.* We investigated the role of RIC allo-SCT in HL pts relapsing or progressing after auto-SCT. Our study was structured similarly to an intent to treat analysis: only those pts undergoing a HLA-typing immediately after the failure of auto-SCT were included. The cohort of pts having a donor (donor group) was compared with the one not having a suitable donor (no donor group). *Patients and Methods. 187* pts were retrospectively evaluated. One-hundred and twenty-two pts found a donor and 104 (85%) underwent an allo-SCT: 57 identical siblings, 33 MUD,14 haploidentical family donors. Eighteen pts having a donor did not receive allo-SCT:10 for progressive disease, 5 for refusal and 3 for physician decision. Pts not having a donor (n=65) received chemo and/or radiotherapy according to the policy of each center. The two cohorts of patients were well balanced in terms of clinical features. *Results.* The patient median age was 30 years (16-59). The median follow-up was 46 months (range 1-143). For all pts, the median overall (OS) and progression free survival (PFS) were 29 and 14 months. The 2-year OS and PFS were 56% and 29% respectively. The cumulative transplant-related mortality was 14.6 % for the allo group. The 2-y OS and PFS were significantly better in the donor compared to the no donor group (OS 66% vs 41%, $p<0.001$; PFS 37% vs 12%, $p<0001$). In the univariate analysis and in multivariate analysis, PFS and OS were significantly influenced by the availability of a donor ($p<0.001$) as well as the time from autoSCT to relapse ($p<0.001$). In multivariate analysis, considering only the allo-group, PFS and OS were significantly improved by being in complete remission before allo-SCT and the occurrence of cGVHD. *Conclusions.* This is the largest study comparing RIC allo-SCT vs conventional treatment in HL patients failing an auto-SCT. These data demonstrated the efficacy of RIC-allo-SCT and provided evidence of a graft-versus-lymphoma effect. As expected, the attainment of complete remission before allo-SCT and the occurrence of cGVHD improve the outcome.

BEST-06

RELATIONSHIP BETWEEN TRANSLOCATION T(4;14)(P16;Q32) AND ACHIEVEMENT OF COMPLETE RESPONSE (CR) WITH VELCADE-THALIDOMIDE-DEXAMETHASONE AS INDUCTION THERAPY IN PREPARATION FOR AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT) IN MULTIPLE MYELOMA: ANALYSIS OF GENE EXPRESSION PROFILE

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The recurrent immunoglobulin translocation t(4;14)(p16;q32) occurs in 15% of Multiple Myeloma patients and has been frequently associated with poor prognosis in patients treated either with conventional or high dose chemotherapy. Recently we reported that the presence of this chromosomal alteration raises a rather positive impact on the response to Velcade-Thalidomide-Dexamethasone (VTD) induction therapy (Cavo et al., ASH 2008). In the present study, 199 newly diagnosed MM patients who were randomly assigned to receive VTD in preparation for subsequent double ASCT were analyzed for the presence of t(4;14). In addition, the differential gene expression of $CD138^+$ enriched plasma cells obtained at diagnosis from patients carrying or not t(4;14) was evaluated by means of expression microarray, in order to investigate the molecular mechanisms underlying the response to therapy. Overall, 16% of patients carried t(4;14). On an intention-to-treat basis, the rate of CR and near CR (nCR) to VTD was higher in t(4;14) positive as compared to t(4;14) negative patients (46% vs. 28%, respectively; $p=0.05$). By comparing the lists of genes differentially expressed in responders (e.g. those who achieved CR+nCR) and non responders (NR) according to the presence or absence of t(4;14), we found that the differential expression of 3719 genes characterized CR+nCR vs. NR patients in the t(4;14) positive subgroup. At the opposite, the differential expression of 3182 genes characterized CR+nCR vs. NR t(4;14) negative patients. The intersection of the two lists of genes showed that only 271 genes were common to the two groups of differentially expressed genes. The presence of t(4,14) significantly affected development and differentiation signalling pathways in CR+nCR patients. These findings were associated with the deregulated expression in CR+nCR patients carrying t(4;14) of genes involved in the regulation of Wnt signalling pathway (e.g. MMP7, FZD7, WNT10A, WNT2B, WNT6 and WNT9A), cell cycle progression (e.g. MDM2, CDKN1A and SMAD2) and Hedgehog signalling pathway (GAS1, fused). We suggest that affection of pathways related to development and differentiation in patients carrying t(4;14) might predispose them to more favourably respond to VTD induction therapy. Moreover, novel prognostic factors can be identified, able to more precisely predict the response to VTD induction therapy.

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BEST-07

RITUXIMAB INDUCTION AND MAINTENANCE CHEMOIMMUNOTHERAPY IMPROVE OUTCOME IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

Del Poeta G.,[1] Del Principe M.I.,[1] Maurillo L.,[1] Ragusa D.,[1] Buccisano F.,[1] Giannì L.,[1] Luciano F.,[1] Zucchetto A.,[2] Perrotti A.P.,[1] Venditti A.,[1] Gattei V.,[2] de Fabritiis P.[1] Amadori S.

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The treatment goal of B-CLL has shifted from symptom palliation to the attainment of maximal disease control with purine analogs and monoclonal antibodies. This chemoimmunotherapy resulted in more complete responses (CR) and longer response duration, remaining only a minimal residual disease (MRD) detectable by flow cytometry. We treated in first line 115 B-CLL symptomatic patients (pts), median age 61 years, with six monthly courses of intravenous or oral fludarabine at conventional doses and then with four weekly doses (375 mg/sqm) of rituximab (rtx). Fourteen pts had a low Rai stage, 98 an intermediate stage and 3 a high stage. We defined as high risk pts having at least two of these markers: unmutated IgVH, CD38>30%, ZAP-70>20%, intermediate/unfavorable cytogenetics (trisomy 12 or del11q or del17p). Based on NCI criteria, 88/115 (76%) pts achieved a CR, 23/115 (20%) a partial remission (PR) and 4/115 (4%) no response or progression. Ten pts underwent grade 3 (WHO) infective lung toxicity, 1 patient acute fatal B hepatitis and 2 pts progressed towards Richter's syndrome. Hematologic toxicity included mainly neutropenia (grade 3 and/or 4 in 56 pts) and thrombocytopenia (grade 3 and/or 4 in 8 pts). Fifty pts either in CR with B-CLL bone marrow cells >1% (MRD^+, n=16 pts) or in CR MRD negative, but with B-CLL peripheral cells going up >1000/microl within 1 year after induction (n=19 pts) or in PR (n=15 pts), underwent consolidation and maintenance therapy with four monthly cycles of rtx at 375 mg/sqm followed by twelve monthly low doses of rtx (150 mg/sqm). The median follow-up duration was 52 months. All treated pts experienced a long progression-free survival from the end of induction treatment (51% at 6 years). Nevertheless, CLL pts that underwent consolidation and maintenance therapy (n=50) showed a longer response duration vs MRD^+ not consolidated pts (n=15; 53% vs 9% at 6 years; $p<0.00001$, Figure). Noteworthy, persistently MRD negative (>1 year) pts (n=43) showed a very long response duration (76% at 6 years, Figure). Overall survival (OS) was shorter in MRD^+ not consolidated pts (0% vs 86% at 15 years; $p=0.01$). Of note, within the high risk subset (n=41), consolidated pts (n=18) showed a significantly longer response duration (56% vs 0% at 2.5 years, $p=0.001$) vs MRD^+ not consolidated pts (n=9). Therefore, consolidation and maintenance therapy may improve response duration in B-CLL, also within the high risk subset, thus potentially increasing OS.

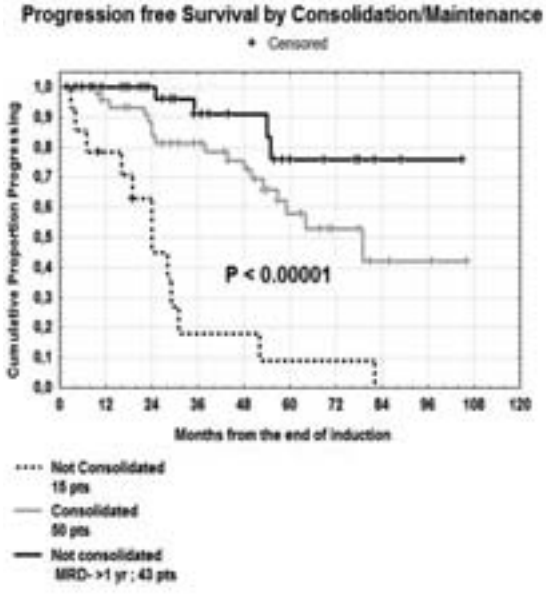

Figure.

BEST-08

FACTORS INFLUENCING THE OCCURRENCE OF SECONDARY MYELODYSPLASTIC SYNDROME/ACUTE LEUKEMIA FOLLOWING HIGH-DOSE THERAPY AND AUTOGRAFT: A GITIL (GRUPPO ITALIANO TERAPIE INNOVATIVE NEI LINFOMI) SURVEY IN 1,347 LYMPHOMA PATIENTS

Tarella C.,[1] Passera R.,[3] Magni M.,[4] Rossi A.,[6] Gueli A.,[2] Benedetti F.,[7] Patti C., Parvis G.,[9] Ciceri F.,[10] Gallamini A.,[11] Cortelazzo S.,[12] Corradini P.,[5] Zoli V.,[13] Leone M,[8] Bosa M.,[9] Barbui A.,[6] Di Nicola M.,[4] Boccadoro M.,[2] Sorio M.,[7] Gianni A.M.,[4] Rambaldi A.[6]

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Introduction. High-dose (hd) therapy with stem cell autograft is an effective treatment for both non-Hodgkin s (NHL) and Hodgkin s Lymphoma (HL). However, the occurrence of secondary malignancies, particularly myelodysplastic syndromes/acute leukemias (sMDS/AL), is a critical issue, representing a major cause of failure in patients potentially cured after hd-chemotherapy. Aim of the study To evaluate: frequency-cumulative incidence-risk factors, of sMDS/AL in a large series of lymphoma patients, treated with the hd-sequential (HDS) chemotherapy approach, followed by peripheral blood progenitor cell (PBPC) autograft. *Patients and Methods.* Data have been collected on 1,347 lymphoma patients treated in the last two decades at 11 Centers, associated to GITIL. All patients received either the original or modified HDS regimens; PBPC were usually collected after hd-cyclophosphamide, or, in a subgroup, after a 2nd round of mobilization with hd-Ara-C. The series included 1,024 B-cell NHL, 234 HL and 89 T-cell NHL; there were 695 high-grade, 278 low-grade and 140 mantle-cell lymphoma; median age was 46 yrs; 57% were male. Overall, 640 (47%) patients received HDS front-line. Most patients were autografted with PBPC (median $CD34^+$ cells: $8x10^6$/kg), few received BM cells; a TBI-conditioning regimen was employed only in 79 patients. *Results.* At a median follow-up of 7 yrs, the 5 and 10 yr Overall Survival (OS) projections are 64% and 56%, respectively. Overall, 53 (3.9%) patients developed s-MDS/AL, with a cumulative incidence of sMDS/AL of 3.1% at 5 yrs and 4.6% at 10 yrs. Median time of s-MDS/AL occurrence was 3.2 yrs since autograft. In univariate analysis, age >45 yrs., male sex and autograft with PBPC of the 2nd mobilization round had a significantly higher risk of sMDS/AL; a trend for a higher incidence was observed in patients receiving HDS with Rituximab ($p=0.092$) and in those presenting with BM involvement ($p=0.075$); on multivariate analysis, age >45 yrs. and autograft with PBPC of the 2nd mobilization round were the only parameters associated with sMDS/AL occurrence (SDHR: 2.74, $p=0.004$ and 2.44, $p=0.002$, respectively). *Conclusions*. The incidence of sMDS/AL observed following HDS is among the lowest reported so far in lymphoma patients treated with hd-therapy and autograft. Moreover, the study suggests that the type of graft employed may be critical for sMDS/AL development. Further analysis are required to understand the sMDS/AL association with the male gender.

BEST-09

EFFECT OF MTOR INHIBITOR RAD001 IN CELLS HARBORING THE JAK2V617F MUTATION

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Somatic mutations in JAK2 and MPL with constitutive activation of JAK2/STAT pathway have been described in chronic myeloproliferative neoplasms (MPN). On the contrary, little is known about phosphatidylinositol 3-kinase(PI3K)/Akt signalling, particulary of the mammalian target of rapamycin (mTOR). mTOR is often abnormally activated in cancer and represents an attractive target for anticancer therapy. We used RAD001, a specific mTOR inhibitor, to evaluate involvement of this pathway in MPN. The IC_{50} of RAD001 in JAK2V617F$^+$ HEL cell line was 0.12 μM, significantly lower than BCR-ABL$^+$ JAK2V617F - K562 cells (13 μM). The IC_{50} of murine Ba/F3 cells transfected with murine JAK2V617F (0.01 μM) was significantly lower than in Ba/F3 cells transfected with wild-type JAK2 (2.6 μM). A reduction of phosphorylated mTOR and 4EBP1 (the main down-stream effector of mTOR) after RAD001 in HEL cell line and in Ba/F3 was demonstrated by Western Blotting. We also found a constitutive activation of mTOR pathway in Ba/F3-V617F cells, as suggested by the phosporylated 4EBP1 in the absence of IL3 as compared to Ba/F3 WT cells. A reduction of phosphorylated STAT5 by Western Blotting and FACS analysis was found in Ba/F3 after treatement with RAD001. Clonogenic assays were performed using mononuclear or CD34$^+$ cells from control subjects or PV and PMF patients: an higher sensitivity to RAD001 of MPN cells compared to control subjects was demonstrated for BFU-E,CFU-GM and CFU-Mk. EEC from PV patients were also most sensitive to a RAD001 (IC_{50} 0.002 vs 0.009 μM). We also found that the ratio of V617F$^+$ versus wild-type BFU-E and CFU-GM colonies in heterozygous PV patients was significantly reduced after the addition of RAD001. In conclusion, we provided evidence for a constitutive activation of mTOR pathway associated with V617F mutation in Ba/F3 cells and for a significant sensitivity to RAD001 of both JAK2V617F mutated cell lines (HEL, Ba/F3) and colonies from MPN patients compared to control subjects. Indirect effects on STAT5 phosphorylation were demonstrated after RAD001 in Ba/F3.These data suggest involvement of mTOR in MPN pathogenesis, possibly mediated by constitutive JAK2V617F activation, and a possible relevance for treatment.

BEST-10

LOSS OF MISMATCHED HLA HAPLOTYPE IN LEUKEMIA UPON ALLOREACTIVE T CELL SELECTIVE PRESSURE FOLLOWING HAPLOIDENTICAL STEM CELL TRANSPLANTATION

Vago L.,[1] Perna S.K.,[1] Zanussi M.,[1] Mazzi B.,[1] Barlassina C.,[2] Lupo Stanghellini M.T.,[1] Perrelli N.F.,[1] Cosentino C.,[2] Torri F.,[2] Angius A.,[3] Forno B.,[1] Casucci M.,[1] Bernardi M.,[1] Peccatori J.,[1] Corti C.,[1] Bondanza A.,[1] Ferrari M.,[1] Rossini S.,[1] Roncarolo M.G.,[1] Bordignon C.,[4] Bonini C.,[1] Ciceri F.,[1] Fleischhauer K.[1]

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Background. Hematopoietic Stem Cell Transplantation (HSCT) from partially-matched family donors is a promising therapy for nearly all patients suffering from hematologic malignancies at high risk of relapse. Donor T cell infusion associated with HSCT is a useful tool to promote post-transplantation immune reconstitution and control minimal residual disease. *Methods*. In five of seventeen patients who suffered clinical relapse of acute myeloid leukemia or myelodysplastic syndrome following haploidentical transplantation and adoptive transfer of donor T cells, we documented and characterized *de novo* mutant variants of the original disease. The genomic rearrangements were studied by Human Leukocyte Antigen (HLA) typing, Short Tandem Repeat (STR) mapping, and Single Nucleotide Polymorphism (SNP) arrays. The post-transplantation immune response against the original and the mutated leukemia was analyzed by Mixed Lymphocyte Cultures. *Results*. Following transplantation, donor-derived T cells mediate a robust patient-specific Graft-versus-Leukemia effect. In turn, this immune response exerts a selective pressure on leukemia *in vivo*, prompting the appearance of mutant variants of the original disease. These *de novo* mutants are characterized by loss of the mismatched HLA haplotype, due to acquired uniparental disomy of chromosome 6p. By losing the HLA molecules targeted by donor T cells, leukemia evades immunosurveillance, a phenomenon responsible for up to one third of clinical relapses documented in our patient series. *Conclusions*. Our data provide the first characterization of a biological mechanism that is frequently responsible for immune escape of leukemia from donor T cells infused in the context of haploidentical HSCT, ultimately leading to clinical relapse.

BEST-11

SHORT TERM CHEMOIMMUNOTHERAPY WITH RITUXIMAB (R)-FND +/- R MAINTENANCE AS FIRST LINE TREATMENT IN ELDERLY PATIENTS WITH ADVANCED FOLLICULAR LYMPHOMA: A PROSPECTIVE RANDOMIZED TRIAL BY INTERGRUPPO ITALIANO LINFOMI

Boccomini C., Ladetto M., Gamba E.,[1] Alvarez I., Baldini L., Ceccarelli M., Chiappella A., Corradini P., De Renzo A., Di Raimondo F., Evangelista A., Gallamini A., Guarini A., Hohaus S., Liberati A.M, Mantoan B., Orsucci L., Parvis G., Petrini M., Pinto A., Pogliani E., Pozzi S., Pulsoni A., Rigacci L., Russo E., Tarella C., Tucci A, Zaja F. and Vitolo U. on the behalf of Intergruppo Italiano Linfomi (IIL)

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Background. In order to maintain efficacy and reduce toxicity of the treatment in elderly follicular lymphoma (FL) patients, we designed a study with a short chemo-immunotherapy. *Methods.* From January 2004 to December 2007, 241 patients (age 60-75) with untreated advanced stage FL were enrolled by 33 IIL centres. Treatment plan was: 4 courses of R-FND (standard doses of Rituximab, Fludarabine, Mitoxantrone, Dexamethasone) every 28 days followed by 4 weekly Rituximab; responding (CR+CRu+PR) patients were randomized between Rituximab maintenance every 2 months for 4 doses or observation. Qualitative and quantitative PCR monitoring for IgH/Bcl-2 rearrangement on bone marrow (BM) was performed at diagnosis, after R-FND and R consolidation and during maintenance/observation. *Results.* 234 patients were eligible for the study: median age was 66 yrs; advanced stage II 14%, stage III 21% and stage IV 65%; BM involvement and B symptoms were documented in 55% and 18% respectively. FLIPI score was: Low 11%, Intermediate 34%, High 55%. One and 2 or more comorbidities were present in 36% and 23% of the patients. Qualitative PCR analysis for IgH/Bcl-2 was performed in 222 patients at diagnosis and 51% were positive. Two hundred and two patients were randomized between maintenance or observation: 32 were not because of: stable/progressive disease (15), adverse events (11) or other causes (6). Overall response at the end of treatment was 86% with 69% CR and 17% PR; PCR negativity at the end of treatment was 74%. Rituximab consolidation was able to induce CR in 36/88 PRs (41%) and to increase PCR negativity from 61% to 74%. With a median follow-up of 18 months, two-years OS and PFS were 92% and 73%, respectively (Figure). Two-years PFS rates according to FLIPI score were 85% for low/intermediate and 65% for high risk (p=0.0002). A total of 1119 courses were delivered; the most frequent CTC grade 3-4 toxicity was neutropenia in 25% of the courses, with only 13 serious infections. One patient developed secondary acute myelogenous leukemia. Two toxic deaths occurred: 1 HBV reactivation and 1 Steven Johnson syndrome. Conclusions: a short term chemo-immunotherapy R-FND + Rituximab consolidation is safe and effective with a good 2-yr PFS rate also in patients with high risk FLIPI score. PCR negativity was achieved in the majority of the BCL2-rearranged patients. Final results of the study will provide insights on the role of Rituximab maintenance after R-chemotherapy.

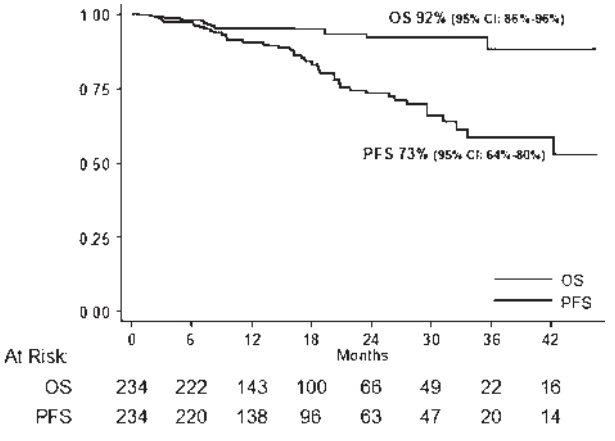

Figure.

BEST-12

AN ANTHRACYCLINE FREE REGIMEN BASED ON CONTINUOUS SEQUENTIAL INFUSION OF FLUDARABINE AND CYTARABINE FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML). RESULTS FROM A PHASE II STUDY ON 130 PATIENTS

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The combination of fludarabine (F) with cytarabine (ARA-C) +/- G-CSF has been proven to be effective in refractory or relapsed AML. In this study, we investigated the efficacy and toxicity of a regimen including F + ARA-C administered as sequential continuous infusion (CI-FLA) in a series of untreated non-M3-AML patients aged over 60 years. F at loading dose of 10 mg/sqm over 15 min at day 0, and after three hours and half ARA-C at a loading dose of 390 mg/sqm over 3 hours were given; at the end, F at 20 mg/sqm/ci/24 hours for a total of 72 hours and ARA-C at 1440 mg/sqm/ci/24 hours for a total of 96 hours were started. G-CSF was added at day +15 at a dose of 5 microg/kg. Patients achieving CR were programmed to receive an additional course of CI-FLA. After consolidation, G-CSF was given from day 15 in order to mobilize $CD34^+$ cells. Between June 2001 and June 2008, 130 patients received the treatment. Median age was 68 years (range 61-81). In 59 patients (45%) an antecedent MDS preceded overt AML. Cytogenetic analysis showed normal karyotype in 69 patients, unfavourable karyotype in 45 cases, no mitoses in 16 cases.Overall, 86 (66%) patients achieved CR, all but two following one course. There were 20 induction deaths (15%), while 24 patients (19%) were refractory. The median number of days to neutrophil > $0.5x10^9$/L and platelet > $20x10^9$/L was 19 (7-34) and 19 (9-38), respectively. Patients needed a median of 3 platelet units (0-19) and 7 blood units (1-38), respectively. Most patients required broad spectrum empiric antibiotic therapy, while intravenous antifungal treatment was needed in 15 % of them. Documented infections occurred in 18 patients (14%). Sixty patients out of 86 (70%) were eligible for consolidation, given at a reduced schedule of 2 and 3 days of F and ARA-C, respectively. Sixty-one patients were evaluated for mobilization, collection being successful in 46 (75%). Median number of $CD34^+$ cells/kg collected was $6.8x10^6$ (2-60.3), median number of apheresis being 2 (1-2). Overall, 38 (29%) patients received autologous stem cell transplantation (ASCT). DFS and OS are 11 and 13 months, respectively. Survival at 5 years is projected to 20%. In conclusion, this study demonstrates that CI-FLA is an effective and well-tolerated regimen for elderly patients with AML, with extremely encouraging results as to CR achievement, $CD34^+$ cell collection and ASCT feasibility. Data compare favorably with conventional anthracycline/ARA-C based therapy.

ORAL COMMUNICATIONS

Non-Hodgkin's Lymphoma I

C001

LONG-TERM FOLLOW-UP OF RITUXIMAB AND INFUSIONAL CYCLOPHOSPHAMIDE, DOXORUBICIN, AND ETOPOSIDE (CDE) IN COMBINATION WITH HAART IN HIV-RELATED NON-HODGKIN'S LYMPHOMAS (NHL)

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Background. The combination of Rituximab plus chemotherapy (CT) is more effective than CT alone in the treatment of high grade NHL. *Objective.* To report the long-term follow-up of CDE plus Rituximab in HIV-NHL. *Methods.* In June 1998, we started a phase II study using infusional CDE (Cyclophosphamide 187.5 mg/m^2/day, Doxorubicin 12.5 mg/m^2/day and Etoposide 60 mg/m^2/day) administered by continuous intravenous infusion for 4 days every 4 weeks and Rituximab 375 mg/m^2 i.v. on day 1. HAART was given concomitantly with CT. *Results.* Seventy-four patients (pts) have been enrolled. The median CD4$^+$ cell count was 161 (range 3-691) and the median Performance Status was 1 (range 0-3). Diffuse large B-cell NHL was diagnosed in 72% of pts and Burkitt in 28%. Seventy per cent of pts had advanced stage (III-IV) disease and 57% of pts had an age-adjusted international prognostic index >2. Fifty-two out of 74 pts (70%) achieved a complete remission (CR), 4/74 (5%) had a partial remission and 18 pts progressed. With a median follow-up of 61 months, only 17% of CRs have relapsed and 41/74 pts are alive. The overall survival, disease free survival and time to treatment failure (TTF) at 5 years were 56%, 81% and 52%, respectively. Only one secondary tumor (acute leukemia) has been observed. No case of late pulmonary or cardiac toxicity has been reported. *Conclusions.* The combination of Rituximab and CDE in HIV-NHL treated concomitantly with HAART is very active. CR rate (70%) and TTF at 5 years (52%) are comparable to those observed in high grade NHL of the general population. Our data confirm that in HAART era a high proportion of HIV-NHL can be cured.

C002

PRALATREXATE SHOWS SYNERGISTIC ACTIVITY WITH THE PROTEASOME INHIBITOR BORTEZOMIB IN *IN VITRO* AND *IN VIVO* MODELS OF LYMPHOID T-CELL MALIGNANCIES

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Pralatrexate (PDX) is an antifolate with greater affinity for the reduced folate carrier (RFC-1). Bortezomib (B) induces apoptosis by inhibiting the 26S proteasome in hematologic malignancies, including non-Hodgkin's lymphoma. We investigated the *in vitro* and *in vivo* activity of PDX±B in a panel of T cell lymphoma and leukemia cell lines including CTCL (H9 and HH) and T-acute lymphoblastic leukemia. Cell viability in *in vitro* cytotoxicity assays was determined using CellTiter-GloTM. Drug interactions were analyzed using the Calcusyn or GraphPad softwares with a combination index (CI) or relative risk ratio (RRR) <1 showing synergism. Apoptosis and caspase 8-9 activation were evaluated by using Yo-Pro-1 plus propidium iodine or active caspase 8-9 staining kits followed by FACSCalibur acquisition. In the *in vivo* xenograft study, SCID beige mice were injected with 2×10^7 HH cells via subcutaneous route and treated with PDX at 15 mg/kg or B at 0.5 mg/kg or the combination of the two drugs on days 1, 4, 8, and 11. In the cytotoxicity assays, PDX+ B at 48 hours showed synergism in H9 (CI≤0.38), P12 (CI≤0.513) and PF382 (CI≤0.352). When the same cell lines were treated with PDX±B (both drugs at 2-6 nanoM) for 48 hours, all the combination groups showed significantly more apoptosis than the single groups and controls. H9: B alone: 26-53%, PDX: 22-58%, B+PDX: 69-89%, RRR≤0.9; P12: B alone: 40-57%, PDX alone: 24-49%, B+PDX: 64-87%; RRR ≤ 0.9; PF382 B alone 72%, PDX alone 65%, B+PDX 94%; RRR≤0.65. The combination of PDX+B also induced a significant increase in caspase 8 and 9 activation compared to the single drugs in H9 (RRR ≤0.81 and ≤0.74 respectively).When the same combinations were explored in peripheral blood mononuclear cells from healthy donors, the combination groups did not show any significant apoptosis compared to bortezomib given alone. In the *in vivo* xenograft study 6 out of 10 mice achieved complete remission (CR) in the combination cohort. No significant weight loss or death was observed in any of the cohorts. Collectively, the data suggest that pralatrexate and bortezomib are synergistic in *in vitro* and *in vivo* models of human T-cell lymphoma without additional toxicity compared with single agents. The combination of bortezomib and pralatrexate may represent a new active regimen for the treatment of T-cell lymphoma and is currently being considered for Phase 1-2 studies.

C003

A PHASE II TRIAL OF RITUXIMAB PLUS CHOP CHEMOTHERAPY FOLLOWED BY YTTRIUM 90 IBRITUMOMAB TIUXETAN (ZEVALIN®) FOR PREVIOUSLY UNTREATED ELDERLY DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS

Fina M.,[1] Tani M.,[1] Stefoni V.,[1] Gandolfi L.,[1] Broccoli A.,[1] Fanti S.,[8] Venturini F.,[1] Pellegrini C.,[1] Derenzini E.,[1] Alinari L[1]., Marchi E.,[1] Quirini F.,[1] Rossi G.,[2] Angelucci E.,[3] Gaidano G.,[4] Petti M.G.,[5] Martelli M.,[6] Vitolo U.,[7] Zinzani P.L.,[1] Baccarani M.[1]

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In 2008 we published a phase II trial abuot the combination of 6 cycles of CHOP plus Zevalin for previously untreated elderly patients with diffuse large B cell lymphoma. The CCR was 95% with OS at 2 years of 95% and PFS at 2 years of 75%. The results of this study support a further evaluation of Yttrium 90 Ibritumomab Tiuxetan (90Y-IT) in combination of chemotherapy. We conducted a prospective, single-arm, non-randomized, phase II trial with CHOP plus Rituximab followed by Zevalin reducing the number of CHOP cycles from 6 to 4 but introducing Rituximab. The rational is to utilize all the therapeutic approaches (chemotherapy, immunotherapy and radioimunotherapy) reducing conventional chemotherapy and probably related toxicity. Patient elegibility was represented by: patients older than 60 years with biopsy proven, untreated, bidimensionally mesurable stage II, III or IV DLBCL. Expression the CD-20 antigen; WHO performance status of 0 to 2. patients were treated with standard CHOP chemotherapy plus Rituximab every 21 days for 4 cycles. Patients were restaged 4 to 6 weeks after completion of 4 cycles of R-CHOP chemotherapy. Patients achieving CR, PR or SD after chemotherapy were eligible for consolidation with 90Y-IT provided the granulocyte count was greater than 1500/microl, the platelet count exceded 100.000/microl and the bone marrow examination at the completion of chemotherapy demostrated no more than 25% involvement with lymphoma. All patients were to receive a single dose of 90Y-IT 14.8 MBq/kg (0.4 mCi/kg). Fifty-five patients have been enrolled: 26 were male and 29 female; the median age was 70 years (range 60-83); 17 were stage II, 38 were stage III-IV. Fifty-one patients had completed the R-CHOP treatment and the overall response rate was 91% including 30 (59%) of patients in CR and 17 (32%) in PR. Treatment was well tolerated; grade 3-4 AEs are comparable with previous experience and the most common grade 3-4 AEs was neutropenia. At this time 43 patients had just received 90Y-IT and 37 are evaluable. Of these patients 28 (76%) are in CR and 4 (11%) are in PR. In particular, 5/12 (42%) patients converted from PR to CR after treatment with 90Y-IT. These preliminary data indicate that radioimmunotherapy (RIT) appears highly effective and feasible as "consolidation" after 4 cycles of immunochemotherapy, improving quality of response without any cumulative toxicity.

C004

IMPACT OF TIME-DEPENDENT VARIABLES ON THE OUTCOME OF SPLENIC MARGINAL ZONE LYMPHOMA: A DYNAMIC MODEL ON 84 PATIENTS

Rattotti S.,[1] Arcaini L.,[1] Pascutto C.,[1] Passamonti F.,[1] Merli M.,[1] Rizzi S.,[1] Lucioni M.,[2] Boveri E.,[2] Rumi E.,[1] Astori C.,[1] Paulli M.,[2] Lazzarino M.[1]

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Splenic marginal zone lymphoma (SMZL) is a low grade B-cell lymphoma, characterized by splenomegaly, lymphocytosis and bone marrow (BM) infiltration. Cytopenias can be present at onset or can develop during the disease: noteworthy anemia belongs to the SMZL score (Blood 2006). We evaluated the dynamic changes of clinical and laboratory parameters and the impact of their modifications on the outcome of disease in a series of 84 pts (38 M, 46 F; median age 62 yrs). 5-years overall-survival (OS) was 70 per cent and median progression-free-survival (PFS) was 30 months. Diagnosis of SMZL was established according to WHO classification 2008 and the proposed diagnostic criteria (Leukemia 2008). We studied peripheral cytopenias, LDH, β2-microglobulin and serum albumin levels (as categorical and continuous variables), degree of splenomegaly and BM infiltration (as continuous variables). PFS was analyzed by univariate and multivariate Cox proportional hazards regression models, with time-dependent covariates. The correlation among clinical and laboratory features was tested with multiple regression model (non-parametric Spearman correlation). In univariate analysis, PFS was influenced by the decrease of hemoglobin (p=0.003) and platelets (p=0.001), by the increase of LDH (p=0.001) and β2-microglobulin levels (p=0.005) (as continuous variables). As time-dependent covariates in multivariate Cox model, decrease of platelets (p<0.0001) and raising levels of LDH (p=0.002) are predictive of a shorter PFS. We applied a multiple regression model to evaluate the relationship of cytopenias with other features of SMZL. Decrease of platelets is directly related with degree of splenomegaly (p<0.0001), with increase of β2-microglobulin (p=0.0001) and reduction of albumin levels (p=0.03). There isn't relationship between platelets level and degree of BM infiltration (p=0.1). Levels of hemoglobin and albumin are highly related variables (p=0.0001), but anemia is related neither with splenomegaly (p=0.3) nor with BM infiltration (p=0.1). In this dynamic model, PFS resulted directly related to time-dependent modifications of lymphoma indexes (LDH, β2-microglobulin), hemoglobin and platelets levels. Thrombocytopenia is related to lymphoma mass: its aetiology seems mainly linked to hypersplenism. Anemia isn't directly caused by hypersplenism or BM infiltration: its relationship with albumin levels could be explained by a progressive dysregulation of cytokine expression.

C005

BORTEZOMIB (B) AND RITUXIMAB (R) COMBINATION IS EFFECTIVE AND SAFE IN THE TREATMENT OF RELAPSED/REFRACTORY INDOLENT NON FOLLICULAR AND MANTLE-CELL NON HODGKIN LYMPHOMA: A PHASE II MULTICENTER STUDY BY INTERGRUPPO ITALIANO LINFOMI (IIL)

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Combination of Bortezomib (B) and Rituximab (R) showed *in vitro* synergistic activity in Mantle Cell Lymphoma (MCL) and Marginal Zone Lymphoma (MZL) cells. The aim of the study was to evaluate safety and efficacy of B and R in relapsed/refractory indolent non follicular and mantle cell lymphoma patients (pts) not eligible to HDC and ASCT. The primary endpoint was to achieve at least ORR >40%. From September 2006 to March 2008, 54 patients were enrolled. Clinical features were: 29 males and 25 females; median age 68 years (range 50-74); 20 lymphocytic/lymphoplasmacytic (LL), 10 MZL and 24 MCL; 45 stage III-IVdisease; IPI score 0-1 36 pts and score 2-3 18; 10 pts had 1 and 44 > 2 prior lines of therapy; 17 pts were R-naïve and 37 R-pretreated; 23 pts had refractory disease (<1 yr from the last therapy) and 31 were in relapse (> 1 yr). The treatment plan was: one course of 4 weekly doses of R (375 mg/sqm) and B (1.6 mg/sqm IV bolus) followed by 2 courses of 4 weekly IV bolus of B (1.6 mg/sqm) as single agent. Responding (CR and PR) and stable disease pts were retreated with further 3 courses with the same schedule. Histological diagnosis was centrally rewiewed. ORR was 25/54 (46%, 95% CI 33.7-59.4): 14 CR and CRu (26%) and 11 PR (20%). ORRs by histology were: 7/20 (35%) in LL, 5/10 (50%) in MZL and 14/24 (58%) in MCL respectively. The ORR was not adversely influenced by R-pretreatment: 18/36 (50%) in R-preatreated pts and 6/17 (35%) in R-naïve. Relapsed pts showed better ORR then refractory ones: 19/30 (63%) and 5/23 (22%) (p=0.002). PFS and OS data are pending. A total of 251 courses were delivered with a median of 4.6 courses per pt. Thirthy-two completed the treatment plan and 22 did not because of: 15 PD during treatment and 7 AE (1 toxic death due to interstitial pneumonitis). Grade 3-4 CTC haematological toxicity was rare with neutropenia in 4% of the courses and thrombocytopenia in <2%. The most frequent extraheamatological toxicity was: grade II and III neurotoxicity in 13 and 5 respectively (recover or return to grade I in all of them but one); infections were detected in 3 (pneumonitis), grade III costipation in 2 and grade > III diarrhea in 5. The combination of R and B is effective and safe in relapsed/refractory indolent non follicular lymphoma and MCL. Major activity was observed in MCL and MZL. This combination may offer a salvage non-chemotherapy treatment in pts not eligible for intensive approaches and warrants future studies. *Disclosure.* Bortezomib was free provided by Jansen-Cilag who gave a research grant to support the study.

C006

COMPARISON BETWEEN DIFFERENT PROGNOSTIC SCORES IN MANTLE CELL LYMPHOMA (MCL) IN THE RITUXIMAB (R) ERA: MANTLE CELL INTERNATIONAL PROGNOSTIC INDEX IS A BETTER PREDICTOR OF THE OUTCOME THAN IPI

Chiappella A.,[1] Puccini B.,[2] Rossi M.,[3] Ferrero S.,[4] Arcaini L.,[3] Audisio E.,[1] Baldi I.,[5] Boccomini C.,[1] Botto B.,[1] Chitarelli I.,[6] Frairia C.,[1] Freilone R.,[1] Ladetto M.,[4] Novero D.,[7] Paulli M.,[8] Rigacci L.,[2] Vitolo U.[1]

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Background. A new prognostic clinical index (MIPI) and a biological one with cell proliferation (Ki-67) evaluation (MIPIb), was shown to give a more reliable estimation of outcome (Hoster 2008). *Aims.* to validate MIPI and MIPIb on a retrospective group of MCL patients treated with R-chemotherapy. *Patients and methods.* Between 1996 and 2009, 136 MCL at diagnosis entered into the study. Histology was centrally reviewed. Clinical characteristics were: median age 62 (37-85) years, 77% stage IV and 15% with blastoid variant. First-line treatments were: R-high-dose chemotherapy in 48 patients, R-fludarabine based chemotherapy in 22, R-CHOP-like in 50 and others R containing regimens in 16. Ki-67 evaluation was evaluable in 93 patients. Overall Survival (OS) and progression-free survival (PFS) curves were estimated both overall and stratified by MIPI, MIPIb and IPI score. Differences between curves were tested using the 2-tailed log-rank test. In order to quantify the predictive discrimination of MIPI, MIPIb and IPI scores, univariate logistic models (with death and progression event as binary outcomes) were fitted and the area under the receiver operating characteristic (ROC) curves (c-index) was estimated.

Table 1.

	2-year OS (%)			p	2-year PFS (%)			p
	LR	IR	HR		LR	IR	HR	
MIPI (n=120)	97	87	51	<.00001	85	80	36	<.0001
IPI (n=122)	88	95	65	.004	79	84	46	.0029
MIPIb (n=93)	95	64	48	.0002	85	47	32	.0001

Results. According to MIPI 42 patients (31%) were at low-risk (LR, 0-3), 36 (26%) at intermediate-risk (IR, 4-5), 42 (31%) at high-risk (HR, >5) and 16 missing. MIPIb was calculated on 93 patients: 70 patients (75%)

were at LR (0-5.699), 7 (8%) at IR (5.7-6.499), 16 (17%) at HR (>6.5). According to IPI 38 patients (28%) were at LR, 40 (30%) at LIR, 44 (32%) at IH-HR and 14 missing. Responses were as follows: complete 74, partial 29, no response 22, not yet evaluable 11. With a median follow-up of 26 months, 2-year OS was 80% (95%CI:71%-87%) and 2-year PFS was 67% (95%CI:57%-75%). Two-year OS and 2-year PFS rates according to MIPI, IPI and MIPIb are shown in Table 1. The c-index for death and failure event were 73% and 65% for MIPI, 66% and 64% for IPI respectively; for MIPIb, assessed in a subsample, were 67% and 62%. *Conclusions.* MIPI score was more predictive than IPI for death event and is more accurate to predict different OS in MCL. New therapeutic strategies are warranted to improve the outcome of MCL namely in HR group.

Acute Leukemias I

C007

MULTICENTER PROSPECTIVE CLINICAL TRIAL WITH LOW DOSE GEMTUZUMAB-OZOGAMICIN PLUS FLUDARABINE, CYTARABINE, IDARUBICIN (GO-FLAI) AS INDUCTION THERAPY OF CD33-POSITIVE ACUTE MYELOID LEUKEMIA (AML) PATIENTS YOUNGER THAN 65 YEARS.

Candoni A.,[1] Martinelli G.,[2] Gherlinzoni F.,[3] Papayanidis C.,[2] Toffoletti E.,[1] Simeone E.,[1] Ottaviani E.,[2] Gottardi M.,[3] Iacobucci I.,[2] Paolini S.,[2] Michelutti A.,[1] Lama B.,[2] Calistri E.,[3] Visani G.,[4] Isidori A.,[4] Piccaluga P.P.,[2] Damiani D.,[1] Russo D.,[5] Baccarani M.,[2] Fanin R.[1]

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Introduction. The role and safety of Gemtuzumab-ozogamicin (GO) target-therapy in first-line induction chemotherapy of AML patients younger than 65 years has not yet been defined. Here we report the preliminary results of a multicenter clinical trial combining low dose of GO with FLAI (Fludarabine, Cytarabine, Idarubicin) regimen. *Patients and methods.* The primary goal of this study was to evaluate the efficacy and the safety profile of FLAI plus GO as induction regimen. Ninety-four consecutive AML patients were included. All patients were younger than 65 with a median age of 50 years (range, 20-65) and CD33 expression exceeded 20% in all cases. The M/F ratio was 48/46, and 69/94 (73%) of patients were poor-risk at diagnosis. The induction regimen (GO-FLAI) included fludarabine (30 mg/sqm) and Ara-C (2 g/sqm) on days 1-5, idarubicin (10 mg/sqm) on days 1, 3, and 5 and GO (3 mg/sqm) on day 6. Hematopoietic stem cell transplant (HSCT) was planned for all high risk AML patients in first complete remission (CR) after consolidation with intermediate doses of Ara-C and idarubicin (ID-AC and IDA). Cytogenetic, multidrug-resistance phenotype, FLT3 and NPM mutation status, WT1 quantitative expression analyses, were performed at diagnosis in all patients. Quantitative WT1 gene expression, cytogenetic (in positive cases) and specific molecular marker analyses were performed after induction to detect and follow Minimal Residual Disease. *Results.* Patients were evaluated for response rate, treatment-related adverse events, overall survival (OS) and disease free survival (DFS). After induction with GO-FLAI, CR rate was 84% (76 of 91 evaluable pts); two patients achieved partial remission and 13 were resistant (Overall response rate 86%). There were only 3 cases of death during induction (DDI 3%). In the setting of patients who achieved a cytological CR after GO-FLAI, the mean of WT1 dropped from 6463±5501 copies/104ABL (at diagnosis) to 117±225 copies/104ABL after induction therapy [$p<0.05$]. The haematological and extra haematological toxicity of GO-FLAI was manageable; 56% of patients experienced transient and reversible GO infusion-related adverse events (especially fever and chills), but no cases of veno-occlusive disease occurred during chemotherapy or after HSCT. After a median follow-up of 12 months (range 1-40), 78/94 (83%) patients are alive (72/78 in CR). The probability of 2-year OS and DFS was 75 and 71%, respectively. Allogeneic and autologus HSCT was performed in 49 (52%) and 12 (13%) patients, respectively. *Conclusions.* The current study confirms that GO-FLAI is an effective and well tolerated induction regimen for CD33 positive AML patients younger than 65 years, with a high complete response rate, good disease debulking, favourable safety profile, low DDI.

C008

IN ACUTE MYELOID LEUKEMIA, THE USE IN INDUCTION OF STANDARD DOSE ARAC IS ASSOCIATED WITH A BETTER QUALITY OF RESPONSE AS COMPARED TO AN INDUCTION REGIMEN CONTAINING HIGH DOSE ARAC

Maurillo L.,[1] Buccisano F.,[2] Spagnoli A.,[2] Del Principe M.I.,[2] Neri B.,[1] Ceresoli E.,[2] Cefalo M.,[2] De Angelis G.,[2] Di Caprio L.,[2] Del Poeta G.,[1] De Fabritiis P.,[1] Lo Coco P.,[2] Amadori P., [2]Venditti A.[2]

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The clinical advantage of high-dose (HD) cytarabine (ARA-C) in induction chemotherapy for acute myeloid leukemia (AML) is still controversial. The purpose of our study was to explore the impact on the "quality of response", of an induction regimen containing standard dose (SD) ARA-C versus HDARA-C, by measuring minimal residual disease (MRD) once CR was achieved. MRD was determined by multiparametric flow cytometry on bone marrow samples collected at the end of induction and consolidation therapy. The threshold for MRD negativity was set below a number of 3.5×10^{-4} residual leukemic cells. We evaluated 110 patients with *de novo* AML, enrolled sequentially in DCE arm of AML10 (n=40) and in AML12 (n=70) EORTC/GIMEMA randomized trials, between 1995 and 2007. In DCE arm of AML10, induction treatment combined ARA-C (100 mg/m^2 day 1-10), etoposide (50 mg/m^2 day 1-5), and on days 1,3,5, daunorubicin (50 mg/m^2). In AML12 trial, patients received the same treatment of DCE arm except for ARA-C dose that was 100 mg/m^2 day 1-10 or 3000 mg/m^2/q12 hrs on days 1,3,5 and 7, according to randomization. As consolidation, all patients received ARA-C (500 mg/m^2/q12 hrs day 1-6) and daunorubicin (50 mg/m^2, day 4-6). Median age was 44 yrs (range 18-60), 64 males and 46 females. Seventy-five patients were treated with SDARA-C regimen and 35 with HDARA-C regimen. The two groups were well balanced in terms of FAB distribution, WBC count, cytogenetics, FLT3 and NPM1 mutation and Pgp-170 expression. After induction, we observed a significantly (p=0.04) higher frequency of MRD negativity in SDARA-C arm versus HDARA-C arm (82% vs 18%, respectively). After consolidation, this figure was confirmed (84% vs 16%, p=0.021). At this stage, 3 further patients treated in the SDARA-C arm, became MRD negative, whereas none of the HDARA-C arm did so. Overall, median level of MRD was significantly lower in SDARA-C group both after induction (1.1×10^{-2} vs 6×10^{-2}, p=0.023) and consolidation (7×10^{-3} vs 2.9×10^{-2}, p=0.021). We identified 4 different groups of patients based on combination of MRD status after consolidation and ARA-C schedule delivered. At 5 years DFS for SDMRDneg, HDMRDneg, HDMRDpos and SDMRDpos was 67%, 33%, 24% and 12%, respectively (p<0.0001). In conclusion, the use of SDARA-C in induction, is associated with a better "quality" of response due to an early leukemic burden clearance, as demonstrated by the more frequent achievement of MRD negative status compared to HDARA-C schema.

C009

SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ARRAYS IDENTIFIED FREQUENT GENOMIC ABNORMALITIES IN GENES INVOLVED IN DRUG TRANSPORT AND INHIBITION OF APOPTOSIS IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS

Iacobucci I.,[1] Ottaviani E.,[1] Testoni N.,[1] Salmi F.,[1] Lonetti A.,[1] Guadagnuolo V.,[1] Papayannidis C.,[1] Ferrari A.,[1] Paolini S.,[1] Astolfi A.,[1] Baldazzi C.,[1] Piccaluga P.P.,[1] Candoni A.,[3] Cilloni D.,[4] F Messa F.,[4] Arruga F.,[4] Russo D.,[1] Lama B., [5] Lo Coco F.,6 Pane F.,7 Saglio G.,[4] Baccarani M.,[1] Martinelli G.[1]

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Introduction. Acute myeloid leukemia (AML) is a heterogeneous disease with various chromosomal aberrations. The karyotype at diagnosis provides important prognostic information that influences therapy and outcome. However, using conventional chromosome banding techniques alone, karyotype abnormalities are detected in only half of all cases. *Aims.* To identify novel genomic regions of interest in normal karyotype AML and to identify novel candidate regions and disease-related genes in patients with complex karyotypes using genome-wide SNP-array. *Patients and methods.* Samples from 70 AML patients with FAB-M0, M1, M2, M3, M4, M5, miscellaneous cytogenetic abnormalities and normal karyotype were examined by SNP arrays (Human Mapping 250K NspI and SNP6.0, Affymetrix). Fluorescence in situ hybridization, quantitative PCR and nucleotide sequencing were used to confirm genomic alterations. Results: A wide spectrum of different genetic lesions (gains/losses) involving complete chromosome arms (del 16q, i(13q10), del 3p, del 7p, monosomy 9) or submicroscopic genomic intervals were identified in a substantial proportion of cases (55%) with a prevalence of gains respect to losses. Focal genetic alterations were detected at the breakpoints of previously cytogenetically identified chromosomal translocations, such as t(2;3)(p22-23)(q26-27) and t(1;11)(p32;q23). Hemizygous deletions were identified at 2q33.3-q34 involving ERBB4 (v-erb-a erythroblastic leukemia viral oncogene homolog 4 avian), at 9p21.3- p21.2 (CDKN2A-2B), at 12p13 (ETV6), at 17q12 (NF1) and 21q21.2 (RUNX1). Most frequent gains affected the oncogene MYC at 8q24 (4.33 Mb), the ABC transporters genes at 17q24, PTPRM at 18p11 and ERG at 21q22. Other recurring genetic lesions were uncommon and were identified only in single cases. Some lesions affected regions with a single gene, such as: ETAA1, FIGN, STK32B, PRAGMIN, PCM1, GLIS3, MRGPRX1, SESN3, BCL2L14 or lacking annotated genes. Marked differences in the combination of copy number anomalies were identified across the different genetic subtypes of AML. *Conclusion*. These data demonstrated that, in contrast to adult acute lymphoblastic leukaemia (ALL), AML is characterized by relatively few recurring copy number alterations, and that spectrum of genetic anomalies is significantly associated with AML disease subtype. Supported by: European LeukemiaNet, AIL, AIRC, FIRB 2006, Fondazione del Monte di Bologna e Ravenna, PIO project 2007, Strategico di Ateneo.

C010

COMPREHENSIVE RISK STRATIFICATION OF ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA BY INCLUDING MINIMAL RESIDUAL DISEASE ASSESSMENT IN PROGNOSTIC ALGORITHMS

Buccisano F., Maurillo L.,[1] Del Principe M.I., Apruzzese A., Del Poeta G.,[1] Panetta P., Irno Consalvo M., Ammatuna E., Fraboni D., Ottaviani L., Sarlo C., Quaresima M., Giannì L., Campagna S., Ditto C., Postorino M., Lo Coco F., Arcese W., Amadori S., Venditti A.

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Prognostic stratification of patients with acute myeloid leukemia (AML) relies on cytogenetic/genetic risk assessment at diagnosis. We have shown by flow cytometry that minimal residual disease (MRD) negativity (bone marrow residual leukemic cells $\leq3.5\times10^{-4}$) at the end of consolidation therapy is independently associated with a significantly longer relapse free survival (RFS) and overall survival (OS). The aim of the study was to develop a comprehensive risk stratification approach integrating information derived from baseline cytogenetic/genetic assessment with those inherent the status of MRD. One hundred and 68 patients affected with non-M3 AML were treated according to the EORTC/GIMEMA protocols AML10/AML12 (age <61yrs) or AML13/AML15 (age >61 yrs). At the end of consolidation, 47 and 121 patients were MRDneg and MRDpos, respectively. Those MRD$^-$ had a significantly longer OS and RFS either in univariate or multivariate analysis (p<0.001 for both). The combined analysis of post-consolidation MRD status, FLT3-ITD and karyotype allows two populations of patients to be recognized (Figure 1): 1) high-risk: with FLT3-ITD mutation, unfavorable-risk karyotype and favorable/intermediate karyotype MRDpos at the post consolidation time-point. For this subgroup the clinical outcome is very poor with long-term OS and RFS below 20%; 2) low-risk: MRDneg at the post consolidation time-point with no FLT3-ITD mutations and/or unfavorable karyotype. This category has a favorable outcome with a long term RFS higher than 60%. Based on these observations, we propose that allogeneic transplant is recommended, not only for poor-risk karyotype or FLT3 positive AML, but also for good/intermediate and FLT3 unmutated categories not gaining MRD negativity, being this option able to provide a superior chance to prolong RFS. Patients belonging to MRDneg good/intermediate and FLT3 unmutated categories, who can

experience a long term survival approaching 60-70%, may have their life expectancy jeopardized by the choice of a therapeutic strategy, such as allogeneic transplant, with a disadvantageous risk/benefit ratio. Thus, the combined evaluation of baseline prognosticators (gene mutations, cytogenetics) and parameters inherent the quality of response (MRD), is useful to refine risk assessment of AML and to facilitate the decision making process in the direction of tailored options which take into account the actual clinical risk of the patients.

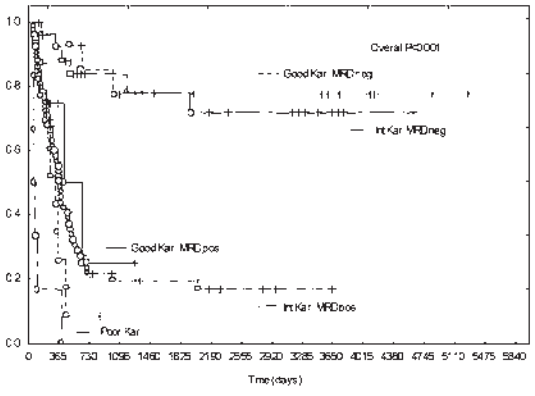

Figure 1. Risk assessment according to FLT3, karyotype and MRD status.

C011

MULTIDRUG RESISTANCE AND APOPTOSIS REPRESENT SYNERGIC MECHANIMS OF THERAPY FAILURE IN ACUTE MYELOID LEUKEMIA

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Background. Gene expression profiling confirmed that multidrug resistance (MDR) factors (P-glycoprotein [PGP], lung resistance protein [LRP], multidrug related protein [MRP]) and apoptosis (bcl-2, bax) are involved in AML poor outcome. The availability of third-generation MDR inhibitors (zosuquidar) and apoptotic molecules (aurora kinase inhibitors), prompted us to strengthen the clinical significance of MDR and apoptosis in AML.

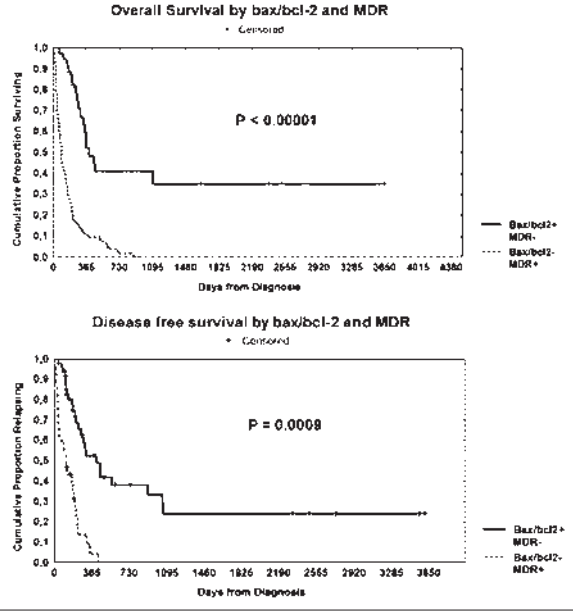

Figure.

Aims. The aims of our research were: 1) to confirm that both bax/bcl-2 (apoptosis) and MDR have relevant clinical impact; 2) to verify their additive and independent prognostic value. *Patients and methods.* We investigated 420 non-M3 AML pts, median age 58 years, treated with intensive chemotherapy regimens. MDR and bax/bcl-2 were performed by flow cytometry, setting cut-off at 20% (PGP), 5% (LRP), 10% (MRP) and 0.35 (bax/bcl2). *Results.* Two hundred-forty four pts (58%) had higher bax/bcl-2, 267 (63%) were PGP+, 223 (53%) LRP$^+$ and 216 (51%) MRP$^+$. Concurrent positivity for PGP, LRP and MRP defined MDR$^+$ subset (112 pts). A significant lower complete remission (CR) rate was found in pts with lower bax/bcl-2 (40% vs 70%, p<0.00001) or higher MDR (35% vs 74%, p<0.00001). Overall survival (OS) and disease free survival (DFS) were shorter in pts with lower bax/bcl-2 (0% vs 20% at 4 years, p<0.00001 and 0% vs 18% at 3 years, p=0.0001) or higher MDR (0% vs 28% at 3 years, p<0.00001 and 5% vs 36% at 2 years, p=0.0001). Interestingly, bax/bcl-2 and MDR showed additive prognostic impact, since higher bax/bcl-2 plus lower MDR identified pts at better prognosis with regard to CR (90% vs 25%, p<0.00001), OS (46% vs 0% at 1 year, p<0.00001) and DFS (41% vs 0% at 2.5 years, p=0.0009) (Figure). Noteworthy, also PGP, LRP and MRP showed additive prognostic impact with regard to OS, since PGP+LRP+MRP$^+$ pts showed a worse outcome vs PGP$^+$ alone (0% vs 10% at 3 years), LRP$^+$ alone (0% vs 8% at 3 years) or MRP$^+$ alone (0% vs 4% at 3 years). The superior independent prognostic value of bax/bcl-2 over MDR was confirmed in multivariate analysis with regard to CR (bax/bcl-2: p=0.0001; PGP: p=0.004), OS (bax/bcl-2: p=0.0001; LRP: p=0.02) and DFS (bax/bcl-2: p=0.0004; PGP: p=0.03; LRP: p=0.03). *Conclusion.* Apoptosis and multidrug resistance are synergic prognostic factors in AML. Multivariate analysis demonstrates that apoptosis has a more relevant clinical impact and therefore therapeutic strategies have to target first apoptosis and then MDR in AML.

C012

ACUTE PROMYELOCYTIC LEUKEMIA (APL) IN PATIENTS AGED OVER 60 YEARS: A MULTICENTER EXPERIENCE OF 34 CONSECUTIVE UNSELECTED PATIENTS.

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Treatment of APL have evolved resulting in cure rate of 75-80% in young-adults. However, the prognosis of older APL patients remains poorer, due to considerable toxicity of either induction or consolidation therapy. Here, we describe a cohort of 34 consecutive APL patients aged over 60 years, with particular emphasis to patients managed outside of a clinical trial, because of comorbidities at diagnosis. In all cases, APL diagnosis was confirmed by molecular identification of the PML/RARa hybrid gene and karyotypic detection of the t(15;17). Median age was 70 years. According to WHO scale, 2 (6%) patients were classified as performance status (PS) 0, 12 (35%) as PS 1, 16 (47%) as PS 2, and 4 (12%) as PS 3. Twenty-seven patients (84%) were affected by a least one concomitant disease requiring specific treatment. According to Sanz prognostic model, 10 patients (29%) were classified as low risk, 17 (50%) as intermediate and 7 (21%) as high risk. Twenty-three patients (68%) fulfilled inclusion criteria of the GIMEMA AIDA protocol and were actually given the programmed treatment, while 11 (32%) received a personalized approach. Causes of exclusion by the protocols were: poor PS in 4 patients, severe cardiomiopathy in 7 and death occurring within 48 hours from diagnosis in 5. Five patients presented with two concomitant reasons for exclusion. In all frail patients, poor PS was judged as unrelated to APL. Median age was 69 years for patients on protocol as opposed to 75 years for the remaining ones (p=0.02). Six patients (18%) died within two days from diagnosis; among these, only one was on protocol. In frail subjects induction and consolidation depended on physician attitude; in particular, 10 patients received only ATRA, while one patient received ATRA + IDA (at reduced dose of 6mg/sqm) because of severe concomitant leukocytosis. Overall, CR was achieved in 68% of cases; CR rate was 74 % for patients on protocol as opposed to 54 % for those out protocol. Most frequent causes of death was cerebral hemorrhage. Patients accrued into AIDA protocol achieved longer survival (median not reached vs. 10 months, p:0.01). In conclusion, our data show that about 30% of older APL patients are not eligible to accrual in multicenter clinical trials; furthermore, in this subset the possibility of early death is substantial. However, when CR is achieved, a personalized consolidation approach can be adopted with possibility of achieving long-term disease control.

Cytogenetics and Laboratory

C013

TET2 DELETION IS AN EARLY EVENT AFFECTING CD34+ HAEMATOPOIETIC PRECURSORS IN AML

Nofrini V.,[1] Crescenzi B.,[1] La Starza R.,[1] Pierini V.,[1] Varasano E.,[1] Gianfelici V.,[2] Testoni N.,[3] Paolini S.,[3] Romoli S.,[1] Beacci D.,[1] Pierini A.,[1] Falzetti F.,[1] Arcioni F.,[1] Martelli M.F.,[1] Mecucci C.[1]

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Background. TET2, a candidate tumor suppressor gene at chromosome 4q24, is reported to be involved in haematological diseases but its role in normal and malignant haematopoiesis remains unclear. *Aim.* To characterise 4q rearrangements by FISH and study haematopoietic lineages affected by cytogenetic or cryptic del(4)(q24) by Fluorescence Immunophenotype and Interphase Cytogenetics as a Tool for Investigations of Neoplasms (FICTION) in patients with haematological diseases. *Materials and methods.* We recruited 8 patients, 1 with leucopenia and normal marrow morphology; 3 with chronic myelomonocytic leukaemia (CMML); 4 with acute myeloid leukaemia (AML). All had cytogenetic or cryptic del(4)(q24). An interstitial deletion was present in 3. The 4q24 band was involved in reciprocal or unbalanced chromosome rearrangements in 5. FISH was performed on bone marrow samples from all patients with 22 DNA clones mapping from 4q21 to 4q26. FISH screened for cryptic del(4)(q24) in 95 patients with diverse haematological diseases: 10 with the 5q- syndrome, 11 with JAK2-positive CMPD, 8 with Ph$^-$ CMPD, 21 with AML, 30 with MDS, 13 with CMML and 2 with MDS/MPD. FICTION on peripheral blood cells from 2 patients (1 CMML in evolution;1 AML) used clone RP11-16G16 labelled in green combined with these monoclonal antibodies labelled in red: anti-CD34 and -CD133 for haematopoietic precursors; anti-CD33, -CD13 and -CD14 for myelomonocytic cells; anti-glycophorin A for erythroid precursors; anti-CD19 and -CD20 for B cells; anti-CD3 and -CD7 for T cells. From 21 to 100 cells were analyzed for each antibody. The cut-off for RP11-16G16 deletion was established at the upper limit from normal controls. *Results.* FISH detected a 4q24 deletion in all 8 cases and identified a common deleted region corresponding to clone RP11-16G16 which encompasses the TET2 gene. Upon screening for cryptic del(4)(q24), all 95 patients were negative. In both cases FICTION showed RP11-16G16/TET2 monoallelic deletion in the following subpopulations: CD34$^+$, CD133$^+$, CD33$^+$, CD13$^+$, CD14$^+$, CD7$^+$, and CD20$^+$. Erythroid precursors, CD19$^+$ and CD3$^+$ cells were involved in the patient with AML. In the other case CD3$^+$ cells were diploid. *Discussion.* Our studies demonstrate: - monoallelic loss of a critical region at 4q24 affects the TET2 gene; - RP11-16G16/TET2 cryptic deletion is rare; - del(4)(q24) may be an early event in MDS/AML, affecting CD34$^+$ haematopoietic precursors in AML.

C014

GENE EXPRESSION PROFILE OF HUMAN NORMAL HEMATOPOIETIC CELLS EXPOSED TO DIOXIN

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an agonist of the aryl hydrocarbon receptor (AhR), a member of the erb-A family that also includes receptors for steroids, thyroid hormones, peroxisome proliferators and retinoids. When bound to dioxin, the AhR binds to DNA and alter the expression of genes including cytokines and growth factors. TCDD has a large number of biological effects such as long-lasting skin disease, cardiovascular disease, diabetes and cancer. An increase in Hodgkin's disease, non-Hodgkin's lymphoma and myeloid leukemia risk was observed after 15 years in the population exposed to dioxin after the 1976 accident in Seveso, Italy. We studied the effect of *in vitro* TCDD exposure on clonogenic growth of CD34$^+$ hematopoietic progenitor cells obtained from healthy volunteers and mobilised by G-CSF. The dioxin dose capable of inhibiting the growth of 50% of the colonies in semisolid medium (IC50) was 20 nM. Furthermore, we analyzed the gene expression profiles of the CD34$^+$ cells before and after exposure to 10 nanomolar TCDD by means of HG-U133A arrays (Affymetrix). Supervised analysis (DNA-Chip Analyzer, dChip 2008) of 4 treated versus 4 untreated samples identified 126 upregulated and 95 downregulated genes significantly involved in cell-adhesion and angiogenesis processes (13%) and also in transcription regulation (12%). Regulation of cell cycle and proliferation (9%), immune response (8%), calcium binding and ion transport (8%), synaptic transmission and visual perception (8%), signal transduction (8%) and tissue development and differentiation (7%) were other consistently modulated functions. Several genes related to fertility and to protein, lipid and carbohydrate metabolic processes, together with genes associated to generation of precursor metabolities and energy resulted also differentially expressed. Interestingly, some proto-oncogenes (9/126, 7%), implicated in specific chromosomal/molecular alterations characterizing several hematological malignancies, such as ABL2, ARHGAP26, ETO and GAS7, were found upregulated after TCDD exposure. Overall, the inhibition of clonogenic potential and the modulation of gene expression induced by TCDD exposure on the CD34$^+$ normal progenitor cells may suggest a role of TCDD in the neoplastic transformation of hematopoietic stem cells supporting the epidemiologic data of increased hematologic cancer risk in the population exposed accidentally to the substance.

C015

MUTATIONAL ANALYSIS OF TET2 IN MYELOID MALIGNANCIES

Varasano E.,[1] Gianfelici V.,[2] Matteucci C.,[1] La Starza R.,[1] Pierini V.,[1] Barba GL.,[1] Crescenzi B.,[1] Nofrini V.,[1] Gorello P.,[1] Brandimarte L.[1] Martelli M.F.,[1] Mecucci C.[1]

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Background. TET2, a putative tumor suppressor gene at chromosome 4q24, codes for two predicted proteins generated by alternative splicing events (long and short isoforms). TET2 undergoes deletions and/or mutations in polycythemia vera (16%), essential thrombocythemia (5%), primary myelofibrosis (17%), systemic mastocytosis (29%), myelodispasia (MDS) (14%) and acute myeloid leukemia (AML) developing from MDS/Chronic MyeloproliferativeDisorders (33%). *Aims.* To investigate whether the second TET2 allele is mutated in patients with monoallelic deletion. *Methods.* We analyzed TET2 mutations in 9 patients with MDS/AML bearing 4q24/TET2 deletions as shown by cytogenetic and FISH studies. Mutations in all coding TET2 exons (NC_000004.10) were detected by PCR-based denaturing high performance liquid chromatography (DHPLC) using a WAVE-MDTMSystem (Transgenomic, Omaha, NE) equipped with a DNASep Cartridge. PCR primers amplified 300-600 bps amplicon sequences. PCR amplicons overlapped all exons to ensure complete coverage. Annealed PCR fragments were injected onto the DNASep HT cartridge for analysis. Electropherograms from patients were compared with normal controls. and abnormal chromatographs underwent direct bidirectional sequencing using ABI prism 3130 (Applied Biosystem). Nonsense and frameshift mutations were detected using Finch TV version 1.4.0. Retrotranscription-PCR (RT-PCR) using specific primers for long and short isoforms investigated mRNA mutations. *Results.* DHPLC profiles were abnormal in 7/9 patients. In 3 patients (33%) direct sequencing detected known TET2 polymorphisms (2 in exon 6; 1 in exon 11). In 4 patients (44%) 2 mutations emerged in exon 3 (nonsense: c.1410C>A, p.C470X; frameshift: c.2222delA,p.N741FsX) and in exon 11 (nonsense: c.4546C>T, p.R1516X; frameshift: c.5342delCA, p.C1780fsX6). Mutations were confirmed in 3/4 cases with available RNA using RT-PCR and direct sequencing (NM_001127208). All mutations produced predicted truncated proteins. *Conclusions.* Our study confirm TET2 loss-of-function mutations are implicated in MDS and AML. Since both alleles were either deleted or mutated in all cases, we hypothesize complete loss of wild-type TET2 is needed for disease pathogenesis. *in vivo* and/or *in vitro* functional studies will elucidate the putative leukemogenic role of predicted TET2 mutated proteins.

C016

HIGH SENSITIVITY OF FLOW CYTOMETRY IMPROVES DETECTION OF OCCULT LEPTOMENINGEAL DISEASE IN HEMATOLOGIC MALIGNANCIES

Del Principe M.I., Quaresima M., Irno-Consalvo M., Fraboni D., Ammatuna E., Panetta P., Pupo L., Gumenyuk S., Di Caprio L., Franceschini L., Postorino M., Del Poeta G.,[1] Maurillo L.,[1] Buccisano F., Lo Coco F., Amadori S., Venditti A

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The morphologic examination of cerebrospinal fluid (CSF) fails to demonstrate malignant cells in up to 45% of patients in whom leptomeningeal disease is thought to be present. Flow cytometry (FCM) is considered to be more sensitive than cytomorphology, but the clinical implications of positive FCM without positive cytomorphology (CM) is not clear. The aims of our study were: 1) to assess the diagnostic accuracy of FCM versus conventional CM and 2) to evaluate the clinical implications of single FCM positivity (FCMpos/CMneg). Since March 2008, CSF samples from 60 patients were obtained after informed consent was given. Thirty-seven males and 23 females, median age 54 years (range 17-75). The haematological diagnoses were acute myeloid leukemia (AML=23), B–lymphoblastic leukemia (B-ALL=14), T-lymphoblastic lymphoma (T-LBL=6), diffuse large cell-NHL (DLC-NHL=16) and plasmacell leukemia (PL=1). Morphological examination was performed on cytospin preparations stained with May-Grunwald Giemsa. Immunophenotyping was performed by four-color immunofluorescence assay. The minimal number of clonally restricted and/or phenotypically aberrant cells to define CSF infiltration by FCM, was 10 clustered events. We found a total of 20/60 cases (33%) FCM positive (AML=9, B-ALL=4, T-LBL=5, DLC-NHL=2): 6 cases were FCMpos/CMpos, while 13 (65%) were FCM^+/CM^-, 1 was FCMpos/CMpos but leptomeningeal localization was unconvincing due to suspected peripheral blood contamination. Of 13 FCM^+/CM^- patients, 8 were AML, 4 T-LBL, 1 B-ALL. Four of these 13 (31%), developed overt CNS disease (T-LBL=3, B-ALL=1), in spite of intrathecal administration of prophylactic liposomal ARA-C. Of note, none of $FCMpos/CM^-$ AML patients experienced overt CNS disease, although no intrathecal prophylaxis was given in these cases. None of FCM negative patients experienced an overt CNS disease. In conclusions, as compared to CM, FCM significantly improves the detection power of leptomeningeal occult localization, allowing clinicians to select patients candidate for pre-emptive intrathecal therapy and additional craniospinal radiotherapy, being prophylaxis not sufficient to prevent CNS disease. In AML, it does not appear clear the clinical relevance of a FCM^+/CM^- condition and possible peripheral blood contamination should be carefully evaluated. On the other hand the schedule of ARA-C dosing might also have a role in preventing overt CNS leukemia in FCM^+/CM^- AML patients.

C017

VARIABILITY OF VERY HIGH PLATELET COUNT: A POSSIBLE CAUSE OF ERROR IN DECISION TREATMENT OF ESSENTIAL THROMBOCYTHEMIA

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Objectives. Quality of high levels platelet count is mandatory for diagnosis, prognosis and treatment of MPNs, in particular in ET. Very high platelet count are associated with increased thrombotic and hemorrhagic risk in ET, frequently found in cases with clonally derived thrombocytosis and JAK2 V617F mutation. Moreover, the international Guidelines for ET treatment fix platelet count as a basic make-decision parameter, besides age and thrombo-hemorrhagic events, to treat with platelet lowering drugs: platelet count needs to be reduced in patients >60ys with a history of major thrombosis/major bleeding, or with a platelet count >1,500×10^9/L, as well as in patients between 40-60 years with platelet count >1,000×10^9/L with cardiovascular risk factor or familial thrombophilia. The target platelet count in patients treated with platelet-lowering therapy is 400-600×10^9/L. Although automated blood cell counters do provide accurate platelet counts in the normal range and even at very low levels, with coefficient of variation below 5%, no data are available on the quality of automated platelet counts at the very high levels seen in ET and some other MPS. *Methods.* We analyzed 31 peripheral blood samples from different patients with platelet count above 600 ×10^9/L with four different routine automated blood cell counters: one dual angle laser (DAL) optical method and three electrical impedance methods. Two of the impedance analyzers could also measure an optical platelet count, used for internal control. Eleven samples were also tested by the ICSH flow-cytometric reference method using anti-CD61 monoclonal antibodies. *Results.* Platelet counts obtained with different methods showed excellent correlation, with r2 higher than 0.9 in all cases, with an evident constant bias depending on the different analytical methods. The DAL method consistently provided results higher than the three impedance methods (p<0.005). The mean of all 31 results was 1,095×10^9/L for DAL and varied between 888-935 for the three impedance methods, with a mean difference ranging from 174-239×10^9/L platelets. Most importantly from a clinical standpoint, 19 of the 31 results were higher than 1,000 with the dual angle optical analyzer, while only 8-10 of them were above 1,000 with the impedance analyzers. Differences tended to increase proportionally to platelet counts. Results obtained with the optical channels of the impedance analyzers did correlated extremely well with the respective impedance counts and, consequently, were significantly lower (p<0.001) than the DAL *Results.* Results obtained with the reference flow-cytometric method correlated poorly with automated platelet counts, especially at the highest levels: differences, however, were lesser on average with the DAL results. *Conclusions.* This study underlines that the problem of analytical variability and incomplete inter-method transferability of high platelet counts is relevant, especially for treatment options in patients with ET and thrombocytemic MPN. In our small series about 50% of patients presenting with more than 1,000×10^9/L platelets with one method, potentially needing a platelet lowering treatment, showed a platelet count below this threshold with three counters based on a different method. Discrepancies could be ascribed to difficulties of counting very large platelets or, more probably, to method-dependent coincidence error.

C018

ANALYSIS OF SEROUS EFFUSIONS AND BRONCHOALVEOLAR LAVAGES FROM PATIENTS WITH HEMATOLOGIC NEOPLASM: COMPARISON OF FLOW CYTOMETRY AND CYTOMORPHOLOGY WITH RETROSPECTIVE CLINICAL ASSESSMENT IN 84 CASES

Cesana C.,[1] Scarpati B.,[1] Brando B.,[2] Volpato E.,[1] Bertani G.,[1] Ferri U.,[1] Faleri M.,[3] Nosari AM.,[1] Scampini L.,[1] Barba C.,[1] Cantoni S.,[1] Lando G.,[1] Nichelatti M.,[1] Morra E.,[1] Cairoli R.[1]

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The differential diagnostic potential of flow cytometry (FC) and cytomorphology (CM) in the analysis of (i) body cavity fluids (BCF) according to different diagnostic settings and (ii) bronchoalveolar lavages (BAL) from patients with hematologic neoplasm (HN) is largely undetermined. Among 1,003 BCF other than cerebrospinal fluid analyzed by four-color FC between 2002 and 2009, we selected those with (i) suspected or known HN at the time of withdrawal, (ii) CM performed on the same sample, and (iii) availability of follow-up findings for retrospective clinical assessment (RCA). FC and CM results (positive or negative for neoplastic cells) were compared to RCA. Uncertain results by CM were excluded from the analysis. Inter- and intra-method comparisons were performed by means of ROC curve analysis and Chi Square test, respectively. Eighty-four samples, 57 serous effusions (SE) and 27 BAL, submitted for suspected (38%) or disclosed (staging: 11%, suspect of relapse/progression: 51%) HN were selected for analysis. During a median follow-up of 4 (interquartile range: 1.7-12.6) months, 24 SE and 8 BAL were found positive by RCA. Overall, 100% specificity was detected for both methods; as compared to CM, FC retained significantly higher sensitivity (87.5% vs 46.2%) and negative predictive value (NPV)(92.9% vs 77.8%)(p=0.0006). The highest FC accuracy (100% sensitivity, 100% NPV) was displayed in the analysis of T-cell precursor non Hodgkin Lymphoma (NHL)/leukemia and of T-cell differentiated NHL. As compared to CM, FC retained significantly higher sensitivity in the subsets of B-cell differentiated NHL (85.7% vs 37.5%; NPV 87% vs 65.5%)(p=0.0006) and B-cell precursor

NHL/leukemia (86.2% vs 41.7%; NPV 83.3% vs 57.6%)(p=0.0005). Similarly, FC displayed a better diagnostic value than CM in the analysis of samples submitted in the suspect of relapse/progression (sensitivity 90% vs 41.2%; NPV 92% vs 67.7%)(p=0.0002). Although FC accuracy in the BAL setting was lower than that displayed in the SE setting (sensitivity 75% vs 91.7%; NPV 90.5% vs 94.3%)(p=0.08), immunophenotyping detected neoplastic cells in 6 out of 8 samples from patients affected by B-cell (n=4) or T-cell (n=4) differentiated NHL, whereas CM gave 100% false negative *Results.* FC is the best diagnostic tool for detecting neoplastic cells in BCF from patients with T-cell lineage lymphoproliferative diseases; a stricking diagnostic advantage is moreover suggested for FC in the analysis of BAL.

Myeloma I

C019

SUPERIOR COMPLETE RESPONSE RATE AND PROGRESSION-FREE SURVIVAL AFTER AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT) INCORPORATING VELCADE-THALIDOMIDE-DEXAMETHASONE (VTD) COMPARED TO THALIDOMIDE-DEXAMETHASONE (TD) IN NEWLY DIAGNOSED MULTIPLE MYELOMA

Cavo M.,[1] Pantani L.,[1] Patriarca F.,[2] Petrucci MT.,[2] Tacchetti P.,[1] Galli M.,[2] Di Raimondo F.,[2] Crippa C.,[2] Bringhen S.,[2] Montefusco V.,[2] Narni F.,[2] Offidani M.,[2] Spadano T.,[2] Olivero B.,[2] Pescosta N.,[2] Caravita T.,[2] Cellini C.,[2] Ledda A.,[2] Falcone A.,[2] Agostini P.,[2] Cavraro M.,[2] Masini L.,[2] Nozzoli C.,[2] Ballanti S.,[2] Derudas D.,[2] De Stefano V.,[2] Pisani F.,[2] Califano C.,[2] Callea V.,[2] Catania G.,[2] Nozza A.,[2] Palmieri S.,[2] Peccatori J.,[2] Baraldi A.,[2] Fattori PP.,[2] Pasini S.,[2] Musto P.,[2] Grasso M.,[2] Ballerini F.,[2] Cangialosi C.,[2] Catalano L.,[2] Morandi S.,[2] Ronconi S. [2] Baccarani M.[1]

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The Italian Myeloma Network GIMEMA designed a phase III study to prospectively compare VTD with TD as induction therapy before, and as consolidation after, melphalan (200 mg/m^2)-based ASCT for symptomatic MM pts with less than 66 years of age. Velcade up-front was given at the standard dose and schedule; in both arms, the daily dose of thalidomide was 200 mg, while the total dose/cycle of dexamethasone was 320 mg. Primary study end point was the rate of complete response (CR) and very good partial response (VGPR) following three 21-d cycles of induction therapy. A total of 474 pts were enrolled in the study and 460 of these (226 randomized to VTD and 234 to TD) were evaluated for primary study end point on an intention to treat basis. In comparison with TD, VTD effected a significantly higher rate of at least VGPR (29% vs 61%, p<0.001), including a fourfold increase in CR rate (p<0.001). Remarkably, no pt treated with VTD had disease progression, as compared to 4.5% of pts on TD (p=0.002). Overall, the discontinuation rate of primary therapy was 4.4% with VTD vs 10.2% with TD (p=0.01). After the first ASCT, the rate of at least VGPR was significantly higher in VTD arm compared to TD arm of the study (77% vs 58%, p<0.001). Benefit from VTD in comparison with TD was statistically significant also in terms of postASCT CR rate (p<0.001). A per protocol analysis of 212 pts who actually received ASCT(s) and two 35-d cycles of consolidation therapy with VTD or TD showed that the rate of CR was furtherly improved of 12% with VTD as compared to 5% with TD. Overall, the postconsolidation CR rate was 57% in VTD arm and 47% in the control arm (p=0.005). After a median follow-up of 15 months, progression-free survival (PFS) for the 226 pts randomized to VTD was significantly superior to that of pts assigned to TD (24-month projected rate: 90% vs 80%, p=0.009), while OS curves were almost superimposable. We conclude that, in comparison with TD, VTD as induction therapy for newly diagnosed MM effected a fourfold increase in the rate of CR before ASCT. Superiority of VTD to TD in terms of CR/VGPR rate was maintained also after ASCT(s) and postASCT consolidation therapy, a gain which ultimately resulted in a significantly longer PFS. In the novel agents era, new highly effective regimens such as VTD have a dramatic impact on both preASCT and postASCT clinical outcomes.

C020

LONG-TERM RESULTS OF THALIDOMIDE AND DEXAMETHASONE (THAL-DEX) AS SALVAGE THERAPY OF MULTIPLE MYELOMA AT FIRST RELAPSE

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Based on the well known activity of thal-dex in advanced phases of MM, we designed a prospective phase II study of this combination as salvage treatment at first relapse after autologous stem-cell transplantation (ASCT) or conventional chemotherapy. By study design, thal was started at the dose of 100 mg/daily for two weeks and then escalated to 200 mg/daily, provided that the initial tolerance was acceptable. Otherwise, thal was continued at the initial dose of 100 mg/daily until progression. Dex was given at a monthly dose of 160 mg. No thromboprophylaxis was administered to the first 60 patients who entered the study. However, after the awareness of the thrombogenic activity of thal-dex

in newly diagnosed MM, fixed low-dose warfarin was instituted in the subsequent 40 patients. A total of 100 patients were enrolled; their median age was 62 years. Median time between diagnosis of MM and the start of thal-dex therapy was 34 months. In 72% of the patients first line therapy included ASCT, either single (30%) or double (42%); the remaining 28 patients had previously received conventional chemotherapy. 59% of the patients received a fixed thal dose of 100 mg/daily over the entire period of the study. Thal-dex was given for a median of 14 months. The most frequent adverse events were constipation (42%, grade III 8%), peripheral neuropathy (58%, grade III 5%), bradycardia (20%, grade III 0%) and skin rash (11%, grade III 1%). VTE was recorded in 7 patients (3 out of thromboprophylaxis), at a median of 8 months (range 3-11) from the start of thal-dex therapy. Discontinuation of thal due to toxicity was recorded in 8 patients after a median of 12 months. On an intention to treat basis, 46% of patients achieved at least a PR, at a median time of 3 months from the start of thal-dex treatment. The median duration of response (DOR) was 28 months, while the median time to next therapy was 15.5 months. With a median follow up of 25 months, median OS, TTP and PFS were 43, 22 and 21 months, respectively. TTP and PFS were significantly longer for patients responding to thal-dex therapy (34 months vs 15 months, p=0.005, and 28 months vs 12 months, p=0.001, respectively). In conclusion, low dose thal-dex was an effective treatment of MM at first relapse, yielding DOR, OS and EFS comparable to those reported with other novel agents when used in the same setting of patients. Low-dose thal-dex was generally well tolerated, as reflected by the long stay on treatment in the absence of progression (median: 25 months) and a low discontinuation rate (8%)due to adverse events.

C021

CONSOLIDATION WITH BORTEZOMIB, THALIDOMIDE, AND DEXAMETHASONE IN PATIENTS WITH MYELOMA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: CLINICAL AND MOLECULAR RESULTS

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Background. PCR monitoring of Minimal Residual Disease (MRD) in Multiple Myeloma (MM) has shown persistence of MM clone after autologous Stem Cell Transplantation (ASCT), while in allo-SCT Molecular Remission (MR) can be obtained and is associated with an improved outcome. Aim of this study was to investigate the antitumor effect of a consolidation treatment with Bortezomib/Thalidomide/Dexamethasone (VTD) in patients achieving a good clinical response after ASCT. *Patients and methods.* Thirty-nine newly diagnosed MM patients achieving at least Very Good Partial Response (VGPR) after ASCT and with a molecular marker were enrolled in this study. Each VTD cycle consisted of: Bortezomib 1.6 mg/m^2 as IV injection once weekly (days 1, 8, 15, and 22) followed by a 13-day rest period (days 23-35); Thalidomide at the initial dose of 50 mg/day PO once daily, with increments up to 200 mg; Dexamethasone 20 mg/day PO once daily, on days 1-4, 8-11, and 15-18, followed by a 17-day rest period (days 19-35). A total of four VTD cycles were planned. MRD was assessed on Bone Marrow samples at diagnosis, study entry, after two VTD courses, after four VTD courses and then every 6 months. PCR analysis was carried out using IgH-R-derived patient-specific primers as already described (Ladetto *et al.*, Biol Bone Marrow Transpl 2000). *Results.* Thirty-nine patients were enrolled and were evaluable at study entry. Thirty-three patients (85%) were in VGPR and 6 (15%) in CR. One patient was PCR negative. At the end of VTD, the CR rate increased to 49% and 6 patients (18%) became PCR negative. Thirty-one patients (80%) completed the 4 VTD courses. Nine patients went off-study (6 adverse events, 2 consent withdrawal and 1 died for progressive disease). Twenty-one patients (54%) had at least one grade 3-4 toxic event (the most common were fatigue and infections). After a median follow-up of 42 months (range, 27-75), 5 patients (13%) had died and 11 (28%) had relapsed or progressed. No clinical relapse has been so far observed in MR patients. Estimated median PFS is 60 months and 3-year OS is 89%. Quantitative PCR has been performed on 20 patients: median tumour bulk at diagnosis was 157000 (35-925000) IgH-R/10^6 diploid genomes (dg), it shrunk to 440 (3-420000) following auto-SCT and to 17 (0-113000) following VTD. *Conclusion.* VTD consolidation is active on residual plasma cells surviving ASCT and improves the quality of remission in MM patients even in case of optimal response to ASCT.

C022

BORTEZOMIB, MELPHALAN, PREDNISONE AND THALIDOMIDE (VMPT) VERSUS BORTEZOMIB, MELPHALAN AND PREDNISONE (VMP) IN ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS: A PROSPECTIVE, RANDOMIZED, PHASE III STUDY

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Background. In newly diagnosed myeloma patients (pts) the combination of VMP was superior to MP. In relapsed-refractory pts the 4 drug combination of VMPT induced a high proportion of CR. *Aims.* In this trial, we compared VMPT and VMP. The primary end point was PFS. *Methods*. Pts (N=511) older than 65 years were randomly assigned to receive VMPT or VMP. Initially, pts were treated with nine 6-week cycles of VMPT (V 1.3 mg/m^2 days 1,4,8,11,22,25,29,32 in cycles 1-4 and days 1,8,22,29 in cycles 5-9; M 9 mg/m^2 days 1-4; P 60 mg/m^2 days 1-4 and T 50 mg days 1-42, followed by V 1.3 mg/m^2 every 15 days and T 50 mg/day as maintenance) or VMP (at the same doses and schedules previously described without maintenance). In 2007, the protocol was amended: both VMPT and VMP schedules were changed to nine 5-week cycles and V schedule was modified to weekly administration (1.3 mg/m^2 days 1,8,15,22 in cycles 1-9). *Results.* Pts characteristics were similar in both groups, median age was 71 years. 221 pts for VMPT and 229 pts for VMP were evaluated. The VGPR rate was higher in the VMPT group (51% versus 42%, p=0.06), including a CR rate of 35% in the VMPT group and 21% in the VMP group (p<0.0001). In the subgroup treated with weekly infusion of bortezomib, VGPR was 50% for VMPT and 39% for VMP (p=0.06), including 32% CR for VMPT and 20% for

VMP (p=0.01). After a median follow-up of 14.8 months, the 2-year PFS was 76.1% in the VMPT pts and 70.0% in the VMP pts (p=0.13). In pts who received weekly infusion of bortezomib, the 2-year PFS was 76.8% in the VMPT pts and 75.5% in the VMP pts (p=0.50). Factors predictive of longer PFS were age ≤75 years (p=0.006) in VMPT but not in VMP and the achievement of VGPR in both groups (p=0.02 and p=0.004). The 3-year OS was 89.6% in the VMPT pts and 88.6% in the VMP pts (p=0.81). The incidence of grade 3-4 adverse events was similar in the VMPT group and in the VMP group: neutropenia (28% vs 28%), thrombocytopenia (19% vs 16%), peripheral neuropathy (13% vs 13%), infections (12% vs 7%), and gastrointestinal complications (5% vs 7%), respectively. The weekly infusion of bortezomib significantly decreased the incidence of grade 3-4 peripheral neuropathy to 6% and 7%. Conclusion: VMPT is superior to VMP in terms of response rates. Longer follow-up is needed to assess their effects on PFS and OS. The weekly infusion of bortezomib significantly reduced the incidence of peripheral neuropathy. These data will be updated for the meeting.

C023

FRONT-LINE CHEMOTHERAPY COMBINATIONS INCLUDING BORTEZOMIB IMPROVE RESPONSE RATE AND MAY POSITIVELY AFFECT SURVIVAL IN PRIMARY PLASMA CELL LEUKEMIA

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Aim of the study. To evaluate the role of chemotherapy combinations including bortezomib as first line treatments in primary plasma cell leukemia (PPCL), a rare variant of multiple myeloma, characterized by very poor prognosis. *Methods*. A retrospective survey performed in 21 hematologic Italian Institutions of previously untreated patients with PPCL who had received bortezomib associated with other agents for the initial treatment of their disease. *Results.* Twenty-nine unselected and previously untreated PPCL patients were enrolled, twenty-five of them are currently evaluable (M/F ratio 1.2; mean age 61 years, range 47-82). Circulating plasma cells ranged from 15 to 88% (mean 37%). Median WBC count was 18.3×10^9/L (range 2.7-81). Variable degrees of renal impairment were observed in 11 patients (44%). Five patients had concomitant extramedullary disease (20%). Cytogenetic/FISH abnormalities were observed in 12 out of 16 evaluated patients (75%), the most frequent being complex caryotype and del13q. Bortezomib was generallt given using the standard schedule of 1.3 mg/sqm days 1, 4, 8, 11, with an interval of 10 days between cycles. Nine patients received bortezomib in combination with dexamethasone and thalidomide (VTD), six with dexamethasone alone (BD), four with doxorubicin and dexamethasone (PAD), two with oral melphalan and prednisone (VMP), two with doxorubicin, dexamethasone and vincristine (PAD-V), one with melphalan, prednisone and thalidomide (VMPT) and one with cyclophosphamide and dexamethasone (BCD). A total number of 92 cycles was administered (mean 3.7, range 1-9). After bortezomib containing induction therapy, five patients underwent double (n.3) or single (n. 2) autologous stem cell transplantation (AuSCT), 3 patients underwent AuSCT followed by reduced intensity (RIC) allogeneic stem cell transplantation (Allo-SCT, one with an unrelated donor), while one patient underwent myeloablative Allo-SCT. One patient failed to collect peripheral blood stem cells. According to the International Uniform Response Criteria, ten PR, two VGPR, and eight CR were achieved (overall response rate: 80%). Renal failure improved or completely disappeared in 10/11 patients. After a median follow-up of 17 months, 13 patients are alive (52%): eleven out of them remain in remission phase, two relapsed after 16 and 31 months, respectively. Ten out of the 12 deceased patients did not receive stem cell transplantation. Grade 3-4 hematological, neurological, infectious and renal toxicities occurred in 3, 5, 4 and 1 patients, respectively.

Conclusions. These findings suggest that chemotherapy combinations including bortezomib are feasible and effective first line induction treatments for PPCL, in particular in patients eligible for stem cell transplantation.

C024

TARGET-RELATED THERAPEUTIC STRATEGIES IN MULTIPLE MYELOMA

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Pre-clinical studies have identified pathways and key targets for multiple myeloma (MM) cell survival, proliferation and chemoresistance. We have previously demonstrated that the selective MEK/ERK inhibitor PD0325901 exerts a potent growth-inhibitory action in pre-clinical MM models with effects mostly related to the arrest of cell cycle progression rather than to apoptosis induction (Blood 2006, 108: 254). More recently, we have gained evidence that ABT-737, a Bcl-2/Bcl-xL inhibitor (kindly provided by Abbott Laboratories), shows a potent *in vitro* growth-inhibitory and pro-apoptotic activity on MM cell lines and on primary CD138+ bone marrow cells from MM patients, regardless of the disease status (Haematologica 2008; 93[suppl. 2]: P195). Since it has been recently reported that statins induce apoptosis in MM cells, by regulating several signaling pathways, including the MEK/ERK module, we here analyzed the activity of PD0325901 in combination with ABT-737 or with Mevinolin on cell proliferation and apoptosis in MM cells. Both PD0325901/ABT-737 and PD0325901/Mevinolin co-exposure resulted in a synergistic arrest of cell growth, with combination indexes (CI), as measured by isobologram analysis (Chou–Talalay method), of 0.12 and 0.15, respectively, for KMS27. As single agent, PD0325901 mainly exerted cytostatic effects without affecting apoptosis, while ABT-737 or Mevinolin induced apoptosis only at high concentrations. However, a greater than 50% net apoptosis induction was observed when combined treatments were used at concentrations that induced minimal apoptosis as single agents. Mitochondrial membrane depolarization was similarly enhanced by the combination strategies. Conversely, in the PD0325901 resistant MM cell line ARH-77, ABT-737 and Mevinolin were still able to induce apoptosis, but their effects were not significantly potentiated by MEK inhibition. In conclusion we demonstrated in MM cell lines a remarkable synergistic pro-apoptotic activity by the simultaneous disruption of MEK/ERK and Bcl-2 or mevalonate signaling. These results support the role of these pathways as prime targets for the molecular therapy of MM and highlight the concept that targeting of multiple signaling pathways may induce highly synergistic antineoplastic effects. Additional analyses on primary MM cells are warranted to determine the efficacy of these combinations for clinical use.

Chronic Myeloid Leukemia I

C025

CHROMOSOME ABNORMALITIES ADDITIONAL TO THE PHILADELPHIA CHROMOSOME AT THE DIAGNOSIS OF CHRONIC MYELOID LEUKEMIA. PATHOGENETIC AND PROGNOSTIC IMPLICATIONS.

Zaccaria A.,[1] Testoni N.,[2] Valenti A.M.,[3] Luatti S.,[2] Tonelli M.,[3] Marzocchi G.,[2] Cipriani R.,[1] Baldazzi C.,[2] Giannini B.,[3] Stacchini M.,[2] Gamberini C.,[2] Castagnetti F.,[2] Rosti G.,[2] Azzena A.,[4] Bernardi F.,[5] Cavazzini F.,[6] Cianciulli A.,[7] Dalsass A.,[8] Donti E.,[9] Giugliano E.,[10] Gozzetti A.,[11] Grimoldi M.G.,[12] Manna M.,[13] Modaferri B.,[14] Piovani G.[15] Ronconi S.,[16] Santoro A.,[17] Santulli B.,[18] Spedicato F.,[19] Zanatta L.,[20] Baccarani M.[2] on behalf of the GIMEMA WP on CML

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Background. In previous papers, we reported that additional chromosome abnormalities (ACAs) present at diagnosis in Chronic Myeloid Leukemia (CML) patients, could persist after the disappearance of the Ph during Imatinib therapy, thus demonstrating the secondary origin of the Ph in these cases.[1,2] We tried to verify the real incidence and the possible prognostic value of this finding in a larger population of patients. *Patients and Methods.* Thirty-six patients were the object of this study. Twenty-one were collected among the 506 ones included into three multicentric national trials (CML 021, CML 022, CML 023) of the GIMEMA Working Party (WP) on CML; moreover, 15 more patients, not included in the trials, were collected from 9 of the 54 Cytogenetics Laboratories included in the GIMEMA WP. All of them were treated either initially or during the course of the disease with Imatinib. Karyotypes were performed according to methods described in a previous paper[3] and chromosomes were classified according to ISCN 2005.[4] *Results*. Table 1a shows the distribution of the patients according to Sokal Score and their response to Imatinib. We divided the patients into 3 groups: 1) patients in which ACAs persisted after therapy in Ph negative cells; 2) patients in which ACAs were always present together, and disappeared with, the Ph in case of response; 3) patients with the same characteristics of group 2 and no response, with persistence of ACAs together with the Ph. Table 1b shows the type of chromosome abnormalities and their outcomes. *Discussion.* The persistence of ACA in Ph-negative cells during Imatinib treatment (patients n.1 to n.6) suggests that they occurred as primary events in a genetically unstable cell.[1,2] In the other patients, the occurrence of the Ph chromosome was clearly a primary event, further on documented by the detection of metaphases with Ph as the sole abnormality in 5 patients. Moreover, based on the data reported here, we can point out that: 1) The occurrence of ACA as a primary event is a rather unusual phenomenon. 2) Sokal Score maintains its unfavourable prognostic value in patients in which Ph occurs as a primary event and not in those in which it occurs as a secondary one.[5,6] 3) The type of ACAs may influence the outcome of the disease, possibly inducing resistance to Imatinib due to the activation of different pathways of disease progression.[7]

Table 1. a) Distribution of the patients according to response to Imatinib and Sokal Score. b) Correlation between chromosome abnormalities and cytogenetic response.

a)

	Patients (N°)	*Sokal score (H/I/L)*	*Complete cytogenetic response (CCR) (N°)*	*Continuous CCR (months)*	*Molecular Relapse*	*Evolution acute leukemia blastic crisis*
1° GROUP	6	5/1/0	5	4 (31+ - 61+)	0	1
2° GROUP	22	5/7/10	21	19 (3+ - 48+)	2	0
3° GROUP	8	5/1/2	0	0	/	2

b)

Chromosome abnormlity	*Patients (N°)*	*Sokal score (H/I/L)*	*CCR (N°)*	*Continuous CCR*
-Y	12	4/4/4	9	8
+8	5	2/1/2	4	2
Single	9	5/1/3	7	7
>2 Chromosome changes	6	3/1/2	0	0
5q-	2	2/0/0	1	1
+19	2	0/1/1	1	1
+Ph	2	0/1/1	1	1

C026

PHASE II MULTICENTRIC EXPLORATIVE STUDY OF INTERMITTENT IMATINIB (IM) TREATMENT (INTERIM) IN ELDERLY PATIENTS WITH PH+ CHRONIC MYELOID LEUKEMIA (CML) WHO ACHIEVED A STABLE COMPLETE CYTOGENETIC RESPONSE (CCGR) WITH STANDARD IM THERAPY

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Background. Standard therapy with Imatinib (IM) significantly prolongs the survival of Ph+ CML patients who obtain a complete cytogenetic response (CCgR). Elderly patients (i.e. > 65 yrs) have similar cytogenetic responses and survival, but they usually show a low compliance. *Aims*. The aim of the study is to investigate if CCgR that has been achieved with standard (daily administration) IM therapy can be maintained with the same dose of IM given intermittently (INTERIM). The study population is represented by elderly patients (>65 years old) with Ph+ CML and with stable CCgR after at least 2 years of standard IM therapy (daily administration). *Methods*. IM is given at the same dose that

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was given at the time of enrollment by the following intermittent schedule: 1 week on / 1 week off for the 1st month; 2 weeks on / 2 weeks off for the 2nd and 3rd month; 1 month on / 1 month off from the 4th month thereafter. The CgR status will be evaluated at baseline (by conventional cytogenetics on bone marrow and FISH on peripheral-blood) and every 3 months during the study (only by FISH on peripheral-blood). Quantitative molecular assessment of BCR-ABL transcript by RQ-PCR on peripheral blood is due at baseline and every 3 months during the study and mutational analysis of ABL will be performed in case of loss of CCgR. If FISH documents a variation of the baseline value of more than 1% in two consecutive examinations, evaluation of marrow cells metaphases will be performed to confirm the loss of CCgR and to check for additional cytogenetic abnormalities. In case of loss of CCgR INTERIM will be stopped and standard therapy (daily administration) will be resumed. After 12 months, the patients who are in continuous CCgR are advised to continue the intermittent study schedule and to be followed indefinitely. *Results.* One-hundred and fourteen patients have been considered eligible, but 17 (15%) refused to enter into the protocol. Out of 97 enrolled patients, 84 started INTERIM, 4 patients (4%) went off the study for major protocol violation before the 3rd month and, at present, 80 patients are ongoing. Of these 80 patients, 40 and 17 completed the 3rd and 6th month, respectively. At the 3rd month, all the 40 evaluable patients maintained the CCgR. As detected by RQ-PCR, 37/40 (92.5%) maintained at least major molecular response (MMR) and 3/40 (7.5%) showed at least a 1 log increase of BCR-ABL ratio. At the 6th month, all the patients maintained the CCgR; as detected by RQ-PCR, 15/17 (88%) maintained at least a MMR, 1 patient maintains a stable disease (2 logs increase with respect to baseline) and 1 patient is not evaluable. *Conclusions.* These preliminary data suggest that IM given intermittently can be sufficient to maintain the CCgR in those patients who have a stable CCgR, previously achieved with standard IM therapy. *Acknowledgments*. This work was supported in part by CML-Leukemia Net

C027

HETEROGENEOUS MECHANISMS AT THE BASIS OF 5'BCR/3'ABL FUSION GENE GENERATION IN CHRONIC MYELOID LEUKEMIA

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The t(9;22)(q34;q11) is found in more than 90% of patients with chronic myelocytic leukemia (CML). About 5-10% of CML patients show variant translocations, involving one or more chromosomes in addition to 9 and 22. In a subset of CML patients the 5'BCR/3'ABL fusion gene is generated by cryptic rearrangements such as nonreciprocal insertions between chromosomes 9 and 22. Moreover, microdeletions on the der(9) chromosome or on the third derivative chromosome involved in variant t(9;22) have been recently described and appeared to be a valuable prognostic factor. To our knowledge, an accurate frequency assessment of different mechanisms at the basis of fusion gene generation or of concomitant chromosomal rearrangements other than the t(9;22) has never been performed. The aim of this study was to perform a detailed molecular cytogenetic characterization of a large series of CML patients to unveil the biological heterogeneity of CML. 404 CML patients in chronic phase were analyzed by conventional cytogenetic analysis and by FISH experiments with "home-brew" probes specific for ABL and BCR genes. Breakpoints characterization and deletions size definition were carried out with additional bacterial artificial chromosome (BAC) and Phage P1-derived artificial chromosome (PAC) probes selected according to the University of California Santa Cruz (UCSC http://genome.ucsc.edu/index.html; March 2006 release) database. Our study identified 43 (10.6%) out of 404 CML cases showing heterogeneous chromosomal mechanisms accountable for generation of the 5'BCR/3'ABL fusion gene. These cases could be classified in three main groups: i) cases with variant chromosomal rearrangements other than the classic t(9;22) (8.9%), showing a "masked der(9)" (7.4%) or a "masked Ph" chromosome (1.5%); ii) CML cases with cryptic insertions of ABL into BCR, or vice versa (1.5%); iii) CML cases bearing additional concomitant chromosomal rearrangements (1.2%). This study provides an outline of the frequency and molecular features of the most relevant cytogenetic groups identified in CML patients. A clear division has been made between cases with variant t(9;22) rearrangements, cases with cryptic insertions, and patients with additional concomitant chromosomal rearrangement other than the presence of 5'BCR/3'ABL. However, the biological significance and the prognostic impact of the cytogenetic molecular heterogeneity occurring in the generation of the 5'BCR/3'ABL fusion gene remain to be clarified.

C028

NO IMPACT OF AGE ON OUTCOME OF EARLY CHRONIC PHASE, PH-POS CML, IMATINIB TREATED PATIENTS: A NATIONWIDE ANALYSIS ON 559 CASES OF THE GIMEMA CML WP

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Background. Age was a consistent poor prognostic factor in CML before the introduction of imatinib (IM). The efficacy and tolerability of IM reduced the impact of age on outcome in early and late chronic phase (ECP; LCP) patients. However, few data are available for older pts in ECP and still the allocation to IM of these pts is not a widely accepted practice. *Aim.* To asses the effects on the responses and outcome of IM in ECP, older pts. *Methods.* We performed an analysis by age groups of 3 concurrent clinical trials of the GIMEMA CML Working Party (CML/021, Clin Trials Gov. NCT00514488; CML/022, NCT00510926; and the observational trial CML/023). Overall, 559 pts have been enrolled between 04/2004 and 04/2007 and treated with IM 400 mg (76%) or 800 mg (24%) daily; the median age was 52 (extr.18-84) yrs, the median follow-up is currently 42 (extr.1-64) months. WHO has set at 65 yrs the older age: at diagnosis, 444 pts (79%, median age 46 yrs) were <65 yrs (group A) and 115 pts (21%, median age 71 yrs) ≥65 yrs (group B). The same proportion in both groups (23% and 25%, respectively) received 800 mg daily IM front-line. The Sokal risk distribution, as expected, was different in the 2 groups A and B: low in 47% / 9%, int. in 30/72%, high in 23/19%. *Results.* In group A and B, the cumulative CCgR rate and the cumulative MMR rate were 88/84% and 82/81%, respectively. Failures (no CHR at 6 months, no CgR at 6 months, no PCgR at 1 year, no CCgR at 18 months, loss CHR, loss CCgR, progression to accelerated/blastic phase and death) were 68/444 (15%) and 23/115 (20%) for group A and B, respectively. Events (failures, off-treatment for toxicity, refusal and lost to follow-up) were 99/444 (22%) and 34/115 (31%) for group A and B, respectively. The rates of progression to accelerated/blastic phase were the same (5%) in both groups. OS was 95% and 88% (all causes of death) and 97% and 96% (CML related deaths) for group A and B, respectively. All the differences were not statistically significant. *Conclusions.* Our analysis confirms, based on a large pts population and a proper period of observation, that age per se is not a negative prognostic indicator in early CP CML patients IM treated. Not the patient's age, but the presence of relevant comorbidities suggesting in advance a negative cost-to-benefit balance of IM, should be used as treatment choice advise. Acknowledgements: Supported by European LeukemiaNet, COFIN, University of Bologna and BolognAIL.

C029

GENOME-WIDE SCREENING OF CHRONIC MYELOID LEUKEMIA PATIENTS BY SNP ARRAYS: ALTERATIONS ASSOCIATED WITH DISEASE PROGRESSION

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Background. The expression of BCR-ABL gene in Chronic Myeloid Leukemia (CML) is necessary for malignant transformation but the biologic basis of the progression from Chronic Phase (CP) to Blast Crisis (BC) is poorly understood. *Aim.* Identifying the genomic imbalances involved in the transition into BC, before clinical features, may provide diagnostic markers of progression; so we used SNP arrays to perform a high-resolution mapping of BC CML patients genomes. *Methods.* We analyzed 11 patients affected by BC CML disease. Genomic DNA were extracted from bone marrow or peripheral blood mononuclear cells archived both at the time of diagnosis and progression. SNP array-based karyotyping was carried out using Affymetrix GeneChip Human Mapping arrays. Copy number (CN) analysis was performed using Hapmap normal individuals as reference set and two different softwares. *Results.* After exclusion of genomic copy number variations (CNVs), the following results were achieved: five patients showed huge amplifications and deletions, ranging from 30Mb to 160Mb, on chromosomes 9, 7, 3 and 6. We also found several heterozygous micro-deletions and micro-amplifications spreading all over the genome. This analysis has identified abnormalities in genes involved in apoptosis (e.g., GADD45A, FOXO3A, GAS6), DNA damage response (e.g., MYST as known as Hmof, XRCC2), tumor suppression (e.g., C/EBPdelta, LATS1), chromatin regulation (e.g., HDAC9), and genes belonged to ABC transporters (e.g., ABCB1), ras family and transcriptional/translational factors (e.g., ETV1). Moreover were found copy number changes (gain/loss) in genes associated with malignancy, in particular TNFRSF17, MET, IGFBPL1, EVI1, PTENP1. Other alterations affected key pathways including cell cycle regulation and WNT signaling. *Conclusions.* The use of the genomic tool Genome-Wide Human SNP array allowed us to identify, at submicroscopic level, genetic lesions in patients affected by CML in BC. Our results will be further validated by real-time PCR for the altered genes involved key pathways, while sequencing and mutation analysis will be performed on the remaining allele of putative tumor suppressor genes to identify their residual activity. All these validations and the increased number of analyzed patients will provide new insights into the genetic profiling that lead disease progression from CP to BC and consequently new opportunities to develop specific target therapies.

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C030

CD34$^+$ OBTAINED FROM HIGH SOKAL RISK CHRONIC MYELOID LEUKEMIA (CML) PATIENTS (PTS) EXPRESSES GENE PROFILES (GEP) SIGNIFICANTLY DIFFERENT FROM CD34$^+$ OBTAINED FROM LOW AND INTERMEDIATE SOKAL RISK PATIENTS

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CML is a clonal myeloproliferative disease which typically presents in chronic phase (CP), whose malignant progenitor cells proliferate rapidly, still retaining their ability to differentiate, with the disease later evolving to accelerated phase/blast crisis. Even after the introduction of glivec , the calculation of the Sokal and the Euro prognostic scores has remained essential in clinical practice, since allow to stratify CML pts at different evolutive risk at diagnosis, guiding therapeutic decisions. More recently, numerous research efforts are ongoing to gain a better understanding about the intrinsic heterogeneity of CML. Here we present data obtained from GEP experiments aimed at the identification of genes and pathways able to predict and/or to elucidate the disease course of CP-CML pts at the onset of the disease. The study was performed on highly enriched CD34$^+$ cells from peripheral blood obtained from pts with untreated CML in CP. Overall, 34 pts were included in the present analysis. In the initial part of the study, the first 20 pts (the "training set") were successfully assayed for global GEP and microarray data were used to define genes differentially expressed in high (H) (7 pts) vs. low (L) (13 pts) Sokal risk, thus identifying 84 probes set; clustering of their GEP showed an homogeneous pattern in H Sokal risk pts, where up-regulated genes are mainly related to the positive regulation of immune response (CR1, UBASH3A, EREG,) and to the induction of apoptosis (TNFRSF25, Apoe, TIMP1), whereas down-regulated genes are mainly involved in the negative regulation of metabolic processes. One of the most significantly up-regulated gene is PVT1, located on chromosome 8, about 55 kb distal to the c-Myc gene, whose putative protein might activate c-Myc gene transcription. Among the most significantly down-regulated genes is PLCB1, which we recently described as being deleted in myelodysplastic syndromes and in myeloid acute leukemia. In the second part of the study, the 84 probes set were tested on an independent test set of 13 pts, including 4 H, 4 intermediate (I) and 5 L Sokal risk pts. The test set GEP clustering displayed the same trend observed in the training set and, while L and I risk pts resulted quite scattered, H risk pts clustered together. Overall, our data suggests that the expression at diagnosis of a particular array of genes might drive the evolutive risk of CML pts.

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Allogeneic Transplantation I

C031

NON-PERMISSIVE HLA-DPB1 DISPARITY IS A SIGNIFICANT INDEPENDENT RISK FACTOR FOR MORTALITY AFTER UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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The importance of donor-recipient human leukocyte antigen (HLA)-DPB1 matching for the clinical outcome of unrelated hematopoietic stem cell transplantation (HSCT) is controversial. We have previously described an algorithm for non-permissive HLA-DPB1 disparities involving HLA-DPB1*0901,*1001,*1701,*0301,*1401,*4501, based on T cell alloreactivity patterns.

Table 1. Multivariate models for OM, NRM, graft failure, aGvHD and relapse.

	Multivariate models					
	OM[1]	*NRM*[1]	*Graft *failure 2-4*[2]	*aGvHD 3-4*[2]	*aGvHD*	*Relapse†*
10/10 allele matched HLA-DPB1 Permissive	1	1	1	1	1	1
10/10 allele matched HLA-DPB1 Non-Permissive	2.12 (1.23-3.64; *p*=0.006)	3.41 1.45-8.02; (*p*=0.005)	N.A.[3]	1.29 (0.58-2.87; *p*=0.52)	1.82 (0.49-6.73; *p*=0.37)	N.A.[3]
9/10 allele matched HLA-DPB1 Permissive	1.38 (0.71-2.69; *p*=0.33)	2.14 (0.81-5.66; *p*=0.12)	N.A.[3]	1.75 (0.69-4.45; *p*=0.24)	5.01 (1.31-19.2; *p*=0.02)	N.A.[3]
9/10 allele matched HLA-DPB1 Non-Permissive	2.21 (1.28-3.80; *p*=0.004)	3.69 (1.58-8.61; *p*=0.002)	N.A.[3]	2.11 (0.97-4.56; *p*=0.06)	4.15 1.20-14.3; (*p*=0.02)	N.A.[3]
8/10 allele matched HLA-DPB1 Irrespective ¶	2.04 (1.18-3.54; *p*=0.01) 2.13	3.06 (1.30-7.19; *p*=0.01)	N.A.	2.13 (0.97-4.65; *p*=0.06)	2.40 (0.66-8.65; *p*=0.18)	N.A.[3]

[1]Non-permissive mismatches were considered in the combined group of GvH and HvG direction, and confronted with permissive mismatches. Numbers were for 10/10 allele matched pairs: n=140 non-permissive, n=61 permissive; for 9/10 allele matched pairs: n=145 non-permissive, n=54 permissive; for ≤8/10 allele matched pairs: n=137. [2]Non-permissive mismatches were considered only in the GvH direction, and confronted with the combined group of permissive mismatches and non-permissive mismatches in HvG direction. Numbers were for 10/10 allele matched pairs: n=72 non-permissive, n=129 permissive; for 9/10 allele matched pairs: n=85 non-permissive, n=114 permissive; for ≤8/10 allele matched pairs: n=137 [3]N.A.: not applicable since the number of events in each subgroup was too limited to allow statistically meaningful analysis. [3]N.A.: not applicable since the p-value in univariate models was >0.2. Multivariate models include gender, age and CMV status of donor and patient, year of transplantation, use of ATG, disease group, stem cell source, conditioning regimen and use of TBI.

By revisiting the immunogenicity of HLA-DPB1*02, a modified algorithm was developed and retrospectively tested in 621 unrelated HSCT, facilitated through the Gruppo Italiano Trapianto di Midollo Osseo, CSE e terapia cellulare (GITMO) and the Italian Bone Marrow Donor Registry (IBMDR), performed in adult patients affected by onco-hematological diseases. The Kaplan Meier probability of 2-year survival was significantly higher in transplants with permissive as compared to non-permissive HLA-DPB1 mismatches (54.8% versus 39.1%, p=0.005), due to increased adjusted hazards of non-relapse mortality (HR =1.74; C.I. 1.19-2.53; p=0.004) but not of relapse (HR =1.02; C.I. 0.73-1.42; p=0.92). In multivariate analysis (see Table 1), taking 10/10 allele matched permissive pairs as reference, the increase in the hazards of overall mortality by non-permissive HLA-DPB1 disparity was similar in 10/10 (HR=2.12; C.I. 1.23-3.64; p=0.006) and 9/10 allele matched transplants (HR=2.21; C.I. 1.28-3.80; p=0.004), and was observed not only in early but also in advanced stage disease. Moreover, those 9/10 allele matched transplants who were DPB1 permissive had mortality risk comparable to 10/10 allele matched, DPB1 permissive pairs (HR=1.38; C.I. 0.71-2.69; p=0.33), therefore DPB1 permissiveness appears to overcome the unfavorable effect played by one allelic mismatch. These data call for revisiting current HLA matching strategies for unrelated HSCT, suggesting that searches should be directed up-front towards identification of HLA-DPB1 permissive, 10/10 or 9/10 matched donors, present with an approximate probability of 30%.

C032

RAPAMYCIN-BASED GVHD PROPHYLAXIS AFTER T-CELL REPLETE UNMANIPULATED HAPLOIDENTICAL PERIPHERAL STEM CELL TRANSPLANTATION FOR ADVANCED LEUKAEMIAS: PRELIMINARY RESULTS OF THE TRRAMM STUDY

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The only effective chance of cure for patients (pts) with advanced high-risk haematological malignancies is allogeneic stem cell transplantation (SCT). A graft-versus-tumor effect is documented in 20-30% of patients with advanced leukemias transplanted from HLA-matched related or unrelated donor. Unfortunately, ex-vivo T-cell depleted haploidentical (haplo) SCT with CD34-selected cells, showed very poor results, in patients with active disease at transplant (Ciceri, Blood 08), due to lack of early GvL effect secondary to profound T-cell depletion. We investigated the feasibility of infusion of unmanipulated peripheral blood stem cells (PBSC) from haplo donor with a combination of drugs – Rapamycin and Mycophenolate – as GvHD prophylaxis to preserve early T regulatory cells function (TrRaMM study, Eudract 2007-5477-54). Since 2007, thirty-six patients underwent allogeneic transplantation for AML (17 pts), ALL (5 pts), sAML (6 pts), MDS (3 pts), CML-CB (2 pts), NHL (2 pts) or HD (1 pt). The median age was 49 years (range 14-69). At transplant 4 pts were in early phase, while 32 were in advanced phase (26 with active disease). The conditioning regimen included Treosulfan (14 g/mq for 3 days), Fludarabine (30 mg/mq for 5 days) and an *in vivo* T and B-cell depletion, by ATG-Fresenius (10 mg/kg for 3 times) and Mabthera (a single 500 mg dose). All pts received allogeneic peripheral blood cells from an HLA-haploidentical related donor without any *in vitro* positive selection. GvHD prophylaxis consisted of Rapamycin (target level 8-15, till day +60) and MMF (15 mg/kg tid till day +30). All patients engrafted, and all but two were in CR at first marrow evaluation on day +30. 8 pts experienced aGvHD grade 2-4 after withdrawal of immunosuppressive prophylaxis. Sustained (>100/mcl) circulating $CD3^+$ lymphocytes, with a physiologic CD4/CD8 ratio, a conserved distribution of naïve/memory lymphocytes and a predomionant Th1/Tc1 functional profile were detected already at day +30. After a median follow-up of 9 months half pts are alive. Causes of death were disease progression in 11 pts, infections in 6 and aGvHD in 1. The probability of event-free survival at 6 months is 53%. *Conclusions.* ATG + Rapamycin and Mycophenolate proved to be effective as GvHD prophylaxis after myeloablative conditioning and transplantation of unmanipulated peripheral blood stem cells (PBSC) from haploidentical donor in patients with advanced disease.

C033

AN IMMUNOSELECTED CD34+ CELL MEGADOSE INDUCES TOLERANCE AND RAPID IMMUNOLOGICAL RECONSTITUTION IN ATG-FREE HSCT FROM HLA-IDENTICAL SIBLINGS

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Introduction. In conditioning for T-cell-depleted HSCT, ATG prevents rejection and GvHD. Through its *in vivo* T-cell depletion, it delays post-transplant immune reconstitution and increases the risk of infection. As immunoselected $CD34^+$ cells exert a veto effect, enhanced immunosuppression is no longer required to prevent rejection. Here we tested ATG-free conditioning regimens in HLA-identical HSCT. *Patients and methods.* 55 patients (29 relapse; 26 CR) with acute leukemia (26), lymphoma (15), myeloma (9), other diseases (5) were assigned to a TBI or non-TBI based conditioning according to whether they had been irradiated. Regimen A (29 patients, median age 49 years; range 26-63): 8 Gy single TBI, thiotepa 5 mg/kg for 2 days, fludarabine 40 mg/sqm for 4 days. Regimen B (26 patients, median age 58 years;range 20-67): melphalan 140 mg/sqm instead of TBI. Peripheral blood cells were processed by Clinimacs. Grafts contained a median of 8×10^6 $CD34^+$ cells/kg and 1×10^4 $CD3^+$ cells/kg recipient b.w. No post-transplant immunosuppressive, anti-fungal or anti-CMV prophylaxis was given. Chimerism was analysed in peripheral T lymphocytes and granulocytes every month post-transplant. All engrafted (neutrophils>500 and platelets>20,000 at a median of 12 and 11 days, respectively) with full donor chimerism. Median $CD4^+$ cells were 150 and 250/mmc at 3 and 6 months respectively. All but 2/29 patients who received Regimen A developed full donor lymphoid chimerism within 3 months. In all patients who received Regimen B percentages of host-type lymphocytes decreasing until disappearance at 24 months post-transplant. One patient who was DRB1 mismatched developed grade II aGvHD. No evaluable patient developed chronic GvHD. Eleven patients died of non-relapse causes, including 1 CMV+ recipient of CMV- graft who died of CMV pneumonia; 22 relapsed; 22 survive disease-free at a median of 22 months (range 5-45). Of the 26 patients in CR at transplant, 11/21 in the Regimen A group and 5/5 in the Regimen B group survive disease-free. *Conclusions.* Extensive ex vivo T cell depletion prevents GvHD. ATG-free conditioning regimens are followed by specific tolerance, rapid immunological reconstitution and fewer infections without need for anti-infective prophylaxis. After Regimen B, expansion of a host population with a wide protective repertoire may have reduced the infection rate. With no GvHD, immunosuppressors or recurrent infections, these elderly adults enjoy an exellent QoL.

C034

ALLOGENIC HEMOPIOETIC STEM-CELL TRANSPLANT FOR PATIENTS WITH PRIMARY MYELOFIBROSIS: A PREDICTIVE TRANSPLANT SCORE BASED ON TRANSFUSION REQUIREMENT , SPLEEN SIZE AND DONOR TYPE

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Forty six patients with primary myelofiobrosis (PMF) (median age 51), underwent an allogeneic hemopoietic stem cell transplant (HSCT) following a thiotepa based reduced intensity conditioning regimen. The median follow up for surviving patients is 3.8 years . In multivariate analysis independent unfavourable factors for survival were red blood cell transfusions > 20 , a spleen size >22 cm and an alternative donor: 24 patients had 0-1 unfavourable predictors (low risk) and 22 patients had 2 or more negative predictors (high risk). The overall actuarial 5 year survival of the 46 patients is 45%. The actuarial survival of low risk and high risk patients is respectively 77% and 8%(p<0.0001) : this is due to a higher transplant related mortality (TRM) for high risk patients (RR 6.0, p=0.006) and a higher relapse related death (RRD) (RR 7.69; p=0.001). In multivariate COX analysis the score maintained its predictive value (p=0.0003) , also after correcting for donor -patient age and gender, Dupriez score , IPPS score pre-transplant and splenectomy. In conclusion, PMF patients undergoing an allogeneic HSCT, may be scored according to spleen size , transfusion history, and donor type: this scoring system may be useful to discuss transplant strategies.

C035

ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) FOLLOWING A REDUCED-INTENSITY CONDITIONING (RIC) REGIMEN IN RELAPSED LYMPHOMAS: 5-YEAR FOLLOW-UP OF THE PHASE II STUDY OF THE GRUPPO ITALIANO TRAPIANTO DI MIDOLLO OSSEO (GITMO)

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Data concerning long-term activity and toxicity following RIC allo-SCT in lymphomas are limited. We report the results of a prospective multicenter phase II trial: 194 relapsed/refractory lymphomas received the same RIC regimen followed by allo-SCT from matched sibling donors. Histologies were non-Hodgkin's lymphomas (NHL) [indolent (LG-NHL, n=69), including follicular lymphoma (FL, n=29), chronic lymphocytic leukemia (CLL, n=36), other (n=4); aggressive (HG-NHL, n=86), including B-cell phenotype (n=42); T-cell phenotype (n=28), mantle cell lymphoma (MCL, n=16)] and HL (n=39). At last follow-up (median 64 months), 116 pts are alive (59%) and 78 died [n=47 for disease progression, n= 30 for non-relapse mortality (NRM), n=1 not assessable]. The 5-year crude cumulative incidence (CCI) of NRM was 15% and was not influenced by previous autologous SCT (p=0.43) or by disease status (p=0.20). The incidence of acute and chronic GVHD were 34% and 55%, respectively. The percentage of pts, still receiving immunosuppressive therapy, decreased from 43% at 3-year to 28% at 5-year. The percentage of pts dying for chronic GVHD did not increase significantly from 3- to 5-year of median follow-up. The incidence of second tumors was 4% (n=6 alive, n=2 death), oral cavity and skin were mainly affected. The 5-year OS and PFS were 62% and 70% for LG-NHL and 61% and 59% for HG-NHL, respectively. The CCI of relapse, PFS and OS in HL at 5-year were 80%, 17%, and 36%,respectively. Disease status significantly influenced outcome in HG-NHL and HL [chemosensitive versus chemorefractory: 73% versus 32% (p<0.001) for HG-NHL; 64% versus 0% (p<0.002) for HL]. Pts with FL and CLL and those affected by HG-NHL of B and T-cell phenotype were well balanced as pre-transplantation characteristics. We observed a trend for an higher CCI of relapse for CLL pts (37% versus 18%, p=0.05), the 5-year OS was not significant different between FL and CLL pts (71% versus 51%, p=0.10). The OS, PFS and CCI of relapse were not significantly different in HG-NHL of B- and T-cell origin [OS: 68% versus 56% (p=0.43); PFS: 63% versus 57% (p=0.49); CCI of relapse: 32% versus 39% (p=0.39)], respectively. At multivariable analysis of OS, refractory disease and occurrence of grade II to IV GVHD were adverse prognostic factors (hazard ratio, HR=2.57, p<0.001; HR=1.65, p<0.040). Chronic GVHD did not influence the outcome. Pts with relapsed lymphomas could achieve durable remissions with limited long-term toxicity.

C036

IMMUNE CORRELATES WITH CLINICAL OUTCOMES IN PATIENTS UNDERGOING HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIAS

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The profound immunodeficiency that often follows allogeneic hematopoietic transplantation (HCT), leads to severe infectious complications, high incidence of disease relapse and reduces the overall efficacy of HCT. In particular, haploidentical HCT, the only curative option for patients with high-risk leukemia lacking HLA-matched donor, is complicated by a high-rate infectious mortality associated with the delayed immune reconstitution (IR) secondary to the procedures for prevention of severe graft-versus-host-disease (GvHD). A dynamic qualitative and quantitative assessment of IR is a promising measure to predict such complications and guide a patient-specific modulation of alloreactivity. We analyzed T cell dynamics in 32 patients undergoing haplo-HCT for acute leukemias. To facilitate immune recovery, 22 patients received suicide-gene transduced donor lymphocytes at day +42 after a T-cell depleted HCT, in the absence of post-transplant immunosuppression (TK007), while 10 patients received a unmanipulated graft and a rapamycin-based GvHD prophylaxis (TrRaMM). Patient samples were collected at day 30, 90 and 180 after HCT. The number of circulating CD3+ lymphocytes/microlitre and different T cell subsets were quantified by flow cytometry, whereas TCR repertoire was evaluated by spectratyping analysis. Moreover, we monitored Cytomegalovirus (CMV)-specific immune responses by gammaIFN ELISPOT. We observed that while the number of circulating lymphocytes correlated with clinical outcome in the patients treated with TK cells, no correlation could be detected in patients exposed to pharmacologic immunosuppression. On the contrary, in both groups an inverse correlation between the n. of CMV-specific T cells and the number and severity of CMV reactivations was documented. Early recovery of naïve T-lymphocytes (CD62L+CD45RA+) and wide and polyclonal TCR repertoire was significantly associated with long-term clinical remissions. Preliminary data showed that rapamycin-based immunosuppression expanded CD4+CD25+FoxP3+CD127low/- T regulatory cells. In this cohort, we found that the higher number of Tregs is associated with a reduced incidence of GvHD. These findings suggest that immune monitoring after allo-HCT is feasible and allows characterization of relevant and predictive biomarkers for clinical outcome.

Cell Therapy

C037

RAPID DIFFERENTIATION OF LEUKEMIC CELLS INTO IMMUNOSTIMULATORY DENDRITIC CELLS: AN EFFICIENT STRATEGY FOR IMPROVING THE GRAFT VERSUS LEUKEMIA EFFECT OF ALLOGENEIC HSCT

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Allogeneic hematopoietic transplantation (allo-HSCT) is the only curative option for patients affected by high-risk acute myeloid leukemia (AML). This is largely due to the ability of allogeneic immune system to eradicate leukemic stem cells. In order to improve the efficacy of the allogeneic immune system against leukemia, we exploited the unique ability of myeloid blasts to differentiate into leukemic dendritic cells (LDC). We observed that a short (48h) exposure to calcium ionophore A23187 and IL-4 is able to induce LDC differentiation in a large proportion (86%) of *de novo* and secondary high-risk AML. Compared to original blasts, LDC significantly up-regulate molecules of the immunological synapse (CD86, CD80, HLA-DR, CD54 and CD58) while maintaining an intact leukemic antigenic (c-kit, CD34, WT1) expression profile. This possibly suggests a direct access of leukemic stem cells to the differentiation process. This favourable phenotype correlates with a high T-cell stimulatory capacity, similar to that of healthy mature DC. Most importantly, LDC proved to be significantly superior to the original blasts in promoting the expansion of leukemia-reactive T-lymphocytes from HLA-identical and haploidentical donors. This ability may correlate with the observation that LDC were significantly more potent than blasts in inducing T lymphocytes with a central memory phenotype, characterized by a high proliferative potential. To note, we proved that these LDC-stimulated, central-memory T lymphocytes were able to react against the original leukaemia not only in vitro but also *in vivo*. When infused in NOD/Scid mice transplanted with the original leukaemia, LDC-stimulated T lymphocytes induced long-term (>16 weeks) complete remissions in the majority of mice, suggesting that this approach may be active against leukemic stem cells. These results show that functionally competent LDC can be generated from the majority of high-risk AML and may be used for an integrated allogeneic immunotherapeutic approach.

C038

INDUCTION OF PREFERENTIALLY EXPRESSED ANTIGEN OF MELANOMA (PRAME)-SPECIFIC IMMUNITY BY PRAME-OVERLAPPING PENTADECAPEPTIDES IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES, INCLUDING CHRONIC MYELOGENENOUS LEUKEMIA

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Several groups, including ours, provided evidence that the cancer testis antigen PRAME is a potential target for adoptive T-cell or vaccine therapy of many hematologic malignancies and solid tumors. PRAME-specific T cells (PRAME-CTL) can be detected in patients with hematologic malignancies and we have shown that they can be generated and expanded ex-vivo, using artificial antigen presenting cells (aAPC) (K562 cell line genetically modified to express the HLA-A*02, CD80, CD40L) (Quintarelli *et al.*, Blood 2008). So far, four PRAME-derived epitopes have been identified by a proteosome mediated digestion assay. However, this strategy may fail to identify of putative peptides generated *in vivo* rather than the proteosome major cleavage site. We have now adopted an alternative method that uses a peptide-library consisting of 135 synthetic pentadecapeptides, overlapping by 11aa, spanning the entire PRAME protein. We evaluated whether novel HLA-A*02 restricted $CD8^+$ T-cell responses to multiple immunogenic epitopes can be iden-

tified and used to generate polyclonal PRAME-CTL lines from patients with hematologic malignancies. CD8+ T lymphocytes from 21 HLA-A*02 healthy donors and 7 patients with CML were primed with autologous dendritic cells loaded with the entire PRAME-peptide library, and then expanded by weekly re-stimulation with peptide loaded aAPC. Using this approach we consistently generated PRAME-CTLs in 19/21 healthy donors (457±412 SFC/105 cells as assessed by IFNg Elispot assay) and all 7 CML patients (936±136 SFC/105). These PRAME-CTLs were also able to target autologous CML cells (57±6 IFNg SFC/105 when cultured with PRAME+ CML blasts), demonstrating that the same peptides were presented physiologically. A Cr51 release assay confirmed that the PRAME-reactive T cells were cytotoxic, lysing autologous-PHA blasts loaded with the peptides derived from the PRAME-library (63±14% at a 20:1 E: T ratio), but not with irrelevant peptides (<15%). Using pentadecapeptides sub-pools, we found that the responses of our expanded PRAME-CTLs were polyclonal, since they consistently released IFNg in response to 1 to 6 pentadecapeptides pools (59% were specific for 1 or 2 pools, 25% to 3 pools, and 16% to 6 pools). In conclusion, this novel approach allowed us to identify several new immunogenic peptides that should facilitate expansion of polyclonal PRAME-CTLs for adoptive transfer or after vaccine administration to patients with PRAME+ hematological malignancy.

C039

MODULATION OF TRYPTOPHAN CATABOLISM BY ACUTE MYELOID LEUKEMIA CELLS ACTS AS A GENERAL MECHANISM OF IMMUNE TOLERANCE VIA THE INDUCTION OF T REGULATORY CELLS

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Indoleamine 2,3-dioxygenase (IDO) enzyme, which catalyzes the conversion of tryptophan into kynurenine, has been identified as a novel immunosuppressive agent by inhibiting T-cell proliferation and is involved in tolerance induction to tumors. We have recently shown that IDO protein is constitutively expressed in a significant subset of newly diagnosed acute myeloid leukemia (AML) patients, resulting in tryptophan catabolism along the kynurenine pathway and in the inhibition of allogeneic T-cell proliferation. Moreover, we demonstrated that IDO-expressing AML cells are capable to promote the differentiation of new $CD4^+CD25^+Foxp3^+$ T regulatory cells (Treg cells). AML cells can be differentiated into dendritic cells (AML-DCs), which have increased immunogenicity and have been proposed as cellular vaccines against leukemia. Here, we demonstrate that after differentiation into DCs, both IDO- and IDO^+ AML samples show the induction and the up-regulation of IDO mRNA and protein, respectively. IDO^+ AML-DCs are capable to catabolize tryptophan into kynurenine metabolite and, functionally, they inhibit allogeneic T-cell proliferation through an IDO-dependent mechanism. Moreover, IDO^+ AML-DCs increase the number of $CD4^+CD25^+Foxp3^+$ T cells and this effect is completely abrogated by the IDO-inhibitor, 1-methyl tryptophan. Purified $CD4^+CD25^+$ T cells obtained from co-culture with IDO^+ AML-DCs act as Tregs as they inhibit naive T-cell proliferation and impair the complete maturation of normal DCs. Importantly, AML-DC-induced Tregs are capable to suppress *in vitro* a leukemia-specific T cell-mediated immune response. These data identify IDO-mediated catabolism as a general tolerogenic mechanism in AML cells, including AML-DCs and raise several concerns for the use of AML-DCs as cellular vaccine against leukemia.

C040

T CELL RECEPTOR GENE TRANSFER INTO EARLY DIFFERENTIATED T LYMPHOCYTES BY LENTIVIRAL VECTORS FOR SAFE AND EFFECTIVE ADOPTIVE IMMUNE THERAPY OF LEUKEMIA

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Adoptive transfer of T cell receptor (TCR) gene-modified autologous T lymphocytes has been proposed as an attractive strategy to target tumors for which high-avidity tumor-specific T cells are difficult to isolate and expand. Although the generation of tumor specific T cells by gene transfer has been demonstrated, this approach is limited by technical issues (i.e. low and transient transgene expression, poor survival/expansion of gene-modified T cells), and safety hurdles related to the random integration of the expression cassette and inappropriate pairing of exogenous and endogenous TCR chains. To overcome these technical limitations, a codon-optimized, cysteine-modified TCR specific for an HLA-A2-restricted peptide from the Wilms tumor antigen 1 (WT1) was cloned into a third generation lentiviral vector (LV) under the control of a bi-directional promoter. Activation with anti-CD3 and anti-CD28 antibody-conjugated beads (bCD3/CD28) and culture with low dose IL-7/IL-15 supported the efficient transduction of lymphocytes by LV, while preserving an early T cell differentiation phenotype. Under these conditions, the bi-directional promoter was able to sustain stoichiometric expression of WT1-specific TCR chains, at levels appropriate for efficient HLA-A2/WT1 pentamer binding (16%), even in the absence of specific antigenic stimulation. TCR transduced cells were able to specifically produce gammaIFN and exhibited cytotoxic activity against WT1+HLA-A2+ primary leukemic blasts from AML patients. To further improve the safety of the strategy and ensure predictable levels of transgene expression, we developed, for the first time, a protocol for site-specific integration of transgenes in a safe genomic harbor into primary T lymphocytes, by exploiting zinc finger nucleases (ZFNs). Delivery of ZFNs targeting the CCR5 gene and a GFP expression cassette flanked by CCR5 homology arms in primary T lymphocytes resulted in efficient site-specific integration into the CCR5 locus which supported stable transgene expression. The specificity of this targeted integration events was confirmed by extensive molecular analysis on both sorted gene-modified cells and on cell clones. These results suggest that efficient targeted integration in early differentiated T lymphocytes might allow the generation of highly efficient and safe engineered tumor-specific CTLs for adoptive cancer T cell therapy.

C041

FETAL TISSUES FOR SELECTION AND EXPANSION OF MESENCHYMAL STEM CELLS

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Mesenchymal Stem Cells (MSCs) are promising candidates for cell therapy and tissue engineering. Increasing evidence suggests that MSCs isolated from fetal tissues are more plastic and grow faster than adult MSCs. In this study, we characterized human mesenchymal progenitor cells from first-trimester chorionic villi (CV) and amniotic fluid (AF), comparing them with adult bone marrow-derived MSC (BM). We evaluated 10 samples of CV (gestional age 11-13 weeks) and 10 samples of AF(gestional age 15-18 weeks) expanded with animal and human serum until MSCs reached senescence. We only used cells taken from the discarded supernatants of AF cell cultures and from back up cultures of CV samples set for prenatal diagnosis. We studied the differentiation capacity of fetal MSCs into adipogenic, osteogenic, chondrogenic and neurogenic lineages and the expression of Oct-4, Rex-1, Gata-4 and Nestin, from the beginning to the term of culture. We characterized MSCs for cytokine expression profile and immunomodulatory capacity. To evaluate replicative stability we studied the expression of telomerase activity and the telomere length, excluding spontaneous chromosomal alterations by cytogenetic analysis. We isolated homogeneous populations

of spindle-shaped cells expressing mesenchymal immunophenotypic markers from CV and AF, after first passages. They achieved 10 logs of expansion in 60 days, whereas BM only 4 logs in the same culture time. Fetal MSCs maintained an undifferentiated stem cell gene expression profile and a typical MSC morphologic differentiation throughout the period of expansion. Despite their high proliferation capacity, fetal MSCs showed a low expression of telomerase activity and maintained stable their long telomeres. Fetal MSCs and adult bone marrow-derived MSCs showed similar cytokine expression profile during all culture time, in all conditions. A normal karyotype was preserved throughout long-term expansion, suggesting the safety of fetal MSCs. In conclusion, this study suggests that fetal mesenchymal stem cells have functional characteristics comparable with those of adult BM-derived MSC, with a higher proliferation potential, longer telomeres and similar expression of telomerase activity identified throughout the expansion time. Our results indicate that fetal MSCs could be a resource for cell therapy and regenerative medicine, without ethical conflicts. We emphasize that we only used discarded cells from prenatal analysis for all the experiments.

C042

GENERATION OF EPSTEIN BARR VIRUS SPECIFIC CYTOTOXIC T LYMPHOCYTES (EBV-CTLS) RESISTANT TO THE IMMUNOSUPPRESSIVE DRUG TACROLIMUS (FK506)

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Adoptive transfer of autologous EBV-CTLs to hematopoietic stem cell and solid organ transplant recipients is effective for prevention and treatment of EBV$^+$ post transplant lymphoproliferative disorders (PTLD). However, CTL persistence and efficacy can be limited by immunosuppressive drugs, which can often be tapered in patients developing PTLD, but not completely withdrawn due to the risk of graft rejection. One of the most used immunosuppressive agents is FK506 whose effects are dependent on binding of FKBP12 proteins. We hypothesize that EBV-CTLs can be resistant to FK506 by knocking down FKBP12, using a small interfering RNA (siRNA) stably expressed by a retroviral vector. After extensive screening of potential target sequences, we identified by Western Blot that the siRNA named siRNA4 knocked down >90% of FKBP12 in EBV-CTLs. We then generated two retroviral vectors encoding for siRNA4/eGFP and irrelevant siRNA/eGFP (control), respectively. These vectors were used to transduce established EBV-CTLs generated from 7 EBV-seropositive donors. Transduction efficiency was 46±22% for siRNA4 and 55±27% for control-siRNA. We measured the proliferation of transduced CTLs in the presence of FK506, in short term and long term cultures. Using thymidine uptake assay we found that proliferation was significantly inhibited in control (74±2%) but not in siRNA4-CTL (41±4%). Furthermore, siRNA4-CTLs were stimulated weekly with autologous LCL, low dose IL-2 and in the presence or absence of FK506 (5 ng/mL), to assess the drug resistance in long-term cultures. The results confirmed the effect of knocking-down of FKBP12; indeed the proportion of siRNA4-CTLs increased over time in 4 weeks of culture not only as a percentage of GFP+ cells (from 46±22% to 89±5%) but also as absolute count (median fold expansion: 24, range 3-70). In contrast, control EBV-CTLs did not show any selection in culture, since the percentage of GFP$^+$ cells remained unchanged (from 55-57%) and CTLs ceased to proliferate (median fold expansion: 1, range 0-2). In addition, siRNA4-CTLs kept their MHC-restricted killing of autologous LCL (66±22% vs 16±12% for allogenic LCL) and the specific production of IFNgamma in response to EBV-peptides, as assessed by IFNγ ELIspot. In conclusion, we have developed a strategy to induce resistance to FK506 in EBV-CTLs and that may be used as adoptive immunotherapy to improve EBV immune reconstitution in patients at high risk of developing post transplant lymphoma.

Quality of Life - Support Therapy

C043

PICC INSERTION AND MANAGEMENT IN THE HEMATOLOGICAL PATIENT: THE EXPERIENCE OF THE BUSINCO'S DEPARTMENT OF HAEMATOLOGY

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Introduction. Nowadays Central Venous Catheters (CVC) are crucial for a good management of hematological patients (chemotherapy, supportive treatment, apheresis procedure, hematopoietic stem cell transplantation). However these devices can cause clinical complications related to hematological disease itself either to catheters features (most common and dangerous systemic infections and thrombosis). The introduction in the practice of Peripheral Insertion Central Venous Catheter (PICC) has simplified the management of patients mainly for those with high risk of long term CVC-related complications or for which the short-term CVC was inadeguate to all therapeutic plan. *Methods and Results.* In our Department, after a specialized educational course, has been created a PICC team, made by an hematologist physician and two nurses. We decided to put PICC in patient who needed long and discontinuous program of chemotherapy, regardless by the hematological disease either to white cells and platelets counts. It was mandatory a previous evaluation of arms vascular anatomy by ultrasonography. From March 2007 to March 2009 globally 96 PICC have been implanted (84 in hematological patients and 12 in patients with non-hematological neoplasms), in 87 people the catheter insertion has been successful, only in 9 was unsuccessful. Most hematologic patients had Hodgkin Disease (37), 38 other hematological disease and 12 non hematological neoplasms. The median life of PICC was 120 days (1-240). The most implantation procedures has been done under ultrasound guide with radiographic control after all insertions. 7 PICC have been used for myeloma and lymphoma autologous stem cell transplantation. 40 out of 87 PICC are still in situ and in use, 37 have been removed for end of therapy, 10 for other reasons. Only 8 PICC have been removed because of complications catheter-related (only one suspected catheter-related sepsis, not microbiologically confirmed, and 3 venous thrombosis). *Conclusions.* These data encourage the use of PICC in the hematological patient because of ow impact of thrombocytopenia and neutropenia in the implantation and low rates of CVC-related complications. Our experience shows the great advantage of these devices especially in the management of patient undergone to discontinuous chemotherapy in day hospital regimen and submitted to stem cell transplantation by avoiding issues related to insertion and management of CVC with central implantation.

C044

EARLY AND LATE CLINICAL COMPLICATIONS RELATED TO CENTRAL VENOUS CATHETERS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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The introduction in the current clinical practice of central venous catheters (CVC) has improved the management of anti-neoplastic therapies in patients with hematological malignancies. However, several severe complications are related to their use. In this unicentric retrospective study, we evaluated on a large cohort of patients the incidence of CVC-related early and late mechanic, thrombotic and infective complications. From July 1999 to September 2005, 1102 CVC have been implanted at our Institution in 881 patients with hematological malignancies (M/F 503/307, median age 44.4 years, range 1-87), for a total day number of implanted CVC of 142.202. With regard to disease, 359 patients (40.7%) had acute myelogenous leukemia, 225 (25.5%) non-Hodgkin's lymphoma, 90 (10.2%) acute lymphoid leukemia, 88 (10%) multiple myeloma and 119 (13.5%) other malignancies. All CVC (single-lumen Groshong Bard 7 Fr in all but 31 patients, who implanted a double-lumen Groshong Bard 9.5 Fr) were inserted by the same operator via a percutaneous puncture of the subclavian vein. Early mechanic

complications were 79 (7.2%-0.55/1.000 days/CVC); in particular, there were 32 venipuncture impairments (2.9%-0.22/1.000 days/CVC), 27 hematomas (2.4%-0.18/1.000 days/CVC), 16 arterial punctures (1.4% - 0.11/1.000 days CVC), 3 pneumotoraxes (0.27%-0.02/1.000 days/CVC) and 1 air embolism (0.09%-0.006/1.000 days/CVC). As to the late mechanic complications, there were 101 episodes of malfunctioning (9.1%), 24 accidental withdrawals (2.1%), 11 abnormal dislocations (0.99%) and 5 CVC ruptures (0.45%). Early infective complications (<1 week from the CVC implant) were 39 (3.5%-0.3/1.000 days/CVC), more than half (51.3%) due to coagulase-negative Staphylococci. With regard to the late infective complications, 187 episodes of CVC-related sepsis (17%-1.3/1.000 days/CVC) were recorded; coagulase-negative Staphylococci were isolated in 30% of episodes, Pseudomonas species in 25%, Stenotrophomonas maltophilia in 16%, Escherichia coli in 8.1%, Candida species in 4.7% and other bacteria in the remaining 16.2%. There were 29 episodes (2.6%) of symptomatic CVC-related thrombotic complications, with a median interval from the CVC implant of 60 days (range 7-395). Inherited thrombophilia was excluded in all patients with thrombotic events; in addition, 9/39 thrombotic episodes occurred in patients with platelet levels $<50\times10^9$/L. The rate of CVC withdrawal due to CVC-related complications was 26%. In conclusion, the incidence of CVC-related complications in our case series is in the ranges reported in the literature, notwithstanding the thrombocytopenia and neutropenia that frequently coexist in our hematological patients.

C045

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY FOLLOWING RITUXIMAB THERAPY IN HIV NEGATIVE PATIENTS: A REPORT OF 56 CASES FROM THE RESEARCH ON ADVERSE DRUG EVENT AND REPORTS (RADAR) PROJECT

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Rituximab improves outcomes for persons with lymphoproliferative disorders and is increasingly used to treat immune-mediated illnesses. Recent reports describe 2 patients with systemic lupus erythematosusm and 1 with rheumatoid arthritis who developed progressive multifocal leukoencephalopathy (PML) after rituximab treatment. Wem reviewed PML case descriptions among patients treated with rituximab from the Food and Drug Administration, the manufacturer, physicians, and a literature reviewfrom1997m to 2008.Overall, 52 patientswith lymphoproliferative disorders, 2 patients with systemic lupus erythematosus, 1 patientwith rheumatoid arthritis, 1 patient with an idiopathic autoimmune pancytopenia, and 1 patient with immune thrombocytopenia developed PML after treatmentwith rituximab and other agents. Other treatments included hematopoietic stemcell transplantation (7 patients), purine analogs (26 patients), or alkylating agents (39 patients). One patient with an autoimmune hemolytic anemia developed PML after treatmentwith corticosteroids and rituximab, and 1 patient with an autoimmune pancytopenia developed PML after treatmentwith corticosteroids, azathioprine, and rituximab. Median time from last rituximab dose to PML diagnosis was 5.5 months. Median time to death after PML diagnosis was 2.0 months. The case-fatality rate was 90%. Awareness is needed of the potential for PML among rituximab-treated persons.

C046

RISK OF HEPATITIS B REACTIVATION AND EFFICACY OF LAMIVUDINE IN PATIENTS UNDERGOING ALLOGENIC STEM CELL TRANSPLANTATION

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Background. Hepatitis B virus (HBV) positive patients undergoing an allograft and recipients from HBV positive donors are at risk of post-transplant viral reactivation. The role of prophylaxis with lamivudine remains unclear. Design and Methods Ninety-nine patients, with various haematological malignancies undergoing an allograft between 1999 and 2007 with at least 3 months of follow-up entered the study. Patients, median age of 51 years (20-67), were transplanted for multiple myeloma (no.54), acute myeloid leukemia (no.16), chronic lymphatic leukemia (no.11), lymphoma (no.10), chronic myeloproliferative disease (no.7) and aplastic anemia (no.1). The conditioning regimen was myeloablative in 12 patients and reduced intensity in 87; 2 patients received a second allograft from the same donor for disease progression. Stem cell source was peripheral blood in 96 patients and bone marrow in 3. Seventy-six patients had a HLA-matched sibling donor and 23 an unrelated donor. Recipients negative for hepatitis B surface antigen (HBsAg) and anti-hepatitis B core antigen antibodies (antiHBc), with HBsAg-negative donors were defined as at no risk of HBV reactivation (no.72), whereas all the remaining 27 patients were defined as at risk. *Results.* Patients at no risk did not experience HBV reactivation/hepatitis. Among patients at risk, HBsAg-negative recipients from HBsAg-positive donors (no.3) and HBsAg-positive recipients from negative donors (no.2) were treated with lamivudine. None developed hepatitis B after a median follow-up of 21 months (13-30). Twenty-two patients were antiHBc-positive. Twelve/22 were not treated whereas 10 received prophylactic lamivudine for a median of 19 months (range 4-36). Hepatitis developed in 3 untreated patients conditioned with a reduced intensity regimen and in none of those on prophylaxis. Conclusions We observed: 1) a null risk of hepatitis B in recipients serologically negative for HBV, transplanted from HBsAg-negative donors; 2) the efficacy of lamivudine in controlling HBV reactivation in both HBsAg-positive recipients from negative donors and HBsAg-negative recipients from positive donors; 3) a significant risk of HBV reactivation in HBsAg- negative/antiHBc positive recipients and efficacy of prophylactic lamivudine in this setting.

C047

CHANGES IN QUALITY OF LIFE AND PATIENT-PHYSICIAN COMMUNICATION IN MYELODYSPLASTIC SYNDROME

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Background. The chronic nature of myelodysplastic syndromes (MDS), the risk of progressive evolution, advanced age, anemia and the rapid changes in physical status make the MDS patient unique within the cancer population. Quality of Life (QoL) in MDS is undoubtedly compromised by functional, psychological, and disease-specific components. Patients are at continuous risk of complications and must face the reality of a relatively low survival. Comprehension of quality of life (QoL) in MDS is a prerequisite for therapeutic choice. METHODS. We designed an observational study in *de novo* (newly) MDS patients with IPSS risk score ≤2 to evaluate determinants of QoL and correlations

between QoL measures. Clinical and laboratory data were collected at baseline, months 1, 3, 6, 12, and 18 and QoL instruments (QOL-E v.2, LASA scale, and EQ-5D) were completed by patients and physicians (both blind to each other's responses). After diagnosis patients could receive any MDS treatment, based on investigators' judgment. *Results.* Of 148 patients enrolled (mean age 72 years, 56% males), 127 had a follow-up visit after 3 months. Mean Hb increased from 10.5±2.0-10.9 ± 2.2 g/dL (p=0.003) and 8 of 34 previously transfused patients became transfusion-free. Eight patients became transfusion-dependent. Surprisingly, most physicians' and patients' QoL scores correlated significantly, though physicians generally overestimated patients' problems; functional, energy and general QoL scores improved significantly. At univariate analysis, Hb correlated with all QoL-E and LASA scores both at baseline and after 3 months (r = 0.22-0.35, p<0.01). At multivariate analysis, baseline Hb was associated (p<0.01) with better physical, fatigue, energy and activity scores, while after 3 months transfusion-dependence predicted worse physical, functional, energy, activity and general scores. Other independent predictors (p<0.01) of worse QoL scores were Charlson's comorbidity index ≥2, IPSS risk, and female gender, while thrombocytopenia (PLT < 50000 microliters) and neutropenia does not seem to have a relevant impact. Single item scores (representing descriptive details of disturbances and perceptions) and their changes in time will be discussed. *Conclusions.* Therapeutic choice in MDS should be guided by prognostic scores and QoL. Analyses at month 3 after diagnosis show that QoL is influenced by Hb levels and transfusion-dependence, but comorbidities and IPSS risk score also play a major role.

C048

MOBILIZATION AND COLLECTION OF PERIPHERAL BLOOD STEM CELL (PBSC) FOR HIGH-DOSE CHEMOTHERAPY (HDC): SOMETHING NEW? AN ANALYSIS OF 182 PATIENTS (PTS) TREATED IN IEO

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HDC followed by autologous PBSC transplantation is effective in relapsing or refractory malignant lymphoma pts as well as in those with relapsed germ cell tumors. Collection of sufficient numbers of stem cells by apheresis (≥2.0×10^6 $CD34^+$cells/kg) is a prerequisite for HDC. Many factors could influence the mobilization of PBSC and 10-30% of pts are considered poor mobilizers ("relative" with a peripheral $CD34^+$ counts <20 and ≥8/microL, "absolute" with <8/microL). We reviewed data of 182 pts enrolled into a mobilizing PBSC program at our Institute between 2006 and 2009 with the aim to identify those factors associated to PBSC mobilization and to verify if pts considered relative or absolute poor mobilizers could collect a PBSC target by ≤3 apheresis. Pts characteristics were: M/F 105/77, median age 51 yrs (18-77), diagnosis of NHL (96 pts), HL (35 pts), MM (25 pts), LA (3 pts) and solid tumors (23 pts); mean number of chemotherapy lines before mobilization was 2. ESHAP plus G-CSF (n=11) or Peg G-CSF (n=47) and high-dose (4 g/mq) CTX (n=87) represented the main mobilization chemotherapy in NHL/HD/MM pts while the vast majority of pts with solid tumors received ICE plus G-CSF. Apheresis was performed when the $CD34^+$ cell count was ≥8/microL. By evaluating the number of $CD34^+$cells after mobilization, 121 pts (66.4%) were considered good mobilizers (group A), 30 (16.4%) relative poor mobilizers (group B) and 31 (25.6%) absolute poor mobilizers (group C). All pts in group A collected >2.0×10^6 $CD34^+$cells/kg (median 8.2); 24/30 (80%) pts in group B collected at least 2.0×10^6 $CD34^+$cells/kg while the remaining a median of 1.7 CD34+cells. Seven out of 31 (22%) absolute poor mobilizers were able to collect the target number of $CD34^+$ even if the apheresis was started with a count >=5/microL $CD34^+$. According the Two-sided Fisher's exact test, age >60 yrs (p=0.019), number of chemotherapy lines before mobilization >3 (p<0.001), pre-treatment with purine analogs (p=0.008) or Zevalin (p=0.001) were found to be independent factors able to affect PBSC mobilization. In a multivariate analysis, age >60 yrs (p=0.017) and pre-treatment with Zevalin (p=0.006) confirmed a detrimental effect. These results could help to identify those poor mobilizer pts who could be candidates to alternative mobilization strategy; however, also those patients defined relative and a proportion of absolute poor mobilizers could be able to collect a number of PBSC adequate for HDC treatment.

Coagulation Disorders

C049

A PHASE III STUDY OF ENOXAPARIN VERSUS FIXED LOW-DOSE WARFARIN VERSUS LOW-DOSE ASPIRIN AS THROMBOPROPHYLAXIS FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE CONTAINING REGIMENS

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Controversies exist concerning the best prophylactic regimen to reduce the risk of thrombosis (VTE) in newly diagnosed MM pts treated with thalidomide-containing regimens. To address this issue, the Italian Myeloma Network GIMEMA designed a sub-study in the context of 2 phase III studies, one comparing VTD vs TD in younger pts candidates for autologous transplantation and the other comparing VMP vs VMPT in older pts. By sub-study design, pts treated on VTD or TD or VMPT were randomly assigned to receive thromboprophylaxis with LMWH (Enoxaparin, 40 mg/d) or fixed low-dose warfarin (WAR, 1.25 mg/d) or low-dose aspirin (ASA, 100 mg/d) over the induction therapy. Pts randomized to VMP did not receive any prophylaxis and were used as controls. Sub-study end points included incidence of VTE, acute cardiovascular events, sudden death, bleeding and any other serious adverse events. At the time of the present analysis, 703 pts who received at least 3 cycles of induction therapy were evaluated. Of these pts, 164 treated with VMP were the control group, while the remaining 539 pts (of whom, 209 treated with VTD, 211 with TD and 119 with VMPT) were randomized to receive either LMWH (n=178) or WAR (n=180) or ASA (n=181). Baseline pts characteristics and risk factors for VTE were comparable in all sub-groups. Overall, the risk of VTE was 3.9% with WAR vs 4.5% with LMWH vs 5.5% with ASA (p n.s.), whereas it was 1.8% in the control group. Pts receiving Velcade-containing regimens (VTD or VMPT) had a VTE frequency in the range of approximately 3%, as compared to 5.8% for pts on TD (p n.s.). The rates of cardiovascular events were comparable between different sub-groups and the control group. No sudden deaths were reported. The incidence of all grades bleeding was 0.6% with LMWH vs 1.1% with WAR vs 3.3% with ASA (P values n.s.), while it was 3.7% among the controls. In conclusion, no significant relationship was found between the frequency of VTE and different thromboprophylactic regimens, induction treatments containing or not Velcade and pts age (young vs elderly). In comparison with LMWH and WAR, there was a higher, albeit marginal, risk of VTE and bleeding complications associated with ASA prophylaxis. Finally, a finding not previously well recognized, fixed low-dose WAR was not inferior to LMWH in reducing the risk of VTE among newly diagnosed MM pts receiving thalidomide-containing regimens. For these pts, LMWH, WAR and ASA are likely to be effective thromboprophylactic regimens.

C050

CIRCULATING VERSUS PROGENITOR ENDOTHELIAL CELLS ARE ABNORMAL IN PATIENTS WITH DIFFERENT TYPES OF VON WILLEBRAND DISEASE AND CORRELATE WITH MARKERS OF ANGIOGENESIS: A COHORT STUDY OF 74 CASES

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Background. von Willebrand disease (VWD) is the most common inherited bleeding disorder and is caused by quantitative or qualitative defects of von Willebrand factor (VWF). VWF, synthesized by endothelium and magakaryocytes, circulates in plasma and is present in subendothelium and platelets: therefore VWF is an ideal marker for endothelial formation/damage and megakaryocytopoiesis. Circulating (CEC) and progenitor (EPC) endothelial cells have been recently proposed as markers of peripheral and bone marrow-derived angiogenesis. To evaluate the association of CEC/EPC with cellular and circulating VWF, we have measured the number of CEC/EPC together with VWF

and cytokines involved in angiogenesis in a cohort of 74 patients with different VWD types. *Methods.* Seventy-four VWD patients and 20 healthy controls were evaluated. VWD were diagnosed according to ISTH as follows: VWD1 (22), VWD2A (9), VWD2B (19), VWD2M (17), VWD3 (7). CEC ($CD146^+/CD31^+/CD45^-$) and EPC ($CD34^+/CD133^+/CD45^-$) were evaluated by flow cytometry. Serum levels of VEGF, E-selectin, P-selectin, EPO and TPO were determined by ELISA. Both CEC/EPC and cytokines were analyzed in blind. *Results*. CEC, E-selectin, VEGF and EPO tended to be higher in all VWD than in controls. Conversely, EPC were lower in all VWD than in controls. Considering VWD all together, there was a statistically significant difference between VWD and controls in mean levels of CEC, EPC, VEGF, E-Selectin and EPO ($p<0.01$), while no statistically significant difference was found for P-Selectin and TPO. Dividing VWD into types, a statistically significant difference was found for CEC (one-way ANOVA: $p=0.005$), EPC ($p=0.001$), E-selectin ($p<0.0001$), EPO ($p=0.021$) and TPO ($p=0.004$). Considering only VWD1, we found a significant inverse relationship between CEC and VWF:Ag plasma levels ($p=.048$; $R^2=0.19$). The relationship was still significant after adjustment for age, sex and WBC in a multiple linear regression analysis ($p=0.046$). *Conclusions.* Based on these results, we can conclude that CEC are increased in VWD patients, especially in VWD2B and 3: high CEC are associated with increased levels of cytokines involved in angiogenesis (up-regulation). Conversely, EPC are always decreased in VWD patients, especially in VWD1 and VWD2A/2M, suggesting down-regulation of bone marrow-derived angiogenesis. This abnormal results on CEC/EPC in patients with inherited deficiencies of VWF might suggest a major role of VWF in peripheral and bone marrow-derived angiogenesis.

C051

RESIDUAL VEIN THROMBOSIS FOR ASSESSING THE OPTIMAL DURATION OF LOW MOLECULAR WEIGHT HEPARIN AFTER CANCER-RELATED TRHOMBOSIS: THE CANCER DACUS STUDY

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Background. Type and duration of anticoagulation is still matter of debate in cancer patients with acute Deep Vein Thrombosis (DVT) of the lower limbs. Residual Vein Thrombosis (RVT) has been proven to be effective for assessing the optimal duration of oral anticoagulants in non cancer patients (Siragusa S *et al.* Blood 2008:112:511-5). In the present study we evaluate the role of a RVT-based management of anticoagulation with Low-Molecular Weight Heparin in cancer patients with acute DVT.

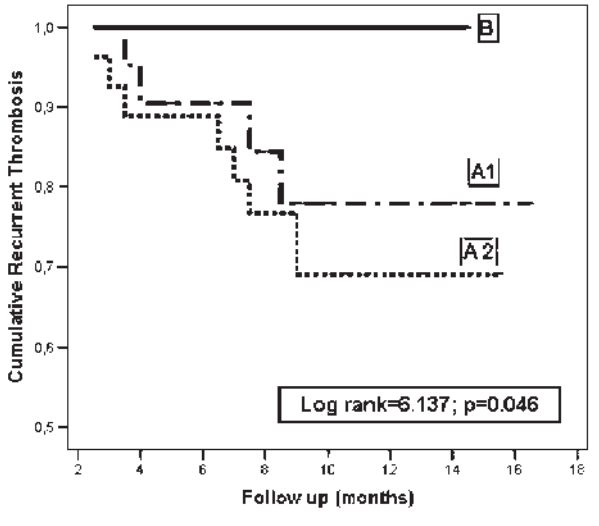

Figure.

Materials and Methods. Cancer patients with a first episode of DVT were treated with LMWH at therapeutic dosage for 1 month followed by dose reduction of 25% in the next 5 months. At this time, they were managed according to RVT findings: those with RVT were randomized to continue anticoagulants for 6 additional months (Group A1) or to stop (Group A2), while patients without RVT stopped LMWH (Group B). Outcomes were recurrent venous thromboembolism and/or major bleeding. *Results.* Over a period of 18 months, 134 patients were evaluated across 12 centers in Italy; total duration of follow-up was 30.5 years and median duration of follow-up was 1.2+0.2 years. RVT was detected in 92 (68.6%) patients; recurrent events occurred in 23.4% of those who discontinued and 15.5% of those who continued LMWH (Figure 1). The adjusted Hazard Ratio (HR) for age and sex (Group A2 vs A1) was 1.58 (95% confidence interval [CI], [UTF-8?]0.85-2.93; $p=0.145$). Of the 42 (31.3%) patients without RVT, one had a recurrence (2.3%) (Figure 1). The adjusted HR (B vs A1) was 4.54 (CI [UTF-8?]2.3-6.66; $p=0.028$). One major bleeding event occurred in each group of patients who stopped (Group A2 and B) and 2 in those who continued anticoagulation. Overall, 31 (23.1%) patients died due to cancer progression after a median follow-up of 13.2 months after randomization. *Conclusions.* The Cancer DACUS is the first study evaluating an individual marker for assessing duration of anticoagulation in active cancer population. This interim analysis shows that absence of RVT identifies a group of patients at low risk for recurrent thrombosis who can safely stop LMWH after 6 months.

C052

LOW DOSE RITUXIMAB AS SALVAGE THERAPY IN ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA

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Rituximab 375 mg/sqm weekly for 4 weeks has significant activity in adults with primary ITP. In this setting, several evidences support the possible use of lower doses of Rituximab. 43 adult patients, median age 41 years, male/female sex ratio 16/27, with previously treated symptomatic ITP were treated prospectively with Rituximab at the fixed dose of 100 mg iv weekly for 4 weeks. Results were evaluated considering: overall and complete responses (OR and CR: platelet level equal to/more than 50 and 100×10^9/L), sustained OR and CR (sOR and sCR: platelet level equal to/more than 50 and 100×10^9/L, respectively at month 6 from the beginning of therapy and discontinuation of any other anti ITP therapy, if present), the time to OR and to CR (TTR and TCR: time necessary to reach a platelet number equal to/more than 50 and 100×10^9/L), the relapse rate, the duration of response (RD: interval between initial response and the loss of the best response previously achieved) and the treatment free survival (TFS; interval between initial response and the necessity of beginning rescue therapy). All patients completed the therapeutic program receiving the 4 infusions of Rituximab, none experiencing relevant short term toxicity. The median $CD20^+$ve lymphocytes absolute count evaluated in 31 patients between day 60 and 90 from beginning of therapy was 0×10^9/L. OR and CR were achieved in 28/43 (65%) and 17/43 (39.5%) patients, respectively. Stepwise logistic regression showed that the rate of CR was inversely associated with weight (OR=0.92, CI95%[0.86;0.98] and was higher in males (OR=0.09, CI95%[0.01;0.65]. No association was found out with OR. 4 patients were not valuable for sOR and sCR due to too short follow up; the rate of sOR and sCR were 16/39 (41%) and 11/39 (28%). In responding patients, the median TTR and TCR were 31 (range: 7-112) and 38 days (range: 7-150), considerably longer than those observed with standard dose in patients with similar characteristics (Haematologica 2003;88:538). None of patients experienced delayed infectious complications or other toxic effects. After a median period of observation in responding patients of 16 months (range: 1-38), 16/28 (57%) patients relapsed, 12 requiring further treatments, with a 24 months projected RD and TFS of 38% and 54%), respectively. In patients with ITP, low dose Rituximab seems to lead mid and long-term response rates similar to standard dose but with slower initial timing of response.

C053

IMPAIRED INTERACTION BETWEEN REGULATORY T CELLS AND DENDRITIC CELLS IN IMMUNE THROMBOCYTOPENIA

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$CD4^+CD25^+$ regulatory T cells (Tregs) are critical in maintaining self-tolerance and preventing organ-specific autoimmune diseases. However, the role of Tregs in the pathogenesis of immune thrombocytopenia (ITP), an immune disorder in which increased platelet clearance is caused by antiplatelet autoantibodies, has not yet been clarified. The purpose of the present study was to investigate whether the interaction between dendritic cells (DCs) and regulatory T cells (Tregs) may play a pathogenetic role in ITP. Forty patients with active disease and 35 healthy subjects were enrolled into the study. We firstly characterized the number (by flow cytometry) and the suppressive activity (by modified allogeneic mixed leukocyte reaction) of Tregs in ITP. We found that the absolute number of Tregs was significantly decreased in ITP patients in comparison to healthy subjects ($CD4^+CD25^{high}Foxp3^+$ T cells (5.46±4.29 vs 11.62±6.98cells/microL; $p<0.01$) and $CD4^+CD25^{high}CD127^{low\text{-}negative}$ T cells (50.99±27.29 vs 80.49±37.71cells/microL; $p<0.02$)). We documented also that in ITP suppressive activity of Tregs was defective as compared to healthy subjects. In parallel experiments we studied the *in vitro* conversion of $CD4^+CD25^-$ T cells, either from ITP patients or healthy subjects, into $CD4^+CD25^+Foxp3^+$ Tregs by co-coltures with mature autologous/allogeneic DCs. The flow cytometry analysis showed that in ITP the low number of circulating Tregs may be partly due to the reduced ability of DCs to convert non-Treg cells into Tregs. We then explored the *in vitro* capability of $CD4^+CD25^+$ Tregs, either from ITP patients or healthy subjects, to inhibit allogeneic DCs maturation by flow cytometry evaluation of the expression of the costimulatory molecules CD80 and CD86. We demonstrated that in ITP patients $CD4^+CD25^+$ Tregs show lower ability to inhibit DCs maturation because they do not affect the expression of CD80 and CD86 molecules. This finding may be related to the lower level of Interleukin-10 and Interleukin-6 in the cocoltures. Taken together, these findings document that the interaction between DCs and Tregs is altered in ITP and suggest that this dysfunction may play a pathogenetic role.

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C054

CHARACTERIZATION OF THE CD47/SIRP-α SYSTEM IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Two mechanisms of platelet destruction have been previously reported in Immune Thrombocytopenia (ITP) patients: antibody-mediated platelet destruction and platelet lysis due to cytotoxic T lymphocytes. Recently, a third mechanism, involving the CD47/SIRP-α system, has been described in a mouse model of immune thrombocytopenia. Experimental evidences in fact demonstrate that *in vivo* the CD47/SIRP-α signaling complex is involved in the prevention of phagocytosis of platelets or red blood cells by macrophages, thereby determining both the life span of the cells and their number in circulation. Despite these findings, the role played by the CD47/SIRP-α system in human ITP has still to be studied in depth. Therefore, our purpose was to evaluate whether alterations of this system may be involved in the pathogenesis of ITP. At variance with fresly isolated platelets, we found that *in vitro* aged platelets from ITP patients show both reduced expression of CD47 antigen and increased apoptosis (as evaluated by PS exposure) in comparison with their normal counterparts. We also phenotypically and functionally characterized SIRP-α expression on DCs from ITP patients and healthy subjects. SIRP-α showed low expression in plasmacytoid DCs; by contrast, it was highly expressed on the surface of immature and mature CD14-derived DCs and on circulating myeloid DCs and monocytes. In addition, we found that in ITP patients SIRP-α expression was significantly reduced in immature/mature CD14-derived DCs and in circulating myeloid DCs in comparison to the normal counterparts. This finding suggest that in ITP the reduced expression of SIRP-α, especially on circulating myeloid DCs, may favour DCs platelet uptake. We then evaluated the role of CD47/SIRP-α system in the platelet phagocytic activity of immature CD14-derived DCs. Fresh and aged normal platelets, pre-labeled with anti-human CD47 monoclonal antibody (20 microg/mL) and PKH26-GL dye, were coincubated with immature CD14-derived DCs. We observed that the CD47/SIRP-α system is capable to affect the platelet phagocytic capacity of immature DCs. In conclusion, the present study demonstrates an aberrant regulation of SIRP-α expression on DCs from ITP patients and suggests that this defect might, in part, contribute to the pathogenesis of the disease.

Supported in part by BolognaAIL (Italian association against Leukemia, Bologna section).

Chronic Lymphocytic Leukemia

C055

INTEGRATIVE ANALYSIS OF DNA COPY NUMBER AND EXPRESSION LEVELS OF MIR-15A/16-1 CLUSTER IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH 13Q14 DELETION

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Hemizygous and/or homozygous loss at 13q14 has been identified as the most frequent genomic alteration in B-cell chronic lymphocytic leukemia (B-CLL). Two microRNAs genes, mir-15a and mir-16-1, are located at 13q14 within a 30-kb region of loss in B-CLL and have been found to be deleted and down-regulated in the majority of cases with respect to normal B-cells. To narrow down the extent of the 13q14 deletion, we investigated the whole genome profile of 100 untreated B-CLL patients in early stage disease (Binet stage A) by means of single nucleotide polymorphism (SNP) arrays (Affymetrix GeneChip® Human Mapping 250K Nsp). To validate the genomic status of mir-15a/16-1 cluster and to correlate the copy number (CN) and expression levels of the two miRNAs, their DNA CN and transcription levels have been detected by quantitative Real Time RT-PCR (Q-RT-PCR) using a custom TaqMan® assay and TaqMan® microRNAs assays (Applied Biosystems), respectively. Del(13)(q14) was present in 44 of our patients, in 34 as a single aberration. Biallelic deletions encompassing the 13q14.2-q14.3 region were found in 11/44 cases (25%). SNP arrays showed that the deletions varied considerably in size, ranging from 291 kb to 56 Mb, with the minimal monoallelic deletion 635 kb long. Notably, the mir-15a/16-1 cluster is located approximately 87 kb upstream to this region, and thus apparently not affected by the deletion. Overall, in our series we found that 4 cases retained one or two copies of the mir-15a/16-1 cluster. We evaluated the miRNA genes DNA CN by means of Q-RT-PCR in the four patients and in a selected panel of 28 cases (10 biallelic deleted, 7 monoallelic deleted, 11 non-deleted cases); the estimated gene CN values showed a good correlation with SNP array data (p=1.37xE-06, Kruskal-Wallis test). As regards the miRNAs expression, we found a significant difference between biallelic and monoallelic deleted B-CLLs (p=0.009 and 0.006 for miR-15a and miR-16, respectively), whereas expression levels associated with the retention of 1 or 2 alleles were not statistically different. Our data confirmed the previous evidence of a short common deleted region at 13q14, narrowing it down to 635 kb of extent. Furthermore we demonstrated that one or two copies of mir-15a/16-1 cluster can be maintained at DNA level in patients with 13q14 deletion. As regards miR-15a and miR-16 expression levels, we found that they are significantly down-regulated only in patients with biallelic deletion.

C056

CD49D EXPRESSION AND SHORT TELOMERE LENGTH INTERACT IN CHRONIC LYMPHOCYTIC LEUKEMIA AND IDENTIFY PATIENTS AT RISK OF PROGRESSION AND SHORT SURVIVAL

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Background. The rationale of the study stems from three considerations: i) CD49d expression and short telomere length (TL) represent novel prognostic markers for chronic lymphocytic leukemia (CLL); ii) both CD49d expression and short TL associate with clinico-biological features of highly proliferating CLL. Aim. To test the impact of the interaction between CD49d expression and short TL on CLL prognostication. Methods. The study was based on a consecutive series of 180 previously untreated CLL. CD49d expression was analyzed by flow cytometry. Peak TL was determined by Southern blot. The best CD49d and TL cut off points for CLL prognostication have been previously identified (Gattei et al, Blood 2008; Rossi et al, Leukemia 2009). *Results.* CD49d expression and telomere length were investigated in a consecutive series of 180 CLL. When treated as continous variables, CD49d expression and telomere length showed a weak association (Spearman's rho=-.152, p=.033).

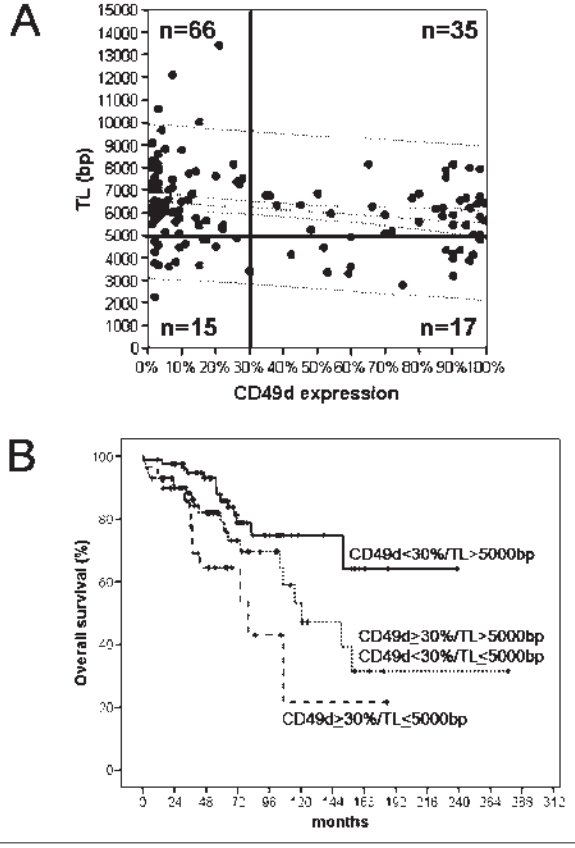

Figure 1.

Clinico-biological variables at diagnosis and outcome were stratified according to CD49d and TL status. For this purpose, CLL were grouped into 3 categories: i) CD49d-low/TL-long (CD49d<30%/ TL>5000bp); ii) CD49d-high/TL-short (CD49d>30%/TL<5000bp); iii) discordant CLL cases (CD49d<30%/TL<5000bp and CD49d>30%/ TL>5000bp). The prevalence of unfavorable variables at diagnosis progressively increased from CD49d-low/TL-long CLL to discordant cases and to CD49d-high/TL-short CLL (Rai stage III-IV: 3.8% vs 15.5% vs 24.1%, respectively, p=0.006; IGHV homology >98%: 19.0% vs 41.4% vs 69.4%, respectively, p<0.001; CD38 >30%: 8.8% vs 39.4% vs 62.1%, respectively,

p<0.001; unfavorable karyotype: 21.3% vs 46.5% vs 65.5%, respectively, p<0.001). Occurrence of markers of rapid cell turnover also progressively increased from CD49d-low/TL-long CLL to discordant cases, and to CD49d-high/TL-short CLL (median β-2-microglobulin: 2.1 mg/L vs 2.7 mg/L vs 3.3 mg/L, respectively, p<.001; median LDH: 323 U/L vs 370 U/L vs 399 U/L, respectively, p<0.001). Time to lymphocyte doubling progressively decreased from low CD49d/long TL CLL to discordant cases, and to high CD49d/short TL CLL (54.2 months vs 28.6 months vs 18.3 months, respectively, p<0.001). Time to progression to a more advanced stage progressively decreased from CD49d-low/TL-long CLL to discordant cases, and to CD49d-high/TL.short CLL (68.9 months vs 34.6 months vs 23.0 months, respectively, p<0.001). Treatment free survival progressively decreased from CD49d-low/TL-long CLL to discordant cases, and to CD49d-high/TL-short CLL (not reached vs 120.5 months vs 79.8 months, respectively, p=0.006) (Figure 1B). Other variables associated with short survival were IGHV homology >98%, presence of del17p13 or 11q22-q23, Rai stage III-IV, and β-2-microglobulin >2.5 mg/L (p<0.05). Multivariate analysis for survival selected the interaction between CD49d expression and TL as independent predictor of survival (HR:2.17; p=0.023) after adjusting for IGHV homology, unfavorable FISH karyotype, Rai stage and β-2-microglobulin. *Conclusions.* The implications of our results are twofold: i) CD49d expression and short TL interaction identifies CLL with rapid tumor kinetics, high risk of progression, and short survival; ii) the interaction between CD49d expression and short TL is an independent predictor of short survival in CLL.

C057

STEROTYPIC B-CELL RECEPTOR AND IGHV4-39 USAGE REPRESENT INDEPENDENT RISK FACTORS OF CHRONIC LYMPHOCYTIC LEUKEMIA TRANSFORMATION TO RICHTER SYNDROME

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Background. Richter's syndrome (RS) represents the transformation of CLL to aggressive lymphoma. Despite the abundance of biological markers available for predicting CLL progression, only few have been shown to be useful for RS prediction. *Aim.* To test the role of stereotypic HCDR3 in RS transformation. *Methods.* The first step of the study consisted of a case-control analysis comparing IGHV gene usage and prevalence of stereotypic HCDR3 in RS (n=69; all DLBCL) versus a control group (n=715) of CLL not transformed to RS. The second step consisted of an actuarial assessment of the impact of IGHV gene usage and stereotypic HCDR3 at CLL diagnosis, on the risk of subsequent transformation to RS in a cohort of 754 CLL, of which 39 had transformed to RS. Cluster analysis was performed by aligning HCDR3 of RS and non-transformed CLL into a database of 2421 HCDR3 of CLL. *Results.* Case-control comparison of IGHV usage documented that IGHV4-39 was the sole gene preferentially utilized in RS compared to non-transformed CLL (p=0.002). Cluster analysis revealed a significantly higher prevalence of stereotypic HCDR3 in RS compared to non-transformed CLL when considering all cases (RS: 34/69, 49.3% vs non-transformed CLL: 152/714, 21.3%; p<0.001), unmutated cases only (RS: 28/48, 58.3% vs non-transformed CLL: 95/276, 34.4%; p=0.002), and mutated cases only (RS: 6/21, 28.6% vs non-transformed CLL: 57/438, 13.0%; p=0.043). Subset 8 utilizing unmutated IGHV4-39/IGHD6-13/IGHJ5 genes was the sole HCDR3 subset preferentially utilized by RS (2/152, 1.3%) compared to non-transformed CLL (5/34, 14.7%, p=0.002). Actuarial univariate analysis revealed higher risk of RS: i) in CLL utilizing stereotypic HCDR3 (5-year risk: 14.2%) compared to CLL without stereotypic HCDR3 (5-year risk: 3.9%) (p<0.00001); and ii) in CLL utilizing IGHV4-39 (5-year risk: 35.4%) compared to CLL utilizing other IGHV genes (5-year risk: 5.6%) (p<0.000001). Bivariate analysis combining stereotypic HCDR3 and IGHV mutation status indicated that stereotypic HCDR3 was not a surrogate of IGHV homology for RS prediction. Multivariate analysis selected IGHV4-39 usage (HR:4.25; p=0.002) and stereotypic HCDR3 (HR:3.08; p=0.002) as independent predictors of RS transformation. The observation that all RS utilizing IGHV4-39 carried stereotypic HCDR3 prompted investigation of the interaction between IGHV4-39 usage and stereotypic HCDR3 in the model. Multivariate analysis selected the interaction between IGHV4-39 usage and stereotypic HCDR3 at CLL diagnosis as the strongest independent predictor of RS transformation (HR:5.13; p=0.001). The relevance of the interaction between IGHV4-39 and stereotypic HCDR3 was confirmed by bivariate analysis. Accordingly, CLL utilizing both IGHV4-39 and stereotypic HCDR3 were identified as the disease category with highest risk of transformation (5-year risk: 68.7%). Neither IGHV4-39 usage nor stereotypic HCDR3 affected the risk of CLL progression occurring without transformation to RS. *Conclusions.* The implications of our results are fourfold: i) RS carry stereotypic HCDR3 at a very high frequency; ii) RS display biased usage of IGHV4-39 with stereotypic HCDR3; iii) stereotypic HCDR3 at CLL diagnosis is an independent risk factor of RS transformation; iv) the combination of stereotypic HCDR3 and IGHV4-39 usage in the same patient identifies CLL with a very high risk of RS transformation.

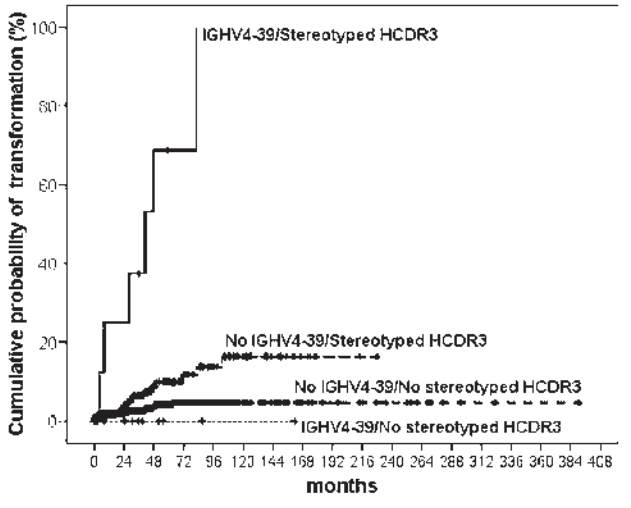

Figure 1.

C058

HAIRY CELL LEUKEMIAS WITH UNMUTATED IGHV GENES DEFINE THE MINOR SUBSET REFRACTORY TO SINGLE AGENT CLADRIBINE AND WITH MORE AGGRESSIVE BEHAVIOR

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Hairy cell leukemia (HCL) is generally responsive to single-agent Cladribine (2CdA) and only a minority of patients are refractory and with poor prognosis. HCL generally express mutated (M) and, in a minority, unmutated (UM) immunoglobulin heavy chain variable region genes (IGHV). In a multicenter clinical trial in newly diagnosed HCL, we prospectively investigated clinical and molecular parameters predicting response and event-free survival (EFS) after single-agent 2CdA. Of 58 HCL, 6 expressed UM-IGHV (UM-HCL) and 52 M-IGHV (M-HCL). Beneficial responses were obtained in 53/58 patients (91%), while treatment failures were observed in 5/58 (9%) patients. Responses correlated significantly with UM-IGHV (5/6 failures, p=0.000001), leucocytosis (3/6, p=0.005), and bulky spleen (4/8, p=0.0007). UM-HCL characteristically had bulky spleen (4/6, p=0.002), leukocytosis (3/6, p=0.01), TP53 defects (2/6, 33%, p=0.03) as documented by mutational analysis and genome wide DNA profile (250K SNP Affymetrix array), and progressed rapidly after first treatment (median EFS 7.5 months, p=0.000000000000004). Our data suggest that UM-HCL identify the minor subgroup refractory to 2CdA and with more aggressive disease presentation. High incidence of TP53 disfunction indicates a potential mechanism of resistance to 2CdA in the UM-HCL group. Overall, our data provide new molecular elements for treatment concerns in HCL

C059

ZAP-70 EXPRESSION AND UNMUTATED IGVH GENES IN B-CLL ARE ASSOCIATED WITH HIGH RISK OF AUTOIMMUNE CYTOPENIAS

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Autoimmune cytopenias, (AIC) particularly autoimmune haemolitic anemia (AIHA) and autoimmune thrombocytopenia (AITP) are not infrequent in B-CLL patients, but factors predicting these events are not definitively identified and influence of AIC on survival is controversial. We evaluated occurrence, risk factors and prognostic relevance of AIC in 260 CLL patients (163 males, 97 females, median age 62 years) selected on the basis of the availability of ZAP-70 expression tested by immunohistochemistry (IHC) on bone marrow biopsy performed at presentation. Of them 198 were Binet stage A (76.1%), 44 Binet B (16.9%) and 18 stage C (6.9%). The median follow-up period was 75 months (12-240). We correlated the occurrence of AIC with ZAP-70 expression, detected by IHC in all patients, IgVH genes status, evaluated in 113 cases, and the majority of known common clinical and biologic prognostic parameters in CLL. AIC occurred in 44 patients (16.9%): 29 (11.2%) AIHA, 14 (5.4%) AITP (4 of them Evans Syndrome), and 1 pure red cell aplasia (PRCA). The 7-year actuarial cumulative incidence of AIC was 17.8+2.9% SE. Patients developing AIC had lower male/female ratio than other patients; at diagnosis, they had higher lactate dehydrogenase, β2microglobulin, thymidine kinase and sCD23 serum levels, more frequent diffuse BM infiltration, unmutated IgVH genes and expression of ZAP-70. Age >65 years, Binet stage C, ZAP-70 expression and IgVH unmutated genes resulted indipendent parameters correlating to development of AIC and to a shorter time to AIC. Moreover CLL patients with both ZAP-70 expression and IgVH unmutated genes presented a 7-years cumulative incidence of AIC of 42%, higher than that of patients discordant for the two prognostic parameters (0%) and of ZAP-70 negative with IgVH mutated genes (7%) (p<0.0001). Treatment did not influence the frequence of AIC, but patients receiving the association of fludarabine and cyclophosphamide (FC) had a lower incidence of AIC. The occurrence of both AIHA and AITP was associated with inferior OS (p=0.039 and 0.050 respectively) at univariate analysis. *Conclusions.* the risk of developing AIC during CLL course is particularly high in ZAP-70 positive patients with unmutated IgVH genes and should be considered when planning treatment and clinical management. We evaluated occurrence, risk factors and prognostic relevance of AIC in 260 CLL patients (163 males, 97 females, median age 62 years) selected on the basis of the availability of ZAP-70 expression tested by immunohistochemistry (IHC) on bone marrow biopsy performed at presentation. Of them 198 were Binet stage A (76.1%), 44 Binet B (16.9%) and 18 stage C (6.9%). The median follow-up period was 75 months (12-240). We correlated the occurrence of AIC with ZAP-70 expression, detected by IHC in all patients, IgVH genes status, evaluated in 113 cases, and the majority of known common clinical and biologic prognostic parameters in CLL. AIC occurred in 44 patients (16.9%): 29 (11.2%) AIHA, 14 (5.4%) AITP (4 of them Evans Syndrome), and 1 pure red cell aplasia (PRCA). The 7-year actuarial cumulative incidence of AIC was 17.8+2.9% SE. Patients developing AIC had lower male/female ratio than other patients; at diagnosis, they had higher lactate dehydrogenase, β2microglobulin, thymidine kinase and sCD23 serum levels, more frequent diffuse BM infiltration, unmutated IgVH genes and expression of ZAP-70. Age >65 years, Binet stage C, ZAP-70 expression and IgVH unmutated genes resulted indipendent parameters correlating to development of AIC and to a shorter time to AIC. Moreover CLL patients with both ZAP-70 expression and IgVH unmutated genes presented a 7-years cumulative incidence of AIC of 42%, higher than that of patients discordant for the two prognostic parameters (0%) and of ZAP-70 negative with IgVH mutated genes (7%) (p<0.0001). Treatment did not influence the frequence of AIC, but patients receiving the association of fludarabine and cyclophosphamide (FC) had a lower incidence of AIC. The occurrence of both AIHA and AITP was associated with inferior OS (p=0.039 and 0.050 respectively) at univariate analysis. *Conclusions.* the risk of developing AIC during CLL course is particularly high in ZAP-70 positive patients with unmutated IgVH genes and should be considered when planning treatment and clinical management.

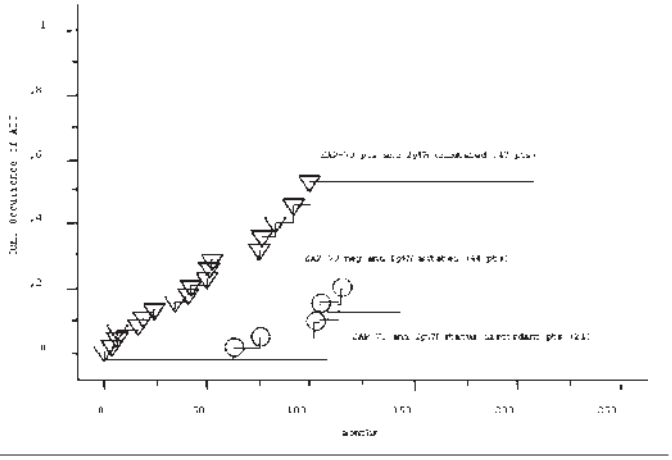

Figure.

C060

EFFICACY AND SAFETY OF A FIRST-LINE COMBINED THERAPEUTIC APPROACH FOR YOUNG CLL PATIENTS STRATIFIED ACCORDING TO THE BIOLOGIC PROGNOSTIC FEATURES: ANALYSIS OF THE GIMEMA MULTICENTER STUDY (LLC0405)

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Eighty-six previously untreated CLL patients ≤60 years, with advanced or progressive disease, from 23 Italian centers, were included in a prospective GIMEMA study. Treatment approach was stratified according to the biologic features of the disease. High risk (HR) patients were defined by the presence of: 1) a 17p deletion (≥20% of analyzed cells), or 2) a 11q deletion with ≥1 additional unfavorable factor (IgVH germline; ZAP-70+, ≥10%; CD38+, ≥7%), or 3) a germline IgVH or mutated VH3-21 and ≥2 unfavorable factors (ZAP^+; $CD38^+$; 6q deletion; trisomy 12). Low risk (LR) patients were defined by the absence of the above mentioned characteristics. For HR patients, treatment consisted of 4 monthly courses of Fludarabine and Campath-1H (FluCam; Flu 30 mg/m^2 iv; Campath-1H 30 mg iv, days 1-3). Patients who achieved a response received a post-induction therapy (reduced intensity PBSCs allogeneic transplant or, in the absence of a sibling donor, an autologous PBSC transplant or, in the absence of a sufficient harvest, Campath-1H sc, 30 mg weekly for a maximum of 12 weeks). For LR patients, treatment included 6 monthly courses of Fludarabine and Cyclophosphamide (FC; Flu 30 mg/m^2 iv and Cy 250 mg/m^2, days 1-3). All patients received Bactrim prophylaxis. Patients treated with FluCam underwent weekly CMV antigenemia monitoring and valacyclovir prophylaxis (2g/8h). Forty-five HR patients (52%) and 41 LR (48%) have been included in the study. Thirty-five HR patients and 23 LR patients have completed the induction therapy. A response was observed in 31 HR patients: OR 89%, CR 34% (MRD negative CR: 23%) and in 23 LR patients: OR 96%, CR 61% (MRD negative CR: 22%). Grade III-IV granulocytopenia was the most common toxicity after FluCam and after FluCy. However, long-lasting cytopenia was observed only in cases treated with FluCy. Asymptomatic CMV reactivation was detected in 3 cases treated with FluCam. Six patients, have died, 4 patients treated with FluCy (febrile granulocytopenia: 2 cases; cerebral hemorrhage: 1; cerebral abscesses of unknown origin: 1) and 2 patients treated with FluCam (disease progression:2). Ten (32%) HR patients who achieved a response have undergone a PBSC transplantation (allogeneic 4, autologous 6). In conclusion, the results of this study, have shown a high CR rate with FluCy given to patients with a LR profile and a considerable response rate with a very few CMV reactivations with FluCam administered to patients with a HR profile.

C061

EFFICACY, SAFETY AND FEASIBILITY OF 5-AZACITIDINE FOR THE TREATMENT OF MYELODYSPLASTIC SYNDROMES IN THE CLINICAL PRACTICE: FINAL RESULTS FROM A RETROSPECTIVE STUDY IN 177 PATIENTS ENROLLED IN THE ITALIAN PATIENT NAMED PROGRAM

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Italian Cooperative Study Group on Azacitidine in Myelodysplastic Syndromes and Acute Myeloid Leukemias

Aims. To evaluate efficacy, safety and feasibility of a treatment with 5-azacytidine (AZA) in patients (pts) with myelodysplastic syndromes (MDS) treated outside of the setting of a clinical trial. *Methods*. Collection of clinical data from a national patient named program registry including 177 MDS pts treated in Italy with AZA between June, 2005, until September, 2007. Updated follow-up: December, 15, 2008. *Results.* Median age was 70 years (range 18-84), M/F ratio 1.55. According to WHO classification, there were 19 RA, 5 RARS, 5 5q- syndrome, 30 RCMD, 44 RAEB-1, 71 RAEB-2, 3 MDS-U. Seventy-four pts were classified as low or intermediate-1 IPSS risk (lower risk cohort), while 103 pts were scored as intermediate-1 or high risk MDS (higher risk cohort). Median time from diagnosis for lower and higher risk pts was 21.5 months (range 1-132) and 6 months (range 1-96), respectively. One-hundred forty-one pts (80%) were transfusion-dependent, 117 (66%) had received prior treatments. AZA was administered as single agent in 122 pts (69%), while in the remaining subjects it was variously combined with erythropoietin +/- G-CSF, valproic acid +/- ATRA, or other drugs. Fifty-eight percent of pts received a standard dose of 75 mg/sqm/d s.c., 42% a fixed dose of 100 mg/d s.c. Single cycle treatment duration was 7 days in 60 %, 5 days in 36%, 10 days in 6% of pts. The median dose of AZA per monthly cycle was 700 mg (range 425-1105 mg). The median number of cycles administered in lower and higher risk pts was 7 (range 1-30) and 4 (range 1-11), respectively. According to the 2006 IWG criteria, overall response rate (ORR), including complete response (CR), partial response (PR), haematological improvement (HI), and bone marrow complete response (M-CR), was 41% (Table 1). ORR was 52% and 43% in lower and higher risk pts who completed at least 4 cycles of treatment, respectively. Thirty-two percent of pts showed stable disease under AZA treatment. After a median follow-up of 15 months, a significant 3-year overall survival (OS) benefit was found in responders versus non-responders (p<0.0014 in lower risk, p<0.00013 in higher risk pts) (Table 1). A better outcome was observed in younger, higher risk pts, without transfusion dependence. Grade 3 or 4 toxicities were myelosuppression (18%), infections (9%), and gastrointestinal (3%) or cardiac (<1%) adverse events. No death could be directly correlated to AZA administration. *Conclusions*. In the clinical practice, AZA may be a feasible, safe and effective therapeutic option for a significant proportion of both lower and higher risk MDS pts.

Table 1

	ORR	*CR*	*PR*	*HI*	*M-CR*	*Response length (median, range)*	*3-year OS*	*3-year OS responders*	*3-year OS non-responders*
Low/Int-1 risk	46%	11%	10%	20%	5%	6 months, 1-32	71%	94%	54%
Int-2/High risk	38%	7%	15%	11%	5%	5 months, 1-21	39%	67%	25%

C062

NOVEL CHROMOSOMAL LESIONS ARE REVEALED IN CYTOGENETICALLY NORMAL MDS PATIENTS BY FISH PROBES DERIVED FROM ARRAY CGH

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The chromosomal pattern is one of the most important parameters to predict overall survival (OS) and the risk of MDS/AML evolution in MDS patients. However, conventional cytogenetics (CC) is not informative in about 40-50% of patients, especially low-risk MDS, who show a normal cytogenetic profile. In these patients CC results can be improved by FISH with probes specific for the chromosomal regions most frequently involved in MDS (Bernasconi et al, 2003). Recently, array CGH (aCGH) studies have provided evidence that chromosomally normal and abnormal MDS patients may harbour novel chromosomal lesions which target regions never affected by CC studies. So, the present study is aimed to establish whether probes derived from a recent aCGH analysis (Starczynowski *et al.,* 2008) were truly able to unmask cryptic lesions in chromosomally normal MDS patients, whether these defects were either chromosomal gains/losses or balanced rearrangements and whether they effected OS and disease evolution. FISH analyses were carried out in twenty-three patients examined between January 2005 and May 2008. There were seven females and sixteen males, whose median age was 64 years (range 24-75). According to WHO classification, 3 patients were classified as RA, 9 as RAEB-1 and 11 as RAEB-2. Considering IPSS score, 4 patients were considered low-risk, 10 intermediate-1 risk and 9 intermediate-2 risk. Median follow-up was eight months (range 1-46). At the time of the analyses no patients have died; 4 had progressed to RAEB-2 and 3 to AML. Our FISH analyses used probes derived from a recent aCGH study carried out in MDS patients. Two criteria were followed to choose our probes: how frequent was their involvement in aCGH studies and which was their Mb position determined using UCSC genome browser on Human Mar. 2003 assembly. All probes, obtained from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA), were labelled and applied as previously reported. We used the following probes: RP11-912d8 (19q13.2); RP11-196p12 (17q11.2); RP11-269c4 (14q12); RP11-351o1 (10q21.3); RP11-144g6 (10q11.2); RP11-122a11 (7q34); RP11-951k18 (5q13.1); RP11-100m20 (4p14); RP11-544h14 (2q33). The cut-off values for interphase FISH (i-FISH) were obtained from the analysis of 300 nuclei from ten normal samples and were fixed at 10%. Twelve patients (52.1%) presented an abnormal FISH pattern: five presented a 19q13.2 deletion, three a 14q12 deletion, two an amplification of band 4p14, two a defect of band 10q21.3, two a potential amplification and one a deletion of band 10q11.2, two a deletion of band 5q13.1 and one a deletion of band 17q11.2. Four of these patients harboured two or more defects. An abnormal FISH pattern was observed in 2/3 RA patients, in 4/9 RAEB-1 and in 6/11 RAEB-2 and in 2/4 IPSS low-risk, in 6/10 intermediate-1 risk and in 4/9 intermediate-2 risk MDS patients. When cryptic defects were correlated with disease progression, such a complication occurred in the only 2 RA patients with an abnormal FISH pattern and in 3 of the 5 RAEB-1/RAEB-2 patients with an abnormal FISH pattern. However, additional studies are warranted to estimate the prognostic relevance of these cryptic lesions. In conclusion, our data suggest that FISH probes derived from aCGH studies i) reveal novel chromosomal lesions affecting unsuspected regions in about 52% of chromosomally normal MDS patients; ii) these lesions mostly consist of chromosomal gains/losses; iii) an abnormal FISH pattern seems to correlate with disease progression.

C063

ROLE OF LIPID SIGNALLING PATHWAYS IN THE RESPONSE TO ERYTHROPOIETIN IN LOW RISK MDS PATIENTS

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Nuclear lipid metabolism has widely been implicated in cell growth, differentiation and neoplastic transformation. An impaired regulation of the PI3K/Akt axis is often associated with hematologic malignancies, including acute and chronic leukemias. Our group previously demonstrated not only that Akt is activated in high-risk MDS patients, but also that there is an inverse correlation between PLCβ1 expression and Akt activation (Follo MY *et al*., Leukemia 2008). Moreover, recent results proved that PLCβ1 mono-allelic deletion is associated with a higher risk of AML evolution (Follo MY *et al.*, JCO 2009). Erythropoietin (EPO) treatment is currently used in the therapy of low risk MDS patients, to compensate and counteract their ineffective erythropoiesis. However, little is known about the molecular mechanisms underlying the effect of this drug in MDS and the reasons why some patients do not respond to this treatment, or subsequently lose response. The activation of the EPO receptor has been linked to the activation of the PI3K/Akt axis, which in turn is linked to both PLCβ1 and PLCgamma1 signalling, so that EPO could affect cell proliferation and apoptosis. In this study we further investigated the role of PLCβ1 and Akt in MDS, focusing on low risk MDS patients treated with EPO. We studied 16 patients (IPSS risk low or intermediate-1), 8 of them (50%) showing a favourable response to EPO. We firstly examined the presence of PLCβ1 mono-allelic deletion in these MDS patients by FISH analysis. Subsequently, we quantified PLCβ1 mRNAs. Finally, we investigated the degree of Akt activation, as well as PLCβ1 protein expression, before and during EPO treatment. Our data show not only that 31% (5/16) of our low risk MDS patients displayed the PLCβ1 mono-allelic deletion, but also that 3 of the patients bearing the deletion were refractory to EPO treatments, thus confirming the possible involvement of PLCβ1 in the EPO signalling. Moreover, in responder patients we observed an increase in activated Akt levels. Taken together, our results indicate that the Akt and PLCβ1 pathways are critical for cell survival and proliferation in MDS patients treated with EPO. Therefore, our data strengthen the concept that the signal transduction pathways could become in the future an important target for the development of innovative strategies for MDS treatment.

C064

DEFERASIROX TREATMENT INDUCES A NF-KAPPAB ACTIVITY MODULATION IN A SUBGROUP OF MYELODYSPLASTIC AND MYELOFIBROTIC PATIENTS WITH CHRONIC IRON OVERLOAD

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Background. Iron overload is a critical issue in low-risk myelodysplastic syndrome (MDS) patients due to the high transfusion requirement during disease history. Iron chelation therapy (ICT) is now recommended in order to preserve organ functions and improve survival. We previously reported 4 cases (3 MDS and 1 myelofibrotic patients) showing an haemoglobin improvement during ICT with deferasirox. Our *in vitro* data demonstrate that this drug induces an NF-kappaB inhibition in leukemic cell lines and MDS cells and suggest a possible explanation of our *in vivo* observation. Furthermore, a high level of the negative regulator of hepcidn, growth differentiation factor 15 (GDF15), has been reported in patients with ineffective erythropoiesis including MDS patients and in clinical conditions of iron depletion and we hypothesize that could be modulated by ICT. *Aims*. to study NF-kappaB activity and GDF15 expression modulation during ICT in patients affected by MDS and primary myelofibrosis (PMF) treated by deferasirox. *Methods*. After written informed consent, 40 serum samples and mononuclear cells were collected prospectively from 10 patients, 8 were affected by MDS and 2 by PMF. Clinical data and samples were collected before and after 1-3-

6-9 and eventually 12 months of deferasirox treatment. Serum GFD15 and NF-kB activity evaluations were performed by ELISA test. *Results.* Median serum ferritin level was 1350 ng/mL (range 1020-3500) and median haemoglobin value before treatment was 7.5 g/dL (7-8.5). Median deferasirox dosage during treatment was 750 mg/die and median follow up period was 6 months (3-12). Two out of 10 patients showed an haemoglobin improvement during ICT which leads in few months to a transfusion requirement reduction. NF-kappaB activity was significantly higher than in normal controls in 5 out of 10 patients, among them we observed a statistically significant NF-kB activity reduction after deferasirox therapy in 3 out of 5 (including both the patients with an erythroid response. GDF15 level appears to be modulated by ICT very rapidly (after almost one month of treatment). *Discussion*. In our cohort of MDS patients deferasirox therapy modulates GDF15 production probably due to iron burden reduction. In addition, it induces an haemoglobin improvement in a minority of MDS/PMF patients which is associated to a NF-kB high basal activity reduction *in vivo*.

C065

BORTEZOMIB THERAPY IN MYELODYSPLASTIC PATIENTS: THE RESULTS OF A PHASE II TRIAL

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Bortezomib (Velcade, formerly PS-341) is a proteasome inhibitor with documented antitumor activity in multiple myeloma and other lymphoid malignancies. We performed a Phase II study to investigate the efficacy and safety of bortezomib in patients with myelodysplastic syndromes (MDS). Ten patients were enrolled in the study: there were 6 females and 4 males, median age was 64 years (range 44-70), median duration of MDS phase was 12 months. According to WHO classification, 5 patients were refractory cytopenia with multilineage dysplasia (RCMD), 1 patient was pure refractory anemia (RA), 1 patient was RCMD with ringed sideroblasts (RCMD-RS), 3 patients were refractory anemia with excess of blasts type 1 (RAEB-1). According to IPSS stratification, 5 patients were classified as low risk, 3 patients as intermediate-1 and 2 patients as intermediate-2 risk. All patients but one received prior erythropoietin therapy (in 1 patient plus steroid therapy) without efficacy. Eight out of 10 patients required transfusional therapy at the time of enrolment (median 4 units per month). Six patients presented cytogenetic aberrations at baseline; 9/10 patients displayed elevated WT1 expression level in bone marrow and 7/10 also in peripheral blood. Bortezomib was administered at the dose of 1.3 mg/mq with a 1, 4, 8, 11 day schedule, every 28 days, for a maximum of 8 cycles. Four patients discontinued therapy after 1, 3, 3 and 4 cycles respectively, due to vascular disease not related to the drug in the first two cases and to disease progression in the other two (both patients with intermediate-2 risk), whereas 6 patients performed all planned eight cycles. Haematological toxicity was recorded in all patients: grade 3/4 neutropenia in 4 patients and grade 3/4 thrombocytopenia in 6 patients. Non-haematological side effects were recorded in 7 patients: diarrhoea in 1 patient, fever in 3 patients, skin rash in 2 patients and pneumonia in 1 patient. All events were limited and no life-threatening. As to response, 3 patients (50% of evaluable cases) achieved a minor erythroid response and 3 patients had a stable disease. As to outcome, 7/10 patients are still alive at a median follow-up of 24 months. After therapy, bone marrow WT1 levels decreased in the patients with minor erythroid response (from median level of 184 copies at diagnosis to a median of 17 copies at the end of treatment), and in 1 patient with stable disease. In patients with stable disease, median WT1 copies number in bone marrow cells was 659 copies at diagnosis and 890 copies at the end of treatment, while it was 736 copies at diagnosis and 788 copies at the end of treatment in peripheral blood. No modifications were observed as to % of bone marrow blasts and of cytogenetic changes with respect to pre-treatment findings. In conclusion, bortezomib used alone in MDS seems to have some efficacy in terms of haematological improvement and appears to also affect the WT1 gene expression, which is typically increased in these diseases.

C066

A STANDARDIZED MORPHOLOGICAL PANEL FOR THE ASSESSMENT OF BONE MARROW DYSPLASIA IMPROVES ON THE ACCURACY OF WHO CLASSIFICATION IN MYELODYSPLASTIC SYNDROMES

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WHO proposal for myelodysplastic syndrome (MDS) classification, that was recently revised, introduced uni- versus multilineage dysplasia as a diagnostic criterion in MDS with <5% bone marrow (BM) blasts, increasing the prognostic value of this classification. However, a structured and reproducible approach for the precise definition and quantification of BM dysplasia is still lacking, and the relationship between cytopenia and dysplasia needs to be clarified. In order to identify a panel of reproducible morphological criteria useful for a correct application of WHO classification, we retrospectively examined in detail the cytological features of BM smears from 429 MDS patients previously classified according to FAB criteria, 214 patients with hyporegenerative anemia and 74 healthy subjects. By counting 100 cells for the erythroid and granulocytic lineages and at least 30 megakaryocytes and classifying them for their dysplastic changes, a panel of dysplastic features showing a better sensitivity and specificity for MDS identification was developed. Some morphological abnormalities were associated with poor outcome and total granulocytic or megakaryocytic dysplasia showed a significant independent unfavorable prognostic value (p=0.0004 and p<0.0001 respectively). Also the degree of granulocytic or megakaryocytic dysplasia was of prognostic relevance. The morphological panel was employed to reclassify MDS patients by WHO proposal using the 10% threshold to record dysplasia in each lineage, with a between-investigators and within-investigator agreement of 92% and 95% respectively; 301 MDS cases were correctly reclassified, 45 were unclassifiable for inadequate BM smears and 83 belonged to other hematopoietic neoplasms. On univariate analysis, percentage of BM blasts, multilineage dysplasia and two or more cytopenias were associated with worse outcome but multivariate analysis failed to confirm the prognostic value of cytopenias. All patients with multilineage dysplasia had a significant worse outcome, independently of the number of cytopenias (p=0.001). In conclusion, we confirmed the significance of multilineage dysplasia correlation with high-grade MDS. The definition of BM dysplasia with a standardized morphological panel that improves the objectivity and reproducibility of microscopic analysis is useful for a correct application of WHO classification as well as for the differential diagnosis between MDS and other cytopenias.

Erythroid Disorders

C067

EFFECT OF DIHYDROARTEMISININ ON HUMAN ERYTHROID CELL DIFFERENTIATION: IMPLICATIONS FOR MALARIA TREATMENT IN PREGNANCY

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Malaria in pregnancy causes anemia, low birth weight of the baby and increased mortality of both mother and infants. Recommended therapy is consisting of Sulphadoxine-Pyrimethamine, but resistance is increasing, therefore an alternative and safe treatment is urgently needed. Artemisinin combination therapy is the first line treatment; however artemisinin derivatives showed animal embryo-toxicity when treatment is performed on certain days of gestation. Our aim was to investigate the effect of Dihydroartemisinin (DHA), the metabolite of artemisinin, on an *in vitro* model reproducing human erythropoiesis and to characterize the target erythroid stage, in order to predict the window of susceptibility to DHA in human pregnancy. $CD34^+$ cells from peripheral blood of healthy volunteers were cultured for 14 days with a specific medium containing erythropoietin to induce erythroid differentiation. DHA at 0,5 or 2 μM, according to the dosages of previous animal experiments, was added for the first time at day 0 (isolated stem cell), at day 2 (early erythroid progenitors), at day 4 (early progenitors and pro-erythroblasts), at day 7 (basophilic erythroblasts) or at day 11 (polychromatic erythroblasts) and then continuously every 3 days, because of its short half life. Cells growth and viability were evaluated by trypan blue exclusion; erythroid differentiation was investigated by FACS analysis of Glycophorin A expression, by morphological analysis and by globin gene expression analysis. DHA added on stem cells or early erythroid progenitors (day 0 and 2 of culture) caused a transient inhibition of both cell growth and differentiation ($p<0.05$) up to day 7, but then the treated cells started growing and completed their erythroid differentiation. When DHA was added on more differentiated basophilic erythroblasts (day 4 and 7 of culture) a significant and long lasting decrease in proliferation and erythroid differentiation was observed up to day 14. With DHA added on mature stages (day 11 of culture), no effects on erythroid differentiation were observed. These data suggest that DHA specific target is the basophilic erythroblast since DHA added at this stage causes a significant inhibition of erythroid differentiation. Based on these *in vitro* results, we hypothesize that DHA could affect human primitive erythropoiesis, which occurs during the late phase of human secondary yolk sac erythropoiesis (weeks 4-8 of gestation), when foetal blood is formed of only primitive erythroblasts. This means that if the treatment with DHA is performed during the first trimester of human pregnancy, toxic effects on embryo could be expected.EU Antimal Project 18834 is acknowledged.

C068

DIAMOND-BLACKFAN ANEMIA: GENOTYPE-PHENOTYPE CORRELATION IN PATIENTS WITH RPL5 AND RPL11 MUTATIONS

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Diamond-Blackfan anemia (DBA) is a rare, pure red blood cell aplasia of childhood due to an intrinsic defect in erythropoietic progenitors. About 40% of patients display malformations. Anemia is corrected by steroid treatment in more than 50% of cases; non-responders need chronic transfusions or stem cell transplantation. Defects in the *RPS19* gene, encoding the ribosomal protein (RP) S19, are the main known cause of DBA, and account for more than 25% of patients. Mutations in *RPS24, RPS17*, and *RPL35A* described in a minority of patients show that DBA is a disorder of ribosome biogenesis. Gadza *et al.* and Cmejla *et al.* have reported involvement of two new genes (*RPL5, RPL11*) encoding for RPs of the large subunit in a considerable percentage of DBA patients. Here we report the screening of *RPS14, RPS16, RPS24, RPL5, RPL11*, and *RPL35A* in 92 Italian patients negative for *RPS19* mutations (36/128). We identified 12 heterozygous RPL5 mutations in 92 patients (13%); these include nonsense mutations, donor splice-site mutations, deletions or insertion of 1-5 nucleotides causing frameshift and one missense mutation. We identified 12 heterozygous RPL11 mutations in 92 probands (13%), the 92% of them (11/12) are deletions of 1-47 nucleotides. We also found two *RPS24* heterozygous mutations while no alterations were detected in *RPS14, RPS16* and *RPL35A*. None of these changes were found on the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/) and all are expected to alter the genetic information drastically and to cause haploinsufficiency. Interestingly, an increased risk of somatic malformations was shown in patients mutated in RPL5 (83%) or *RPL11* (73%) if compared to patients mutated in RPS19 (43%) or without molecular alterations (29%). Specifically we observed a close association between craniofacial anomalies and RPL5 mutations and between hand malformations and *RPL11* mutations (Figure 1). About 20% of Italian DBA patients displayed mutations in RPL5 or *RPL11*, and only 1.6% in RPS24. Genotype-phenotype data suggest that mutation screening should begin with *RPL5* and *RPL11* in DBA patients with a malformative status, and specifically in those who present craniofacial aberrations or hand abnormalities.

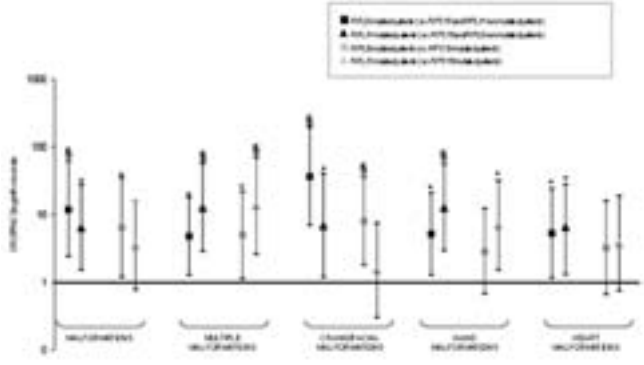

Figure 1. Malformative status of RPL5 and RPL11-mutated patients. Associations between malformative status and RP gene mutations are assessed with odds ratio and 95% CI calculated from logistic regression; OR are drawn on a logarithmic scale. RPL5 and RPL11-mutated patients are compared to both RPS19-mutated patients and non-mutated patients. The Wald test is used to test the statistical significance of each association, the p value is indicate as (*) $p<0.05$; (§) $p<0.01$.

C069

IRON METABOLISM IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS ON ECULIZUMAB: FROM PERPETUAL HEMOSIDERINURIA AND RENAL SIDEROSIS TO POSSIBLE LIVER OVERLOAD DUE TO EXTRAVASCULAR HEMOLYSIS

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PNH is characterized by chronic complement-mediated intravascular hemolysis (IH) and consequent perpetual urinary iron loss; thus, even in presence of large transfusional requirement, PNH patients are prone to develop iron deficiency rather than iron overload. Eculizumab (Ecu) has proven effective for the treatment of IH in PNH patients; however, in some cases residual C3-mediated extravascular hemolysis may impair Hb normalization. We evaluated iron metabolism in 5 untreated PNH patients and 17 who were receiving Ecu, combining biochemical parameters with a semiquantitative T2* MRI technology, which gives a signal intensity (SI) of renal cortex, liver and spleen. Taking all patients together (regardless they were or were not on Ecu) there was a significant correlation between liver SI and serum ferritin ($p<0.001$), while kidney SI correlated with the presence of hemosiderinuria (HS, $p<0.001$). All

untreated PNH patients showed significant renal cortex siderosis with normal SI in liver and spleen, consistent with ongoing IH and HS. In contrast, the 17 PNH patients on Ecu showed a different and heterogeneous pattern. All patients showed a normal renal SI, with the exception of 2 cases who have recently started Ecu and had still detectable HS and 1 experiencing Ecu breakthrough. More interestingly, the majority of patients showed increased hepatic SI, with moderate in 4 and severe iron overload in 5 cases; some patients also showed increased splenic SI. There was a statistical correlation between hepatic SI and serum ferritin. Looking for possible causes of iron overload, only a single patients was receiving transfusions due to partial response to Ecu; patients with suboptimal hematological response were more likely to develop severe hepatic iron overload (p=0.02). Looking to other clinical and laboratory data, while there was no relationship with LDH level, liver SI correlated with both absolute reticulocyte count (p=0.02) and % of C3+ PNH RBCs (p=0.02), both considered markers of extravascular hemolysis. In conclusion, we show by T2* RMI that untreated PNH patients have significant renal siderosis, which tends to disappear during Ecu treatment; such blockade of urinary loss may render PNH patients susceptible to liver iron overload resulting from transfusions. We also provide evidence that changes in iron metabolism during Ecu treatment may be linked to the recently documented C3-mediated residual extravascular hemolysis, and may deserve specific care.

C070

SAFETY AND EFFICACY OF SUBCUTANEOUS ALEMTUZUMAB AS IMMUNOSUPPRESSIVE TREATMENT FOR APLASTIC ANEMIA, PURE RED CELL AND WHITE CELL APLASIA: RESULTS FROM A PROSPECTIVE PHASE II STUDY

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Acquired bone marrow failure syndromes (BMFS), such as aplastic anemia (AA), pure red cell (PRCA) and white cell (PWCA) aplasia, share a common cellular immune-mediated pathophysiology. We investigated an experimental immunosuppressive therapy (IST) based on the anti-CD52 antibody alemtuzumab (ALE). This was a phase II prospective trial which included a total of 25 patients (11 SAA, 12 PRCA and 2 PWCA) who received ALE as subcutaneous injection of 3-10-30-30-(30) mg (total dose 103 mg for SAA, 73 mg for PRCA and PWCA), in consecutive days, followed by oral cyclosporine A (CyA, 1 mg/kg). Among these, 15 (5 SAA, 9 PRCA and 1 PWCA) had not received any previous IST. The treatment was administered on outpatient basis, with the exception of patients requiring hospitalization for clinical reasons. All patients completed the treatment without serious adverse events (AE); due to relapse, a total of 63 courses of ALE were administered.

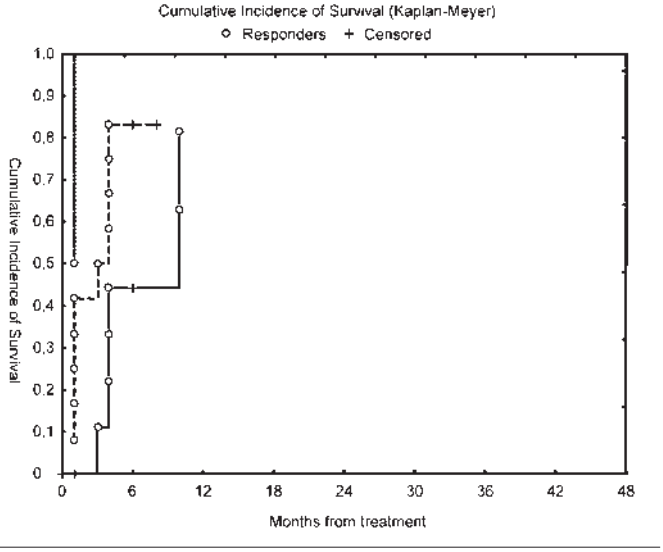

Figure 1.

The most frequent AE was injection-related fever and/or cutaneous rash (28%); transient worsening of blood counts (especially neutropenia) was observed in 40%. Given the major concerns about infectious risk, all patients received adequate prophylaxis, including valganciclovir (VG) and bactrim. With a median follow up of 17 months, infectious events were infrequent and clinically mild. No CMV disease nor EBV-related disease or lymphoproliferative disorders were observed, even if 4 patients developed late asymptomatic CMV reactivation (promptly cleared by VG reintroduction). ALE led to complete lympho-ablation in all patients, which lasted for several months (especially for CD4). There were 3 CR and 3 PR in the 11 SAA, 8 CR and 2 PR in the 12 PRCA; both PWCA achieved a CR. The cumulative incidence of response were 81%, 84% and 100% in SAA, PRCA and PWCA, respectively (Figure 1). Responses were rapid in PRCA and PWCA patients (range 1-4 months), while took longer in the SAA setting (3-10 months). Notwithstanding low-dose CyA, relapses were frequent and quite early (5 of 7 SAAs, 5 of 8 PRCAs and 1 of 2 PWCA). ALE (as either single 30 mg injection or complete courses) retained great efficacy even in patients experiencing multiple relapses, demonstrating easiness of retreatment. This is the largest study investigating ALE as IST for immune-mediated BMFS. We provide evidence that ALE-based IST is feasible and manageable in patients suffering from SAA, PRCA and PWCA, paving the way for systematic investigation in comparison to standard IST.

C071

MOLECULAR CHARACTERIZATION OF HAEMOGLOBIN VARIANTS IN LOMBARDIA

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Hemoglobinopathies are hereditary defects of haemoglobin (Hb) structure. Up to now about 1000 haemoglobin variants have been described. Haemoglobin variants may be unstable, have different oxygen affinity, be responsible for heterogeneous clinical pictures or can be clinically silent.

Table.

β Variants	*n.*	*Origins*
Hb S β 6(A3) Glu>Val	111	Italy, Senegal, Nigeria, Ghana, Morocco
Hb C β 6(A3) Glu>Lys	14	Italy, Burkina Faso, Ivory Coast, Ghana
Hb Lepore WB (delta fino a 87; β da 116)	14	Italy
Hb E β 26(B8) Glu>Lys	6	Thailand, Bangladesh, Philippines
Hb Monroe β 30(B12) Arg>Thr	4	Italy
Hb Camperdown β 104(G6) Arg>Ser	3	Italy
Hb O-Arab β1 21(GH4) Glu>Lys	3	Italy
Hb Knossos β 27(B9) Ala>Ser	2	Egypt
Hb Andrew-Minneapolis β 144(HC1) Lys>Asn	1	Italy
Hb San Diego β 109(G11) Val>Met	1	Italy
Hb Abruzzo β 143(H21) His>Arg	1	Italy
Hb Heathrow β 103(G5) Phe>Leu	1	Italy
Hb M-Milwaukee-2 β92(F8) His>Tyr	1	Italy
Hb Neapolis β 126(H4)Val>Gly	1	Italy
Hb G-San José β 7(A4) Glu->Gly	1	Italy
Hb D-Ibadan β 87(F3) Thr>Lys	1	Italy
Hb D-Los Angeles β 121(GH4) Glu>Gln	1	Italy
Hb Austin β 40(C6) Arg>Ser	1	Italy
Hb City of Hope β 69(E13) Gly>Ser	1	Italy
α Variants	n.	Origins
Hb O-Padova α2 30(B11) Glu>Lys	2	Italy
Hb Icaria α2 142, Stop>Lys	2	Italy
Hb Constant Spring α2 142, Stop>Gln	1	Asia
Hb G-Philadelphia α2-1 68(E17) Asn>Lys	1	Nigeria
Hb Hasharon α2-1 47(CE5) Asp>His	1	Italy
Hb Southern Italy α2 130(B7) Ala>Pro AND α2 26(H13) Ala>Thr		1 Italy
Hb Rampa α2 95(G2) Pro>Ser	1	Italy
Delta Variants	n.	origins
Hb A2-Yialousa δ 27(B9) Ala>Ser	9	Italy, Albania
Hb A2' δ 16(A13) Gly>Arg	2	Ghana
Hb δ 4(A1) Thr>Ile	1	Italy
Hb A2-Puglia δ 26(B8) Glu->Asp	1	Italy
Hb A2-Troodos δ 116(G18) Arg>Cys	1	Italy
Hb A2 Shepherds Bush δ 74(E18) Gly>Asp	1	Italy

Among all the haemoglobin variants, HbS, C and E are the most frequent and represent an important healthy problem, being widespread in Africa (HbS, HbC) and South-East Asia (HbE). In this study we report the molecular data regarding the haemoglobin variants observed in our Hereditary Anemia Centre, the Reference Centre in Lombardia. The molecular analysis have been done for preconceptional genetic counselling or for the definition of haematological phenotype. For each sample we evaluated blood cell count and Hb fractions. The molecular analysis of the globin genes has been carried out through direct DNA sequencing and GAP-PCR. Since 2003 192 carriers of haemoglobin variants have been identified: 88% had β chains variants, 8% had delta chains variants and 5% had α chains variants. Forty-six percent of subjects were homozygotes or double heterozygotes for two variants and 33% had combined α or β-thalassemic trait. The majority of carriers were immigrants, mainly from Central Africa and South East Asia. Nineteen different β structural variants were identified; the most common were HbS (66%), HbC (8%), Hb Lepore (8%), HbE (4%). Among the β variants we discovered: 5 variants with thalassemic phenotype (Hb Lepore, HbE, Hb Monroe, Hb Knossos and Hb Neapolis), 4 rare variants with increased affinity (Hb Andrew-Minneapolis, Hb San Diego, Hb Abruzzo and Hb Heathrow) and 1 very rare responsible for methemoglobinemia (Hb M-Milwaukee-2). Six α variants were identified, 4 on the gene α2 and 2 on the hybrid gene α2-α1, resulting from 3.7 Kb deletion on the α cluster. We also registered the rare chromosome called Hb Southern Italy that originates from the coexistence of two known mutations, HBA2:c.79G>A (Hb Caserta) and HBA2:c.391G>C (Hb Sun Prairie), occurring on the same α2 globin gene. Among the 6 delta variants, Hb A2 Yialousa is the most common (60%), while Hb A2 Shepherds Bush is a new haemoglobin variant which has never been described before. These data suggest that, due to immigration during last 10 years, the haemoglobinopathies became a diagnostic issue in Italy and particularly in northern Italy, where haemoglobinopathies were uncommon.

C072

THE IMPACT OF PREVIOUS OR CONCOMITANT PEG-INTERFERON THERAPY IN DEFERIPRONE-INDUCED AGRANULOCYTOSIS AND NEUTROPENIA: A RETROSPECTIVE STUDY OF A SINGLE UNIT OF "MICROCITEMIA"

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The cure of iron overload and Hepatitis C virus (HCV) still represent significant problems for adult patients (pts) with thalassemia. Iron chelation therapy has been often based on Deferiprone (DFP) alone or in association (alternate and combined) with desferioxammine (DFO) while IFN-treatments alone (Pegylated interferons) have been used to treat concomitant HCV Hepatitis. DFP and IFN can cause neutropenia while DFP is responsible also for agranulocytosis. We evaluated retrospectively the incidence of neutropenia and agranulocytosis in patients treated with DFP and in the subset of patients also pretreated or cotreated with IFN. Between 2001 and 2008, 66 pts were treated with DFP; among these pts, 36 were transiently treated with DFP in combination with DFO for a total therapy exposure of 58 patient-years (median = 1.3 patient-year) while 61 patients were treated with DFP alone or in alternate regimen with DFO for a total therapy exposure of 204 patient-years (median = 2.5 patient-year). A subset of 25 pts were treated (at least for 3 months) also with PEG-IFN (6 were cotreated while 19 were pretreated). Overall, we observed three agranulocytosis and 26 episodes (in 13 patients) of neutropenia, 10 (38%) of them required DFP discontinuation. Overall, the incidence of neutropenia and agranulocytosis was 0.15 and 0.02/100 patient/year, respectively. However, the incidence of neutropenia and agranulocytosis was lower (0.2 and 0.02/100 patient/year, respectively) in patients treated with DFP alone or in alternate combination in comparison to them observed (0.6 and 0.1/100 patient/year, respectively) in patients tretaed with combined regimen. All three cases of agranulocytosis were related to the use of PEG-IFN: two patients (one cotreated, the other pretreated) were under combined regimen, the other patient was under alternate regimen of DFP administration. In 9 patients out of the 25 patients pretreated or cotreated with PEG-IFN, 77% (20/26) of episodes of neutropenia were observed, including 80% (8/10) of the episodes which required DFP discontinuation. The odds ratio for neutropenia in patients treated with IFN was 8.53. Out of 36 patients treated with combined regimen, 11 patients were pretreated or cotreated with PEG-IFN; 4 developed 5 episodes of neutropenia. The odds ratio for neutropenia in patients treated with IFN was 4.19. Total therapy exposure in these 11 patients was 13 patient-years and the overall occurrence of haematological side effects was 4.8/100 patient/year. Our data, suggest that PEG-IFN treatment could play a role in increasing the incidence of DFP-induced haematological side effects.

Non Hodgkin Lymphoma II

C073

SAFETY AND EFFICACY OF BENDAMUSTINE WITH OR WITHOUT RITUXIMAB IN THE TREATMENT OF HEAVILY PRETREATED PATIENTS. A MULTICENTER RETROSPECTIVE STUDY

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Bendamustine is an alkylating agent with a nitrogen mustard group and a purine like benzimidazol group. Recently this drug was introduced in Italy. We analized all pts treated in fourteen haematological Italian centers with Bendamustine alone or in combination with anti-CD20 antibody. Pts who have received at least one complete cycle were evaluated for response and toxicity, pts with ongoing therapy were not evaluated for response but were evaluated for toxicity. The treatments consisted of: Bendamustine 60-90 mg/m^2 days 2,3 alone or in combination with Rituximab 375 mg/m^2 day 1, every 21 or 28 days. 135 pts were analized, median age was 67 (range 31-87), 92 were male, 49 chronic lymphatic leukaemia 29 indolent non-follicular lymphoma, 20 diffuse large B cell lymphoma, 22 follicular lymphoma, 13 mantle cell lymphoma, 2 Peripheral T cell lymphoma. Pts were heavily pretreated the median number of previous treatments was 3 (range 1-8), 65 pts have experienced more than three chemotherapy schemes. One hundred and three pts were previously treated with Rituximab and 22 have performed an autologous transplantation. The Bendamustine pre-treatment condition was: 58 relapsed pts, 29 with refractory disease and 48 with a progressive disease after partial response. The median number of Bendamustine cycles was 4 (range 1-11). One hundred and eight pts were evaluable for response: 30 (28%) complete remission, 59 (55%) partial response or stable disease with an overall response rate of 83% and 19 non responders. Interestingly we observed that all evaluable patients with mantle cell lymphoma obtained a response (5 CR;4 PR), 22/23 (4 CR; 18 PR) indolent non follicular lymphoma and 13/15 (6 CR;7 PR) follicular lymphoma obtained a response, 34/39 CLL obtained a response and 8/18 (4 CR;4 PR) DLBCL obtained a response to therapy. With a median period of observation of 7 months (1-36) 78% of pts are alive. In this group of heavily pretreated pts 551 cycles were performed: the extrahematological toxicity was really mild and the hematological toxicity was trombocytopenia grade 3-4 in 13 patients and neutropenia grade 3-4 in 22 pts. In conclusion this retrospective study shows that treatment with Bendamustine alone or in combination with Rituximab is a safe and efficacy regimen in a subset of pluriresistent patients. This data shows also that the best results could be obtained in indolent lymphoma and incouraging data in mantle cell lymphoma.

C074

A PHASE II TRIAL OF R-FM (RITUXIMAB, FLUDARABINE AND MITOXANTRONE) CHEMOTHERAPY FOLLOWED BY YTTRIUM 90 (90Y) IBRITUMOMAB TIUXETAN (90Y-IT) (ZEVALIN®) FOR UNTREATED FOLLICULAR LYMPHOMA (FL) PATIENTS

Tani M.,[1] Alinari L.,[1] Broccoli A.,[1] Derenzini E.,[1] De Renzo A.,[3] Fanti S.,[4] Fina M.,[1] Gandolfi L.,[1] Marchi E.,[1] Pellegrini C.,[1] Pulsoni A.,[2] Quirini F.,[1] Stefoni V.,[1] Venturini F.,[1] Zinzani P.L.[1] Baccarani M.[1]

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In 2008 we published a multicenter non-randomized phase II trial of fludarabine and mitoxantrone plus 90Y-IT in untreated patients with FL. By the end of the entire treatment regimen 95% of the patients achieved complete remission (CR). With a median follow-up of 30 months, 3-year PFS was estimated to be 76% and 3-year OS 100%. On the basis of these results we are currently conducting a prospective, multicenter, non-randomized, phase II study of R-FM followed by 90Y-IT in untreated patients with FL in which the number of fludarabine and mitoxantrone cycles has been decreased to four and in which rituximab is administered before each cycle. The rationale of the trial is to use different forms of treatment and to reduce the use of conventional chemotherapy and its related toxic effects. Patients eligibility is represented by: age more than 18, stage II-IV, FL grade I-II, WHO performance status 0-2. Patients are treated with standard FM chemotherapy plus rituximab every 28 days for 4 cycles. Patients are restaged 4 to 8 weeks after completion of immunochemotherapy and those achieving at least a partial response are eligible for 90Y-IT. All patients receive a single dose of 90Y-IT 14.8 MBq/kg. At the time of the analysis we enrolled 55 patients. 25 patients were male and 30 female; the median age was 56 years (range 26-84); 12 patients were stage II, 13 stage III and 30 stage IV; 11 patients had a bulky disease. 46 patients completed the induction chemotherapy, all except 2 were eligible for the consolidation treatment with 90Y-IT and 38 patients were restaged after the entire treatment regimen. After the R-FM chemotherapy, the overall response rate was 97,8% (45/46) including 34 (78.2%) CR and 9 (19.6%) partial remissions (PR). Time to event analyses, including TTP and duration of response are pending further follow-up. Treatment was well tolerated grade 3-4 haematologic AEs (mostly neutropenia) were seen in 50% of the patients. Among the 38 patients (9 PR and 28 CR) subsequentially treated with 90Y-IT and reassessed for the response. In particular, among 9 PR patients 8 (88.9%) improved their remission status from PR to CR. 90Y-IT toxicity included mostly grade 3-4 neutropenia and thrombocytopenia and was comparable to the literature data. These preliminary data indicate that radioimmunotherapy appears highly effective and feasible as "consolidation" after short (4 cycles) immunochemotherapy, improving quality of response without any cumulative toxicity.

C075

MOLECULAR SIGNATURE DETERMINED BY IMMUNOHISTOCHEMISTRY IN YOUNG PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) WITH POOR PROGNOSIS, TREATED WITH RITUXIMAB AND HIGH-DOSE CHEMOTHERAPY (HDC) PLUS AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT): NON GERMINAL CENTER B-CELL (NON-GCB) PROFILE DOES NOT HAVE AN UNFAVORABLE OUTCOME COMPARED TO GCB

Chiappella A.,[1] Novero D.,[2] Tucci A.,[1] Facchetti F.,[3] Cabras M.G.,[1] Angelucci E.,[1] Bottelli C.,[1] Botto B.,[1] Ciccone G.,[4] Falchi L.,[1] Frairia C.,[1] Levis A.,[1] Liberati A.M.,[1] Orsucci L.,[1] Perrone T.,[1] Pogliani E.M.,[1] Rota-Scalabrini D.,[1] Salvi F.,[1] Tonso A.,[1] Inghirami G.,[2] Vitolo U.[1]

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Introduction. In Rituximab (R) era, the prognostic value of specific molecular signature GCB and non-GCB was confirmed in studies conducted by gene profiling analysis, but data with immunohistochemistry analysis are controversial. *Patients and methods.* from June 2002 to December 2005, 97 patients <61 years with newly diagnosis of DLBCL with poor prognosis (age adjusted-International Prognostic Index 2 or 3), were enrolled into a phase II GIMURELL trial (Vitolo U, Haematologica, in press). Treatment was: 4 courses of dose-dense R-MegaCEOP14, followed by R-HDC and ASCT (2 R-MAD + BEAM and ASCT). Tissue microarray (TMA) blocks were created from all cases and they were studied in immunohistochemistry; sections were stained with antibodies to CD20, CD3, CD10, CD138, Ki67, Bcl6, Bcl2, MUM1 and cyclinD1. Patients were classified as GCB or non-GCB according to the Hans' algorithm. Samples were scored positive for CD10, Bcl-6 and MUM-1 if 30% or more of tumor cells were stained. Cases with the coexpression of CD10 and MUM-1 were classified as GC-activated according to Chang and grouped with GCB subgroup for purpose analysis. Crude Kaplan-Meier overall (OS) and progression-free (PFS) survival curves were estimated both overall and stratified by TMA features. Differences between curves were tested using the 2-tailed log-rank test. *Results.* all samples were centrally reviewed and 3 were excluded because of follicular grade IIIa in 2 and mantle cell blastoid variant in one. Nine-

ty-four samples were studied in immunoistochemistry; overall 69 were evaluable, 25 were not, due to inadequate pathological materials. Thirty-seven patients were classified as GCB, 24 as non-GCB and 8 as GC-activated. Patient characteristics were well balanced between GCB and non-GCB subgroups. With a median follow-up of 53 months, in the whole series 4-year OS and 4-year PFS were: 81% (95%CI: 71%-88%) and 74% (95%CI: 64%-82%) respectively. At the same timeframe, 4-year OS and 4-year PFS in GCB vs non-GCB were 77% vs 86% and 65% vs 87% respectively (Figure 1). Conclusions: the scheme with R-dose dense chemotherapy and HDC with ASCT is effective in high risk DLBCL in both GCB and non-GCB subgroups. Namely, in this analysis, non-GBC profile as determined by TMA does not represent a poor prognostic feature. More detailed biological analysis are needed to further characterize these patients.

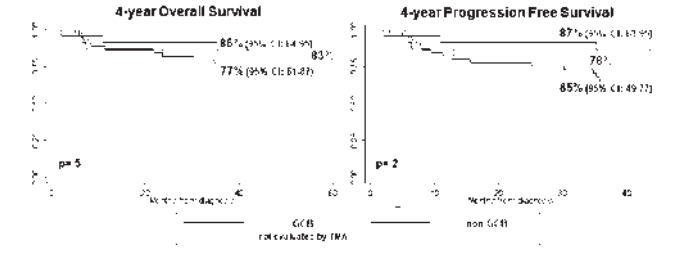

Figure 1.

C076

YTTRIUM-90 IBRITUMUMAB TIUXETAN RADIOIMMUNOTHERAPY IN PRETREATED NON-HODGKIN'S LYMPHOMA (NHL): EXPERIENCE ON 57 PATIENTS

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Background. On the basis of the historical data regarding the role of radioimmunotherapy in pretreated NHL, we reviewed our clinical database on this issue. *Patients and Methods.* Between July 2005 and June 2008, 57 patients were treated with Yttrium-90 Ibritumumab Tiuxetan. Twenty-four were males and thirty-three females with a median age of 53 years (range 33-78), and all of them had been previously treated by at least one rituximab-containing chemotherapy. The median number of pretreatments was 3 (range 1-9). Forthy-six patients had stage III-IV disease (31 patients had bone marrow involvement); 6 patients had bulky disease (≥10 cm). According to the histology, 53 were follicular lymphoma (FL), 1 mantle cell lymphoma, 1 marginal zone lymphoma, and 1 small lymphocytic lymphoma. All patients received rituximab 250 mg/m^2 on days 1 and 8, and either 0.4 mCi/kg of Yttrium-90 Ibritumumab Tiuxetan on day 8 (maximum dose, 32 mCi). *Results.* The overall response rate was 93% (53/57) and the complete response rate was 70% (40/57). In particular, actually 26/40 (65%) patients who obtained a CR are in continuous CR (CCR) with a median follow-up of 13.4 months (range 2.8-35.5 months). Among these long-term responders, four patients are in CCR after at least 24 months. All CCR patients were FL and 21/26 were in stage III-IV before radioimmunotherapy treatment; according to the stage status before Yttrium-90 Ibritumumab Tiuxetan, 12/26 were heavily pretreated (≥3 prior treatments) and, in particular, two patients had prior autologous stem cell transplantation. Toxicity was primarily hematological and mostly transient; no grade 4 non-hematological toxicity was observed. *Conclusions.* This study confirms the safety and high efficacy of Yttrium-90 Ibritumumab Tiuxetan radioimmunotherapy in heavily pretreated FL patients with the possibility to have a subset of long-term responders.

C077

POST-CHT BRAIN IRRADIATION IN PRIMARY CNS LYMPHOMAS (PCNSL): IMPACT ON SURVIVAL OF DIFFERENT FIELDS AND DOSES

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Background. High-dose methotrexate (HD-MTX)-based chemotherapy (CHT) followed by whole-brain irradiation (WBRT), the conventional approach to PCNSL, is associated with relevant neurotoxicity. To avoid or reduce irradiation volumes and doses have been proposed to minimize this complication, but no studies focused on RT parameters exist and the best RT schedule remains to be defined. *Methods*. The impact on outcome of different RT fields and doses was assessed in a mono-institutional series of 85 HIV- pts with PCNSL treated with upfront CHT containing MTX 3.5 g/m^2 every 3 weeks, alone or in combination, followed by WBRT. Pts were stratified according to the response to CHT for analysis of WBRT dose and tumor bed (TB) dose. *Results.* Response after CHT was complete (CR) in 37 (44%) pts and partial (PR) in 17 (20%); 24 pts experienced PD and 7 died of toxicity. Thirty-three of the 37 CRs were referred to consolidation RT; a WBRT ≥40 Gy (3-yr FFS 64% vs. 64%; p=0.31) and a TB dose ≥45 Gy (3-yr FFS 62% vs. 68%; p=0.49) were not associated with a significantly better outcome. The 17 pts in PR after CHT were referred to complementary RT; a WBRT ≥40 Gy was not associated with better outcome (3-yr FFS 25% vs. 33%; p=0.49), while a TB dose ≥45 Gy was associated with a significantly better OS (3-yr OS 44% vs. 17%; p=0.03). Sixteen of the 24 pts with PD after CHT received immediate salvage RT, with 3 CRs and 4 PRs; 9 pts experienced PD. All responders but one experienced relapse and died; WB and TB doses had no impact on survival. *Conclusion.* In PCNSL pts, RT parameters should be chosen on the basis of response to primary CHT: WBRT with 36-40 Gy is advisable in responders, with the addition of a TB boost of 9-14 Gy in pts in PR. Salvage WBRT is inefficacious in pts with PD and substitution with immediate salvage CHT should be investigated.

C078

RADIOIMMUNOTHERAPY WITH 90YTTRIUM ZEVALIN FOLLOWED BY BEAM CONDITIONING REGIMEN (Z-BEAM) AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR THE TREATMENT OF HIGH RISK RELAPSED/RESISTANT NON HODGKIN'S LYMPHOMA (NHL): A SINGLE INSTITUTION EXPERIENCE

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High dose chemotherapy (HDC) and ASCT is an effective treatment for relapsed NHL. Radiolabelled immunotherapy with 90Yttrium Zevalin as single agent delivers targeted irradiation without TBI toxicity. Standard dose Zevalin (0.4 mCi/kg) combined with conventional BEAM (Z-BEAM) is a promising condition regimen for the treatment of high risk relapsed/resistant NHL. We evaluated the feasibility and the efficacy of Z-BEAM in a group of relapsed/refractory pts treated in a single institution. Between October 2006 and December 2008 fourteen pts were treated with Zevalin (day –14) followed by standard dose BEAM (day –7 to –1) and ASCT. Patients were included into the study and considered at high risk of failure if: progression or early relapse (<1 year) from previous therapy or multiple relapses. Rituximab + DHAP/ICE was used as debulking and mobilizing schedule. Clinical characteristics were as follows: 9 refractory and 5 early or multiple relapse; 5 grade I-II follicular, 6 PML/DLBCL, 2 MCL; 4 stage II and 10 stage III-IV; 8 had bulky disease and 6 bone marrow involvement; 7 LDH level above normal. Five pts received only one previous therapy and 9 were treated with 2 lines before Z-BEAM, all containing Rituximab. Only 2/14 pts received a reducted dose of 0.3 mCi/kg Zevalin because of low platelets counts. Response status before Zevalin was: 3 CR (21%), 6 PR (42%), 3 SD (21%) and 2 PD (16%). At the end of treatment response status was: CR 8(57%), PR 5(36%) and PD 1(7%). Overall response rate was converted from 63% to 93%. Median CD34$^+$ cells infused was 7.26 106/kilograms (range 4.43-8.9).All pts engrafted with median time to platelet and neuthrophils count higher than 20x10^9/L

and 0.5x10^9/L of 11 and 10 days respectively. Febrile neutropenia occurred in 10/14 pts. One pulmonary Aspergillosis and 7 bacteriemia were documented. One experienced an intestinal perforation during aplasia and one cardiac failure was documented in a woman previously treated with cumulative antraciclines doses and mediastinal radiotherapy. With a median follow up of 16 months progression free survival (PFS) is 86%. Two refractory pts before Z-BEAM showed a subsequent progression and two pts relapsed: all four died of lymphoma. No toxic deaths were recorded. In this group of pts with high risk relapsed/resistant NHL Z-BEAM+ASCT is able to achieve a good response with engraftment and toxicity not different from standard BEAM. This approach needs to be tested in a larger multicenter study.

Chronic Myeloid Leukemia II

C079

NILOTINIB 800 MG DAILY IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA: 12-MONTHS RESULTS OF A PHASE 2 TRIAL OF THE GIMEMA CML WORKING PARTY

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Background. Imatinib (IM) 400 mg daily is the standard treatment for CML in early chronic phase (ECP). The cumulative rate of CCgR in the IRIS trial for the IM 400 mg arm was 51%, 69% and 87% at 6, 12 and 60 months, respectively. One-fourth of all the IM treated pts fail to obtain a stable CCgR due to resistance/ intolerance. Nilotinib has a higher binding affinity and selectivity for Abl with respect to IM and is highly effective in IM resistant patients, across every disease phase. AIMS To investigate the safety and the efficacy of nilotinib 400 mg BID in ECP, Ph-pos CML patients, the GIMEMA CML WP is conducting an open-label, multicentric, phase II trial (ClinicalTrials.gov.NCT00481052). *Methods.* The primary endpoint is the CCgR rate at 1 year; the kinetic of MR is studied by Q-PCR baseline and after 1, 2, 3, 6, 9 and 12 month. RESULTS 73 patients have been enrolled from 20 Centres between 6/07 and 2/08. Median age 51 years (18-83 yrs), 45% low, 41% interm. and 14% high Sokal risk. Median follow-up 15 months (12-24 m.). CCgR rates at 3, 6 and 12 months (ITT), 78%, 96% and 96%. MMR rates (BCR-ABL: ABL ratio ≤0.1%, International Scale), after 1, 2, 3, 6, 9 and 12 months are 3%, 21%, 52%, 66%, 73% and 81%. One pt progressed at 6 months to ABP with T315I mutation. Nilotinib mean daily dose during the first 12 months was between 750 and 800 mg daily in 64% of the patients, between 600 and 749 mg daily in 10%, between 400 and 599 mg in 18%. 35 (48%) received the full dose of imatinib without any interruption during the first 12 months while 38 patients (52%) had at least 1 treatment interruption; AEs were mostly grade 1 and 2 and manageable with appropriate dose adaptations: the most frequent biochemical AEs were bilirubin increase (53% all grades, 16% grade 3), s-GPT increase (42% all grades, 8% grade 3) and lipase and amylase increase (29% and 18%, all grades, 8% and 4% grade 3+4, respectively); 1 pt went off treatment after 6 months due to recurrent episodes of amylase and/or lipase increase (no pancreatitis). Hematopoietic toxicity g. 3-4 was recorded in 5 pts (7%). *Conclusions.* The results that have been achieved in these unselected patients and within a multicentric trial, strongly support the hypothesis that in ECP, Ph-pos CML patients the response to nilotinib is faster than the response to IM standard and high dose.

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C080

BCR AND BCR-ABL REGULATION DURING MYELOID DIFFERENTIATION IN HEALTHY DONORS AND CHRONIC PHASE-BLAST CRISIS CML PATIENTS

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Chronic Myeloid Leukemia (CML) is caused by the BCR/ABL hybrid gene, the product of the Philadelphia (Ph) chromosome. In spite of all the extensive research in the field, the molecular mechanisms leading from Chronic Phase (CP) to Blast Crisis (BC) are not yet understood. However, both the presence and the levels of BCR/ABL seem to be important for CML progression. BCR/ABL is under the transcriptional control of BCR promoter. Here we focused on the gene expression control of BCR and BCR/ABL upon myeloid differentiation in healthy donors (HD), CP and BC patients. Using Q-PCR, we analyzed BCR and BCR/ABL expression in HD and CML patients in haematopoietic stem cells (HSCs), common myeloid (CMPs) and granulocyte-monocyte progenitors (GMPs). BCR showed a downregulation during myeloid maturation in HD (7 samples, HSCs [0.83] vs CMPs [0.15] and vs GMPs [0.13], p=0.0006 and p=0.0012, respectively). In CP patients (10 samples), BCR and BCR/ABL were downregulated upon committement to differentiation (BCR: HSCs [0.3] vs CMPs [0.1], p=0.0039, and vs GMPs [0.04], p=0.0003; BCR/ABL: HSCs [3.16] vs CMPs [0.8], p=0.0003, HSCs [3.1] vs GMPs [0.5], p<0.0001). However, while BCR levels in HSCs were lower in CP compared to HD (HSC samples [0.83 vs 0.3]: p=0.0031, CMPs [0.15 vs 0.1] p=0.36; GMPs [0.13 vs 0.04]: p=0.7), the expression of BCR/ABL in CP was higher than the one of BCR measured in CP-CML (HSCs: p=0.0031) and in HD samples (HSCs: p<0.0001). In BC samples (7 patients), the decrease of BCR and BCR/ABL was less evident and did not reach statistical significance. Thus in BC the downregulation of BCR/ABL during myeloid maturation is impaired, but this decreased regulation is also shared by BCR. Moreover, we detected a tendency for higher expression of BCR/ABL in BC than CP in all the subpopulations under study, which reached statistical significance only in GMPs (GMPs [2.2 BC vs 0.5 CP]: p=0.0115). In conclusion, the expression of BCR showed a physiological downregulation during myeloid maturation in HD and CP-CML patients. A similar pattern was detected for BCR/ABL, thus suggesting that the two genes may be under a similar transcriptional control. In BC this mechanism seems to be impaired for BCR/ABL and also for BCR. This suggests that an abnormal transcriptional control acting *in trans* on BCR and BCR/ABL promoters could be the cause of the loss of BCR/ABL downmodulation upon myeloid differentiation in BC.

C081

IMATINIB LONG TERM EFFECTS STUDY: THREE YEARS OF FOLLOW-UP AND ASSESSMENT

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Imatinib is an effective therapy for chronic myeloid leukemia (CML). Durable complete cytogenetic responses (CCyR) are reported in the majority of patients, with a benign side effect profile. ILTE is an independent and global study on a retrospective cohort and includes 31 centers in Europe, North/South America, Africa and Asia. Consecutive patients with Ph$^+$ CML who started imatinib between 01 September 1999 and 31 December 2004 were eligible if they were in Complete Cytogenetic Response (CCyR) after two years of imatinib treatment. Study endpoints were (a) survival, (b), serious adverse events (SAE, including second cancers), (c) toxicities not qualifying as SAE (NSAE) but judged by the referring physician as substantially impacting quality of life, (d) loss of CCyR, and (e) development of PCR negativity. A total of 957 patients were enrolled. The median age of eligible patients was 50 (range 15-92) years; the median follow-up is 2.9 years (excluding the first 2 years of treatment). As of Dec. 31 2007, 2565 person years were available for analysis. Eleven deaths were observed (3 CML related), with a standardized rate of 0.4/100 person years and an observed/expected ratio of 0.48 (95% CI = 0.24-0.85; p<0.05).Second cancers were documented in 27 patients,with an observed/expected ratio of 1.22 (95% CI = 0.81-1.13; p=NS).More frequent types of second cancers were prostate carcinoma (37%) and squamous cell (14,8%). One-hundred SAE were recorded, with 21% being considered related to imatinib. Among the 570 NSAE recorded (0.65/patient) the most frequent types were "edema, cramps, skin fragility, diarrhea"; 71% of them were related to imatinib. A total of 12 patients (1.4 %) discontinued imatinib because of toxicities during the period of observation. 34 patients lost CCyR, corresponding to a rate of 1.4/100 person years, with stable or increasing rates over time. Finally, 215 patients (39.7%) developed durable (>1 year) PCR negativity. In conclusion, the report shows that CML patients on imatinib die rarely of CML related causes, do not appear to have substantially higher second cancer rates than the general population, have mortality rates lower than expected in an age/sex matched population and do not show new types of imatinib-related adverse events. They also experience a low but steady rate of loss of CCyR and develop PCR negativity in approximately of cases. Follow-up and further analysis up to Dec 31 2008 is ongoing and will be presented.

C082

VARIANT PHILADELPHIA TRANSLOCATION IN EARLY CHRONIC PHASE OF CHRONIC MYELOID LEUKEMIA: CYTOGENETIC - MOLECULAR CHARACTERIZATION AND CORRELATION TO IMATINIB MESYLATE THERAPY (A GIMEMA WP ON CML ANALYSIS)

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Background. At diagnosis, variant Philadelphia (Ph) translocation occurring in Chronic Myeloid Leukemia (CML), have been reported in 5-10% of patients (pts). Some studies have reported that have no impact on prognosis after Imatinib Mesylate (IM) treatment. Variant translocation could be three or four-way translocation (involving chromosomes 9, 22 and 1 or 2 additional chromosome respectively), associated or not with genetic deletions. Two mechanisms could generate the variant translocations: a one-step mechanism (chromosome breakage was on 3 different chromosomes simultaneously), or a two-step mechanism (t(9;22) translocation is followed by subsequent translocation involving additional chromosomes). AIM To investigate the role of occurrence of variant translocations on the response to IM in early chronic phase (CP) CML pts. METHODS A sub-analysis of 531 evaluable CML pts in early CP, have been performed within 3 simultaneously running trials of the GIMEMA WP on CML (CML/021; CML/022; CML/023). Median observation time was 42 months. Monitoring: hematologic, continuously; CC, FISH and molecular analysis were performed at baseline and then every 6 months by local or reference labs. *Results.* At enrollment, 28pts

(5.3%) had variant translocation: 2pts (7.1%) had a four-way translocation; 26pts (92.9%) had a three-way translocation. Twenty-four pts showed a one-step mechanism and 4 a two-step. In 6pts (21.4%) translocation was associated with deletion of adjacent genetic sequences, which have been characterized by FISH and SNPs assays. One case showed a Ph masked; and one carried an additional chromosome abnormality: t(7;19)(q21;p13). Pts, with or without variant translocation, were similar for age, Sokal risk and IM dose. During followup, 25pts achieved complete cytogenetic response (CCgR; 89.3% vs 91.3% in pts without variant) and major molecular response (89.3% vs 84.7%); in 5 treatment was unsuccessful (17.8% vs 15.9%). The 2pts with four-way translocation and 5 of 6pts (83.3%) with deletions reached CCgR. Two pts with a two-step mechanism failed therapy. *Conclusions.* In our large series of pts in early CP treated with IM therapy, we found no difference in cytogenetic and molecular response rates between pts with or without variant translocations. Some studies suggested that two-step mechanism in the formation of variant translocation was similar to or was in essence a clonal evolution, in association with a poorer prognosis, but it will be to confirme.

C083

OCCURRENCE OF PLEURAL EFFUSIONS DURING DASATINIB TREATMENT IN ELDERLY CHRONIC MYELOGENOUS LEUKEMIA PATIENTS

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Pleural effusions are the most common extra-haematological toxicity observed during Dasatinib (DAS) treatment: their occurrence often requires treatment discontinuation and impairs the otherwise high efficacy of DAS. We revised 86 patients with Chronic Myelogenous Leukemia (CML) in chronic phase treated in 15 Italian Centers, who received DAS when aged >60 years after being resistant/intolerant to Imatinib (IM), in order to find the most important prognostic factors for pleural effusions. There were 52 male and 34 female, Sokal risk score at diagnosis was low in 19 patients, intermediate in 35, high in 14 and not evaluable in 18; median age at DAS start was 69.9 years (IR 65.5-73.9), median interval from diagnosis to DAS therapy was 85.7 months (IR 46.5 -119.4), 44/86 patients (51.1%) had received an IFN-based therapy before IM. As to IM treatment, all patients had initially received the 400 mg daily standard dosage and 49/86 patients (56.9%) had increased the dosage to 600-800 mg/day; on the whole, median period of IM treatment was 54.3 months (IR 29.2-64.6). Starting dosage of DAS was 140 mg in 38 patients, 100 mg in 43 patients and <100 mg in the remaining 5 patients. During treatment, 23/86 patients (26.7%) presented a pleural effusion (grade 2 in 10 patients and grade 3-4 in 13 patients, according to WHO scale); in 5 patients, there was a concomitant pericardial effusion. All 23 patients with pleural or pericardial effusion discontinued DAS; however, DAS was successfully restarted in all but one patient, who died from cardiac tamponade. The interval between discontinuation and restart of DAS treatment was <6 weeks in 16/22 patients (72.8%). The predictive role for pleural effusions of several characteristics (sex, age, Sokal risk, smoke attitude, concomitant cardiological or pulmonary diseases, concomitant diuretic treatment, interval from diagnosis to DAS, CHR at DAS, DAS initial dosage) was evaluated in univariate analysis; only Sokal risk at diagnosis (p=0.006) and presence of concomitant pulmonary disease (p=0.005) were significant. It is worth of note that DAS initial dosage, reported in other trials as an important predictive factor for such complication, did not reach statistical significance in our series. In conclusion, pleural effusions were frequently reported in our unselected population of elderly patients but did not represent a cause of permanent DAS discontinuation; however, a complete pulmonary evaluation before treatment could be useful to tailor the treatment with DAS in elderly patients.

C084

CHRONIC MYELOID LEUKEMIA AS SECOND MALIGNANCY; A RETROSPECTIVE MULTICENTRIC STUDY

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Background. There are few are studies focusing on the survival of patients with CML occurring after a prior malignancy. Moreover, it is not clear if the previous exposure to an antiblastic treatment can modify the clinical course of a "secondary-CML" and its response to tyrosine-kinase inhibitors. *Aim.* To examine the incidence, the presenting features and the outcome of CML diagnosed after a prior malignancy. *Methods*. All incident cases of CML diagnosed during the last 10 years were reviewed and those occurring after a prior malignancy were included in the present analysis. *Results.* Among the 569 pts with CML, 47 (8.2%) were diagnosed following a previous malignancy. Of them, 40% were male with a median age at the time of diagnosis of the primary malignancy and of CML of 52 yrs (22-84) and 60 yrs (29-86) respectively. The most common preceding malignancy was breast cancer (23.4%) followed by colon/rectum (21.3%), prostate (8.5%), lymphoma (8.5%). The median time from primary malignancy to CML was 54 months (range 2-328). Only two pts showed additional cytogenetic abnormalities besides Ph chromosome. 31 pts received treatment with Imatinib and all attained at least an haematological response at 3 months (48.4% Major or Complete Cytogenetic response and 6.4% molecular response); at 12 months 54% achieved a molecular response (complete 9.1%) and 31.8% a Cytogenetic Response (Complete 27.3%) and at 18 months the percentage of complete molecular responses raised up to 31.8% with only two pts (9%) losing response or progressing. With a median follow-up of 28 months only one out of 37 pts treated with Imatinib and 3 out of 10 who received alternative therapies died of CML . According to the treatment received for the primary neoplasm, 19 pts had received chemotherapy and/or radiotherapy while 28 surgery with or without hormonal therapy. The median age at primary tumor diagnosis was similar in the two groups (52 vs 57 yrs) while in the former group the median age at CML diagnosis was significantly lower (57 vs 65 p=0.04) and a trend toward a shorter interval between first neoplasm and CML diagnosis was detected (62 vs 103 months; p=0.06). *Conclusion*. CML secondary to other neoplasms emerges from this study as a well-defined entity with biological, clinical and prognostic features paralleling those of primary CML and in which the role of a prior chemotherapeutic treatment seems to be related to the progression of the leukaemic clone more than to its rise.

Allogeneic Transplantation II

C085

EFFECT OF DONOR-RECIPIENT MINOR HISTOCOMPATIBILITY ANTIGENS MISMATCHES ON REDUCED INTENSITY ALLOGENEIC TRANSPLANT OUTCOMES

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Minor histocompatibility antigens (mHAs) mismatches between full HLA-matched donor and recipient might impact allogeneic stem cell transplantation (alloSCT) outcomes. We aimed to assess whether in 52 consecutive patients allografted for lymphoid malignancies mHAs mismatches could influence acute or chronic GVHD (aGVHD, cGVHD) and overall and progression free survival (OS, PFS). All patients received allogeneic peripheral blood stem-cells after reduced intensity conditioning. GVHD prophylaxis included methotrexate and cyclosporine with or without mycophenolate mofetil. Nine patients had chronic lymphocytic leukemia (17%), 13 Hodgkin's lymphoma (25%) and 30 multiple myeloma (58%). Median age was 51 years (range 17-65), 29 patients were male (56%), 19 patients (37%) were in complete, 27 (52%) in partial remission, 6 had progressive disease (11%). Karnofsky performance status (PS) was >80% in 41 patients (79%). Siblings and unrelated donors (40 and 12, 77% and 23%) were matched at allelic level for HLA-A, -B, -Cw, -DRB1 and -DQB1. Allelic mHAs were assessed by PCR with sequence-specific primers for 14 autosomic mHAs and H-Y. OS and PFS were analyzed with Kaplan-Meier method. The impact of age, sex, disease, PS and mHAs mismatches on grade ≥2 aGVHD and extensive cGVHD was assessed by logistic regression. Median follow-up was 42 months (2-83). One-, 2- and 3-year OS and PFS were 83%, 79% and 74% and 58%, 47% and 43% respectively. Donor-vs-recipient (DR) mHAs mismatches did not significantly impact OS and PFS, which were significantly affected by disease status at transplant (p<0.001 and p=0.003) and PS (≤80 vs >80, p<0.001 and p=0.001). The presence of at least one DR mHAs mismatch, the number of DR mHAs mismatches and the presence of DR hematopoietic-restricted mHAs mismatches significantly increased aGVHD incidence (p=0.02, p=0.02, p=0.01 respectively) whereas broad DR and H-Y mHAs mismatches did not. DR LB-ADIR mHAs mismatches increased aGVHD (p=0.04) whereas DR HA-1, HA-2 and HA-8 mHAs mismatches did not. Recipient-vs-donor (RD) mHAs mismatches, disease status al PS did not significantly affect aGVHD. The presence of at least one RD mHAs mismatch was the only factor which significantly increased cGVHD (p=0.02). In conclusion the study shows that DR mHAs mismatches significantly increase aGVHD in full HLA-matched reduced-intensity allogeneic transplantation. Assessing mHAs mismatches may be useful to tailor patient-specific GVHD prophylaxis.

C086

METHODS OF ARTIFICIAL INTELLIGENCE APPLIED TO GENE EXPRESSION DATA FOR EARLY DIAGNOSIS OF ACUTE GRAFT VS HOST DISEASE

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Rationale. Acute Graft-Versus-Host Disease (AGVHD) is the major complication after allogeneic haematopoietic stem cell transplantation (HSCT) in which functional immune cells of donor recognize the recipient as "foreign" and mount an immunologic attack. At present, the diagnosis of aGVHD is merely based on clinical criteria and biopsy. There is no definitive diagnostic blood test for aGVHD, although a lot of blood proteins have been described as potential biomarkers in small studies. *Aim*. To use standard methods of computational intelligence to validate a novel and not invasive diagnostic method of aGVHD in HSCT patients at onset of clinical symptoms. *Methods*. For this purpose, according to governative research project: "Predictive and prognostic value for graft vs. host disease of chimerism and gene expression", we performed gene-expression profiles (GEPs) on peripheral blood analyzing 47 genes associated to allo-reactivity in 59 patients submitted to HSCT. We used 26 samples from aGVHD (YES) patients that were taken at the time of diagnosis and we selected 33 samples from patients that didn't experienced aGVHD (NO). All together YES/NO patient groups comprised a validation set. GEP results were collected in experimental data base. On this data we have applied 2 feature selection algorithms combined with 2 different classifiers: a Correlation-based Feature Selection (CFS) algorithm combined with an Artificial Neural Network (ANN) and also a wrapper method combined with the Naive Bayesian classifier for selecting the most important features (genes) for the aGVHD diagnosis. *Results.* From comparative computational methods was evident that wrapper was less robust of CFS approach. Infact, on the training data set accuracy wrapper was 90% , accuracy CSF was 96%. Howewer, for the classification aim wrapper with naïve Bayes technique gave the same results of ANN, with 97% of accuracy. In patients with aGVHD level expression of immune gene pattern showed a different behaviour: BCL2A1, CASP1, CCL7, CD83 were up-expressed than reference normal value (it's assumed to be = 1). In contrast, CXCL10, EGR2, FAS, ICOS, IL-4, IL-10, SELP, SLP1, STAT6 was always down-regulated during aGvHD. In aGVHD (NO) group the transcriptosome always showed very high value. *Conclusion*. This is a preliminary and the first study which tackles both computational and biological evidence for the involvement of a limited number of genes for diagnosis of aGVHD. Directions for further studies are outlined.

C087

THIOTEPA BASED CONDITIONING REGIMEN IN 374 PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTS FROM RELATED OR UNRELATED DONORS

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Background. Following the experienced of the Perugia group, who first introduced thiotepa (THIO) in allogeneic stem cell transplants (HSCT), we have developed several conditioning regimens, including THIO , cyclophopshamide (CY), Fludarabine (FLU), melfalan (MEL) and low dose TBI , mainly for patients above the age 45. *Aim of the study.* assess the outcome of patients undergoing an allogeneic HSCT with a THIO based conditioning regimen. *Patients.* 374 patients were allografted with a THIO based regimen, between 1994 and 2005, from HLA identical siblings (n=221) , family partially mismatched (n=67) or unrelated (n=86) donors. Median patient age was 48 years (range16-.67). The stem cell source was unmanipulated in all cases , either bone marrow (n=276) or peripheral blood (n=98). The conditioniong regimens were classified as reduced intensity (n=177) (THIO+CY or THIO+FLU) or intensified (n=197) (THIO +CY with MEL or TBI 200r). The disease was in 1stCR (n=221) or more advanced phase (n=153). Diagnosis were as follows chronic myeloproliferative disease (n=123), acute leukemia (n=120), myelodisplasia (n=46), other (n=85, including lymphoma and myeloma). All, patients received cyclosporin methotrexate GvHD prophylaxis. Alternative donor transplants received additional anti-thymocyte globulin in the conditioning. The median follow up for surviving patients is 5 years (range 1-12 years). *Results.* The overall actuarial 10 year survival is 40%, (57% vs 30% in CR1 or >CR1 disease). The cumulative incidence (CI) of transplant related mortality (TRM) at 10 years is 28% (19% vs 32% for CR1 or >CR1 disease); TRM for CR1 patients grafted from identical siblings (n=94) is 13%. Acute GvHD grade III-IV was seen in 6% of sibling HSCT and 12% of alternative donor grafts. The CI of relapse related death (RRD) at 10 years was 31% (22% vs 36% in CR1 or >CR1). There was no effect of patient age, nor of stem cell source on survival. In multivariate analysis on survival, significant predictors were disease phase (RR 2.4 of death for patients3 beyond CR1) and intensity of the conditioning (RR 1.66 for intensified regimens). These two variables (disease phase and conditioning intensity) were also significant predictors of TRM. Only disease phase predicted RRD. *Conclusions.* This 12 year study in a relatively large number of adults, shows that THIO based conditioning regimens produce encouraging long term survival, with a low incidence of GvHD and low toxicity, when used in combination with CY or FLU alone, especially in patients with early disease. The addition of MEL or TBI reduces RRD, but increases significantly TRM and does not improve survival . Disease phase remains a major predictor of outcome.

C088

LEUKEMIA RELAPSE AFTER ALLOGENEIC TRANSPLANTS FOR AML: PREDICTIVE ROLE OF WT1 EXPRESSION AND PROTECTIVE EFFECT OF WT1 DRIVEN DONOR LYMPHOCYTE INFUSIONS

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WT1 expression was studied in marrow cells from 82 acute myeloid leukaemia , before and after an allogeneic hemopoietic stem cell transplant : 53 patients were in first remission (CR1) and the median age was 44 years. Pre-transplant, 51 patients were WT1- (copy numbers/ 10^4 ABL transcripts ≤180), and 31 were WT1+: their relapse risk was 13% vs 62% (p<0.0001) and their actuarial 3 year survival , 72% vs 27% (p<0.0001). Post-transplant, 6 patients were WT1+ at first assay , and all relapsed. Of 76 WT1- patients on day+30, 56 remained negative, whereas 20 became WT1+ with a marrow in CR: hematologic relapse occurred respectively in 21% vs 50% (p=0.01. In multivariate analysis, WT1 level pre-transplant was the strongest predictor of relapse and survival. Of the 20 WT1+ patients with marrow in CR, hematologic relapse occurred in 3/10 given donor lymphocyte infusions (DLI), compared to 7/9 not given DLI (p=0.03) : survival was respectively 67% vs 14% (p=0.03). In conclusion, WT1 expression is a strong predictor of relapse and survival in patients with AML, and may be used to drive pre-emptive DLI.

C089

KIR POLYMORPHISM IN REDUCED INTENSITY HLA-ID SIBLING STEM CELL TRANSPLANTATION: IMPACT ON CLINICAL OUTCOME

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In the setting of unmanipulated non myeloablative (RIC) hematopoietic stem cell transplantation (HSCT) the possbility of biologically significant donor Killer cell immunoglobilin- like receptors (KIRs)/HLA-C genomic (KIR-ligand) mismatched is potentially important. To define the role of KIRs and KIR-ligands polymorphism we retrospectively analyzed 37 patients who underwent to HSCT from HLA identical siblings for myeloid (n=16) or lymphoid (n=21) malignancy.

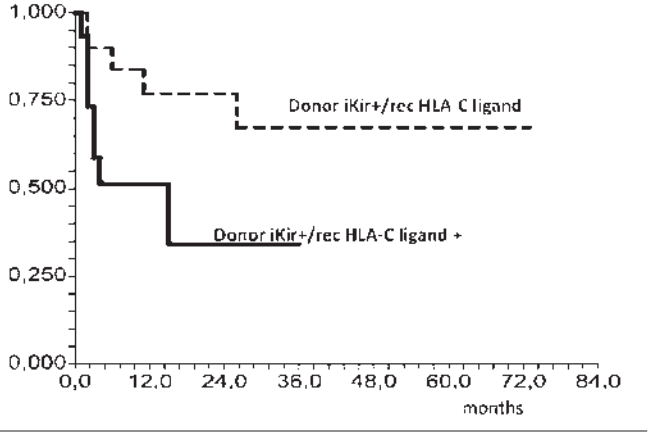

Figure 1.

Patients and donor KIR genes and ligands polymorphisms were determined by PCR-SSP (14 genes plus 2 pseudogenes) and analyzed with different transplantation endpoints. 15/37 (40%) pairs were heterozygote for C1/C2 groups to confirm a polymorphisms in KIR genes also in a HLA-identical sibling setting. 12/37 (32%) and 9/37 (24%) were homoygotes respectively for C1 and C2 groups therefore lacking the KIR-ligand C1 for donor inhibitory KIR2DL2/3 and 20 the KIR-ligand C2 for donor inhibitory KIR2DL1. We cannot find any correlation with acute or chronic GVHD and OS. On the contrary the absence of patients' HLA-C ligand for the corresponding donor inhibitory Kir genes was associated to a better disease free survival (DFS) (9 months versus 3 months, chi square 3,9; p=0.04, logrank test). Moreover the absence in the recipient of group C1 ligand was associated to a higher probability of CMV reactivation ((chi square 4.14, p=0.038). Finally the presence in the donor of at least one Kir inhibitory receptor significantly improved DFS, (chi square 3.9, p=0.039), irrespective to the presence of its HLA class I ligand in the recipient. Despite the small cohort of patients our data suggest a significant impact of inhibitory Kir on disease progression/relapse. Further studies are ongoing to assess the role of activating Kir genes and to evaluate the impact on survival of Kir haplotype. If confirmed in larger series and in unrelated setting, the assessment of Kir genoptype may be used in a more sophisticated donor selection.

C090

NON T-CELL DEPLETED BONE MARROW TRANSPLANT FROM HAPLOIDENTICAL RELATED DONOR IN HAEMATOLOGICAL MALIGNANCIES

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Haploidentical bone marrow transplantation (BMT) is an alternative treatment to patients with high-risk hematologic malignancy lacking a HLA-matched donor and those urgently need transplantation. We used a haploidentical-BMT protocol without ex vivo T cell depleted based on the knowledge that marrow grafts have 10 times fewer lymphocytes compared to peripheral blood stem cell grafts and granulocyte colony-stimulating factor (G-CSF) donor priming reduce the incidence of acute GvHD. *Materials and Methods.* 40 patients (median age of 32, 12-63) with advanced disease or leukemia with poor prognostic features underwent unmanipulated haplo-BMT: 22 with AML, 9 with ALL, 3 with CML, 3 with Hodgkin lymphoma and 3 with plasmacell leukemia. Status at disease: 22 early (first or second complete remission), 18 advanced (progressive or refractory disease). All pairs of donors and recipients were identical for one HLA haplotype and incompatible at 2 or 3 loci.The myeloablative conditioning regimens used were different; antithymocyte globuline, cyclosporine, metotrexate, mycophenolate mofetil and basiliximab were used for GvHD prophylaxis. Donors were primed with filgrastim at 4 micrograms/Kg/d for 7 consecutive days. Bone marrow cells were harvest on the 8 day and were infused unmanipulated. *Results.* the median dose of total nucleated, $CD34^+$ and $CD3^+$cells was $7x10^8$/Kg (1.01-28.7), $2.3x10^6$/Kg (1.17-6.0) and $23.3x10^6$/Kg (9.7-66.6) respectively. 1 patients had a primary graft failure and 5 patients died early prior to engraftment. In the remaining 34 patients, engraftment was seen with median time to granulocyte and platelet recovery of 22 and 27 days respectively; acute GvHD was grade 0 in 17 patients (50%), grade I in 9 (26%), grade II in 7 (20%) and grade IV in 1 (3%). In 29 evaluable patients, chronic GvHD was limited in 3 (10%) and extensive in 1 (3%). Transplant-related mortality at 6 months for early and advantage stage was 22% and 35% respectively. After a median follow up of 18 (3-42) months, 8 patients relapsed; 11 patients (50%) in the early stage and 4 (22%) in advanced phase are now living in haematological remission. The 1-year Kaplan-Meyer probability of disease-free survival is 45% for all patients. *Conclusion.* The high engraftment rate, low incidence of grade II-IV acute GvHD and an acceptable TRM suggest that G-CSF-primed marrow grafting along with sequential immunosuppression could provide an excellent alternative for patients who lack matched donors.

Autologous Transplantation

C091

FINAL ANALYSIS OF THE MULTICENTER PHASE II "BOLOGNA 2002" STUDY INCORPORATING THALIDOMIDE-DEXAMETHASONE (THAL-DEX) INTO UP-FRONT DOUBLE AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT) FOR MULTIPLE MYELOMA

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We report here on the final analysis of the multicenter phase II "Bologna 2002" study which incorporated thal-dex into double ASCT with melphalan (200 mg/m^2) as front-line therapy for younger multiple myeloma (MM) patients. By study design, thal (200 mg/d) and dex (160 mg/month) were administered from the outset until the second ASCT. 378 patients were enrolled in the study; of these, 357 who were followed for a median of 43 months, were analyzed on an intention to treat basis. The most frequent grade III-IV adverse events during induction phase were peripheral neuropathy (3%), constipation (9%) and VTE (13%). 13% of patients discontinued induction treatment, 8% of them due to toxicity and 5% due to progression. TRM after the first and second ASCT was 0.5% and 2%, respectively. The ≥ VGPR rate increased from 33% after induction therapy to 66% after the second ASCT. Median duration of VGPR was 59 months. Median TTP and PFS were 68 and 47 months, respectively. The 6-year projected OS rate was 55%. 90% to 80% of the patients were screened for the presence at diagnosis of del(13q)(45%), t(4;14) (14%) and del(17p) (6%) on purified bone marrow plasma cells. In a multivariate analysis, the absence of del(13q) was significantly related to attainment of at least VGPR after the second ASCT (p=0.003), as well as to extended TTP (p=0.007), PFS (p=0.001) and OS (p=0.007). Additional variables predicting for prolonged OS were attainment of ≥ VGPR after the second ASCT (p=0.01) and low β2-m (p=0.001). PFS was favorably influenced by attainment of ≥VGPR postASCT (p=0.025), low β2-m (p=0.01) and hemoglobin concentration >10 g/dL (p=0.007). A case match comparison of "Bologna 2002" study with the previous "Bologna 96" study of double ASCT confirmed the benefits from the addition of thal-dex in terms of rate (p=0.001) and duration (p<0.001) of at least VGPR, TTP (p<0.001) and PFS (p=0.001). Attainment of ≥VGPR was a major determinant of favorable postASCT outcomes. Poor prognosis conferred by del(13q) was not overcome by thalidomide, consistently with previous results in refractory MM patients and patients receiving maintenance therapy after double ASCT.The limited number of patients carrying t(4;14) and del(17p) precluded a careful analysis of the independent prognostic relevance of these high-risk cytogenetic abnormalities.

C092

SUPERIOR OUTCOME WITH VELCADE-THALIDOMIDE-DEXAMETHASONE (VTD) COMPARED TO THALIDOMIDE-DEXAMETHASONE (TD) INCORPORATED INTO AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT) FOR MULTIPLE MYELOMA (MM) IS NOT AFFECTED BY POOR PROGNOSTIC FACTORS, INCLUDING HIGH-RISK CYTOGENETIC ABNORMALITIES

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Complete response (CR) is a major determinant of prognosis in MM, particularly in the autotransplantation setting. In an attempt to increase the rate of CR before ASCT as a way to improve postASCT outcomes, the Italian Myeloma Network GIMEMA conducted a phase III study comparing VTD with TD administered before and after double ASCT in newly diagnosed MM. In the present analysis we evaluated the outcomes of 460 pts, of whom 226 randomly assigned to VTD and 234 to TD, in relationship to their characteristics at diagnosis. All analyses were intent to treat. In comparison with TD, VTD as induction therapy effected a threefold increase in CR+nCR rate (p<0.001). Superiority of VTD to TD was maintained across all sub-group analyses according to low-risk and high-risk prognostic factors, including high-risk cytogenetic abnormalities such as del(13q) (39% vs 12%, p<0.001), t(4;14) (40% vs 8.5%, p<0.001) and del(17p) (27% vs 0%, p=0.03). Variables associated with achievement of CR+nCR in the two arms that retained statistical significance when assessed by multivariate analysis included randomization to VTD (p<0.001), light chain only subtype (p<0.001), IgA isotype (p<0.001) and Hb>10 g/dL (p=0.01). Randomization to VTD was closely associated with increased CR+nCR rate also after ASCT(s) (p<0.001) and remained statistically significant (p<0.001) in the multivariate analysis, along with light chain only subtype (p<0.001) and IgA isotype (p=0.005). After a median follow-up of 15 months, progression-free survival (PFS) for the 226 pts randomized to VTD was significantly superior to that of patients assigned to TD (24-month projected rate: 90% vs 80%, p=0.009). Superior PFS with VTD vs TD was maintained across all sub-group analyses, including sub-groups with cytogenetic abnormalities. In particular, the 24-month projected rate of PFS for pts carrying del(13q) was 87% with VTD vs 68% with TD (p=0.03), while the corresponding values for t(4;14) positive pts were 85% vs 72%, respectively (p=0.04). We conclude that randomization to VTD up-front was the strongest and independent factor associated with increased rates of CR+nCR before and after ASCT. Superior CR rates with VTD vs TD pertained in both low-risk and high-risk sub-groups, including high-risk cytogenetic sub-groups. In comparison with TD, incorporation of VTD into ASCT resulted in significantly longer PFS , a benefit which remained statistically significant even in pts with high-risk cytogenetic abnormalities.

C093

BORTEZOMIB-DOXORUBICIN-DEXAMETHASONE INDUCTION BEFORE AUTOLOGOUS TRANSPLANTATION, FOLLOWED BY LENALIDOMIDE CONSOLIDATION-MAINTENANCE IN UNTREATED MYELOMA PATIENTS

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Background. New drugs have been introduced as induction prior to autologous stem cell transplant (ASCT) and as consolidation/maintenance thereafter to improve complete response (CR) rates in myeloma patients. *Objectives*. This is a phase II study in which we evaluate a sequential approach with bortezomib plus doxorubicin and dexamethasone as induction prior to reduced intensity autologous transplantation, followed by lenalidomide as consolidation-maintenance. Primary endpoints were safety (incidence of grade 3-4 adverse events) and efficacy (response rate). *Materials and Methods.* Newly diagnosed multiple myeloma (MM) patients, aged 65-75 years, were eligible. The induction included four 21-day cycles of bortezomib (1.3 mg/m^2 days 1,4,8,11), pegylated-liposomal-doxorubicin (30 mg/m^2 day 4) and dexamethasone (40 mg: days 1-4, 8-11, 15-18, cycle 1; days 1-4, cycles 2 to 4) (PAD). Autologous transplantation was tandem Melphalan 100 mg/m^2 (MEL100) followed by stem-cell support. Consolidation included four 28-day cycles of lenalidomide (25 mg/day days 1-21) plus prednisone (50 mg every other day) (LP), followed by maintenance with lenalidomide alone (10 mg/day days 1-21 every 28 days) (L) until relapse. *Results*. One-hundred and two patients have been enrolled. In a per-protocol analysis, PAD induced 58.5% at least very good partial response (VGPR), including

12.8% CR. After MEL100 autologous transplantation 82.0% of patients achieved at least VGPR and 38.6% CR; further improvement in response rate was obtained after LP-L consolidation-maintenance: 86.0% at least VGPR and 66.0% CR. After a median follow-up of 20.3 months, the 3-year progression-free survival (PFS) was 68.8%, the 3-year time to progression (TTP) was 74.7% and the 3-year overall survival was 86.3%. By exploratory subgroup analyses, patients with high risk cytogenetic profile - including del17 or t(4;14) or t(14;16) – and patients with standard cytogenetic profile had similar TTP (HR 0.49; 95% CI, 0.13-1.81; p=0.28). During PAD, main grade 3-4 adverse events included thrombocytopenia (16.7%), neutropenia (9.8%), peripheral neuropathy (15.7%), and pneumonia (9.8%). During LP-L grade 3-4 adverse events were neutropenia (16.5%), thrombocytopenia (6.3%) and cutaneous rash (3.8%). *Conclusion.* Bortezomib as induction, followed by lenalidomide as consolidation-maintenance induced high response rate and prolonged 3-year PFS and TTP, overcoming the adverse prognostic impact of cytogenetic abnormalities.

C094

SHORT-TERM THALIDOMIDE INCORPORATED INTO DOUBLE AUTOLOGOUS STEM-CELL TRANSPLANTATION IMPROVES OUTCOMES IN COMPARISON WITH DOUBLE AUTOTRANSPLANTATION FOR MULTIPLE MYELOMA

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Remarkable activity of thalidomide-dexamethasone in all phases of MM, formed the basis for the design of phase II Bologna 2002 study incorporating thalidomide-dexamethasone into melphalan-based double ASCT for younger pts with newly diagnosed MM. By study design, thalidomide 200 mg daily was administered from the outset until the second course of melphalan 200 mg/m^2. To assess potential benefits from this trial design, 135 pts who were enrolled in the study were retrospectively analyzed with an equal number of pair mates who were randomly assigned to the double ASCT arm of the previous Bologna 96 study. Case-matching was performed with respect to the following pt characteristics at baseline: age (± 2 years), disease stage according to the Durie-Salmon system and serum β2-microglobulin levels (±1 mg/dL). Instead of thalidomide-dexamethasone, pts accrued into Bologna 96 study received VAD as induction therapy and melphalan 120 mg/m^2 plus busulfan 12 mg/kg before the second ASCT. At the time of the analysis, the median follow-up times for pts enrolled on Bologna 2002 and Bologna 96 studies are 45 and 62 months, respectively. On an intention-to-treat basis, the addition of thalidomide to double ASCT effected a significant improvement in the rate (68% vs 49%, p=0.001) and duration (62% vs 33% at 4 years, p=0.0006) of at least very good partial response (VGPR), time to progression (TTP) (61% vs 41% at 4 years, p=0.0008) and progression-free survival (PFS) (51% vs 31% at 4 years, p=0.001). A trend was also noted for extended overall survival (OS) among thalidomide-treated patients (69% at 5 years vs 53% for the control group), although the difference between the two groups was not statistically significant (p=0.07). Benefit with thalidomide in increasing the rate of VGPR or better response, TTP and PFS was confirmed in a multivariate analysis. Attainment of at least VGPR was an additional good-risk factor for PFS and OS. Median survival after relapse was 24 months for patients on thalidomide added to double ASCT versus 25 months for the control group. Overall, 17% of patients discontinued thalidomide, including 8% due to drug-related adverse events. It is concluded that, in comparison with double ASCT, the addition of thalidomide up-front to double ASCT improved clinical outcomes. Short-term thalidomide was generally well tolerated and had no adverse impact on postrelapse survival.

C095

POST-TRANSPLANT EVALUATION OF BONE MARROW HEMATOPOIETIC FUNCTION IN PATIENTS RECEIVING HIGH-DOSE YTTRIUM-90-IBRITUMOMAB TIUXETAN (ZEVALIN) WITH AUTOGRAFT

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High-dose (hd) Zevalin® radioimmunotherapy (RIT) followed by tandem peripheral blood stem cell (PBSC) reinfusion is a well tolerated myeloablative regimen for the treatment of NHL. The risk of long-term hematopoietic damage and therapy-related myelodysplasia and acute myeloid leukemia (t-MDS/AML) is a matter of concern. The present study aimed to investigate bone marrow (BM) hematopoietic abnormalities after hd-Zevalin. From July 2004 through December 2007, 54 consecutive NHL patients (M/F=33/21; median age 64 yrs, range 26-76 yrs) receiving hd-Zevalin (1.2 mCi/kg) with tandem stem-cell support as myeloablative consolidation following sequential hd-chemotherapy were enrolled in this study. Disease histology included diffuse large B cell (n=20), follicular (n=20), mantle cell (n=8), marginal zone (n=3), small lymphocytic (n=1) lymphomas and Richter Syndrome (n=2). At study entry, 37 patients had relapsed or refractory disease, whereas 17 patients were chemotherapy-naive. BM and peripheral blood (PB) mononuclear cells (MNC) collected prior to Zevalin® and at 6-month intervals thereafter were analyzed for (i) frequency of BM erythroid BFU-E, myeloid CFU-GM, and multilineage CFU-mix, (ii) telomere length (TL), (iii) cytogenetic abnormalities. With a median follow-up of 33 months (range, 9-58) from autograft, a total of 215 samples have been analyzed. As compared to pre-transplant values, a significant reduction in BM CFCs was detected 6 months after Zevalin® (median incidence per 5 x 104 MNCs were as follows, BFU-E: 45 vs 28, p≤0.04; CFU-GM: 61 vs 34, p≤0.0005; CFU-mix: 0.8 vs 0.5, p≤0.2). CFCs recovered to pre-transplant values 12 months after Zevalin®, and showed sustained growth values at subsequent time-points. Similar values (p=NS) of TL were obtained on pre-transplant MNCs (median: 5.893 bp, range 4,107-8,353) and MNCs collected at 6-12 months post-transplant (median: 5,829 bp, range 3,133-8037). Three patients (5.5%) have developed t-MDS/AML associated with chromosomal abnormalities including deletion and monosomy of chromosome 7 and deletion of chromosome 5. In this cohort of elderly and heavily pretreated patients, hematopoietic function seems comparable to that of patients receiving conventional myeloablative chemotherapy. No increase in cytogenetic abnormalities was detected up to 36 months post-transplant. A careful monitoring is still ongoing in order to verify at long-term whether hd-Zevalin® might increase the risk of t-MDS/AML.

C096

A PHASE II RANDOMIZED STUDY COMPARING ONE FIXED-DOSE OF PEGFILGRASTIM VERSUS FILGRASTIM AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL SUPPORT

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Purpose. The aim of this phase II randomized trial was to demonstrate the non-inferiority of one fixed dose pegfilgrastim (PEG) compared to daily filgrastim (FIL), in patients receiving high-dose chemotherapy (HDC) and peripheral stem cell support (PBSC). *Patients and methods.* 80 patients were enrolled in a phase II open-label randomized study. In experimental arm, PEG was administered at fixed dose (6 mg) at day +1 after autologous stem cell infusion. In the standard arm, FIL was administered at weight-based daily dose (5 mcg/kg/d) from day +1. The composite primary end point was the duration of severe neutropenia (absolute neutrophil count, ANC <0.5x10^9/L) and the number of days to achieve an ANC >0.5x10^9/L. *Results.* PEG was non-inferior to FIL for both variables included in the primary composite end point. The median days of ANC less than 0.5x10^9/L and the median time to reach an ANC more than 0.5x10^9/L were days 6 vs 6, and 11.5 vs 10.7, respectively in the FIL and PEG. No differences were observed in the median time to reach an ANC more than 1.0x10^9/L (mean days 12 vs 12), number of patients with fever (61% vs 54%), patient with documented infections (32% vs 26%), median duration of antibiotic therapy (median days 5.9 vs 4.5, range 0-13 and 0-30). *Conclusions.* This phase II randomized study shows that PEG was not inferior to FIL in terms of hematological reconstitution (primary end point). PEG could be safely used after PBSC infusion.

Table 1. Not inferiority analysis. Mean value, upper delta limit and mean differences with 95% confidence interval for the non inferiority evaluation.

	FIL arm Mean (CI 95%)	*PEG arm Mean (CI 95%)*	*Upper Δ limit*	*Mean difference (CI 95%)*
Days with ANC < 500x10^9/L	5.97 (5.23;6.71)	6.2 (5.51;6.89)	1.66	0.22 (-0.77;1.22)
Time to reach ANC >500x10^9/L	11.53 (9.80;13.26)	10.75 (9.32;12.18)	3.65	-0.78 (-2.97;1.42)
Time to reach ANC >1000x10^9/L	12.16 (10.27;14.04)	11.98 (8.98;14.97)	5.85	-0.18 (-3.70;3.34)
Days with fever	1.63 (0.87;2.38)	0.95 (0.51;1.39)	1.45	-0.68 (-1.54;0.19)

Hodgkin's Lymphoma

C097

COMBINATION OF THE HISTONE DEACETYLASE INHIBITOR GIVINOSTAT WITH THE ALKYLATING AGENT MECLORETHAMINE DEMONSTRATES CLINICAL ACTIVITY AND SAFETY IN HEAVILY PRETREATED PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA (HL): PRELIMINARY RESULTS OF A PHASE II TRIAL

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HL patients with refractory disease or relapsing after autologous stem cell transplant (SCT) have very poor prognosis. Givinostat (ITF2357, Italfarmaco spa, Italy) is an orally bioavailable hydroxamate inhibitor of histone deacetylases (HDACs). Preclinical data demonstrating a synergistic activity of Givinostat with the alkylating agent Meclorethamine established the rationale for this ongoing phase II study aimed to determine activity and safety of the sequential Givinostat and Meclorethamine therapy. Patients with HL who have failed second- or subsequent-line salvage chemo-radiotherapy were enrolled. Eligibility criteria included prior treatment with autologous and/or allogeneic SCT, at least one target lesion ≥2 cm, and platelet counts of at least 75,000/μL. Givinostat (50 mg QID, per os, days 1-3) followed by Meclorethamine (6 mg/sqm, intravenously, day 4) was dosed in 3-week cycles until disease progression or appearance of clinical significant toxicity, but for a maximum of 12 cycles. Tumor responses were determined by computed tomography (CT) and positron emission tomography (PET) scan. To date, 26 patients have been enrolled (22 males and 4 females; median age, 33 years; range, 21-61 years), including 8 patients enrolled in a preliminary compassionate use trial, and 18 patients of a planned 23 enrolled in this ongoing phase II trial. Prior to study entry, patients received a median of 5 (range 2-7) lines of treatment with autologous SCT performed in 20 (77%) and an additional allogeneic SCT in 7 (27%) patients. At study entry, 9 patients had relapsed and 17 refractory HL. To date, patients received a median of 4 cycles (range, 1-11) of Givinostat/Meclorethamine and are evaluable for response. Best response to therapy included 4 (15%) complete remissions (CR) and 6 (23%) partial remissions (PR), for an overall response rate (ORR) of 38%. In addition, 6 (23%) patients achieved stable disease (SD) for ≥4 months, while 10 (39%) patients progressed. Overall, therapy was well tolerated without significant adverse events. No prolongation of QT/QTc interval has been detected over 114 therapy cycles. Hematological toxicities included grade 1-2 anemia (77%), neutropenia (30%), and thrombocytopenia (46%). Preliminary results from this ongoing trial suggest that Givinostat in combination with Meclorethamine demonstrates significant anti-tumor activity in heavily pretreated relapsed/refractory HL and is well tolerated.

C098

TANDEM AUTOLOGOUS/REDUCED-INTENSITY ALLOGRAFT FOR RELAPSED/REFRACTORY HODGKIN'S LYMPHOMA: EARLY ALLOTRANSPLANT AFTER INTENSIVE CYTOREDUCTION MAY MAXIMIZE GRAFT VS LYMPHOMA EFFECT?

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Background. Hodgkin's Lymphoma (HL) patients with disease at the time of autolgous transplantation (ASCT), have high probability of progression after ASCT. Reduced-intensity conditioning allotransplant (RICT) aims to exploit graft vs lymphoma (GvLy) effects while reducing conditioning-related toxicity. RICT is considered as the last therapeutic option and is usually offered to HL patients failing ASCT and in this contest GvLy responses might be insufficient. *Aims.* we pionered that offering RICT as an earlier option after intensive cytoreduction (ASCT) may allow GvLy reaction to be better exploited (Carella et al. JCO 2000; 18:3918). *Patients and methods.* twentyseven HL patients (14M/13F) underwent RICT preceeded by autografting (ASCT). Median age at diagnosis was 27 (range 15-44), median n° of chemotherapy lines was 2.5 (range 2-4). All but one patient had disease at ASCT. Twelve patients were chemosensitive and 15 chemorefractory at ASCT and high-dose therapy consisted of Melphalan 200 mg/mq (n=7) and BEAM (n=20). The time interval between ASCT and RIC was 3 months (range 1.3-6.7).

RIC consisted of fludarabine-cyclophosphamide (n=12) or fludarabine-melphalan (n=15). *Results.* the median time to neutrophils and platelets recovery was 10 days and 16 days, respectively. Chimerism studies indicated 100% donor-derived engraftment. Seven patients developed aGVHD (grade II-IV) and 9 cGVHD (2 limited and 7 extensive). At the last follow up 17 patients (63%) were alive, 12 (70.6%) in RC and 5 (29.6%) with disease. Ten patients expired (37%), 7 of disease progression, 1 of aGVHD, 1 of cGVHD and 1 of infection. With a median follow-up of 46 months (6-117 months), median OS was 47 months. *Conclusions*. These encouraging results suggest that GvLy may have a role on residual disease after ASCT. A prospective study based on genetic randomization (ASCT vs ASCT followed by RICT) would help to answer this important issue.

C099

THE EARLY AND INTEREMEDIATE-TERM TOXICITY TO PRIMITIVE HEMATOPOIETIC PROGENITOR CELLS OF THREE CHEMOTHERAPY REGIMENS FOR ADVANCED HODGIN'S LYMPHOMA

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The early stem cell reservoir can be impaired by a few cycles of chemotherapy and this impairment may persist after normalization of peripheral cytopenias. We directly evaluated the damage caused to marrow progenitor cells by three currently used chemotherapy regimens for advanced Hodgkin's lymphoma. Bone marrow samples from 37 patients randomly treated according to either the ABVD, COPPEBVCAD or BEACOPP schedale (GISL HD-2000 trial) were taken a few days before the start of chemotherapy and 30 days and 6 months after its completion. Samples were cryopreserved, thawed in a single session and cultured for five week to detect long-term culture-initiating cells (LTC-IC). Statistical differences were evaluated by the Wilcoxon's test. On the basis of the numbers of LTC-IC detected and of their relative variations with respect to the pre-treatment values, the ABVD regimen was associated with least early reduction and the best late recovery of LTC-IC (3.8±0.9; 2.4 ±0.8; 3,4±0.5, respectively). COPPEBVCAD produced the greatest early damage but recovery was nearly complete by 6 months (3.2±0.9; 1.2 ± 0,8; 2.4± 1,0). BEACOPP caused intermediate early toxicity which persisted at 6 months, with significant difference from pre-treatment values (3.2±1,3; 1.8±1.0; 1.9±1.0). Per cent variations with respect to the pre-treatment valus are illustrated in the Figure 1. The different late toxicity exerted on marrow progenitors by these chemotherapy regimens should be carefully weighed in relation to both the expected early response rate and subsequent possibility of rescue in case of first treatment failure.

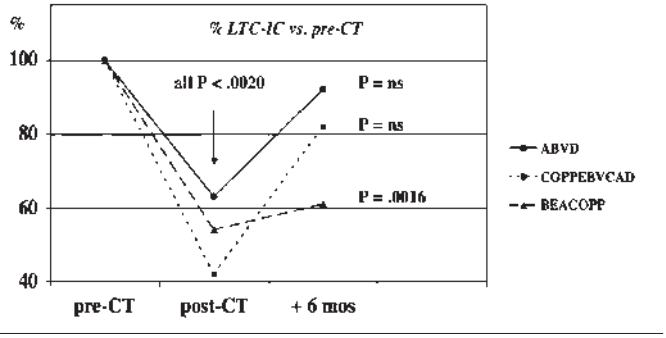

Figure 1.

C100

PREGNANCY OUTCOMES IN WOMEN TREATED FOR HODGKIN'S LYMPHOMA

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Purpose. The aim of this study was to evaluate the pregnancy outcomes in women with Hodgkin's Lymphoma (HL) diagnosis, treated from 1972 to 1999 at Department of Radiotherapy and Hematology of University of Rome " La Sapienza". *Materials and Methods.* We have retrospectively studied 101 female patients that have conceived after treatment for HL. Median age at diagnosis was 24 years old (range 9-34). Stage at diagnosis was: stage I-IIA in 63%, IIB in 21% and III-IV in 16%, respectively. Eight (8%) patients were treated with chemotherapy alone (ABVD, ABVD/MOPP, MOPP), 33 (32%) with radiotherapy alone as supradiaphragmatic radiotherapy (SupRad: mantle irradiation) or as infradiaphragmatic radiotherapy (InfRad:spleen and/or para-aortic field and/or inverted Y field with or without SupRad) and sixty (60%) with chemotherapy and radiotherapy (SupRad and/or InfRad).Ormonal therapy was administrated to thirty-one patients (30.7%). *Results.* All patients achieved a complete remission after first line therapy. The median follow-up was 127 months (range 25-282 months). One-hundred 1 patients reported 147 pregnancies. We observed 134 deliveries (2 of them twin births) after a median time of 55 months (range 4-278 months) from the end of therapy. Twelve women (12%) experienced 13 miscarriages after a median time of 50 months (range 12-108) from the end of therapy. We recorded 10/134 (7.5%) premature births and 12/136 babies (8.8%) were underweighted at the time of birth. We recorded 2 cases of congenital malformations (gastroschisis, megaureter). No cases of cancer in children were observed after a median follow-up of 164 months. No statistical correlations between pregnancy outcomes and therapeutic approaches were found. In particular the InfRad radiotherapy showed no statistical association with miscarriages, premature birth and low birth weight at term. *Conclusions*. In our series, the incidence of worse pregnancy outcome seems not correlated with previous chemotherapy and/or radiotherapy approach.

C101

IN COMPARISON WITH ABVD, BEACOPP CAN CURE ADVANCED-STAGE HODGKIN PATIENTS WITH A 50TH PERCENTILE HIGHER TUMOR BURDEN

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In Hodgkin's lymphoma (HL) the tumor burden at diagnosis has proved to be the relatively best prognostic determinant before the start of therapy. Here we studied its relationship with chemoresistance, as clinically expressed by incomplete response to first-line therapy or by early relapse (within 12 months). We evaluated 222 patients with advanced-stage HL (IIB, III, IV) enrolled in two distinct and parallel clinical trials, i.e. the FM-GITIL-ILL study – with two chemotherapy arms, ABVD (A) 6-8 cycles vs. BEACOPP (B) 8 cycles – and the GISL HD-2000 trial – with three arms, two of which involving (A) 6 courses vs. (B) courses). In both studies the first 4 cycles of (B) were administered according to its escalated schedule and radiotherapy was delivered finally on sites of initial bulky disease or on residual masses. Ninety-five patients were treated in the FM-GITIL-ILL study, 44 with (A) and 51 with (B), 127 in the GISL study, 71 with (A) and 56 with (B). No differences were found in the clinical characteristics among trials and chemotherapy regimens. Median follow-up was 36 months. The volume of tumor burden was calculated from the slices of the total body staging TC and was normalized to body surface area (relative tumor burden, rTB). Events of chemoresistence (E) were considered 25 responses less than complete remission after chemotherapy (and radiotherapy, if planned) and 20 early relapses. The measured rTB was 185.9±140.9 ccm/sqm (range: 3,9-694.5) in the 115 (A) patients, 178.5±138.8 ccm/sqm (2,5–619.2) in the 107 (B) ones. The rTB was confirmed to be the only

statistically significant factor when matched against chemoresistance events in logistic regressions including stage, constitutional symptoms, histologic type, bulky mass, performance status, extranodal involvement, hemoglobin, serum albumin, erythrocyte sedimentation rate, and LDH. The probabilities of (E) in relation to the number of patients at risk and the amount of rTB for (A) and (B) treatment, respectively, were as follows: < 100 ccm/sqm, 0.08 vs. 0.00; 101-200 ccm/sqm, 0.17 vs. 0.09; 201-300 ccm/sqm, 0.35 vs. 0.30; 301-400 ccm/sqm, 0.50 vs. 0.33; 401-500 ccm/sqm, 0.50 vs. 0.40; 501-600 ccm/sqm, 1.00 vs. 0.50; 601-700 ccm/sqm, 1.00 vs. 1.00. The logistic regression of (E) related to rTB and treatment estimates that the hazard of resistance increases by a factor 2 every 77 ccm/sqm of rTB for both treatments, but – risks being equal – B can cure rTB of about 89 ccm/sqm higher than A (Figure 1). In other words, the regimen (B) shows the same response rate at 12 months as the regimen (A) while treating patients with a rTB about 50% higher than the mean of rTB in advanced-stage patients (about 180 ccm/sqm). However, the low values of R2, (0.548 for A and 0.515 for B, in spite of the present new confirmation of rTB as the relatively best prognosticator), and the clearer clinical advantage of B in patients with low rTB suggest that a number of other unconsidered factors – besides rTB and the currently known prognostic factors – are involved in the clinical manifestation of chemoresistance.

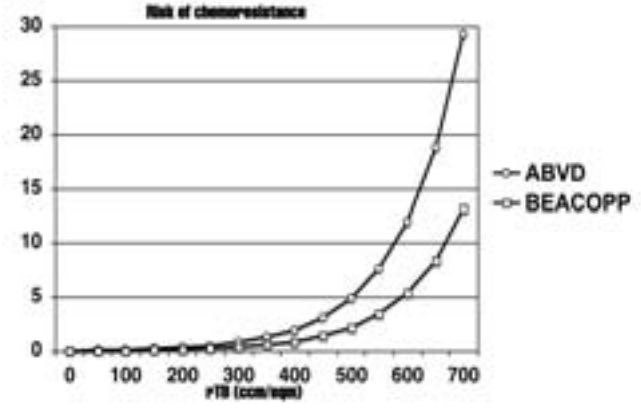

Figure 1.

C102

PREDICTIVE ROLE OF EARLY INTERIM FDG-PET IN HODGKIN LYMPHOMA

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Background. Hodgkin lymphoma (HL) is a curable malignancy with a long-term survival of around 80%. FDG-PET is a noninvasive imaging modality widely used in lymphoma patients. Early PET assessment of response to therapy is a routine part of management in HL patients, and an independent, strong predictor of progression-free survival. *Patients and Methods.* 178 patients, with a diagnosis of HL, underwent to an early PET evaluation during their course of chemotherapy and were considered eligible for the study. 85 patients (48%) were male and 93 (52%) female; the median age at diagnosis was 33 (13-78) years. 6 patients (3%) had stage I disease; 106 patients (60%) stage II; 34 (19%) stage III and 32 (18%) stage IV (bone marrow involvement in 5 cases). B-symptoms were detected in 81 patients (46%). A mediastinal bulk was detected in 54 cases (30%). The majority of patients (173, 97%) underwent to ABVD as first line therapy; 5 received BEACOPP chemotherapy (3%). Early PET evaluation was performed after the second course of therapy. Results were classified into complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) according to International Workshop standardized response criteria. PET scan was performed again at the end of the first-line treatment. 44 patients have been addressed to a second-line therapy, in presence of PR, PD or relapsing disease; in particular, 39 patients received an autologous stem-cells transplantation (ASCT), and 3 an allogeneic bone marrow transplantation (ABMT). *Results.* At a median follow up of 41.85 (5.23-141.77) months, 152 patients are alive and in CR; 7 in PR; 3 alive with SD and 7 present a PD. 9 patients have died. 150 patients presented with a negative PET after 2 cycles, and 28 with a positive one (26 in PR, 1 with SD and 1 with PD). More specifically, of the 178 initial patients, 150 (84%) had a negative early PET and 28 (16%) a positive early PET. Of those with a negative PET, 135 (90%) experienced a continuous CR, while among those with a positive early PET, none obtained at least a stable CR. Of this unfavourable group of patients, 9 (32%) reached, and still maintain, a CR after ASCT. These results are fully described in Table 1. *Conclusions.* Our experience indeed confirms the highly predictive value of a negative early PET during the therapy for HL. Moreover we may suggest the potential role of ASCT in inducing a CR in around one-third of those unfavorable patients with a positive early interim PET.

Table 1.

	PET+2 Result	Response to 1st line	Status
NEGATIVE PET+2	CR 150	CR 136	135 CR 1 died (after disease relapse)
		PR 9	7 CR (6 after ASCT, 1 after radiotherapy) 1 PR (after ASCT) 1 died (after ASCT)
		PD 5	1 CR (after ASCT) 1 PR (after ASCT) 1 SD (after gemcitabine) 2 died (after ASCT)
POSITIVE PET+2	PR 26	CR 8	4 CR (after ASCT) 2 PR (after ASCT) 2 PD (after ASCT)
		PR 8	3 CR (after ASCT, ABMT, radiotherapy) 2 PR (after ASCT) 1 PD (after ASCT, ABMT) 2 died (after ASCT, ABMT)
		SD 3	1 CR (after ASCT) 1 PR (after ASCT, ABMT) 1 PD (after ASCT)
		PD 7	2 SD (after ASCT) 2 PD (after ASCT) 3 died (after ASCT)
	SD 1	PR 1	1 CR (after ASCT)
	PD 1	PD 1	1 PD (after ASCT)

Acute Leukemias II

C103

IKZF1 (IKAROS) DELETIONS ARE INDEPENDENT ON THE PHILADELPHIA CHROMOSOME AND ARE ASSOCIATED WITH AN IMPAIRED B-CELL DIFFERENTIATION AND POOR OUTCOME IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Recent genome-wide analyses in B-ALL have identified a high frequency of DNA copy-number abnormalities, but the biological and prognostic implications of these abnormalities have not been defined. In order to address this issue, a cohort of 149 adult ALL patients (106 BCR-ABL1-postive, 3 ALL1/AF4, 1 EA/PBX, 39 negative for known molecular rearrangements) were analyzed with the use of single-nucleotide–polymorphism (SNP) microarrays (Affymetrix 250K NspI and SNP6.0), FISH, transcriptional profiling, and resequencing of samples obtained at diagnosis. Lesions varied from loss or gain of complete chromosome arms (trisomy 4, monosomy 7, loss of 9p, gain of 1q) to micro-alterations targeting genomic intervals in which the minimal common region of change involved one or few genes. Lesions in genes involved in early B-cell differentiation (60%) and cell cycle regulation (40%) were observed with relatively high frequency (60%). The most frequent somatic copy number alteration was deletion on 7p12 of IKZF1 (75% in BCR-ABL1 positive and 58% in BCR-ABL1-negative ALL), which encodes the transcription factor Ikaros required for the earliest stages of lymphoid lineage commitment. FISH analysis using a pool of fosmid probes for IKZF1 and genomic quantitative PCR confirmed SNP results. Among the 149 patients, the entire IKZF1 locus was deleted in 19 (13%); in 84 (56%) additional patients, a subgroup of exons or the genomic region immediately upstream of IKZF1 was deleted. In 48 of them, there was a deletion of coding exons 4 through 7, which results in expression of a dominant-negative isoform, Ik6, which cytoplasmatic localization. Using gene-set enrichment analysis to compare the gene-expression signatures of patients with IKZF1 deletion versus not-deleted patients, we identified a unique signatures independent by BCR-ABL1 and characterized by down-regulation of B-cell lineage genes (e.g. VPREB1, VPREB3, IGLL3, BLK) and up-regulation of genes involved in cell-cycle progression (STK17B, SERPINB9, CDKN1A). We next investigated whether the IKZF1 deletions associated with a poor outcome. Univariate analysis showed that the IKZF1 deletion negatively influenced the cumulative incidence of relapse (p=0.0103) and disease-free survival (p=0.0229). *Conclusion.* Deletion of IKZF1 is an important event in the development of B-progenitor ALL which significantly influences clinical outcome.

European LeukemiaNet, AIL, AIRC, FIRB 2006, Strategico di Ateneo, GIMEMA Onlus.

C104

COMBINED BCL-2 AND MTOR SIGNAL TRASDUCTION INHIBITION IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Recent pre-clinical therapeutic approaches in acute lymphoblastic leukemia (ALL) are currently investigating, among others, small molecule inhibitors capable of targeting key regulators of survival and proliferation, such as Bcl-2 and PI3K/AKT/mTOR. We have previously demonstrated that ABT-737 (kindly provided by Abbott Laboratories), a Bcl-2/Bcl-xL (BH3 mimetic) inhibitor, exerts a potent cell growth inhibition and apoptosis induction on ALL cell lines and primary samples (Blood, 2007; 110:53a). We have also identified on primary and secondary ALL cells a resistant phenotype mostly characterized by Mcl-1 overexpression. Since it has been reported that Temsirolimus (CCI-779), an mTOR inhibitor, induces growth arrest and apoptosis in preclinical models of primary ALL, we aimed in this study to investigate the effect of the simultaneous exposure to ABT-737 and CCI-779 on ALL cells. Exploring CCI-779 activity in ALL cells as a single agent, a biphasic dose response was observed in the MOLT-4 cell line (IC50: 9865 nM), with a flat curve at concentrations (35-55% of inhibition) ranging between 1 nM and 5000 nM, and a more pronounced growth inhibition at concentrations ≥10000 nM. The CEM cell line, as observed also with ABT-737, proved resistant (IC50: 47780 nM) showing only inhibition of cell cycle progression. We then evaluated the impact of the simultaneous inhibition of Bcl-2 and mTOR. Our results showed that in MOLT-4 cells CCI-779 was able to potentiate the cytotoxic effects of ABT-737, as demonstrated by the MTT-based assay. Striking apoptosis induction was observed combining 50 nM of ABT-737 with 5000 nM of CCI-779. In fact, annexin V-positive cells increased from 9.82% (vehicle) to 18.85% with 50 nM ABT-737, to 16.65% with 5000 nM CCI-779 and to 63.95% with both inhibitors. In the CEM cell line, resistant to ABT-737, the simultaneous treatment with ABT-737 and CCI-779 reduced the S-phase from 41.4±0.14% (vehicle) to 24±13.01% (10000 nM of CCI-779) and to 18.9±5.02% (ABT-737/CCI-779 combination). Preliminary data obtained on primary ALL cells showed that in a sample less sensitive to ABT-737 the combination with CCI-779 induces apoptosis from 14.5% (vehicle), to 28.6% (50 nM of ABT-737), to 33.3% (5000 nM of CCI-779), and to 71.8% (ABT-737/CCI-779 combination). Our study shows that CCI-779 can potentiate the effect of ABT-737 on ALL cells. The molecular mechanism that regulates responsiveness to the inhibitor combination is under evaluation.

C105

THE THERAPEUTIC RESPONSE AND CLINICAL OUTCOME OF ADULT PATIENTS WITH ALL1(MLL)/AF4 FUSION POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA ACCORDING TO THE GIMEMA EXPERIENCE

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We report the results achieved in adult ALL1(MLL)/AF4 positive ALLs treated according to the GIMEMA 0496 and LAL2000 trials, active from October 1996 to December 1999, and from January 2000 to September 2004, respectively. These two protocols differed for post-induction treatments that, in the 0496 trial, consisted of two courses with high dose Ara-C (2 gr/m^2 every 12 hours as 3-hour infusion on days 1 and 2) and Etoposide, followed by 3 years of maintenance treatment, whereas in the LAL 2000 protocol included a consolidation course with high dose Ara-C (two daily doses at 3 gr/m^2, days 1, 2, 3 and 4) and Mitoxantrone (10 mg/m^2, days 3, 4 and 5) followed by an allogeneic or autologous HSCT, based to the availability of a HLA compatible donor. By contrast, both protocols included an identical 4 drugs induction (prednisone [PDN], vincristine [VCR], daunorubicin [DNR], and asparaginase [ASP]) with high dose DNR (270 mg/m^2). Twenty-five and 21 adults with ALL1(MLL)/AF4 positive ALL were enrolled in the GIMEMA 0496 and LAL 2000 trials, respectively. After induction, a CR was achieved in 22 (88%) and 18 (90%) patients (p= n.s.) entered into the two protocols. Among these, relapses occurred in 16/22 (72%) and 10/18 (55%) patients, respectively (p=n.s.). In particular, 4/10 relapses of the LAL 2000 group occurred before transplant (median time to relapse: 1.6 months; range: 1.2-5.8 months). At 36 months overall and disease free survival rates were 31.8% (C.I. 95%: 25.4-39.6) and 32.9% (C.I. 95%: 26.6-40.7), 27.3% (C.I. 95%: 22.6-32.9) and 28% (C.I. 95%: 23.5-33.4) in 0496 and LAL 2000 trials, respectively. Thirteen patients in the LAL 2000 protocol received a transplant (5=autologous and 8=allogeneic). Analysis of the clinical outcome of patients showed that relapses

remained the main failure raison that occurred in a total of 26/40 (65%) responders. In addition, incidence of relapses were higher in patients receiving chemotherapy than in those transplanted (78% vs 46%, respectively; p=0.07). This data achieved in a numerous group of patients with a rare ALL, suggest that this leukemic subtype remains a disease with an adverse prognosis that should be treated very intensively. In addition, the observed similar poor outcome of the two patient groups seems to be due to a lower antitumoral activity of our induction-consolidation treatments, not including either cyclophosphamide nor high dose MTX, more than to a putative inefficacy of transplant procedures.

C106

EFFICACY AND CLINICAL OUTCOME OF PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED WITH SECOND GENERATION TYROSINE KINASE INHIBITORS (TKIS): THE BOLOGNA EXPERIENCE

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Background. Approximately 30% of adult acute lymphoblastic leukemia (ALL) patients are characterized by the presence of the Philadelphia (Ph) chromosome, which derives from a reciprocal translocation t(9;22)(q34;q11) and results in a chimeric BCR-ABL oncogene. The prognosis of this subset of patients treated with standard therapies, including multi-agent chemotherapy, Imatinib, and allogeneic stem cell transplantation, is still dismal, due to a high risk of relapse. Dasatinib and Nilotinb are second generation TKIs developed to overcome the problem of resistance to Imatinib in relapsed Ph$^+$ leukemias. *Design and Methods.* We retrospectively evaluated the single center experience on therapy efficacy of Dasatinib, Nilotinib, and experimental third generation TKIs, administered as second or subsequent line of therapy on 25 relapsed Ph$^+$ adult ALL patients. All patients were previously treated with Imatinib. The median age at time of diagnosis was 50 years (range 18-74), 17 patients were male and 8 female. Ten patients presented a BCR-ABL P190 fusion protein and corresponding fusion transcript, the remaining a BCR-ABL P210. Nineteen patients received Dasatinib, 2 patients Nilotinib and the remaining 4 patients were treated with third generation TKIs. Fourteen patients (56%) were in first relapse, and 7 (28%), 3 (12%) and 1 (4%) were in second, third and fourth relapse, respectively. A mutational analysis was performed in all the patients before TKIs (9 wild type, 16 mutated, including T315I) and at the time of subsequent relapse; gene expression profiling, SNPArray (6.0 Affymetrix chip), and Ikaros deletions were also analyzed. *Results.* 13 out of 25 patients (52%) obtained a haematological response (HR) (11 patients treated with Dasatinib, 1 patient with Nilotinib and 1 patient with a third generation experimental TKI). 10 patients obtained also a cytogenetic response (CyR) and 6 patients a molecular response (MolR). With a median follow up of 10.8 months (range 2-29 months), median duration of HR, CyR and MolR were 117 days (range 14-385 days); progression free survival were 162 days with Dasatinib and 91 days with Nilotinib. Overall survival was 25.8 months. Interestingly, in 6 out of 9 wild-type patients, treated with Dasatinib, the mutational analysis showed the emergence of T315I or F317I mutation at the time of relapse. *Conclusion.* Second and third generation TKIs represent a valid approach in relapsed Ph$^+$ adult ALL patients; the subsequent relapse is often associated to the emergence of mutation, conferring resistance to TKIs.

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C107

5-AZACITIDINE FOR THE TREATMENT OF PATIENTS WITH ACUTE MYELOID LEUKEMIA: RETROSPECTIVE ANALYSIS OF 63 PATIENTS ENROLLED IN AN ITALIAN PATIENT NAMED PROGRAM

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Azacitidine (AZA) has shown effective in patients with high risk myelodysplastic syndromes, including RAEB-T which now meet criteria for AML, by WHO. These findings have prompted the question of a possible efficacy of AZA in patients with AML. Among a total of 246 patients treated in 31 different Italian Institutions since 2005, 63 AML, according to WHO criteria, were selected. Median age 74 years (range 29-87), 31 patients males, 32 females. Among 47 patients with evaluable cytogenetics 12 (25%) had poor risk and 35 (75%) intermediate karyotype (including 29 normal). Secondary AML were 14 (25%). Median time from diagnosis to AZA initiation was 5 months (range 0-72). Twenty-four elderly patients (38%), not eligible for intensive chemotherapy, received AZA as front-line treatment. Thirty-nine patients (62%) were pre-treated with one or more lines of chemotherapy (n=15 and 24, respectively); most of the pre-treated patients (23 out of 39) received high dose chemotherapy including autologous or allogeneic stem cell transplantation, the remaining low dose chemotherapy (n=13) or growth factors (n=3). The median number of monthly AZA cycles administered was 4 (range 1-22); 41 patients (65%) received AZA 100 mg/d fixed-dose subcutaneously, 22 (35%) 75 mg/sqm/d subcutaneously. A seven-day per month schedule was delivered to 50 patients (79.4%), while 12 (19%) received AZA for more than 7 days. One patient received the drug for 5 days (1.6%). The most relevant observed toxicities (grade 3-4) were myelosuppression (21%) and infections (21%, 1 disseminated fungal infection, 4 pneumonia and 3 septic shocks). The overall response rate (ORR) was 35% (22/63): complete remission (n=8, 12.7%), partial response (n=5, 8%), bone marrow complete response (n=4, 6.3%) hematologic improvement (HI, n=5; 8%). ORR was significantly higher in untreated patients compared to pre-treated (p=0.003) and in patients with normal compared to abnormal cytogenetics (p=0.03). The median time from diagnosis to treatment was significantly shorter in responders (RE) than in non-responders (NR) (1.5 vs 6.5 months, p=0.01). One-year actuarial probability of overall survival (OS) was 49.1%, 24.2% and 6.5% for RE, patients with stable disease (SD) and NR, respectively (p<0.001). In conclusion: 1) AZA is safe and effective, mostly in untreated patients and with normal karyotype; 2) AZA significantly prolongs OS in RE compared to NR patients.

C108

METHYLATION PATTERN OF THERAPY-RELATED MDS/AML

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DNA methylation is one of the major epigenetic changes in human cancers, leading to silencing of tumor suppressor genes, with a pathogenetic role in tumor development and progression in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Methylation of key promoter regions, induced by cytotoxic therapy together with complex genetic changes, is important in the biology of therapy-related (t-) MDS/AML. We were interested in the characterization of the methylation pattern of AML and MDS *de novo* and therapy-related. We studied 388 patients (179 females, 209 males), of a median age of 66 years (range 16-98 years). There were 106 MDS, 208 *de novo* AML and 74 t-MDS/AML. Using a methylation-specific PCR, we studied the promoter methylation status of e-cadherin (CDH1), TBSP-1, COX-2, DAP-Kinase 1 and GSTP1. These genes have been shown to be involved in the malignant transformation, interfering with angiogenesis, interaction with micro-environment, apoptosis and xenobiotic detoxification. We found no associations between promoter hypermethylation and gender or age at the time of initial diagnosis. In patients with MDS, there were no associations between hypermethylation and clinical characteristics, including IPSS score, WHO classification and cytogenetics. DAPK1 was more frequently methylated in t-MDS/AML when compared to *de novo* MDS and AML (40% vs 15.6% and 24.4%, p=0.004), while methylation of CDH1 was similar in t-MDS/AML and AML (50.8% and 52.8%), but less frequent in *de novo* MDS (29%) (p=0.001). In the t-MDS/AML group, we found that the methylation pattern appeared to be related to the primary tumor, with DAPK1 more frequently methylated in patients with a previous lymphoproliferative disease (60% vs 31%, p=0.008). On the other hand, methylation of CDH1 was associated to radiotherapy for the primary malignancy (84.5% vs 43%, p=0.02). GSTP1, COX-2 and TBSP-1 hypermethylation were rare and were not characteristic of t-MDS/AML. In 356 patients studied for concurrent methylation of several promoters, t-MDS/AML were significantly more frequently hypermethylated in 2 or more promoter regions than *de novo* MDS or AML (17% vs 11.5% and 9.5%, p=0.035). Chemotherapy and individual genetic predisposition have a clear role in t-MDS/AML development; the identification of specific epigenetic modifications may explain complexity and genomic instability of these diseases and give the basis for targeted-therapy.

Chronic Myeloproliferative Disorders

C109

THE JAK2V617 MUTATION INDUCES CONSTITUTIVE ACTIVATION AND AGONIST HYPERSENSITIVITY IN BASOPHILS OF POLYCYTHAEMIA VERA

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JAK2V617F mutation has been shown to affect several aspects of granulocyte function in patients with PV, while there is no information concerning basophils. We found that the V617F allele burden in immunomagnetically purified basophils was comparable to granulocytes, while JAK2 mRNA content was significantly higher in PV basophils than in PV granulocytes (p<0.0001), normal basophils (p=0.02) and granulocytes (p=0.01), that was not due to a preferential representation of mutated RNA since the proportion of wild-type and V617F-mutated JAK2 mRNA transcripts was consistent with quantitative genotyping. However, total amount of JAK2 protein, measured by flow cytometry and western blot, was comparable between PV and normal basophils and granulocytes. This situation is reminescent of the increased levels of PRV-1 mRNA without greater protein content typical of PV granulocytes. CD63 is expressed on basophils outer membrane following their activation; we found that both median frequency and absolute number of $CD63^+$ basophils was greater in PV pts than in controls (p=0.01, p=0.003), as well as the expression level of CD63 measured as MFI (p=0.04). We found a greater percentage of activated basophils in PV pts suffering from pruritus compared to those without (p=.009), and in pts with >50% mutated allele compared to those with <50% (p= .02), with a linear correlation with V617F burden (r=0.76, p<0.01). Transmission electron microscope analysis showed that, compared to healthy counterpart, PV basophils had increased number of granules (p=0.005) most of which were empty (p<0.001). We analyzed basophil ex-vivo activation in response to the fMLP peptide and IL-3. Using progressive dilutions of fMLP in IL-3-primed cells, the increase of $CD63^+$ basophils was significantly greater in PV pts than in controls, particularly in case of >50% V617F allele (p<0.01); using sub-optimal amounts of IL3 and a fixed dose of fMLP, the response of PV cells resulted significantly greater than in control cells at all concentrations employed, overall pointing to an hypersensitivity of mutated cells. Preincubation with the JAK2 inhibitor AZD1480 (kindly provided by Astra Zeneca Ltd) prevented the ex-vivo activation of PV basophils which proved to be even more sensitive than normal cells. These data indicate that constitutively activated and hypersensitive basophils circulate in PV, underscoring a role of JAK2V617F in their abnormal function and, putatively, in pathogenesis of pruritus.

C110

LONG TERM PATIENT-ADJUSTED MAINTEINANCE SCHEDULE OF MEPOLIZUMAB IS SAFE AND EFFECTIVE IN HYPEREOSINOPHILIC SYNDROME (HES)

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Background. Steroids are currently the treatment of choice of FIP1L1/PDGFRα negative HES. However, such approach has limited efficacy and possible side effects if chronically used. Mepolizumab (M) is an anti-IL-5 monoclonal antibody recently shown to be effective in reducing eosinophils and controlling symptoms. However, long term results have not been assessed yet. *Aim.* We aimed to assess the long term effects of M in treatment-requiring HES patients. *Methods.* We treated 7 patients with HES refractory or intolerant to steroid. M was administered at the dose of 750 mg ev monthly until response. Maintenance therapy was subsequently given according to clinical indications at the same dose. Four patients had a prevalent respiratory involvement while 3 patients had a prevalent peripheral hypereosinophilia. Two patients had also cardiomyopathy and 1 had a eosinophilic gastritis; 6/7 patients had previously failed therapy with imatinib. *Results.* Five out seven patients achieved a response: 4/4 with respiratory involvement

obtained a remission of the symptoms. Notably, one patient with chronic rhinitis also recovered from the associated anosmia after six infusions. 1/3 patients with peripheral hypereosinophilia showed a drastic eosinophil reduction after the first M administration. The other two patients were resistant. Duration of response was variable among patients, ranging from 4 to 16 weeks (mean 10.2). Notably, all patients re-achieved signs/symptoms remission when re-treated and, interestingly, clinical remission duration after maintenance courses was comparable, for each patients, to that recorded after induction. The mean number of maintenance cycles was 7.6 (range 3-17). With a median follow-up of 26 months (range 7-52) no patient has lost response. Neither M-related adverse events nor allergic reactions during infusion were reported. *Conclusions.* Though in a limited series our study confirmed that M is safe and effective in controlling symptoms in HES patients. Notably, sensitivity to M was maintained after repeated cycles and no acquired resistance as well as late toxicity were recorded. Finally, response duration was variable among patient but, being consistent in each case, a patient-adjusted maintenance schedule might be used for symptoms recurrence prevention.

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C111

CONTRIBUTION OF BONE MARROW DERIVED CELL ANGIOGENESIS IN BCR/ABL NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES

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Background. Circulating cells derived from bone marrow (BM) have been reported to induce and modulate angiogenesis in condition of ischemia and/or cancer. Endothelial progenitor cells (EPCs) are BM-derived stem cells that can home into the sites of active neo-vascularization and are found increased in setting of enhanced angiogenesis. Other subsets of hematopoietic BM-derived cell expressing angiopoietin or VEGF receptors have been recently characterized in experimental models of angiogenesis. Increased angiogenesis is thought to have a central role in bcr/abl negative chronic myeloproliferative disorders (CMPDs). To assess the role of BM-derived cells in this setting, we evaluated the level of EPCs, Tie2 and VEGFR-1 expressing circulating cells in a cohort of bcr/abl negative CMPDs. We additionally evaluate microvessel density (MVD) on BM biopsies and a set of cytokines related to angiogenesis. *Methods.* We analyzed 88 patients classified as follows: ET (25), PV (18), CIMF (45) and 25 controls. EPCs ($CD34^+/CD133^+/CD45^-$), $VEGR1^+/CD45^+$ and $TIE2^+/CD45^+$ cells were evaluated by flow cytometry. Serum concentration of VEGF, Ang-1, Ang-2 and bFGF were assessed by ELISA kits. Forty-one BM biopsies were available; MVD was evaluated using the "hot spots" method. *Results*. Bcr/abl negative CMPDs were characterized by elevated EPCs (p=0.0005), bFGF (p=0.02) and BM-MVD (p<0.0001). No difference was found regarding the other cytokines evaluated. Absolute and percent number of $VEGFR1^+/CD45^+$ were higher in CMPDs in respect to controls (p=.01) while $TIE2^+/CD45^+$ cell number was not different. $TIE2^+/CD45^+$ cells positively correlated with serum VEGF (p=0.03), Ang-1 (p=0.04) and bFGF (p=.02). Most of the $TIE2^+/CD45^+$ cells expressed CD14. $TIE2^+/CD45^+/CD14^+$ correlate with VEGF (p=0.03), Ang-1 (p=0.0004) and they were negatively correlated with BM-MVD (p=0.007), while the $TIE2^+/CD45^+/CD14^-$ cells do not. *Conclusion.* CMPDs have an abnormally enhanced angiogenesis as shown by high EPCs, VEGF, bFGF and BM-MVD. In this group of patients $VEGFR1^+/CD45^+$ cells are found increased while the $TIE2^+/CD45^+$ are correlated with serum markers of angiogenesis and BM-MVD. These preliminary data put forward the concept that different subset of cells can contribute to the pro-angiogenic phenotype of bcr/abl negative CMPDs. It may be therefore interesting to further explore the role of these cells in this setting of pathological maturation and proliferation of the hematopoietic BM compartment.

C112

JAK2 V617F MUTATION IN ACUTE MYELOID LEUKEMIA SECONDARY TO PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Transformation to acute myeloid leukemia (AML) is a known complication of myeloproliferative disorders (MPDs). Recently, Theocharides *et al.* have published an interesting report about the high incidence (around 70%) of JAK2 negative blasts from patients affected by secondary AML derived from JAK2 mutated MPDs. We collected, by cell sorting, blast cells and mature cells from total bone marrow of 25 patients newly diagnosed of secondary AML [14 derived from primary myelofibrosis (PMF); 5 from polycythemia vera (PV) and 6 from essential thrombocythemia (ET)], and 3 patients affected by MPDs with excess of blast (>5%) (2 from PV and 1 from ET). All the samples were genotyped for JAK2-V617F mutation by ASO PCR and the measurement of the allele burden was performed in Real Time PCR. At MPD diagnosis, JAK2-V617F was detectable in 17 of 28 patients (8 of 15 PMF; 7 of 7 PV and 2 of 6 ET). All patients had received cytoreductive treatment with HuOH. In our cohort of patients we found that JAK2-V617F mutation was still present at the blast transformation in both compartments (blasts and mature cells) in 13 of 17 JAK2 mutated MPDs. Four of 17 patients developed JAK2-V617F negative AML starting from a mutated MPD. Interestingly, the negativity for the mutation was confirmed in blast cells but also in the rest of mature-myeloproliferative bone marrow tissue. Surprisingly we also described a case of JAK2-V617F mutated AML from a wild type MPD but even in this case the positivity occurred in mature and blast compartments. The remaining 10 wild type JAK2 MPDs maintained the same JAK2 status during blast crisis. No differences in the allele burden were found before and after leukemic transformations in both cell compartments comparing the two groups of patients. Two JAK2 positive AML from JAK2 positive MPD (1 ET and 1 PV) achieved CR after induction treatment while the others did not respond to the therapy. According to our preliminary results, in contrast to the previous study, we conclude that JAK2-V617F positive MPD yields rarely a JAK2-V617F negative AML. Furthermore we wanted to underline how any modifications in the JAK2 integrity or the persistence of the previous status involved the entire bone marrow during leukemic transformation suggesting that the leukemic hit could take place in a common ancestor precursor able to modify entirely the genomic signature of the disease.

C113

DYSREGULATED EXPRESSION OF MICRORNA-16 CONTRIBUTES TO ABNORMAL ERYTHROPOIESIS IN PATIENTS WITH POLYCYTHEMIA VERA

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Dysregulation of miRNAs might contribute to the pathogenesis of myeloproliferative neoplasms. In an analysis of miRNAs in $CD34^+$ cells from PV pts we found abnormally increased levels of miR-16 (p<0.0001). There are two miR-16 genes, miR-16-1 (in a cluster with miR-15a on chr 13) and miR-16-2 (in a cluster with miR-15b on chr 3), which differ in their pre-miR; increased expression was specific for miR-16 since levels of miR-15a and miR-15b were unchanged. Expression of miR-16 were independent of JAK2V671F burden. Sequencing the entire pre- and mature miR-16-1/miR-15a and miR-16-2/miR15b regions disclosed no abnormality. We also determined gene copy number by RT-PCR, but found no evidence of miR-16-1/2 copy number changes. By performing knock-down experiments using siRNAs specific for miR-16-1 or miR-16-2 we determined that abnormally high mature miR-16 levels in PV $CD34^+$ cells was due to miR-16-2 overexpression. We determined the

kinetics of miR-16 during cultures of CD34+ cells induced to differentiate along erythroid lineage; miR-16 levels started to increase progressively from day 6 reaching a maximum on day 12-14; at any time point considered, the miR-16 levels measured in PV cells were significantly greater than controls. To address the function of miR-16 in erythroid differentiation, we over-expressed (Amaxa technology) mature miR16 in normal CD34+ cells both at the beginning of culture and at day 6, when progenitors were switched from a proliferative to a differentiative (plus Epo) culture phase. In cells transfected at day 6 the percentage of CD36+/GPA+ cells in the presence of EPO at least doubled; notably, also in the absence of EPO GPA+ cells, virtually absence in mock-treated cultures, were generated. Normal cord-blood CD34+ cells transfected with pre-miR-16 produced significantly increased number of CFU-e and BFU-E compared to control transfected cells (p=<0.02) while myeloid colonies were unaffected. Also, significantly more erythroid colonies were produced in the absence of Epo. Finally, knocking-down miR-16-2 in PV CD34+ cells resulted in significant reduction of Epo-independent erythroid colonies. Overall, these data indicate dysregulated expression of miR-16 in CD34+ and in in-vitro generated erythroid cells from PV patients, and point to miR-16-2 as being specifically involved; we also provided evidence for a role of miR-16 in normal erythroid differentiation and for its involvement in the abnormal erythropoiesis of PV.

C114

RESPONSE TO DASATINIB IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS WITH D816V KIT MUTATION: 9 ITALIAN CASES

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Systemic mastocytosis (SM) is a rare disease in which the fundamental molecular alteration is the presence of a gain-of-function point mutation (D816V) in the c-kit protooncogene, resulting in uncontrolled mast cells (MCs) proliferation and presence of severe constitutional symptoms derived by organopathy produced by MCs infiltration (C-Findings), or from the realise of mediators. No therapies exist to slacken the progression. Aggressive systemic mastocytosis (ASM) and Indolent Systemic mastocytosis (ISM) are characterized by different spectrum of alterations, and necessity of therapy. For its pathogenesis, SM belongs to the group of Ph negative chronic myeloproliferative disorders in which the use of tyrosin kinase inhibitor (TKI) is suggested. Imatinib has demonstrated substantial inactivity against this KIT mutant, so the newer molecules targeting mutant tyrosin kinase isoforms, as Dasatinib, may have a role. In the current report we focused on nine patients affected by SM (7 ASM and 2 ISM) referred to several institutions with advanced disease that received dasatinib therapy. The scheduled dose of dasatinib was 70 mg BID, and the evaluation of response was made according to Valent criteria. The median duration of treatment was 9 months (range 2-15). All but one patient reached the scheduled dose. We can noticed 4 major response (MR) with prompt resolution of one of C-findings (ascite, malabsorption, splenomegaly and limphnodes), 2 stable disease, whereas in three patients no MR was evident, but with amelioration of B-findings. We also attained a measurable decrease of the burden of neoplastic MCs by means of tryptase level monitoring in all but one patient. The most commonly toxicity involved gastroenteric tract, with diarrohea and abdominal pain, hypotension and pleural effusion and was recorded during the first three months of therapy. Symptoms resolved or return to mild grade with dose reduction. Two patients are on treatment without any signs or symptoms of disease progression. Five patients interrupted dasatinib for worsening clinical condition and then rapidly progressed. Two patients stopped dasatinib after 5 and 15 months for progression to acute leukaemia, one of them when in MR. In conclusion we think that dasatinib has a strong effect on mastocytosis cells, with measurable reduction of tumor burden, but large studies are needed to optimize the management of therapy and for better results.

Myeloma II

C115

LONG TERM RESULTS OF A COMPARISON OF NONMYELOABLATIVE ALLOGRAFTING WITH AUTOGRAFTING FOR THE TREATMENT OF MYELOMA

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Background. We previously reported on a study where the treatment assignment of 162 newly diagnosed myeloma patients younger than 65 years was based only on the presence/absence of a potential HLA-identical sibling donor (Bruno *et al.*, N Engl J Med). *Design and Methods.* We are now reporting results of an update with a median follow up 2 years longer than that reported in the original study. Briefly, up-front treatment plans consisted of a cytoreductive autograft followed by a nonmyeloablative allograft (Auto-Allo) or a second melphalan-based autograft (Double-Auto). Primary endpoints were overall (OS) and event-free (EFS) survivals by intention-to-treat analysis. The 80 patients with a sibling donor were offered a Auto-Allo and the 82 without a Double-Auto after high (140-200 mg/m^2) or intermediate dose melphalan (100 mg/m^2). No maintenance and/or consolidation therapy was allowed by protocol after nonmyeloablative allografting. *Results.* After a median follow up of 6 years, OS and EFS were significantly longer in patients with donors: not reached versus 52 months (p=0.003) and 35 versus 29 months (p=0.008). Median OS was not reached in the 58 (out of 60 enrolled, 97%) patients who completed Tandem Auto-Allo and was 64 months in the 46 (out of 59 enrolled, 78%) who completed high-dose Double-Auto (p=0.03). Furthermore, an extended experience consisting of 100 newly diagnosed myeloma patients treated with Auto-Allo in a prospective phase II clinical trial by the Gruppo Italiano Trapianti di Midollo Osseo (GITMO) confirmed these encouraging findings. After a median follow up of 5 years, OS was not reached and EFS was 37 months. Incidences of acute and chronic GVHD were 38% and 50%, respectively. Complete remission (including molecular remission) was achieved in 53% of patients. Profound cytoreduction (at least very good partial remission) prior to allografting was associated with achievement of post-transplant remission (HR 2.20, p=0.03) and longer EFS (HR 0.33, p<0.01). Interestingly, development of chronic GVHD was not correlated with response duration. Conclusions Overall, the Auto-Allo approach allows prolonged disease free survival in patients with reduced tumor burden at the time of allografting. We are currently investigating the role of "new drugs" and in particular lenalidomide in intensifying pre-transplant cytoreduction and post-transplant graft-vs.-myeloma effects to further improve clinical outcomes especially in patients with poor prognosis.

C116

HYPOXIA AND HYPOXIA INDUCIBLE FACTOR (HIF)-1α IN MULTIPLE MYELOMA PATIENTS: ROLE IN THE ANGIOGENIC SWITCH

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An increase of bone marrow (BM) angiogenesis occurs in multiple myeloma (MM) patients due to the overexpression of several pro-angiogenic factors by MM cells, however the molecular mechanisms involved are not jet completely investigated. Hypoxia is a common feature of solid tumors associated to angiogenesis. Tumor adaptation to hypoxia is mainly due to the hypoxia-inducible factor (HIF)-1α. The effect of hypoxia on MM cells and the role of HIF-1 in MM-induced angiogenesis actually are not known. In this study, first we checked the level of BM oxygen tension in a cohort of MM patients (n°=25) at the diagnosis as compared to healthy donors and MGUS subjects. The mean pO2 ± SD was 52.3±9 mmHg in MM patients similar to that observed in the controls, confirming that MM cells are exposed *in vivo* to hypoxic microenvironment. Thereafter HIF-1 protein expression by MM cells

was checked by immunohistochemistry on bone biopsies of MM patients showing the presence of HIF-1α stabilization at nuclear level in malignant plasmacells into the BM. Interestingly, HIF-1α protein stabilization and activity was observed at nuclear level in purified CD138+ MM cells in about of 28% of MM patients evaluated suggesting that a hypoxia independent stabilization of HIF-1α may occur in MM cells. Consequently the effect of hypoxia and HIF-1α in MM cells was checked silencing HIF-1α by a pool of siRNA. A gene expression profiling evaluation was performed by microarray analysis using Gene Chips U133plus 2.0 (Affymetrix). Data were then validated by real time PCR. We found that hypoxia significantly upregulated the expression of the pro-angiogenic molecules in MM and cells including VEGF, OPN, IL-8 blunted by HIF-1α know-out. Genes belonging to glycolysis and HIF-1α regulating signal pathways were also regulated by HIF-1α in MM cells in hypoxic condition. These observations were confirmed in purified CD138+ MM cells exposed to hypoxia that induced a significant up-regulation of the pro-angiogenic molecules and the modulation of glycolysis and ubiquitin mediated proteolysis signal pathways. In normoxic condition, HIF-1α knock out significantly affected in MM cells either pro-angiogenic molecules as VEGF or several genes belonging to cell cycle regulation. In conclusion our data underline the role of hypoxia in the regulation of the angiogenic signature of MM cells and suggest that HIF-1α could be a potential target in MM.

C117

THALIDOMIDE-DEXAMETHASONE AS INDUCTION THERAPY PRIOR TO AUTOLOGOUS STEM-CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA AND RENAL FAILURE

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Multiple myeloma (MM) patients presenting with renal failure at disease onset should not be excluded from high-dose therapy programs; seeking novel effective and non-nephrotoxic induction regimens is thus mandatory in order to maximize the reduction of tumor burden prior to transplant. Primary objective of this phase II study was to prospectively evaluate the efficacy and toxicity of thalidomide (Thal) –dexamethasone (Dex) as induction therapy in preparation for autologous stem-cell transplantation (ASCT) in previously untreated MM patients with renal failure at diagnosis. Thirty-one patients with baseline creatinine clearance values higher than 50 mL/min, 7 of whom were in chronic hemodyalisis, entered the study. As per protocol design, patients received four months of oral Thal-Dex followed by peripheral blood stem cell (PBSC) collection and subsequent ASCT. After thal-dex induction therapy, at least a partial response (PR) was obtained in 23 patients (74%), including 8 (26%) who achieved a very good partial response (VGPR) or better. An improvement in renal function was more frequently observed in patients achieving a PR or better (82% vs. 37% in patients obtaining failing to achieve PR, p=0.04). Toxicity profile of Thal-Dex was comparable to that observed in patients with a normal renal function. Twenty-six patients underwent PBSC mobilization; in 17 of them (65%) more than 4x10^6 CD34+ cells/kg were collected; a double or a single ASCT were performed in 15 and 7 patients, respectively. Overall, median event-free survival was 30 months, and median survival has not been reached, upon 32 months median follow-up. According to our data Thal-Dex is effective and safe in patients with newly diagnosed MM and renal failure; given the relationship between recovery of renal function and response to induction treatment, more intensive Thal + Bortezomib-including regimens could be potentially useful in order to rescue a higher number of patients.

C118

VD (BORTEZOMIB AND DEXAMETHASONE) AS MAINTENANCE THERAPY IN ADVANCED MULTIPLE MYELOMA PATIENTS RESPONDING TO SALVAGE BORTEZOMIB CONTAINIG REGIMENS

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Background. Several studies have demonstrated the efficacy of combination bortezomid/dexamethasone (VD) in patients with advanced MM but no data are available regarding the maintenance therapy (MT) after salvage treatment. *Patients and methods.* In this trial we tested safety and efficacy of VD as MT (bortezomib: i.v. bolus on days 1 and 15 at 1.3 mg/mq; dexamethasone: orally at 20 mg/d on days 1-2 and 15-16 every 28-day cycle for a total of 6-8 cycles) in patients with advanced MM who responded to salvage therapy. We also assessed the impact of MT on progression-free survival (PFS) and overall survival (OS). *Results.* From October 2004 until April 2008, 49 MM patients have been enrolled. The characteristics of the patients were as follows: 28 males and 21 females, median age was 71 years (IQR:66-75).The median number of prior therapies was 2 (2-3). All patients were in PR after salvage therapy that included bortezomib as single agent or in combination with steroids and/or thalidomide in 39 patients (79.6%). Median time from diagnosis to the first dose of MT was 39 months (IQR:26-56). The median number of bortezomib infusion was 8 (7-12). After a median follow up of 19 months (IQR:13-22), 12 patients died for PD, 1 for IMA and 6 patients for infections. The MT improved the quality of response after salvage therapy as follows: 4 CR, 3 VGPR, 10 PR, 19 SD whereas 13 patients experienced PD. The median time to progression was 17 months (95%CI: 7-38) with a PFS at 1 year of 63% (95%CI: 48-75) (Figure1). The OS at 1 years was 79% (95%IC: 75-88) and the cumulative incidence of death due to PD adjusted for competitive risk event was 12% (95%IC:2-19). In a univariate analysis the response rate to MT was not significantly affected by age, sex, number or type of previous therapy and haemoglobin concentration. Non-dose-limiting toxicities included neuropathy grade 1 (15 pts), HZV reactivation (2 pts), pulmonary infections (2 pts), and gastrointestinal affections (3 pts). Three patients developed a neuropathy grade 2 which required a dose reduction (1.0 mg/mq) of bortezomib. *Conclusion.* The combination bortezomib/dexamethasone as a maintenance therapy in relapse/refractory MM is effective and well tolerated. These preliminary data suggest that bortezomib/dexametasone MT can improve remission duration and also quality of response with an acceptable toxicity.

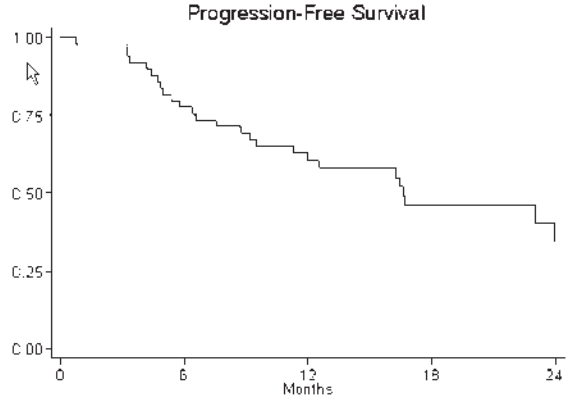

Figure 1.

C119

LENALIDOMIDE, MELPHALAN, PREDNISONE AND THALIDOMIDE (RMPT) AS SALVAGE THERAPY IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background. Immunomodulating drugs represent a novel class of anticancer agent, active in multiple myeloma (MM). Increased understanding of myeloma biology has led to the development of new therapies, with the aim of enhancing the anti-tumour activity whilst reducing the toxic effects of thalidomide. In newly diagnosed MM the addition of new drugs, lenalidomide, thalidomide or bortezomib, to the standard oral melphalan and prednisone (MP) combination significantly increases response rate and event-free survival. In advanced setting, a 4 drug association VMPT further improves response rate. MATERIALS AND METHODS: This is a multicenter open label phase I/II trial, to evaluate efficacy and safety of the association of lenalidomide, melphalan, prednisone and thalidomide (RMPT) in relapsed/refractory MM. Patients (pts) received 6-28 days courses with oral lenalidomide 10 mg/day on days 1-21, melphalan 0.18 mg/kg on days 1-4 and prednisone 2 mg/kg on days 1-4. Thalidomide was given at 50 mg/day (Arm A) or 100 mg/day (Arm B) on days 1-28. Maintenance therapy included Lenalidomide 10 mg/day on days 1-21. Aspirin 100 mg/day was given as a prophylaxis for thrombosis. *Results.* Forthy-four pts with relapsed or refractory MM were enrolled. Median age was 69 years (range 47-80). All pts had been already treated with a median of 2 previous lines of treatment (including autologous and allogeneic transplant, conventional chemotherapy, thalidomide and bortezomib): 26 pts received RMPT as second line of therapy and 18 as third line. After a median of 5 courses, 75% of pts achieved at least a partial response (PR), including 20% very good partial response (VGPR) and 14% near or complete response (CR or nCR). Among 26 pts who received RMPT as second line therapy the PR rate was 73%, including VGPR 23% and CR/nCR 19%. Among pts who received thalidomide 100 mg, the PR rate was 82% (VGPR 23% and CR/nCR 23%) compared to 68% of thalidomide 50 mg. The 1-year-progression-free survival was 51.5%, the 1-year survival from study entry was 72%. Grade 3-4 hematologic adverse events included: neutropenia 63%, thrombocytopenia 33,8% and anemia 34,1%. Grade 3-4 non hematologic adverse events included: infections 22%, neurological toxicity (7%) and fatigue (7%). No thromboembolic events grade 3-4 were reported. *Conclusions.* RMPT is an effective salvage combination therapy with a high proportion of responses and a manageable toxicity profile. No thromboembolic adverse events were reported.

C120

THADD VS THADD PLUS HDT IN ELDERLY PATIENTS WITH DE NOVO MULTIPLE MYELOMA

Offidani M.,[1] Galieni P.,[2] Corvatta L.,[2] Polloni C.,[1] Gentili S.,[1] Visani G.,[2] Alesiani F.,[2] Brunori M.,[2] Catarini M.,[2] Mele A.,[2] Burattini M.,[2] Marconi M.,[2] Samori A.,[2] Centurioni R.,[2] Ferranti M.,[2] Blasi N.,[2] Fraticelli P.,[2] Gini G.,[1] Montanari M.,[1] Leoni P.[1]

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While the role of a sequential therapy including novel agents combination and high-dose therapy (HDT) has been recently explored in elderly patients with MM (Palumbo *et al.*, ASH 2008) no authors have focused on the utility of a consolidation with HDT after an induction with new drugs combination in older patients eligible for transplantation. This remains a burning question considering that in newly diagnosed MM patients CR rates obtained with new drugs are close to those of HDT. In this post-hoc analysis of a prospective trial we compared the outcome of patients treated with ThaDD regimen only and patients receiving ThaDD followed by HDT with the aim to try to address the above issue. Sixty two patients not eligible for HDT received 6 courses of ThaDD regimen only and 26 patients eligible for HDT were treated with 4 courses of ThaDD followed by Mel-200 (16 patients) or Mel-100 (10 patients) and autologous stem cell transplantation. The two groups of patients were matched for the main characteristics as performance status, albumin, CRP, creatinine, ISS (p=0.208) and cytogenetics (p=0.857) but age (median 74 vs 67 years; p=0.001) and beta-2 microglobulin (median 4.2 vs 2.9 mg/L; p= 0.005) favouring the HDT group. After induction at least PR was achieved by 96% and 92% (p=0.474), at least VGPR by 73% and 61% (p=0.291) of ThaDD and ThaDD+HDT group, respectively. After HDT response rate ≥ VGPR raised to 81% and CR rate increased from 26% to 57%. After a median follow-up of 32 months (range 8-60), median PFS and OS resulted 29 and 32 months (p=0.726), 58 and 45 months (p= 0.404) in the standard dose group and HDT group, respectively. Moreover, patients achieving CR after induction had a median PFS of 43 months compared to 22 months in patients that obtained CR just after HDT (p=0.032). ThaDD was fairly well tolerated but either DVT/PE and severe infection occurred in 11 patients (12.5%). Grade 3 peripheral neuropathy developed in 7 patients (8%). Only 2 patients dropped out due to toxicity (1 EP, 1 severe infection). In the HDT group all patients had a complete engraftment, toxicity was as expected and no patients died from procedure. In conclusion, although in elderly patients with newly diagnosed MM ThaDD plus HDT results in increased CR rate, it seems equivalent to ThaDD alone in terms of PFS and OS. Our results suggest that in the new drugs era the role of HDT consolidation should have to be evaluated through wide randomized trials.

POSTERS

Non-Hodgkin's Lymphoma I

PO-001

RITUXIMAB PLUS PEGYLATED LIPOSOMAL DOXORUBICIN IN COMBINATION WITH CYCLOPHOSPHAMIDE: A FIRST LINE THERAPEUTIC OPTION FOR VERY ELDERLY OR UNFIT PATIENTS WITH AGGRESSIVE NON HODGKIN LYMPHOMA

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Purpose. The standard treatment for patients with aggressive B-cell Lymphoma, in particular diffuse large-B-cell lymphoma [DLBCL], is cyclophosphamide, doxorubicin, vincristine and prednisone [CHOP] plus rituximab, a chimeric monoclonal antibody against the CD20 antigen. However, some very elderly patients are not fit enough to tolerate CHOP or they have a comorbidity that exclude antracyclin in the regimen. The overall survival in this subset of patients is very poor. Pegylated liposomal doxorubicin is associated with a lower risk of cardiotoxocity than conventional formulations of doxorubicin, allowing the use of higher cumulative doses The aim of this single institution study was to investigate the outcome of pegylated liposomial plus cyclophosphamide (Cae-CY) and rituximab (R) regimen in patients ≥ 75 years old, with diagnosis of DLBCL and cardiological comorbidity. *Patients and Methods*. In this study, 22 patients aged over 75 years (median 79, range 75-91 years) with aggressive non-Hodgkin's Iymphoma (NHL) (age adjusted International Prognostic Index (IPI): IPI-2 (25%); IPI-3 (35%); IPI-4 (40%)) received pegylated liposomal doxorubicin (25 mg/m^2/day 1), cyclophosphamide (300 mg/m^2 day 1) and rituximab (375 mg/m^2 day 2), q28. *Rusults.* 20 patients completed 6 treatment cycles and were evaluable for efficacy and safety. A complete response was achieved in 14 (70%) patients and a partial response in 2 (10%) patients. 4 patients showed stable disease or progressive disease (20%). With a median follow-up of 18 months, the median time to progression was 12 months. The major toxicity was haematologic: grade 2 leukocytopenia occurred in 6 patients, grade 3 thrombocytopenia in 5 patients, but no grade IV toxicity occurred. There were no episodes of clinically significant bleeding. Three patients developed febrile neutropenia. No significant decrease in LVEF or clinical evidence of congestive heart failure was observed during the treatment or in follow-up. Pegylated liposomal doxorubicin plus cyclophosphamide is an effective and well tolerated regimen for the treatment of aggressive NHL in elderly or unfit patients.

P002

PHASE II STUDY OF INTRATHECAL LONG ACTING LIPOSOMAL CYTARABINE (DEPOCYTE®) IN THE PROPHYLAXIS OF LYMPHOMATOUS MENINGITIS IN HIV-RELATED NON-HODGKIN'S LYMPHOMA

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Around 5% of patients with aggressive non-Hodgkin's lymphoma (NHL) develop central nervous system (CNS) progression or relapse during the course of their disease. Patients with human immunodeficiency (HIV)-related NHL often develop CNS progression despite the use of adequate prophylaxis. Liposomal cytarabine has show a significant activity in lymphomatous meningitis but there are limited data in the prophylactic setting. Between May 2006 and December 2008, we performed a prospective phase II study of intrathecal liposomal cytarabine (Depocyte®) at the dose of 50 mg in 28 patients with HIV-NHL with the aim to evaluate the feasibility and activity of this drug in the prevention of lymphomatous meningitis. Twenty-three patients were males and the median age was 42 years (range 18-64 yrs). As far as the histological subtype of NHL, 64% of patients had a diffuse large B-cell (DLBC) NHL and 36% Burkitt NHL. Stage III-IV was diagnosed in 75% of patients and 79% of DLBC were age-adjusted IPI 2 or more. An extranodal involvement was diagnosed in 64% of patients (gastrointestinal 29%, bone marrow 18%, spleen 18%, bone 14%). Liposomal cytarabine was well tolerated with headache grade I to III being the most frequent side effect in 46% of patients. Less common toxicity (all grade I) included cortical changes (7%), fever (4%), vomiting (4%), hypertension (4%), chills (4%). With a median follow up of 10 months only one patient (4%) with Burkitt lymphoma developed a combined systemic and meningeal relapse. Moreover, in our experience previously the present study, we used methotrexate as practical use in 267 HIV-NHL with a meningeal progression or relapse of 10% (p=0.32). The use of a liposomal formulation allowed to significantly reduce the number of lumbar injections in comparison to the standard schedules (approximately of 50%) with an improvement of quality of life of patients and with a reduction of professional exposure risk for health care staff. In conclusion, in this first prospective study on prophylaxis of lymphomatous meningitis in HIV-NHL reported in the literature, liposomal cytarabine seems safe and active and it reduces of approximately 50% the number of lumbar punctures and exposure risk for health staff as well.

P003

FCR (FLUDARABINE,CYCLOPHOSPHAMIDE,RITUXIMAB) REGIMEN FOLLOWED BY YTTRIUM-90 IBRITUMOMAB TIUXETAN CONSOLIDATION FOR THE TREATMENT OF RELAPSED GRADES 1 AND 2 FOLLICULAR LYMPHOMA (FL)

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Background. FCR regimen has provided encouraging results in FL and Yttrium-90 Ibritumomab Tiuxetan (90Y-RIT) has been reported to be effective in patients with relapsed or refractory FL. Our study investigates the efficacy and safety of 90Y-RIT consolidation in relapsed grades 1 and 2 FL patients, responding to FCR. *Methods.* We have recruited 9 patients median age 63 yrs (range 46-77). All enrolled patients were relapsed patients with histologically confirmed CD20-positive (grade 1 or 2) FL. All patients at relapse received FCR every 28 days: F (25 mg/m^{2}x3 days), C (1 gr/m^{2}day1) and R (375 mg/m^{2}day4) for 4 cycles. Patients were restaged one month after the last course of FCR; who achieved at least a partial remission, with<25% bone marrow involvement, was eligible for Yttrium-90 Ibritumomab Tiuxetan 11.1 or 14.8 MBq/Kg up to a maximum dose 1184 MBq, at 3 months after the completion of FCR. The patients underwent a further rastaging at 12 weeks after 90Y-RIT with total body CT scan, FDG-PET/CT and bilateral bone marrow biopsy. Results: Between August 2005 and April 2009 nine patients have completed the treatment: FCR followed by 90Y-RIT (6 patients at 14.8 MBq/Kg, 3 patients at 11.1 MBq/Kg). All 9 patients were relapsed patients: 1 patient received a prior therapy, 6 patients received 2 prior therapy regimens and 2 patients had received 3 to 5 regimens. After FCR 7 patients obtained CR and 2 PR; after 90Y-RIT treatment the ORR was 100% and CCR was 100% with median follow up of 16 months (range 4-34) and all patients are alive in CR. The most common grade 3 or 4 adverse events were hematologic: grade 3 or 4 neutropenia occurred in 8/9 patients treated with FCR and in 9/9 patients assessable after 90Y-RIT. Grade 3 or 4 thrombocytopenia occurred only after 90Y-RIT in 5/9 patients. Following treatment with 90Y-RIT the median neutrophils nadir was 0.8x10^9/L (range 0.1-0.99x10^9/L) at week 5 ; the median platelets nadir was 49x10^9/L (range 17-81x10^9/L) at week 5. The median duration nadir for both neutrophils or platelets was 14 days. One patient developed herpes zoster infection after 8 months following valacyclovir discontinuation; another patient developed fungal infection. *Conclusions*. Our experience indicate feasibility, tolerability and efficacy of FCR regimen followed by Zevalin® in patients relapsed with grades 1 and 2 FL. Hematologic toxicity occurring with FCR or with radioimmunotherapy are clinically controllable and acceptable in the population composed mainly of patients with a history of prior treatment using rituximab plus chemotherapy. A longer follow up and a larger number of patients with relapsed grades 1 and 2 FL are required to determine the impact of this regimen on long-term duration of response and EFS, but these preliminary results suggest that this regimen could be an option to be used for the treatment in this setting of patients, specially at age of 65-75 and earlier in first relapse.

P004

USE OF LIPOSOMAL DOXORUBICINE IN ELDERLY CARDIOPATHIC PATIENTS AFFECTED BY NON HODGKIN LYMPHOMA

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R-CHOP protocol is the gold standard treatment for Non Hodgkin's Lymphoma and doxorubicine is the drug with the higher antitumoural activity. However, disease (cardiotoxicity, drug-resistence, etc.) and patient characteristics (advanced age, comorbidity) limit the use of that protocol. In frail patients the use of liposomal anthracycline instead of classic one may reduce cardiotoxicity even though manteining the same antitumoral efficacy (i.e. R-COMP regimen). We investigated the use of liposomal doxorubicin in elderly patients affected by NHL with associated cardiopathy. From 2003 to 2008, in our Haematology Unit ASL NA-1 we treated 24 elderly cardiopathic patients affected by NHL. All the patients underwent echocardiographic evalutation of left ventricular ejection fraction (LVEF%) before starting treatment, during treatment and 3, 6, 12, 18, 24 months after the end of therapy. Patients' characteristics were: 16 male and 8 female; median age 69 years (range: 66-80); istology: 14 Diffuse Large B Cell Lymphoma (DLBCL), 4 cutaneous NHL; 3 Mantle Cell Lymphoma (MCL) and 4 Follicular (G3) Lymphoma. At diagnosis LVEF was less than 50% (35-45%) in all patients. One month after the last cycle all patients were subjected to a disease re-staging: 85% of patients were in CR, 12% in PR, 3% in progression disease or resistance. Nobody had a reduction of LVEF; interestingly, 2 out of 24 patients showed an improvement of LVEF. Overall survival (+24 months) was 85%. We confirm the efficacy of R-COMP regimen in the treatment of NHL. Moreover, the use of liposomal anthracycline allows the treatment of cardiopathic patients with aggressive lymphoma. Further studies are warranted to confirm the safety and effectiveness of polichemotherapy with liposomal anthracycline in NHL frail patients.

P005

HEPATITIS C VIRUS POSITIVE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA HAVE EXCELLENT SURVIVAL AFTER STANDARD CHEMO-IMMUNOTHERAPY: RESULTS OF A PROSPECTIVE UNICENTRIC OBSERVATIONAL TRIAL

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Purpose. To define the tolerance to chemo-immunotherapy and the outcome of patients with diffuse large B-cell lymphoma (DLBCL) and hepatitis C virus (HCV) infection. *Patients and Methods.* From February 2004 to June 2008, we performed a prospective observational study enrolling 168 incidental patients with newly diagnosed DLBCL. All patients were tested for HCV antibodies and treated uniformly following internal standard criteria. Inclusion criteria were: no prior treatment for lymphoma, hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) seronegativity, and no evidence of hepatocellular carcinoma.

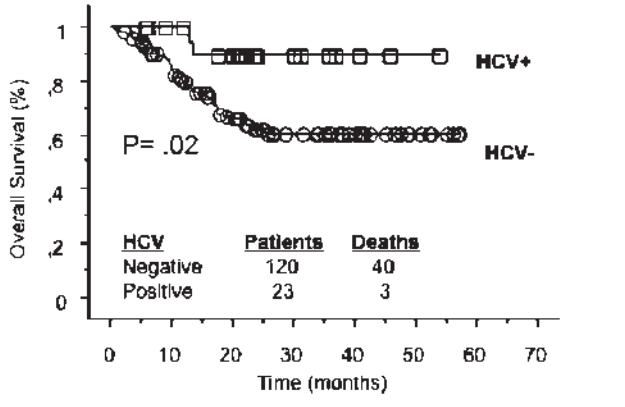

Figure 1. Overall survival of 143 patients treated with curative intent, according to HCV status.

No antiviral treatment was allowed for HCV-positive patients at any time during the study. Eligible patients were stratified according to administered treatment. Primary end-points were the safety assessment and the survival of patients according to HCV status. The HCV infection prevalence among enrolled patients was 18% (31/168). *Results.* HCV-positive patients were treated less intensively than HCV-negative patients because of more frequent co-morbidities (p=0.05). However, the hepatic toxicity of chemo-immunotherapy was low. Patients with HCV infection had a slightly better overall survival (3-year OS 70% for HCV-positive vs 53% for HCV-negative, p=0.18). When patients were stratified according to the intention to cure, the HCV-positive patients had a significantly superior OS than the HCV-negative patients (89% vs 60%, p=0.02), and multivariate analysis revealed a favourable and independent influence of HCV infection on OS. *Conclusion*. HCV-positive patients with DLBCL have an unexpectedly good OS and should not be deprived of standard curative chemo-immunotherapy. Our findings suggest a role for the virus in maintaining lympho-proliferation.

P006

EFFICACY OF RITUXIMAB PLUS BENDAMUSTINE AS SALVAGE REGIMEN FOR ELDERLY PATIENTS WITH INDOLENT LYMPHOPROLIFERATIVE DISORDERS

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Introduction. Bendamustine is a water-soluble, bifunctional chemotherapeutic agent with characteristics of both an alkylator and a purine analog. *In vitro* studies showed the synergistic effect of bendamustine and rituximab against various leukemia and lymphoma cell lines. Clinical trials supported these results by showing the high efficacy of bendamustine plus rituximab combination in relapsed and refractory patients with indolent lymphoma. These results have been found in rituximab-naive, rituximab-pretreated, and rituximab-refractory patients with excellent response rates and toxicity profiles. This study evaluated bendamustine plus rituximab combination treatment in elderly patients with relapsed, indolent B-cell or mantle cell lymphoma. *Patients and Methods.* All patients received rituximab 375 mg/mq intravenously on day 1 and bendamustine 90 mg/mq or 120 mg/mq intravenously on days 2 and 3 of each 28-day cycle for four to six cycles. An additional dose of rituximab was administered 4 weeks after the last cycle. Results: Twelve elderly patients (median age 76 years, range 68-81) has been enrolled in the study; all patients were in advanced stage with symptomatic disease and received median 3 prior line chemo-immunotherapy; 1 patient had concomitant myelodisplastic syndrome; the histologic subtype was 6 follicular lymphoma, 4 lymphocytic lymphoma/chronic lymphocytic leukemia, 1 mantle cell e 1 diffuse large B-cell lymphoma. After a median of 4 cycles, an overall response rate of 74% (16% complete response, 50% partial and 8% stabile disease) was observed. The median duration of response was 9.0 months. One patients died for progressive disease and concomitant fungal broncopneumonia. Most of the non hematologic adverse events were mild (WHO 1-2). Grade 3 or 4 reversible hematologic toxicities included neutropenia (30%) and anemia (12%). Conclusion Bendamustine plus rituximab is an effective combination in very elderly patients with refractory/relapsed indolent and mantle cell lymphoma with an acceptable toxicity profile. These findings are promising and will serve as a benchmark for future clinical trials in this patient population.

P007

ROLE OF TOTAL BODY MAGNETIC RESONANCE IN DETECTING SKELETAL INVOLVEMENT IN ADVANCED STAGE LYMPHOMAS: UN UPDATE FROM A SINGLE CENTER EXPERIENCE

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Introduction. Skeletal localizations are reported in approximately 10-20% of advanced stage malignant lymphomas. Computed tomography (CT) is considered the gold standard for lymphoma staging and evaluation of treatment response; this technique, however, has a limited sensitivity in identifying skeletal localizations. Positron emission tomography (PET) has a higher sensitivity in recognizing bone involvement and

represents a diagnostic tool, complementary to CT. Total body magnetic resonance (MR) may represent an option for detection and better characterization of tumoral bone lesions. We compared MR with conventional imaging procedures in the evaluation of lymphoma patients (pts) with suspected skeletal involvement. *Patients and Methods.* Seven pts with diagnosis of Hodgkin lymphoma and 12 pts with diffuse large B cell lymphoma with clinical signs or radiological findings suggesting skeletal involvement, underwent MR in addiction to standard staging techniques (CT and PET). Total body MR was performed with a body coil (1.5 Tesla) and images were obtained by using fast spin-echo short time inversion recovery and spin-echo single-shot sequences diffusion weighted. In 14 pts MR was performed at diagnosis, in 17 cases as evaluation after treatment, for a total of 31 scans. MR images were compared with those obtained from conventional imaging performed at the same time. *Results*. At diagnosis MR detected skeletal involvement in all cases, while CT and PET in 21% and 88% of cases respectively. Moreover, both CT and PET even when positive, detected a lower number of osseous localizations than MR. Seventeen MR were performed for evaluation after treatment, documenting skeletal disease regression in 15/17 (88%) cases. In these 15 pts PET results were concordant. In the 2 pts with positive MR, PET detected skeletal involvement only in 1 case. In the 3 CT positive pts at diagnosis, CT alterations persisted in the evaluation after treatment, even if MR and PET became negative. Considering CT and PET performed both at diagnosis and after therapy, sensitivity was 26% and 81% respectively. *Discussion*. These data suggest that total body MR can be used in the management of lymphoma pts with suspected skeletal involvement, since in the described group of pts CT failed to evidence skeletal localizations in the majority of cases. PET is often concordant with total body MR, but detects a lower number of bone lesions.

P008

FDG-PET IN THE EVALUATION OF LYMPHOMA IN PATIENTS WITH HIV-1 INFECTION: A SINGLE CENTER EXPERIENCE

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Introduction. Systemic lymphoadenopathy is often seen in patients (pts) with HIV-1 infection. An increased risk of developing non-Hodgkin lymphomas (NHL) is also associated with HIV and evaluation of nodal involvement by lymphoma is not straightforward using conventional imaging. The role of positron-emission tomography using 18F-fluorodeoxyglucose (FDG-PET) in the staging and evaluation of response to treatment of pts with NHL and Hodgkin lymphomas (HL) is well documented in immunocompetent pts, while only limited information is available on lymphomas arising in the setting of HIV infection. We undertook a retrospective analysis of results of PET in a group of HIV pts with lymphoma, both NHL and HL. *Patients and Methods.* Twelve HIV-positive male pts with a diagnosis of lymphoma (7 high grade and 1 follicular NHL, 4 HL) underwent a total of 22 PET scans. PET results were compared with those obtained by computed tomography (CT). Nine PET were performed at staging, 7 at restaging following chemotherapy, and 6 at follow-up. At diagnosis of lymphoma: median CD4 count was 200 cells/mcL (range 98-451); HIV genome was undetectable in 5/9 pts, in the remaining 4 pts, for whom the data was available, viral loads ranged from 103 to 1,452,720 copies/mL; 10/12 pts were already receiving HAART, while the remaining 2 pts started treatment upon diagnosis of lymphoma. *Results.* In the majority of cases (17/22, 77%) results of PET, CT scan and clinical status were concordant, being diagnostic of either lymphoma presence (11/17) or absence (6/17). No false positive results were recorded at follow-up. In 5/22 cases (23%) PET and CT scan were discordant. In 4 cases PET yielded a false negative result; in 2 cases at diagnosis (2 pts with HL) and in 2 cases at follow-up (both in the patient with follicular lymphoma). In the remaining case, a PET-negative CT-positive adenopathy was demonstrated, which proved to be reactive in nature at biopsy. *Discussion.* Similarly to immunocompetent pts, FDG-PET is useful in the evaluation of lymphoma in the setting of HIV infection. In our experience, PET proved to be able to discriminate between reactive and lymphomatous involvement of lymphoid tissue as demonstrated by absence of false positive results in pts evaluated at follow-up. These results need to be confirmed by larger clinical trials.

P009

SAFETY AND EFFICACY OF INTRATHECAL LIPOSOMAL CYTARABINE IN COMBINATION WITH METHOTREXATE AND STEROIDS FOR THE TREATMENT AND PROPHYLAXIS OF LEUKEMIC AND LYMPHOMATOUS MENINGITIS: A SINGLE INSTITUTION EXPERIENCE

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Background. Intrathecal (IT) chemotherapy is an important component of the prophylaxis or treatment of hematologic malignancies in the central nervous system (CNS), especially in patients with acute lymphoblastic leukemia and aggressive lymphomas. The three commonest IT drugs are methotrexate, cytosine arabinoside (Ara-C), and corticosteroids. Liposomal cytarabine (Depocyte) is a sustained-release formulation of cytarabine developed for intrathecal administration, ensuring prolonged cytotoxic drug concentrations of cytarabine in cerebrospinal fluid. Liposomal cytarabine permits to decrease frequency of lumbar punctures, without loss of efficacy, because intrathecal levels of the drug remain cytotoxic for up to 14 days. *Aim.* With the aim to evaluate the safety and efficacy of treatment and prophylaxis schedule with methotrexate, liposomal cytarabine and methyl-prednisolone. *Methods*. From October 2007, 6 patients affected by aggressive lymphomas (HG NHL) (5 cases) and acute lymphoblastic leukemia (ALL) (1 case) underwent CNS prophylaxis with methotrexate 12 mg, liposomal cytarabine 50 mg and methyl-prednisolone 40 mg IT every 15 days in our Institution. Moreover 2 patients (HG NHL 1 pt and ALL 1 pt) have been treated with same schedule for neoplastic meningitis. The clinical and hematological features of the patients were: 6 males, 2 females; median age 60 yrs (range: 32-73). *Results*. After a median follow-up of 10 months a total of 83 lumbar punctures have been administered. In any case a III-IV WHO grade toxicity has been observed. All 6 patients undergone prophilaxis did not show the occurrence of neoplastic meningitis. The remaining 2 patients treated for neoplastic meningitis showed a complete clearance of blasts after a median of 3 lumbar punctures and are still in remission. *Conclusions.* With the limitation of the relatively low number of cases, we can conclude that the simultaneous IT administration of methotrexate, Liposomal cytarabine and corticosteroids is well tolerated and appears to be particularly effective in the prophylaxis for aggressive lymphomas and acute lymphoblastic leukemia patients. Moreover, a long term remission status maybe mantained. Certainly, confirmation in a larger cohort of patients is now warranted.

P010

CLINICAL CHARACTERISTICS AND OUTCOME OF HEPATITIS C VIRUS POSITIVE WALDENSTROM'S MACROGLOBULINEMIA

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Hepatitis C virus (HCV) infection has been associated with increased risk of developing B-cell lymphoproliferative disorders. The potential link between HCV and Waldenstrom's Macroglobulinemia (WM) remains controversial and results of studies analyzing the prevalence of HCV in WM are conflicting. The aim of the current study was to assess the impact of the infection itself in the clinical and biological features and outcomes among WM pts as compared with those with HCV negative WM. We retrospectively analyzed 78 WM patients in which the anti HCV antibody (HCV-Ab) had been determined. Ten of the 78 pts (12.8%) were found to be positive for HCV-Ab. In all the 10 pts HCV-RNA was found to be detectable. Clinical characteristic of pts at diagnosis of WM are reported in the table. When comparing the two groups of patients according to the disease characteristics listed in the table a statistical significative difference was found for the presence of autoantibodies and the PMN level. Furthermore we didn't reveal a difference in outcome defined as progression of disease needing treatment, time from diagnosis to first therapy, overall survival (median OS is not reached in both groups) between HCV^- and HCV^+ pts. Overall 45 pts (57.7%) received treatment for disease progression with schedules including Rituximab in 30 cases. Rituximab was administered even in HCV-RNA positive patients associated to cyclophosphamide and Fludarabine and this did not translate in hepatitis development. During immunotherapy HCV-RNA was strictly monitored and we did not observe any significant flair. In conclusion in our series of pts HCV infection does not seem

to affect prognosis and does not impair the administration of intensive chemotherapy even with monoclonal antibodies.

Table 1.

Characterstic	All pts	HCV-	HCV+	P
Age y median	60	61	57	0.444
Sex M/F %	54/46	54/46	50/50	>0.999
Hb g/dl median	13	13.2	11.15	0.086
PLT x 10^9/L median	252	257.5	176.5	0.065
PMN x 10^9/L median	3.6	3.8	2.65	0.044
IgM mg/dl median	1760	1690	3586	0.179
Bone marrow infiltration:				
≤30%	50.6	45.3	80	0.223
30-80%	26.6	31.25	-	> 0.999
≥80%	22.6	23.4	20	0.672
LDH U/L median	314	313	415	0.523
β^2 m. mcg/mL median	2.3	2.3	3.55	0.265
Albumine g/dL median	4.6	4.6	4.43	0.492
Creatinine mg/dL median	0.9	1.13	0.93	0.371
Splenomegaly %	21.8	19.11	40	0.242
Lymphadenopathy %	23.1	22	30	0.709
Neuropathy %	10.2	8.8	20	0.314
Presence of autoantibondies %	16.6	10.2	60	0.007
Cryoglobulins %	14.1	10.3	40	0.198

P011

ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA (AITL) DEVELOPING AFTER AN EBV-POSITIVE LARGE B-CELL LYMPHOMA. DESCRIPTION OF A CASE

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A rational model to explain the pathobiological features of AITL is based on the neoplastic cell derivation from germinal center follicular helper T-cells, wich promote follicular B-cell differentiation through activation-induced cytidine deaminase. Immune dysfunction and immunodeficiency, common in AITL, are critical to the expansion of large B-cell blasts, often infected by Epstein Barr virus, wich may mimic Reed-Sternberg cells. In patients with AITL an EBV+ B cell lymphoid proliferation, mostly DLBCL, can develop subsequently to original diagnosis. We report a 60-year-old male who developed an AITL after successful treatment of an EBV-associated DLBCL. On december 2005 the patient was evaluated because of cervical and axillary lymphadenopathy. The lymph node biopsy showed atypical large B cells (CD20+, CD79a+, Oct1+, Bob1+) on a background of small T lymphocytes and histiocytes. The large cells variably expressed CD30 and were positive for EBV (LMP1 antigen). JH and TCR rearrangement analysis was negative. Bone marrow biopsy was negative. T- and histiocyte-rich DLBCL, EBV-associated, stage IIIA was diagnosed. The patient received four courses of R-ACVBP followed by MTX-ifo consolidation and obtained a complete remission. Two months later he was admitted because of increasing lymphoadenopathy, hepatosplenomegaly, fever, weight loss and immunological abnormalities including hypergammaglobulinemia and Coombs-positive hemolytic anemia. A new lymph node biopsy showed a diffuse proliferation of large atypical cells associated with numerous blood vessels; they expressed CD2, CD3, CD4, CD5 and LAT; part of them were CXCL13+. CD10 and CD30 were negative. No large B cells nor EBV+ cells were found. Numerous CD21+CD23+CD35+ follicular dendritic cells were identified. TCR analysis showed clonal T-cell population, consistent with AITL. The patient's course was further complicated by invasive pulmonary aspergillosis, CMV reactivation, subacute demyelinating peripheral neuropathy, relapse of hemolytic anemia and by severe thrombocytopenia (PLT<5x10^9/L) refractory to steroids, ivIg, danazol, mtx and rituximab. At last follow-up, after treatment with oral cyclophosphamide and prednisone, there is no evidence of lymphoma, but severe thrombocytopenia persists. Molecular studies on peripheral blood and nodal lymphoid cells are ongoing. The present case highlights the complex relationship between AITL and DLBCL arising in a background of severe immune dysregulation.

P012

PROGNOSTIC VALUE OF RESIDUAL CT SCAN MASS IN AGGRESSIVE LYMPHOMAS PATIENTS WITH PET NEGATIVE AFTER CHEMO+/-RADIOTERAPY

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Background. Positron emission tomography (PET) with 2-[18F]fluoro-2-deoxy-D-glucose (18F-FDG) currently rapresents the most accurate tool for the assessment of treatment response in some lymphoma subsets. In fact, the revised response criteria (Cheson, 2007) for FDG avid lymphoma , e.g. Hodgkin(HL) and diffuse large cell non Hodgkin lymphoma (DLCL) require Pet negativity to define complete remission(CR), independently from the persistence of residual masses of computed tomography (CT scan) . Nevertheless, some reports suggested a slightly lower prognosis among PET patients (pts) with CT scan residual masses. *Aims*. To evaluate the negative predictive value (NPV) of residual CT scan masses in pts with DLCL and HL patients with PET negative at the end of treatment. *Methods.* The analysis was retrospectively conducted in DLCL and HL pts who underwent whole-body 18F-FDG-PET and CT scan after the end of treatment program at least twice at our institution. The NPV was defined as the proportion of patients without progression, relapse, or need for irradiation within 12 months after PET. *Results*. From February 2004 to February 2008, PET negative was observed in 219 pts (128 with DLCL and 91 with HL, respectively). One hundred seventy-seven pts were evaluated after first line treatment program, while 42 pts after salvage therapy program. Residual CT scan disease (PET-/CT scan +) of at least 2.0 cm in the largest diameter was assessed in 139 pts. Ninety-six had only one side with residual mass, while 43 pts more than one sides. As of March 2009, 44 pts relapsed, and 27 of these had previous CT scan positive. The disease-free survival (DSF) was 77.9% for PET-/CT scan- pts and 79.4% for PET- /Ct scan + pts (P=0.722). Among other prognostic factors analyzed, (histology, number or size of masses, first vs salvage treatment program) no correlation with DSF or overall survival (OS) emerged. With a median follow-up of 3 years, the DSF and OS were 79.2% and 89.1 %, respectively. *Conclusions*. In our study we did not observe any significant difference in DFS among PET negative pts with or without CT scan residual masses after lymphoma therapy. This suggest that residual disesase at CT scan is not a prognostic factor for relapse/progression disease. Further analysis will be performed in order to identify a possible dimensional cut-off at CT scan predictive for disease progression. This could imply the submission to consolidative radiotherapy on largest residual masses.

P013

BENDAMUSTIN (BE)+ RITUXIMAB(R) SCHEDULE IN THE TREATMENT OF RELAPSE IN OLDER (>65Y) NON HODGKIN LYMPHOMA (NHL) PATIENTS: PRELIMINARY RESULTS OF A SINGLE CENTER STUDY

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In older low grade (Lg) and mantle cell (MCL) NHL patients, treatment acquired resistance and non neoplastic comorbidities represent the main issue to front up when disease progression/relapse occurs. Thus it is mandatory that treatment overcomes resistance with minimal toxicity. BE, a purine analogue related to alkylating agents, is shown to have a greater activity/toxicity and no cross-resistance with respect to cyclophosphamide. The synergistic association BE + R is coming out an effective schedule in adult Lg NHL and MCL; in 2008 we activated this single center study to test the feasibility, efficacy and safety of this schedule in the treatment of relapse in older heavily pre-treated Lg NHL patients. Treatment program consisted of R (375 mg/mq d.0) + BE (90 mg/mq, d1,2) + Steroids (40 mg t.d. day 1-5) given every 21 days for 6

cycles. Response was evaluated, by CT scan, early (after the 3rd cycle), then at the end of treatment. From February 2008 to February 2009, 11 - 5 males, 6 females, median age 76y (min.65-max.79)- patients entered in the study. Of these, 9 were lgNHL (4 LL/CLL, 2 FL, 2LPL and 1 Malt NHL) and 2 MCL; 4 cases had extranodal disease (brain + liquor, gastric mucosa, skin, retroperitoneum area, respectively), while in 8 comorbidities such as cardiopathy (4), hepatitis B (3), and severe diabetes(1)were present. All patients, on out-patients basis, received BE+R as >/=2 line treatment. As of April 2009, 4 patients are off-therapy, of the remaining 7 still on treatment, 3 completed the 3rd cycle, and 4 the 2nd one, respectively. Of 7 pts early (after 3rd cycle) evaluated, 2 achieved CR, 5 VGPR(>70%); while of the 4 who completed therapy, 2 maintained CR, and 2 VGPR, median response duration was 6 months(min.3-max 8 mo). Among these last cases, the patient (CLL, 76 y old) with CNS lymphoma achieved CR which lasts for 7 months, another one (MCL 71 y-old) underwent ASCT. As toxicity, only 1 pt had WHO >2 grade neutropenia, no pt discontinued treatment. These preliminary results show that BE+R is a feasible, active and safe approach in older heavily pretreated NHL with severe extranodal disease. If these data need to be confirmed in a larger series of patients with more prolonged follow-up, it has to outline this schedule is able to induce an early ORR >70%, furthermore it does not preclude patient from undergoing a successive intensive response consolidation such as autologous stem cell transplant

P014

INTENSIFIED CHOP (ICHOP) +/- RITUXIMAB IN PRIMARY MEDIASTINAL DIFFUSE LARGE B CELL LYMPHOMA (PMBCL): THE ROLE OF DOXORUBICIN/ CYCLOPHOSPHAMIDE DOSE-INTENSITY

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Background. PMBCL is a clinical/biological distinct entity, sharing some characteristics with both classical DLBCL and Hodgkin's lymphoma. MACOP B is considered the treatment of choice. *Aims.* To evaluate the role of doxorubicin/cyclophosphamide dose intensity in this setting of patients. *Methods.* Starting from 1997, we treated PMBCL with an ICHOP regimen including cyclophosphamide 1750 mg/mq with MESNA uroprotection, doxorubicin 75 mg/mq, vincristine 1.4 mg/mq with 2 mg cap, and prednisone 100 mg d 1-5 of each 14-day courses, GCSF from day 7 to day 12. Rituximab (R) 375mg/mq/course was added to ICHOP (R-ICHOP) from 2002. Treatment plan included five courses of ICHOP±R. Cases with unfavourable prognosis according to age-adjusted International Prognostic Index (aaIPI2-3) were submitted to high dose chemotherapy (HDT) and peripheral stem cell rescue. Radiotherapy on involved sites was then delivered to all patients if at least partial remission (PR) was reached. Clinical response was evaluated through CT +/- Gallium scan (14 pts) up to 2002, and thorough CT + PET scan (16 pts) thereafter, according to Cheson criteria. *Results*. up to 2006, 30 pts were treated, with the following characteristics: M/F 10/20, median age 34 years (range 22-53), Ann Arbor stage I: 4, II –IIE:19, III: 1, IV: 6; bulky disease: 29; B symptoms: 14; aa IPI 0-1: 24, 2-3: 6; RICHOP / ICHOP 21/9. After ICHOP±R 15 patients achieved complete (CR) or unconfirmed complete remission (CR-U), 14 PR, 1 stable disease. At the end of the whole program 29/30 pts reached CR and one progressed. Seven pts received HDT, six following ICHOP±R and one after II line chemotherapy for refractory disease. After a median observation time of 60 months 1 patient progressed and 1 patient relapsed, respectively. Both died of lymphoma. One patient with stage IIE IPI 0 relapsed 18 months after completion of ICHOP and RT and died after further 5 treatment lines including alloBMT. The other patient with stage II EB IPI 1, progressed shortly after R-ICHOP and RT and died five months later. Five-yr failure free survival and overall survival are 93.2 and 92.8, respectively. ICHOP±R was well tolerated, with neither toxic death or life-threatening toxicity. No patient interrupted the planned treatment because of toxicity. Hospitalization was required in seven cases due to febrile neutropenia (6), hemorrhagic cystitis (3 cases), and pneumonia (1). Five episodes of grade III-IV mucositis were observed in 4 patients. Of 147 delivered cycles, 25 were delayed (13 pts). *Conclusion.* In PMBCL, the results obtained with the ICHOP protocol are better than standard CHOP and comparable to MACOP-B, emphasizing the role of doxorubicin and cyclophosphamide dose-intensity. In this limited series, the impact of adding rituximab is not clear.

Table 1.

	R-ICHOP	*ICHOP*	*Total*
Patients (N°)	21	9	30
IPI 0-1	16	8	24
IPI 2-3	5	1	6
Response to CT (N°)			
Complete Remission	10	5	15
Partial Remission	11	3	14
Induction Failure	0	1*	1*
Response CT +RT+/- HDT (N°)			
Complete Remission	20	9	29
Partial Remission	0	0	0
Induction Failure	1	0	1
Relapse (N°)	0	1^	1^
5-yr FFP	95.2	88.9	93.2
5-yr OS	95.2	88.9	92.8
Median follow up (range)	52 months	104 months	60 months

** IPI 0; ^ IPI 1.*

P015

CONTRIBUTION OF FLOW CYTOMETRY (FC) TO THE DIAGNOSIS OF MALIGNANT AND NON MALIGNANT CONDITIONS IN LYMPH NODE BIOPSIES

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Lymph nodes are typically evaluated by histologic and immunohistochemical analysis. The use of FC has undoubtedly added an intriguing dimension to the diagnosis of hematologic neoplasms, particularly when applied for the diagnosis of haematological malignancies in peripheral blood and bone marrow. In order to assess the contribution of FC to the diagnosis of lymph node disorders we retrospectively compared the pathological and the FC diagnosis made in 107 consecutive lymph node biopsies. Pathological diagnosis included B-cell Non Hodgkin lymphoma (NHL) (n=55, 51%), Hodgkin lymphoma (HL) (n=15, 14%) and non-hematological disease (n=37, 35%). B-cell lineage was detected by determination of K e L light-chain, sIgG, sIgA, sIgM, CD10, CD5, CD23, FMC7, CD20, CD30, CD22 differently combined with CD19. Other primary antibodies used were CD45, CD3, CD4, CD8, CD25. The FC histograms were reviewed by investigators who were blinded to the histological diagnosis. In 78 of 107 cases K/L ratio resulted evaluable and monoclonal light chain restriction was defined by a K:L ratio included in the range > 3:1 or < 0.3:1. Light-chain determination showed monoclonal restriction in 23/26 low-grade, 11/17 high-grade NHL, 0/7 LH and 5/28 non-neoplastic cases. The diagnostic potential of monoclonal light chain restriction to predict the diagnosis of NHL (low grade NHL+high grade NHL versus non-neoplastic+ HL) was as follows: sensitivity 79%, specificity 85%, positive predictive value 87%, negative predictive value 77%, accuracy 82%. Kappa statistic revealed that the agreement between diagnosis of NHL and monoclonal light chain restriction was of moderate degree (K=0.64, p<0.001). A significantly higher number of CD3-positive were accounted among HL cases (HL=60+23 SD versus non-neoplastic=51+20 versus high-grade NHL=40+25 versus low-grade NHL=36+24, p=0.006) associated with a significantly reduced number of CD19-positive cells (HL=18+15 SD versus non-neoplastic=25+17 versus high-grade NHL=36+26 versus low-grade NHL=48+25, p=0.006) cells were accounted among HL cases. In conclusion, this study confirms that FC performed on fresh lymph node samples is a useful diagnostic tool in patients with NHL considering that few cases left undiagnosed by monoclonal restriction of light chain. Although the potential of FC to recognize HL is low, FC immunophenotype of T lymphocytes seems to have a potential role as diagnostic tool in these patients.

P016

QUALITATIVE AND QUANTITATIVE ASSESSMENT OF BONE MARROW INVOLVEMENT IN THE ENTIRE SPECTRUM OF NON-HODGKIN'S LYMPHOMAS: COMPARISON BETWEEN HISTOLOGY AND FLOW CYTOMETRY IN 572 PATIENTS

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Bone marrow (BM) examination is essential in the staging of non-Hodgkin's lymphoma (NHL) pts. Few studies have compared BM histologic findings with results of flow cytometric (FC) analysis and detailed data about correlation of the results of these two methods in large series comprising different NHLs are lacking. We analysed the incidence and patterns of histologic BM involvement in a series of 822 pts with NHL diagnosed at Division of Hematology of Pavia from 1998 to 2008. For 572 pts a concurrent FC analysis on BM was available and data were compared. We studied BM biopsies of 253 pts with follicular lymphoma (FL), 220 diffuse large B-cell lymphoma (DLBCL), 82 splenic MZL (SMZL), 69 extranodal marginal zone lymphoma of MALT, 23 nodal MZL, 30 lymphoplasmacytic lymphoma (LPL), 33 mantle cell lymphoma, 29 small lymphocytic lymphoma (SLL), 11 primary cutaneous follicle center lymphoma, 11 peripheral T-cell lymphoma, 16 cutaneous T-cell lymphoma and 72 B-cell chronic lymphoproliferative disorders (B-CLPD). Overall, histologic BM involvement was detected at diagnosis in 373/822 (45%) pts. By FC analysis, BM involvement was detected in 207/572 (36%) available samples. A concordance between 2 methods (Spearman's R=0,7, p<0.001) was detected in 459 (85%) cases (33% BM+/FC+; 52% BM-FC-) and a discordance was present in 82 (15%); 64 cases (12%) were BM+/FC- and 18 (3%) BM-/FC+ (with a small clonal population at FC; median 9%). Discordance was more frequent in FL (19% BM+/FC- and 3% BM-/FC+) and in LPL (18% BM+/FC- and 6% BM-/FC+), as summarized in Table 1. The rate of false negative FC exams resulted inversely related to the extent of histologic infiltrate for the whole series (p<0.001) and, specifically, for FL (p=0.01), LPL (p=0.04) and B-CLPD (p=0.03). Patterns were analyzed in 316 BM biopsies. Nodular paratrabecular pattern was associated with FL, sinusoidal with SMZL, interstitial with SLL, diffuse with DLBCL (all p<0.001) and with LPL (p=0.02). FC better overlapped with histology in case of interstitial (p<0.001), sinusoidal (p=0.03) and nodular centrolacunar pattern (p=0.01). False negative FC results correlated inversely with percentage of $CD19^+$ cells (p=0.04). The quantitative assessment of infiltrate detected by the 2 methods resulted significantly overlapping (p<0.001). Our data demonstrate that FC is comparable with histology in qualitative and quantitative assessment of BM involvement for the entire spectrum of NHL, with the exception of FL and LPL

Table 1. Comparison between histological and flow citometry findings.

P017

DOSE-DENSE THERAPY WITH NON-PEGYLATED LIPOSOMAL DOXORUBICIN, CYCLOPHOSPHAMIDE, VINCRISTINE, PREDNISONE AND RITUXIMAB (R-COMP) IS FEASIBLE AND EFFECTIVE IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED AGGRESSIVE B-CELL NON-HODGKIN LYMPHOMA: R-COMP 14 VS R-COMP 21. INTERIM ANALYSIS FROM AN ITALIAN PROSPECTIC MULTICENTER TRIAL

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The toxicity and efficacy of nonpegylated liposomal doxorubicin (MyocetTM) when substituted for conventional doxorubicin in the CHOP 14 or 21 regimen (Doxorubicin, Cyclophosphamide, Vincristine, Prednisone given every 2 or 3 weeks) were evaluated in the treatment of elderly patients with newly diagnosed aggressive B-cell non-Hodgkin's lymphoma. Forty-eight patients with aggressive B cell non-Hodgkin lymphoma at diagnosis were enrolled so far in the study. Patients were split in 2 groups according to the Multidimensional Geriatric Assessment (MDGA). Patients with an Activities of Daily Living (ADL) = 6 were addressed to receive dose-dense R-COMP every 2 weeks (14), whereas patients with an ADL >7 were addressed to receive R-COMP every 3 weeks (21). Starting from this background, 30 patients were enrolled in the R-COMP 21 arm and 18 in the R-COMP 14 arm. The study was planned as a double phase II according to a single-step Fleming design using 3 years event-free survival as primary endpoint. The characteristics of patients enrolled in the study were as follows: the median age was 72 years (range: 67-80) in the R-COMP 14 group and 75 years (range: 66-89) in the R-COMP 21. At baseline 13/18 (72%) patients had stage IV disease in the R-COMP 14 group, whereas 13/30 (43%)in the R-COMP 21. Median performance status and median number of comorbities were comparable between the 2 groups. Thirteen out of 18 (72%) patients had an intermediate or high risk International Prognostic Index score in the R-COMP 14 group, compared to 12/30 (40%) in the R-COMP 21. The median left ventricular ejection fraction (LVEF) before starting chemotherapy was comparable between the two groups (59% vs 60%). A total of 261 cycles of chemotherapy were administered (96 R-COMP 14 and 165 R-COMP 21). Of the cycles administered, 9 (9%) were delayed by haematological toxicity in the R-COMP 14 group and 11 (7%) in the R-COMP 21 group, with a relative dose intensity for the regimens of 91% and 93%, respectively. Toxicity was mainly haematological in both groups. Grade 3/4 neutropenia occurred in 10% and 24% of cycles in the R-COMP 14 and 21 groups respectively, with an incidence of febrile neutropenia of 3% and 5% respectively. It is of note that patients addressed to receive dose-dense chemotherapy were treated with pegfilgrastim on day +2 during the entire study treatment, with a notable reduction in the incidence of both severe and febrile neutropenia. Regarding cardiotoxicity, only 1/18 patients presented a grade II-IV WHO toxicity (atrial fibrillation) in the R-COMP 14 group, whereas 4/30 in the R-COMP 21 group (one congestive heart failure, two ischemic heart failure, one reduction of 20% in the LVEF). All patients were evaluable for response between the two groups. In the R-COMP 14 group, 15/18 patients (83%) obtained a CR, 2/18 (11%) achieved a PR, and 1/18 (6%) did not respond to therapy and rapidly died due to progressive disease. In the R-COMP 21 group, 20/30 patients (67%) obtained a CR, 7/30 (23%) achieved a PR, and 3/30 (10%) did not respond to therapy and rapidly died due to progressive disease. With a median follow-up of 7 months (range 2-12) and 10 (range 4-24) as of January 2009, 15/18 patients (83%) and 20/30 (67%) are alive and disease free in the R-COMP 14 and in the R-COMP 21 group, respectively. In conclusion, the stratification of patients according to the MDGA allows elderly and fit patients with aggressive B-cell NHL with poor prognosis (high IPI score) to receive dose dense chemotherapy which might favorably impact on response rate and survival. The increased response rate obtained with dose-dense R-COMP 14 might impact on long-term event-free survival. A longer follow-up is warranted to better define the impact of dose-dense R-COMP regimen on overall survival of patients with high-intermediate or high IPI score.

P018

TREATMENT, PROGNOSTIC FACTORS AND BIOLOGICAL CHARACTERISTICS OF PRIMARY THYROID B-CELL LYMPHOMAS (PTL): AN IELSG AND IIL STUDY

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PTL are a heterogeneous group of distinct histopathologic entities, whose treatment, prognostic factors and biological characteristics are still matter of research. The aim of this study was to clarify some unsolved aspects of this rare disease. From 1986 to 2006 11 international centers recruited 55 pts with diagnosis of PTL. The prevailing histologic subtype was DLBCL (n=49), whereas only 6 cases had low grade NHL (5 MALT, 1FL). Therefore, the present analysis regarded only the DLBCL-subgroup. The median age at diagnosis was 72 yrs (range 34-90) and M/F were 0.29. 67% had a stage-modified IPI ≥2, mostly due to stage II (51%) and age >60 (75%), whereas only few cases had a poor ECOG-PS (32%), elevated LDH (51%) and >1 extranodal site apart thyroid (10%). Bulky disease (≥10 cm) was present in 28% and pre-existing Hashimoto's thyroiditis (HT) in 33%. 69% of the pts received an anthracyclin containing chemotherapy combined with RT and/or surgery (SX), while the remaining cases were given only CHT (24%), and SX or RT (7%). The CR rate for these 3 subgroups were 90%, 77% and 75%, respectively. Among 42 responders, 5 eventually relapsed (12%), 3 locally and 2 in different sites. After a median follow-up of 3 yrs (range, 0.6-13 yrs), 5-year estimate of OS was 51%, DFS 86% and PFS 46%, respectively. Disease and treatment related mortality caused 9 deaths (18%) and lymphoma-specific survival (LSS) was 86%. By univariate analysis advanced age and poor ECOG-PS negatively influence OS and PFS (p<0.05), while Cox regression analysis performed on the pts >60 yrs (n=37) failed to identify an independent risk factor for OS, PFS and LSS. A previous diagnosis of HT seemed not to influence survival (p=0.1). The analysis of somatic hypermutation of IGHV genes of 17 pts revealed that 76% of the cases were mutated (frequency of mutation >2%). Furthermore 2 cases had a significant clustering of S and R mutations in CDRs and FRs. In conclusion, we confirmed that DLBCL-PTL is a disease of elderly women. These patients benefit of an anthracyclin containing regimen, combined with RT and/or surgery and have a comparable outcome to that of the nodal localized counterpart. However, the usual prognostic factors, including stage-modified IPI failed to predict survival. The analysis of the IgVH-genes suggests that PTL-DLBCL derive from germinal center experienced B-cells and that antigen stimulation may have an important role in the pathogenesis of these NHL.

P019

BENDAMUSTIN IN ASSOCIATION WITH RITUXIMAB (BR)IN THE TREATMENT OF PATIENS WITH RELAPSED/REFRACTORY LYMPHOID NEOPLASM. A GISL RETROSPECTIVE STUDY

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Bendamustine (B) is a purine analogue/alkylator hybrid agent with a unique mechanism of action and in vitro studies have demonstrated synergistic pro-apoptotic effects of bendamustine and the CD20 antibody rituximab (R). The association BR has shown good clinical efficacy and acceptable tolerability in various haematological malignancies in both phase-II and phase-III trials. *Aim.* To asses efficacy and toxicity of the association BR in heavily pre-treated pts with mature B-cell neoplasms in a retrospective muticentre study. Sixty-five patients (pts) with a median number of 3 pre-treatments (1-9) and median age of 67 years (range 26-84). The diagnosis were as follows: Chronic Lymphocytic Leukemia (CLL=32); Mantle cell lymphoma (MCL n=8); Follicular Lymphoma (FL=9); Diffuse large B-cell lymphoma (DLCL=4); Marginal Lymphoma (=3); Splenic Marginal Zone Lymphoma (=3), Lymphoplasmacytoid Lymphoma (n=3), indolent non Hodgkin Lymphoma (n=3). Eight per cent of pts had received anthracyclin and or rituximab containing regimens, 50% Fludarabine and 20% autologous PBSCT. At study entry 30 cases were relapsed disease, 29 progressive disease, 4 resistant relapses and 6 primary refractory. Sixty-three (92%) pts received bendamustine 80-120 mg/m^2 at day 1+2, in combination with rituximab 375 mg/m^2 at day 1 of each cycle given every 21 or 28 days. Forty-seven cases were evaluable for response and toxicity, the others were still on treatment at the time of this analysis. The median number of BR cycles was 5 (range 1-8). Fifteen pts (32%) achieved a CR and 17 (36%) a PR (ORR=68%). The OR and CR rates were quite homogeneously represented in all three major histological groups (CLL:70%; MCL:60%; FL:71%); Interestingly, 3 out five MCL achieved a CR. At a median observation time of 8 months (range 2-35), 66% of pts were alive. Causes of death were as follows: Progression 11, cardiovascular 2, encephalitis 1 and second cancer 2. Toxicity was mild and mostly haematological: grade 3-4 neutropenia, thrombocytopenia, anemia and lymphopenia were recorded respectively in 34%, 17%, 6% and 32% of cases. Our experience compare favourably with that reported in the literature and shows that the association BR has a favourable toxicity profile and is highly effective in the salvage treatment of heavily pre-treated patients with low-grade lymphoproliferative disorders. The promising results obtained in MCL warrant to be explored in a larger series of cases.

P020

ASSESSMENT OF METABOLIC ACTIVITY BY PET-CT WITH F-18-FDG IN PATIENTS WITH T-CELL LYMPHOMA

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Aim. 18F-fluorodeoxyglucose positron emission tomography-computed tomography (PET/CT) is highly sensitive and specific in the imaging of B-cell lymphomas. In contrast, its utility in the diagnostic evaluation of T-cell lymphomas (TL) is less defined. We evaluated the metabolic activity by PET in patients scheduled for chemotherapic treatment and compared the results with those of patients presenting low- and high-grade B-cell non-Hodgkin lymphoma (B-NHL). *Material and Methods*. 20 consecutive patients (7 women; 54.9±16.5 y) with TL underwent a comprehensive clinical, instrumental and haematological evaluation as well as PET/CT prior to receive first line therapies. Diagnosis was made according World Health Organization (WHO) classification of lymphomas. The number and dimensions of involved nodal and extra-nodal sites was evaluated at PET/CT as per tumoral patient burden. The analysis by maximum standardized uptake value (SUVmax) was carried out according to European Organization for Research and Treatment of Cancer recommendations. PET/CT results were then compared with those obtained from 20 age- and sex-matched patients (7 women; 53.6±13.9 y) with low-grade B-NHL and 20 patients (7 women; 54.1±14.5 y) presenting extra-nodal high-grade B-NHL. *Results*. a mean number of 3.8±2 and of 4.7±2 was involved in the group with TL and with low-grade B-NHL, respectively (p=ns) whereas extra-nodal high-grade B-NHL had 1.5±0.4 sites concerned. The mean SUVmax of patients with TL was lower as compared to both low-grade and extra-nodal B-NHL (6.1±4 vs 9.1±6, and vs 8.9±5, respectively; p<0.01). When the SUVmax was assessed only in extra-nodal locations TL group still presented lower values than both other groups (5.3±3 vs 9.5±6, and vs 8.9±5, respectively;

$p<0.05$). SUVmax in nodal locations of patients with TL was lower than in nodal low-grade B-NHL (6.4±3 vs 8.8±6; $p<0.05$). *Conclusion.* Although aggressive clinical behaviour, TL have a low metabolic activity at PET/CT scan in patients with a significant tumour burden. These patients had a lower uptake index in the involved regions than both age- and sex-matched patients presenting low-grade and high-grade B-NHL. Nevertheless, all TL patients had a positive PET scan.

P021

MODIFICATION OF CD20 ANTIGEN EXPRESSION IN PATIENTS WITH INDOLENT CD20+ LYMPHOPROLIFERATIVE DISORDERS AFTER RITUXIMAB THERAPY

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Disappearance of CD20 expression may be observed in some patients with CD20+ lymphoproliferative disorders (LPD) after exposure to Rituximab. This phenomenon is better known in patients with high grade disease, while available data in subjects with indolent LPD (such as B-LLC/SLL, where CD20 expression is characteristically less evident), are quite scanty. With the purpose to evaluate the frequency and the clinical behaviour of the CD20 disappearance on neoplastic cells in CD20+ positive, non aggressive LPD treated with Rituximab, a flow cytometric analysis was performed in 22 consecutive patients seen at our Institution during a one-year period and affected by CD20+ indolent LPD (7 B-CLL, 4 SLL, 4 FL, 3 MCL, 1 MZL, 3 unspecified) with bone marrow and/or peripheral blood involvement. Three out of these patients (13.6%) evidenced the lack of CD20 expression on neoplastic cells after Rituximab. The first patient was a 67 year-old male with SLL, previously treated with chlorambucil and fludarabine. At progression, bone marrow analysis revealed the presence (42%) of a clonal population of CD19+, CD20+, CD5+, CD23+, lambda+ lymphocytes. Six monthly cycles with a R-CHOP-like regimen induced a transient partial response, with marrow persistence (25%) of CD20-, CD19+, CD5+, CD23+, clonal cells, despite subsequent treatments with alemtuzumab and bendamustine. At the time of the last follow up, 13 months after the first dose of Rituximab, marrow neoplastic lymphocytes were 20%, showing, however, re-expression of CD20. The second patient was a 74-year old woman diagnosed in another hospital as having CD20+, CD5+, CD23+, lambda+ SLL. Prior therapies included chorambucil and five R-Flu-Cy cycles. When admitted at our Institution, CT, PET and a new lymph-node biopsy documented a transformation toward a high grade, CD20+ DLBCL (Richter's syndrome). In this circumstance, however, flow cytometry analysis on bone marrow aspirate revealed 50% of "B-CLL-like", CD20- neoplastic cells. The conditions of the patient rapidly worsened and she died soon after. The last patient was a man of 68 years, with CD20+, CD19+, CD5+, CD23+, lambda+ B-CLL, initially treated with Flu-Cy. About three years later the disease progressed with the same phenotype. Six cycles of Chlorambucil + Rituximab were given, achieving a good remission, with minimal (5%) bone marrow infiltration by CD20, CD19+, CD5+, CD23+, CD52+ clonal cells. A consolidation treatment with alemtuzumab was interrupted because of a CMV reactivation. At last restaging (2 months later), flow cytometry analysis was still positive for a few CD19+ CD23+ CD5+, but also CD20+ neoplastic cells. Actually, the process of CD20- LPD relapsing (or resistant) after treatment with Rituximab is still unclear and could have various explanations: internalization, masking,(epigenetic®) down-modulation of CD20 or selection of CD20- clones. Whatever the cause, the three cases we show here suggest that the lack of the CD20 surface expression after Rituximab therapy is a not rare, generally transient (though of variable duration: 5 to 13 months in our experience) and clinically heterogeneous phenomenon in indolent LPD. Careful monitoring of CD20 expression in these patients may be relevant for their appropriate management.

Acute Leukemias I

P022

FLAIE (FLUDARABINE, CYTARABINE, IDARUBICIN AND ETOPOSIDE) INDUCTION CHEMOTHERAPY FOR ADULT ACUTE MYELOID LEUKEMIA. RESULTS OF A PILOT STUDY

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Background. Standard induction chemotherapy for acute myeloid leukemia (AML) is based on the association of cytarabine (Ara-C) and an anthracycline (daunorubicin or idarubicin). Several studies have shown the efficacy and low toxicity of the addition of fludarabine to conventional two-drug induction regimen. Conversely, the inclusion of etoposide to the Ara-C + antracycline schema has led to conflicting results, and no data are available about the inclusion of etoposide in a fludarabine-based induction therapy. *Aims and Methods.* In this phase II trial we tested the efficacy and safety of a Four-drug Induction Regimen with fludarabine, Ara-C, idarubicine and etoposide (FLAIE) in previously untreated AML patients younger than 65 years. Fifty consecutive patients with a diagnosis of *de novo* AML were included in this study, between January 2003 and December 2005. Median age was 51 years (range: 21-63). Overall, 38 patients (76%) were at high risk for the presence of one or more of the following: secondary AML, unfavourable cytogenetics, blast count > $30x10^9$/L, over-expression of P-Glycoprotein. FLAIE regimen consisted of fludarabine (25 mg/sqm/d, days 1-5), Ara-C (2 g/sqm/d, days 1-5), etoposide (100 mg/sqm/d, days 1-5) and idarubicin (6 mg/sqm/d, days 1, 3 and 5). After induction therapy, all patients received two consolidation courses with Ara-C and idarubicin. *Results*. After induction with FLAIE, Complete Remission (CR) occurred in 63% of patients (31 of 49 evaluable cases); three patients (6%) achieved Partial Remission and 15 patients (31%) were resistant. There were one cases of death during induction (DDI 2%). The hematological and extrahematological toxicity of FLAIE was acceptable. Infections occurred in 29/50 (58%) of patients including 18 episodes of bacteremia and 15 cases of pneumonia. Oral mucositis grade II-III WHO was reported in 11/50 (22%) of patients. Median time to neutrophil (>$1x10^9$/L) and platelet (>$50x10^9$/L) recovery was 24 (range 18-42) and 26 days (range 20-45), respectively. Supportive treatment: 13 RBC units (range 10-28) and 9 PLT units (range 5-15). G-CSF was required in 22/50 (44%) of cases. After a median follow-up of 23 months (range 1-68), 19/50 (38%) patients are alive (19/19 in CR). The probability of 1-year OS and DFS were 68 % and 48%. Allogeneic and autologus HSCT was performed in 35 (70%) and 4 (8%) of patients, respectively. *Conclusions*. The FLAIE regimen appeared to have acceptable toxicity but compared to our previous experiences with the induction regimen FLAI (Russo *et al.,* 2001 and 2005), the addition of etoposide did not affected significantly neither the CR rate nor the long-term survival of AML patients. Besides the efficacy of FLAI was not greater than that of standard induction regimens.

P023

PEGYLATED RECOMBINANT FILGRASTIM (PEG-FILGRASTIM) AFTER CONSOLIDATION CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION

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Introduction. Clinical trials are currently expanding the clinical experience with Peg-filgrastim in a variety of solid tumors and hematologic malignancies. Some previous reports showed that Peg-filgrastim was as effective as daily Filgrastim after induction chemotherapy in AML in order to reduce time to recovery from granulocytopenia. Scarce data are available about the role of Pegfilgrastim after consolidation chemotherapy in AML in first complete remission (CR). *Patients and Results.* A total of 44 consecutive AML pts (23M/21F) in first CR (median age 52 yrs, range 25-71) received Peg-filgrastim (6 mg single dose, subcutaneously) 24 hours after completion of consolidation chemotherapy that consisted of cytosine arabinoside 2 g/m^2 for 6 days, plus idarubicin 12 mg/m^2

day 1-3 (35/44), or cytosine arabinoside alone 3 g/m^2 twice day for 4 days (9/44). This is a setting of pts with an expected prolonged and severe neutropenia and all of 44 cases have previously received the same intensive induction therapy with fludarabine containing regimen (FLAI). All 44 pts, as expected, experienced grade IV WHO neutropenia after consolidation chemotherapy. Median time to PMN recovery (first of 2 consecutive days with PMN > 0,5x10^9/L and with PMN > 1x10^9/L) from Pegfilgrastim was 16 and 17 days, respectively. The mean peak value of PMN was 7,2±6,3x10^9/L and occurred after a median of 22 (range 10-43) days from Peg-filgrastim injection. Eighteen of 44 pts (41%) experienced infectious complications during the aplastic phase (3 pneumonias, 13 bacteremias, 1 cystitis and 1 skin abscess) but no deaths infection related were reported. Peg-filgrastim was well tolerated and only 1/44 pts required pain-control medications (osteoalgia). In 40% of cases, without PMN >1x10^9/L after 18 days from Pegfilgrastim, Filgrastim (mean 3 fl/pts) were administered in order to accelerate PMN recovery. In order to collect PBSC, daily evaluation of CD34$^+$ cells was performed in 25 pts and 12 of them (48%) harvested a mean of 4,6±3,2x10^6/Kg CD34$^+$ cells, after a mean time from Peg-filgrastim of 15±6 days. *Conclusions.* These data suggest that Peg-filgrastim: 1) was well tolerated after consolidation chemotherapy in AML in first CR; 2) represent a cost-effective alternative to long–term conventional Filgrastim to overcome severe and prolonged granulocytopenia after consolidation chemotherapy in AML; 3) could permit simplification of PBSC mobilization procedures and harvest. In our experience 48% (12/25) of pts who needed stem cell collection, obtained CD34$^+$ cells mobilization and harvest. This is not a negative result taking into account the underling disease (AML) and the previous induction with fludarabine based regimen that is well know to severly impair PBSC mobilization.

P024

FLT3-ITD ABNORMALITIES AND COMORBIDITIES AS INDEPENDENT PROGNOSTIC INDICATORS OF SURVIVAL IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS

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A dismal prognosis was reported in elderly AML patients due to biological features of disease in itself and to presence of comorbidities. Aim of this study was to evaluate the prognostic impact of comorbidity prognostic score systems applied to our population of patients, as well as of other clinical-biological features. We retrospectively considered the outcome of 120 patients aged > 65 years diagnosed as having AML between January 2001 and December 2005. Comorbidities were evaluated by using Charlson comorbidity index (CCI), Hematopoietic cell transplantation comorbidity index (HCTCI) and a score proposed by Dombret *et al.* in 2007. Median patient age was 67 years. Forty-six patients were treated with intensive chemotherapy and 23 (50%) reached a complete remission. Seventy-four patients received only supportive therapies or low-dose chemotherapy. Multivariate analysis showed the effects of leukocytosis (p=0.0013), antecedent MDS (p=0.011), FLT3 abnormalities (p=0.032), CCI (p=0.0037) and Dombret et al score (p=0.045) as independent prognostic parameters for survival. Based on these variables we were able to stratify patients in low and high risk, with different median overall survival: patients were considered as low risk if they had none or only one of the above mentioned adverse factors, with a median overall survival of 447 days. Patients with two or more adverse factors were categorized as high risk: this subgroup had a median overall survival of 227 days (p=0.001). Comorbidities are independent factors that influence survival. Application of CCI and Dombret et al score may help to better identify patients at diagnosis who can benefit from intensive chemotherapy.

P025

PHASE II STUDY OF BORTEZOMIB AS A SINGLE AGENT IN PATIENTS WITH RELAPSED/REFRACTORY OR DE NOVO ACUTE MYELOID LEUKEMIA UNFIT FOR INTENSIVE THERAPY

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In spite of significant therapeutic improvements over the last 15-20 years, approximately 20% of adult patients with acute myeloid leukemia (AML) is refractory and up to 60-70% relapses following front-line chemotherapy. Refractory/relapsed AML has a very unfavorable prognosis and novel agents are needed to improve outcome for these patients. Bortezomib is a potent inhibitor of the 26S proteasome which has been found to inhibit AML blast survival and interfere with the interaction between AML progenitors and microenviromental niche. The present study was designed to explore the safety and efficacy of Bortezomib given as single agent at the unconventional dose of 1.5 mg/m^2, to patients with AML, unfit for intensive chemotherapy or with relapsed/refractory disease, after previous lines of therapy. Thirteen patients have been included in the study, being 10 evaluable. Nine males and 4 females, median age 70 (range 60-78), 4 and 1 cases were de novo and secondary AML, respectively. Six and 2 patients had refractory and relapsed disease, respectively. Bortezomib 1.5 mg/m^2 was given on day 1,4,8 and 11 of each 21 day cycle, for a maximum of 8 cycles. Bone marrow samples were collected at baseline and, for response evaluation, on day 1 of each cycle. Three of 13 patients died early due to pulmonary infection. Among the remainder, we observed 5 haematological improvement (HI) defined as a reduction of peripheral blast count <25% as compared to baseline and a Hb level increase by 2 gr/dL. One patient had a stable disease lasting for 3 months, the remaining 4 patients had a rapid progressive disease. Median number of delivered cycles until progression was 3, for patients with HI whereas patients with progression were able to receive no more than 1 course of bortezomib. Median overall survival of patients with HI/stable disease was 4 months (range 3-8) versus 2 (range 1-2) of those with progression. Of 10 evaluable patients, 6 developed peripheral neuropathy which was NCI-CTC grade 4 in 2 (neurological bladder, requiring permanent catheterization in 1 instance) and grade 1-2 in 4. In the last, neuropathy resolved promptly after bortezomib discontinuation. In conclusion, bortezomib does have anti-leukemic activity and future clinical trials should focus on its combination with conventional chemotherapy. In this context, a dose reduction would be desirable due to the neurologic complications observed in our study.

P026

GEMTUZUMAB OZOGAMICIN IN COMBINATION WITH INTENSIVE CHEMOTHERAPY FOR RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

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Gemtuzumab Ozogamicin (GO) has moderate activity as a single agent in patients with CD33-positive refractory or relapsed acute myeloid leukemia (AML). We analyzed the safety and efficacy of GO combined with salvage therapy in the treatment of 25 patients with refractory (n=8) or relapsed (n=17) AML. Salvage regimens associated with GO were: ARA-C(100 mg/m^2/day, day 1-7) plus daunorubicine (50 mg/m^2/day, day 1, 3, 5) (3+7 regimen) or ARA-C(2000 mg/m^2/day, day 1-5) plus fludarabine (30 mg/m^2/day, day 1-5) (FLA regimen). GO at a dose of 6 mg/m^2 as a single intravenous infusion was added on day 1, in 3+7 regimen and on day 6, in the FLA regimen. Median age was 58 years (range 25-70), 18 males and 7 females. CD33 expression exceeded 20% in 88% of cases (range 25-90%). Four patients (16%) had a secondary AML. Cytogenetic, classified according to the SWOG criteria, was successful in 19/25 patients and was unfavourable in 6 (24%), intermediate in 13 (52%). Patients were evaluated for response rate, treatment-related adverse events, overall survival and relapse free survival. Tolerability was assessed using the National Cancer Institute Common Toxicity Criteria Version 3.0. Eight (32%) patients achieved a complete remission (3+7+GO=2, FLA+GO=6), 1 (4%) a partial remission (FLA+GO=1) for an overall response rate (CR+PR) of 36%. Fifteen (60%) were resistant and there was only one case of induction death due to sepsis. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was performed in 5 (62%) of 8 achieving CR, the remaining 3 were not transplanted because of age ≥ 65 yrs. Toxicity was acceptable; no cases of sinusoidal occlusive syndrome (SOS) occurred during chemotherapy whereas 1 instance of SOS was observed after allo-HSCT, this was a young female with a history of previous multiple treatments. The most common adverse event was myelosuppression (universal); all patients developed grade 3, 4 or, in 1 instance grade 5, infection toxicity. Other grade 2-3

non-hematologic toxicities included oral mucositis and diarrhea. No treatment–related cardiotoxicity or cerebellar toxicity was observed. Median overall survival duration was 4 months (range 1-30); at 10 months, the survival rate was 32%. Median relapse free survival duration was 8 months (range 4.5-29). In conclusion, our data support the use of GO in combination with conventional salvage chemotherapy in this very high risk category of patients and suggest that, for responders, allo-HSCT is feasible.

P027

A SIMPLE PROGNOSTIC SCORING SYSTEM FOR NEWLY DIAGNOSED ACUTE LEUKAEMIA PATIENTS WITH NORMAL KARYOTYPE: A RETROSPECTIVE ANALYSIS ON 420 CASES

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Background. Cytogenetics is the most important prognostic factor for acute myeloid leukaemia (AML), enabling to categorize AML patients in three risk categories: favourable, intermediate and unfavourable. The post-induction therapeutic strategy, including or not allogeneic-SCT, is not well established in patients with NK, who account for at least the 40-50% of cases and show an extremely variable long term survival, ranging from 20-70%. *Aims*. The aim of the study is to provide a simple prognostic scoring system, to better define the disease-risk in AML patients with NK and to optimally address the issue of post-induction therapy. *Methods.* We retrospectively analyzed 420 AML patients with NK (NK-AML) consecutively treated from 1990 to 2005. Induction regimen was Fludarabine-based in 281 patients (67%) and ICE/DCE in 139 cases. After the first consolidation, patients were addressed to intensification therapy including allogeneic-SCT if aged less then 45 yrs, with a HLA compatible donor and at least one other high risk feature (WBC over $30x10^9$/L, secondary AML, non response to the first induction therapy, multidrug-resistance Pgp over-expression and presence of Flt3-ITD). An allogeneic-SCT was performed in 71/418 cases (17%). By univariate analysis age at least 55 yrs, Pgp positive phenotype and secondary AML significantly affected the CR rate. Moreover, age at least 55 yrs, FAB subtype, secondary AML, WBC count over $16x10^9$/L and no response to the first induction cycle significantly affected disease free survival (DFS) and overall survival (OS). By multivariate analysis, only Pgp over-expression significantly affected the CR rate, whereas age at least 55 yrs, WBC count aver $16x10^9$/L, secondary AML and no response to induction significantly affected the DFS and OS. Univariate and multivariate analysis were conducted using the logistic regression model for CR rates and the Cox proportional hazard model for survival. A numerical score was derived from the regression coefficients of each independent prognostic variable and was 1 for WBC count over $16x10^9$/L, age at least 55 yrs and secondary AML and 2 for no response to the first induction regimen. The prognostic score for each patient was then calculated by totalling up the score of each independent variable. *Results.* The allo-transplanted patients were censored at the time of SCT. Patients could be stratified in low (score = 0), intermediate (score = 1) and high risk group (score at least 2), with a median DFS of 115, 11 and 8, respectively ($p<0.0001$). Similarly, the median OS was 120, 24 and 8 in low, intermediate and high risk group, respectively ($p=0.0001$). *Conclusions*. These preliminary retrospective data suggest that common available clinical variables may still represent a valid approach for determining a prognostic stratification for NK-AML patients.

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P028

P2 RECEPTORS ARE EXPRESSED ON ACUTE MYELOBLASTIC LEUKEMIA CELLS AND THEIR STIMULATION MODULATES LEUKEMIA CELLS FUNCTION

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Extracellular nucleotides ATP and UTP are emerging as ubiquitous molecules involved in a wide variety of biological responses and their biological effects are mediated by specific plasma membrane receptors, P2 receptors (P2R). Previously, we showed that extracellular nucleotides stimulate the proliferation and engraftment potential of normal human hematopoietic stem cells. In this study, we assessed whether P2R are expressed on acute myeloblastic leukemia (AML) cells and whether their engagement modulates leukemic cell functions. By RT-PCR we found in AML the mRNA expression of P2X1, P2X3, P2X4, P2X5, P2X6, P2X7, P2Y1, P2Y2, P2Y4 receptors. The expression of P2X7, P2X4, P2Y1 was confirmed at the protein level. Stimulation of AML cells by extracellular nucleotides (ATP, UTP, BzATP) induced intracellular Ca^{2+} concentration increases. Furthermore we identified a number of genes significantly modulated by ATP treatment. Gene expression profiling revealed that leukemic cells stimulated with ATP underwent a down-regulation of genes involved in cell proliferation and migration whereas those involved in cell cycle inhibition were strongly up-regulated. At the functional level, the clonogenic efficiency of leukemic blasts was significantly inhibited by the addition of ATP and, to a higher extent, by the stable analogs INS415 and INS973. We also observed a pronounced inhibitory effect of triphosphate nucleotides on blast spontaneous migration and in response to CXCL12. To assess the activity of nucleotides on AML cell migration in vivo, NOD/SCID/Gamma-Null mice were sublethally irradiated and intravenously injected with human AML cells incubated with nucleotides or their analogues. Xenotransplant experiments demonstrated that the homing and the engraftment capacity of human AML cells to murine bone marrow was significantly inhibited by pre-treatment with ATP, UTP and INS415 and INS973 analogues. Thus, our data show that purinergic signaling modulates leukemic cells in a opposite way than normal cells. Characterization of P2R expression and function in leukemia may help the better understading of the mechanism of neoplastic transformation and tumor progression.

P029

WILMS' TUMOR 1 (WT1) MUTATIONS IN NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA

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Introduction. Acute myeloid leukemia (AML) is a heterogeneous disease characterized by different recurrent chromosomal aberrations that determine the current risk-group classification. In adult AML approximately 40-50% of cases at diagnosis cannot be characterized by karyotypic aberrations. Several molecular aberrations have been identified in this subgroup, such as internal tandem duplications of the FLT3 gene (FLT3/ITD), mutations in NPM1 and CEBPα. The WT1 gene is known to be overexpressed in myeloid leukemias, and is therefore utilized as a marker for minimal residual disease detection. The gene encodes for a zinc-finger transcription factor involved in the regulation of growth and differentiation. Recently WT1 mutations have been identified in ~10% of adult AML with normal karyotype (NK-AML), and their association with unfavourable prognosis is controversial. To determine the role of WT1 aberrations in our patients , we searched for these aberrations in a well-characterized cohort of adult *de novo* AML patients Results and conclusion: Pre-treatment samples from all patients were studied by

chromosome and molecular analysis including detection of recurring gene fusions and mutation screening of the FLT3 and NPM1 genes. We genotyped 58 AML (40 NK-AML samples and 18 samples with chromosomal aberration) for mutations in WT1 (exons 7,8 and 9). A total of 4 different WT1 mutations (927 ins CGTACGAC, 939 insC, 941 ins TCGG, 1017 insT) were found 4 AML patients (7%). All WT1 mutations clustered in exon 7; in accordance with previous studies, the majority of WT1 mutations in our study were frameshift mutations occurring in exon 7, since this exon encodes the first Zn finger of WT1, these mutations would result in proteins lacking functional domains such as the DNA binding portion, and the nuclear localization signal. We confirmed the known association of WT1 mutation and NK-AML in fact all WT1 mutated cases showed normal karyotype of them 1 case showed additional double mutations of FLT3/NPM1 (ITD or D835), 1 case the FLT3 ITD mutation and 1 case showed the NPM1 mutation A. There was no difference in relapse-free survival in overall survival between patients with WT1mut or without WT1 mutations, however subset analysis showed that the two patients with the genotype WT1mut/FLT3-ITDpos did not achieve complete remission (CR) while two patients with the genotype WT1mut/FLT3-ITDneg are in CR respectively at 6 and 13 months of follow up. Further studies needs to understand the impact of WT1 mutations as prognostic factor in NK-AML

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P030

ANALYSIS OF JAK2 GENE MUTATIONS IN T(8;21) ACUTE MYELOID LEUKEMIA

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Acute Myeloid Leukemia (AML) is considered to result from the cooperation between at least 2 classes of mutations: class I mutations or activating mutations that confer a proliferative and/or survival advantage to hematopoietic cells (exemplified by constitutively activated tyrosine kinases or their downstream effectors), class II mutations or mutations inducing a differentiation stop that result in loss of function of transcription factors that are important for normal hematopoietic differentiation (Core- binding factor with AML1 ETO and CBFb-MY11 or PML-RARA positive AML). In accordance with this model, mutations in the receptor tyrosine kinase including C-KIT and FLT3 genes appear to be the genetic events that cause AML. The activating V617F mutation of JAK2 tyrosine kinase that is a common event in myeloproliferative disorders (MPD) disrupts the JH2 inhibitory regulation of JAK2, leaving the enzyme constitutively active. In addition to the V617F other rare mutations have been reported located in the pseudokinase domain JH2 suggesting a similar constitutive activation of the JAK-STAT pathway. Recent studies showed that JAK2 V617F mutation is found in AML 1-8% of cases and the frequency of mutations in patients with AML1-ETO AML was significantly higher, but few data have been reported at now. So we decide to investigate the incidence of JAK2 mutation V617F in patients with AML and to analyze the JAK2 mutation status in patients with AML1ETO-AML extending the analysis to most of the pseudokinase domain coding region of the gene. *Results.* We studied the JAK2 mutation in 9 patients with *de novo* t(8:21) AML. The JAK2 V617F mutation was found in 1 of the 9 patients with t(8:21) AML (11%), whereas only 1 of the 84 (1.2 %) patients with AML other than t(8:21) had the same mutation (p<0.05). This patient had a preceding myeloproliferative disorder. Mutations in the JAK2 exon 12 have been described in patients with MPD, but no mutations were detected in JAK2 exon 12 of t(8:21) AML. We extended the analysis to most of the pseudokinase domain coding region of the JAK2 gene exon 14-15. Our preliminary results show that no mutation other than V617F were found in the exon 12-14 of JAK2 in the sample analyzed. *Conclusion.* The present study confirmed that the V617F JAK2 mutation is highly associated with t(8:21) AML and might represent a typical activating mutation cooperating with class II.. Further studies are in progress to understand the importance of JAK2 mutations as prognostic factor in t (8:21) AML.

P031

HYDROXYUREA + VINDESINE IN THE TREATMENT OF ELDERLY ACUTE MYELOGENOUS LEUKEMIA (AML) PATIENTS UNFIT FOR INTENSIVE CHEMOTHERAPY

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In order to improve the dismal results obtained in elderly patients with Acute Myelogenous Leukemia (AML) not eligible for Intensive Chemotherapy (IC), we tested the addition of Vindesine (VND) to standard palliative chemotherapy with Hydroxyurea (HU). From 12/2001 to 12/2007, 38 elderly patients [M/F 24/14, median age 77.6 yrs, interquarile range (IR) 74.0-81.6] with newly diagnosed AML and not eligible for standard IC were enrolled into the study. Twelve patients had a previously documented myelodysplastic phase, 4 patients had a PS (WHO) >2 and 5 patients had a documented infection at onset. Median Hb, WBC, PLTS and marrow blasts were 9.0 g/dL, 15.1x10^9/L, 63x10^9/L and 54%, respectively. Clinical criteria for exclusion from IC were age >75 yrs (21 patients), cardiological disease (7 patients), PS >2 (4 patients), renal disease (3 patients) or other organ failures (3 patients). The primary endpoint was to achieve a phase of stable disease (WBC <10x10^9/L for >2 months during treatment). After diagnosis, patients were observed weekly and the association of HU (initial dose 1500 mg/day) and VND (5 mg iv every 15 days) was started in the presence of WBC > 10 x 109/l or a doubling time shorter than a week. Median time from diagnosis to treatment was 13 days (IR 3-49). Three patients (7.9%) achieved a morphological and/or cytogenetic Complete Remission (CR), 2 (5.3%) an haematological improvement (HI), 12 (31.5%) a stable disease and 21 (55.3%) showed a disease progression. As to the 3 patients in CR, 1 with an abnormal karyotype (47 XY,+13) at diagnosis, achieved both morphologic and cytogenetic CR that lasted 14 months; the other 2 patients with normal karyotype at diagnosis achieved CR lasting 4 and 16 months, respectively. Documented infections (14 broncopneumonic episodes, 4 abscesses and 1 bacterial sepsis) were the most common complications during treatment. Median time of hospitalization was 10 days (IR 5-20). Median overall survival was 108 days (IR 55-262), with 14/38 patients (36.8%) surviving > 6 months and 4/38 (10.5%) surviving > 12 months. Median survival of responding patients (CR + HI + stable disease) was 298 days (IR 206-333), median survival of patients with progressive disease was 68 days (IR 48-133) (p=0.0017). After a minimum observation period of 18 months, no patient is still alive, 5 patients were lost to follow-up and 33 died, 24 from disease progression and 9 in stable disease from infections (5), acute myocardial infarction (2), hemorrhage (1). The addition of VND to HU seems to ameliorate the response rate (CR + HI) as compared to HU historical controls, but it does not seem to affect overall survival. The role of VND in this subset of frail patients remains unclear and further studies with different doses and associations might be tested.

P032

QUANTITATIVE ASSESSMENT OF WT1 EXPRESSION: A USEFUL TOOL FOR MONITORING MINIMAL RESIDUAL DISEASE IN ACUTE LEUKEMIA PATIENTS

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Introduction. The Wilms' tumor 1 (WT1) gene encodes a transcription factor important for normal cellular development and cell survival. In recent years, the study of WT1's involvement in malignant cells, including haematological neoplasias, has revealed a potential role as an oncogene. Moreover, its overexpression in leukemias has been used as a molecular marker for the detection of minimal residual disease (MRD). *Aims*. our study aims to verify if quantitative assessment of the WT1 transcript amount can be used as a marker for monitoring MRD and whether WT1 levels may be predictable of clinical outcome. *Patients and Methods.* the WT1 amount was determined in bone marrow (BM) and peripher-

al blood (PBL) samples of patients with AML lacking a molecular marker for MRD detection and in normal control. We also analyzed WT1 levels at sequential time intervals during follow-up to assess the significance of the WT1 expression for clinical purpouse. Samples from 45 AML patients with miscellaneous cytogenetic abnormalities and normal karyotype at diagnosis (36 BM, 9 PBL) and 16 healthy control (4 BM, 12 PBL) were examined for WT1 expression. A total of 201 specimens of AML patients at diagnosis and during follow-up (181 BM, 20 PBL) were detected for quantitative assessment of the WT1 transcript amount. RQ-PCR reaction and fluorescence measurements were made on the ABI PRISM 7900 HT Sequence Detection System (PE Applied Byosystems). *Results.* In healthy controls, the median value of WT1 levels in BM and PBL was 23,08 (range 0-176,70) and 0 (range 0-8,50), respectively. In the total samples of AML patients at diagnosis and during the follow-up BM and PBL specimens showed a median of 276 (range 0-42.553,33) and 540,47 (range 2,18-25.696) respectively. At diagnosis BM and PBL samples had a median value of 3816,50 (range 7,97-23.094,10) and 7397 (range 6,00-25.696), respectively and overexpression of WT1 was observed in 92% of the cases. In 4 patients bearing an additional molecular marker, like AML1-ETO, PML-RARα or CBFbeta-MYH11, we found a complete parallelism between the behaviour of WT1 transcript and the fusion genes. Clinically, the haematological relapse was correlated, in some cases predicted, by the increase of WT1 expression. Moreover, we observed that the persistence of high level of WT1 transcript after induction chemotherapy was associated with imminent relapse. *Conclusion.* 1) WT1 expression may have utility for detection of MRD in a population of patients with AML lacking additional molecular markers; 2) there is a good correlation to other follow-up markers; 3) normal and abnormal WT1 transcript level may be able to detect patients at high risk of treatment failure after induction therapy and quantitative analysis as postremission control can predict imminent relapse.

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P033

TOTAL SERUM TRYPTASE: A PREDICTIVE MARKER FOR KIT MUTATION IN ACUTE MYELOID LEUKEMIA

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Human tryptase is a serine protease abundantly expressed by mast-cells and, in trace amounts, by basophils and myeloblasts. In patients with *de novo* acute myeloid leukemia (AML), elevated tryptase serum values were detected in 39% of cases, mainly in M2 and M4Eo subtypes. To correlate total serum tryptase (ts-try) levels with cytogenetic features, we retrospectively analyzed cryopreserved serum samples collected at onset of disease of 155 AML patients diagnosed in 4 Italian centres between January 2000 and December 2007. The ts-try was quantitatively evaluated by UniCap tryptase fluoroenzyme-immunoassay 72 (Pharmacia, Uppsala, Sweden) using UniCAP 100 instrument. Fifty healthy controls were enrolled for standard range determination and elevated tryptase levels were defined as more than 15 ng/mL. Forty-five percent of patients (70/155) showed elevated ts-try values. With respect of karyotypes, higher ts-try values were demonstrated in 81.5% (22/27) and 75% (15/20) of patients carrying t(8;21) or inv(16) rearrangements, respectively ($p<.01$, Kruskal-Wallis test). Oppositely, euploid karyotypes resulted significantly linked with tryptase levels lower than 15 ng/ml ($p<.001$, Kruskal-Wallis test). There were no correlations between ts-try and age, sex and white-blood cells count at presentation. To assess whether an elevated value of ts-try was significantly associated with KIT mutations, 75 out of 155 patients were submitted to mutational status for the KIT genes. Twenty patients resulted KIT mutated, of whom 11 had t(8;21) and 6 had inv(16) rearrangements. Significantly higher tryptase levels (median 61.5 ng/mL) were found among the 20 KIT-mutated patients compared with values measured in the wild type group ($p<.001$, Mann–Whitney U test). The receiver operating characteristics (ROC) analysis recognized 26 ng/mL as a possible ts-try cut-off to predict KIT mutations, with 85.0% sensitivity, 58.1% specificity and 42.5% positive-predictive value. Multivariate analysis confirmed this cut-point as a powerful marker to predict KIT mutations in AML patients ($p<.001$, likelihood ratio test). Finally, logistic analysis showed that patients with higher ts-try and t(8;21) rearrangement, had an odds ratio for KIT mutations 9.8 times greater than patients with normal karyotype. In conclusion, we propose that checking for ts-try at diagnosis of AML, especially in t(8;21) rearrangement, may be a simple tool to select patients to be addressed to KIT mutation screening.

P034

THE B-CELL MUTATOR ACTIVATION-INDUCED CYTIDINE DEAMINASE IS ALTERNATIVELY SPLICED IN BCR-ABL1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background. BCR-ABL1-positive ALL is the most frequent and prognostically unfavorable subtype of adult ALL, mainly because of genetic instability. Activation-induced cytidine deaminase (AID) introduces single-strand breaks into target DNA and produces immunediversity by inducing somatic hypermutations and class-switch recombinations (CSR) in human immunoglobulin genes (Ig). *Aim.* We investigated the expression of the AID in BCR-ABL1-positive ALL. *Patients and Methods.* 61 adult *de novo* BCR-ABL1-positive ALL patients (pts) were analyzed. AID cDNA, obtained from bone marrow or peripheral blood, was amplified with two pairs of oligonucleotides, the forward primer of each couple conjugated with a fluorescent dye (fluorescein) at its 5' end. PCR products were then loaded on the ABI Prism 3730 DNA Analyzer and the results were plotted with the AbiPrism GeneMapper v3.5 software (Applied Biosystems). *Results*. On the 61 *de novo* adult BCR-ABL1-positive ALL pts, AID mRNA and protein were detected in 36 (59%); their expression correlated with BCR-ABL1 transcript levels and disappeared after treatment with tyrosine kinase inhibitors. Different isoforms of AID were identified: 13/61 (21%) pts expressed the full-length isoform (AID-FL); 19/61 (31%) co-expressed the wild-type and different splice variants. In particular, we found an isoform with a 30 bp deletion of exon 4 (AID-deltaE4a); an isoform characterized by the deletion of the entire exon 4 (AID-deltaE4) which led to a C-terminal truncation due a frameshift. In 2 patients, AIDins3, characterized by retention of intron 3, was expressed. This variant maintained the cytidine domain but lacked the class switch recombination (CSR) domain due a frameshift. Four out 61 Ph^+ ALL patients (7%) expressed the AID deltaE3-E4 isoform without deaminase activity but retaining intact the CSR domain. Since splicing induced alterations in the nuclear export signal (NES) at the C-terminus we found that. AID-FL exhibited predominant cytoplasmic localization, as did the AID-deltaE4a and AID-deltaE3E4 variants whereas the C-terminal truncated AID-deltaE4 showed a slightly increased nuclear localization. *Conclusions.* Our findings show that BCR-ABL1-positive ALL cells aberrantly express different isoforms of AID that may act as mutator outside the Ig gene loci in promoting genetic instability in leukemia cells.

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P035

ARSENIC TRIOXIDE ALONE OR COMBINED TO RETINOIC ACID FOR RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA

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Arsenic trioxide (ATO) is able to induce hematologic and molecular CR in a high rate of patients with relapsed acute promyelocytic leukemia (APL). Thirty patients with relapsed APL were treated with ATO at the dose of 0.15 mg/kg: 20 received ATO as a single agent, whereas 10 received ATO together with retinoic acid (ATRA) at the dose of 45 mg/m^2 for 30 consecutive days. Sixteen patients were treated in 1st hematological relapse, whereas 9 patients were treated for 2nd or 3rd relapse. Five patients were treated in molecular relapse. After the first course of therapy, 21 patients achieved CR with ATO as a single agent (64%) or in association with ATRA (36%); 6 patients died during induction due to disease progression and/or hemorrhagic complications. During the first course, 2 patients experienced differentiation syndrome and 5 patients electrocardiographic alterations. Median days of neutropenia and thrombocytopenia were 18 and 25 days, respectively, in patients treated for hematological relapse. Twenty patients were treated with an additional course of ATO as consolidation therapy: all achieved an hematologic CR and 95% obtained a molecular remission. During the second cycle, QTc prolongation was observed in 5 patients. Other adverse events observed during treatment were mild nausea, neuropathy (1 case), herpes-zoster reactivation (2 cases). Six patients experienced a morphological relapse after a median of 6 months: 2 received gentuzumab ozogamicin, 2 patients intensive chemotherapy and allo-HSCT, and 2 conservative treatments. Two patients experienced an isolated molecular relapse after a median of 8 months and received an allo-HSCT. All patients who experienced a relapse after ATO died of disease progression or complications related to HSCT. Eight patients underwent an allo-HSCT after the second cycle of ATO in molecular remission and 6 are alive at 34 months of follow-up. In conclusion, ATO therapy alone or in association with ATRA was remarkably effective for relapsed APL; however, a post-remission therapy appears necessary to maintain a durable remission.

P036

RASGRP1/APTX RATIO STRONGLY CORRELATES WITH CLINICAL RESPONSE AND SURVIVAL IN AML PATIENTS TREATED WITH TIPIFARNIB-BORTEZOMIB COMBINATION

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Background. Outcome of elderly acute myeloid leukemia (AML) patients is dismal. Targeted-therapies might improve current results by overcoming drug-resistance and reducing toxicity. *Aim.* We conduced a phase II study aiming to assess efficacy and toxicity of Tipifarnib (Zarnestra®, Z) and Bortezomib (Velcade®, V) association in AML patients >18 years, unfit for conventional therapy, or >60 years, in relapse. Furthermore, we aimed to evaluated the predictive value of the RASGRP1/APTX ratio, which was previously found to be associated to treatment sensitivity in patients receiving Z alone. *Methods*. V (1.0 mg/m^2) was administered as weekly infusion for 3 consecutive weeks (days 1, 8, 15). Z was administered at dose of 300-600 mg BID for 21 consecutive days. Response was assessed at the end of each cycle (28 days). Real-time quantitative-PCR (q-PCR) was used for RASGRP1/APTX quantification. *Results.* Seventy-two patients were enrolled and 62 actually initiated the treatment. Median age was 70 years (42-84). 6 patients achieved complete remission (CR) and 2 partial response (PR). 3 patients obtained a hematological improvement (HI), and 3 died during marrow aplasia. 13 had progressive disease (PD) and the remaining showed stable disease (SD). The median time to response was 88 days. Marrow response (CR+PR) was associated with overall survival (OS) (p<0.0001). RASGRP1/APTX was evaluated before treatment initiation on bone marrow (BM) and/or peripheral blood (PB). The median RASGRP/APTX value on BM was 15.3 (15-19.8) in responder (R) patients and 2.2 (0.5-25.9) in non responders (NR), respectively (p=0.00006). Its median value on PB was 31.6 (19.3-35.5) in R and 6.4 (0.5-27.1) in NR, respectively (p=0.00001). Interestingly, no marrow responses were recorded in patients with BM RASGRP1/APTX ratio <15, while the response rate was 73% in patients with ratio >15 (p<0.0001). Finally, RASGRP1/APTX levels correlated with OS (p=0.005). Toxicity was overall mild, the most common being febrile neutropenia. *Conclusion*. We conclude that the clinical efficacy of the combination Z-V was similar to what reported for Z alone. However we could confirm that the RASGPR1/APTX BM or PB level is an effective predictor of response. Though higher RASGRP1/APTX is relatively rare (~10% of cases), Z (±V) may represent an important option in a subset of high risk/frail AML patients.

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P037

ERYTHROLEUKEMIA: A PECULIAR SUBTYPE OF AML? OUTCOME AND PROGNOSTIC FACTORS IN THE GIMEMA EXPERIENCE

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Within the FAB classification, erythroleukemia is characterized by a proliferation of erythroblasts greater than 50% and of myeloblasts greater than 30%. Adult patients with acute myeloid leukemia (AML) consecutively enrolled in the GIMEMA trials AML10 and LAM99p were retrospectively analyzed with the aim of evaluating the epidemiologic features and outcome of erythroblastic leukemia (FAB M6). Among 1675 AML patients, 59 (3.5%) were identified as M6; 39 were males and 20 females the median age was 49 years (range 25.9-60.7), the median WBC count at diagnosis 2.7x10^9/L (range 0.7-41.8), the median Hb level 7.8 gr/dL (range 5.1-11.8) and the median platelet count 38x10^9 (range 6-245). Univariate analysis showed a statistical difference between the M6 cases and the non-M6 series enrolled in these two clinical trials with regard to: incidence of male gender (p=0.03), prevalence of older age (p=0.001), leukopenia at diagnosis (p<0.0001), decreased levels of Hb (p=0.0006), platelet count (p=0.05), peripheral blast count (p<0.0001) and increased PMN count (p<0.0001). A previous myelodysplastic phase was reported in 5.6% of M6 cases compared to 1.7% in the other AML subtypes (p=0.07). Analysis of response to intensive chemotherapy evaluated as ITT, showed that 64.4% of M6 patients achieved a complete remission (CR) compared to 69.6% in the other FAB subtypes (p=0.39); a similar induction death rate (13.5%) was observed in both groups. Overall survival (OS) at 60 months was 29.5% in M6 patients and 34% in the other FAB types (p=0.75); no significant differences were recorded also with regard to disease-free survival (DFS): 44% in M6 vs 39.7% in the other FAB types after 60 months of follow-up. For patients who reached CR, the cumulative incidence of relapse (CIR) did not show sta-

tistical differences: at 60 months 45% in M6 vs 46.9% in the other types, p=089). Also the cumulative incidence of non-relapse mortality (CINRM) at 60 months was similar in both groups: 10.7% in M6 vs 13.4% in the other types, p=0.62. In conclusion, despite the higher incidence of some risk factors - higher age, higher proportion of myelodisplastic pre-phase and citopenias - in this rare form of AML the CR duration and the OS are similar to those observed in the other more frequent forms of AML. Based on this analysis, the prognosis of this form of acute leukemia does not differ from that of the other subtypes.

P038

SEQUENTIAL LOW DOSES OF RETINOIC ACID (LOATRA) +/- VALPROIC ACID (VPA) FOLLOWED BY LOW DOSES ARA-C (LODAC) IN ELDERLY (>65YRS) ACUTE MYELOID LEUKAEMIA (AML), CLINICAL AND BIOLOGICAL RESULTS OF A SINGLE CENTRE STUDY

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The ability of epigenetic therapies to sensitize neoplastic cells to chemotherapy has been recently suggested. Thus we designed two induction treatments with LoDAC preceded by VPA+LoATRA (treatment A) or LoATRA alone (treatment B) for elderly AML patients(pts) or AML pts not eligible for intensive therapy to evaluate safety, efficacy and biological changes occurring in leukemic blasts during treatment. From September 2006 to December 2008 we enrolled 24 consecutive AML pts, clinical and biological characteristics are reported on table 1.Treatment A included VPA(10-30 mg/Kg/die d1-55), LoATRA (25 mg/m^2 d7-55); LoDAC (40 mg td./die sc. d10-16 and d45-51); in treatment B VPA was omitted. After a rest of 20 days, the course was repeated up to a total of 3 courses. Eight pts received treatment A, and 16 treatment B. PB and BM samples for biological studies were weekly collected. Response was evaluated after the first cycle according to IWG criteria for AML and MDS. Of 19 pts evaluable for response, 7 achieved CR, 3 PR and 1 mHI.Of the remaining 8 pts, 2 died during induction and 6 were refractory.

Table 1.

	Treatment A	*Treatment B*	*TOT*
N. pz	8	16	24
M/F	7/1	8/8	15/9
Median Age (44-80) years	68	68	68
De novo AML/ Secondary AML	4/1	10/4	14/5
AML >1 relapses	3	2	5
Median BM Blasts% (range)	50 (20-81)	47 (20-65)	60 (20-81)
Cytogenetics			
Normal	1	10	11
Complex	4	4	8
t(8;21)	1	0	1
t(2;13)	1	1	2
-y	1	0	1
Dup(1)	0	1	1
Molecular Biology			
FLT3	2	2	4
NPM1	1	2	3
AML1/ETO	1	0	1
Negative	2	7	9
Not done	2	5	7

Overall Response Rate(ORR) was 57,8%. In the 11 responders, PB recovery and BM blasts clearance occurred at d 42 (range 35-50) and at d 35 (range 25-50) respectively. No patient had WHO grade 3 or 4 extrahaematological toxicities, all cases were treated on out-patient basis. Biological results showed a significant difference in responders vs no responders occurring before LoDAC administration. In particular, in responders we observed: 1) increasing rate of S phase cells (p=0.02 0.03 at d +7 and +14 respectiovely); 2) increasing MPO/CAE expression (p=0.03 at d+7); 3)progressive decreasing of early myeloid markers (HLA-DR,CD34,CD117) expression and concomitant increase of late myeloid (CD11b, CD15 or CD14) differentiation markers(p<0.05 at d+28). Among 11 responders,7 relapsed in a median time of 7.5months (range 5-15). As of March 2009, 7pts are still alive, 2 in 1st CR(+2mos), 2 in PR (+10,+15mos), 3 in SD (+4,+15 and+17mos); median OS was 10 mos. These two sequential treatments are feasible, well-tolerated and safe, obtaining an ORR 57.8%. Moreover, they are able to induce phenotipical changes in AML blasts that may increase their sensitivity to LoDAC. It is noteworthy that 2FLT3+ pts achieved CR lasting 2+ and 12 mos. Further studies are needed to establish whether or not the increase of MPO expression and S phase cell rate at d7 may be predictive for response.

P039

PRIMARY PLASMA CELL LEUKEMIA: RESULTS OF A RETROSPECTIVE MULTICENTRIC SURVEY

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Aims. To evaluate epidemiological and clinical features, diagnostic procedures, treatments and outcome of patients (pts) with primary Plasma Cell Leukemia (pPCL) collected among GIMEMA centers. Methods: A retrospective multicenter study was conducted between January 2000 and December 2008 in 31 Italian Hematology Divisions. *Results*. 124 PCL were evaluated and 71 (57%) were classified as pPCL. Median age was 62 years (range 32-86); male/female ratio 1.4:1. At diagnosis the median percentage of peripheral blood plasma cells was 30% (10-88) and the median bone marrow plasma cell infiltration was 80% (40-100). Residual bone marrow function was poor, as documented by low hemoglobin level (median value 9.2 g/dL, 4.8-12.9) and platelet count (median count 86x10^9/L, 8-428x10^9/L). Extramedullary disease was described in 15 pts (21%) and included hepatosplenomegaly, testis, bone and muscular localization. At the onset, 30 cases (42%) presented impaired renal function and 10 (14%) had hypercalcemia. In 22 pts (31%) a cytogenetic study was performed, being the karyotype abnormal in 16 (73%); particularly in 13 cases del(13q-) was detected. Seventy pts received front-line therapy (1 died early) which was: in 21 single alkylating agents like melphalan (9) or cyclofosfamide (12); in 36 antracycline-containing regimens like VAD; in 10 bortezomib and dexamethasone; in 3 thalidomide. Bortezomib and thalidomide were also added to conventional front-line treatment in 7 and 11 cases respectively. Twenty-three pts (32%) underwent intensive high dose therapy followed by allogeneic (8) or autologous (15) stem cell transplantation (SCT) as part of front-line therapy. A complete and partial remission was achieved in 16 and 18 pts respectively (overall response rate 48%). The median overall survival (OS) was 11.5 months (range 1-76). Median OS of pts treated with SCT was 21.3 months (3-75), with a statistically significant advantage with respect to non-transplanted pts (median OS 7.3 months, 0.4-76) (p 0.0008). *Conclusions.* pPCL is a highly aggressive lymphoprolipherative malignancy, characterized by a poor prognosis and a low response rate to conventional therapy. The use of high-dose chemotherapy including autologous or allogeneic-SCT may represent an effective therapy able to improve an otherwise very unfavorable clinical outcome.

P040

SERUM LDH AS PREDICTOR OF CLINICAL OUTCOME IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA AND NORMAL KARYOTYPE

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Cytogenetic abnormalities represent the most relevant prognostic factor in acute myeloid leukemia (AML). However, about half of the patients have normal karyotype (NK) and in this subset FLT3 and NPM1 mutations are extremely useful for the prediction of outcome as well as for the selection of post-remission therapy in young adult patients. On the contrary, the situation is less clear in the elderly subjects, in whom the clinical relevance of either FLT3 or NPM1 mutations is still uncertain. In this study, we analyzed the impact of different well recognized prognostic factors in a series of 103 elderly patients (> 60 years) with AML and NK. In particular, age less or more than 70 years, antecedent myelodysplastic syndrome (MDS), presence of concomitant dysplastic changes, WBC count at diagnosis (i.e. more or less 30 or $50x10^9$/L), type of induction chemotherapy (i.e 3+ 7 or FLAG), serum LDH ≤ or > than 500 IU/l) and FLT3-ITD mutations were included into either univariate or multivariate analysis The median age was 68 years (range 61-81), 70 patients had *de novo* AML, while 33 had AML post-MDS. Thirty-three patients (34%) were aged over 70 years. Trilinear dysplastic changes were found in 25 out of 70 *de novo* cases (36%). Twenty-two patients (21%) had FLT3 ITD mutation, while 70 (68%) had s-LDH higher than 500 IU/L. All patients were given intensive induction therapy followed by consolidation based on intermediate dose ARA-C. Overall, complete remission (CR) rate was 62 %; 22 patients were refractory (21%) and 17 died in induction (16%). Overall, no statistically significant impact was found as the impact of different parameters on CR achievement is concerned. In addition, by univariate analysis, no difference was found between FLT3+ and FLT3- patients, *de novo* and post MDS patients, patients with more or less 30 or $50x10^9$/L and age less or more than 70 years on overall survival (OS) and disease free survival (DFS). On the contrary, patients with low s-LDH level achieved significantly longer OS (24 months vs. 7 months) and DFS (38 months vs. 12 months). Finally, in the multivariate analysis, the only parameter found as having major prognostic significance on survival and disease free survival was high serum LDH levels at diagnosis. We conclude that a simple, cost-effective and routinely available tool as serum LDH level represents an important prognostic factor in AML of the elderly patients with normal karyotype.

P041

PERIPHERAL BLOOD CIRCULATING BLASTS LINEAGE ASSESSMENT USING THE ABBOTT CELL DYN SAPPHIRE

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Objectives. The quick identification of the lineage of blasts circulating in peripheral blood in patients presenting with acute leukemia at the first observation is mandatory to start the most appropriate first line treatment, steroids vs hydrossiurea. In emergency the official diagnostics procedures – smear, cytochemistry, bone marrow aspirate, immunophenotyping, genetics, molecular genetics - should be often delayed with a high risk for the patients. We evaluated the possibility to use an integrated hematology analyzer as first automated procedure for the lineage identification of circulating blasts in peripheral blood. *Methods.* We have analysed 20 samples of peripheral blood of patients presenting with a acute leukemia (14 AML, 6 ALL) at the diagnosis by using the Cell-Dyn Sapphire, an automated hematology analyzer incorporating multiple analytical methods to provide the complete blood count. Moreover the system employs immuno-fluorescence analysis technology, similar to that used on a dedicated fluorescence flow cytometer, for analysis of MAb applications. Dako purified monoclonal mouse antibodies to CD34, CD13, CD33 (conjugated with Rphycoerythrin (RPE)) and CD45, CD3, CD19 (conjugated with fluorescein isothiocyanate(FITC)) very used for this study. The FBC was: WBC: mean $27,13x10^9$/L (range 1,1102), Hb: mean 8,9 g/dL (range 7.2-13.9), Plt: mean $71,8x10^9$/L (range 9-210) and all the reports showed a flag for presence of Blasts > 52%. 100uL of patient sample was pipetted into each of the 2 tubes followed by 10uL of anti-CD34 and 5uL of anti-CD45 and a mixture of 10 uL anti-CD13&33 (PE) and -CD3&19 (FITC) monoclonal antibodies respectively. Tubes were then incubated in the dark at room temperature for 20 minutes and were processed. Results were compared with the final diagnosis performed according to the standard diagnostic procedures. *Results*. We found a full agreement in the final lineage assignment of the blast cells between the Cell-Dyn results and the standard diagnostic methods, morphology and flow cytometry. *Conclusions.* Our study indicates the possibility to use the Cell-Dyn Sapphire as a quick, reliable automated procedure procedure to assess the lineage identification of circulating blasts in peripheral blood, as first reliable step in the leukemia diagnostic pathway. Clinicians as well as patients could practically take advantage from this quick (less than 1 hour) method in starting the appropriate first-line treatment without any delay using a routine hematology analyzer.

P042

CEREBROSPINAL FLUID EXAMINATION IN 123 CASES OF HEMATOLOGIC MALIGNANCY: FLOW CYTOMETRY ACCURACY DEPENDS ON THE NUMBER OF ACQUIRED CD45+ CELL EVENTS

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In active leptomeningeal hematologic malignancy (HM), flow cytometry (FC) results on serial cerebrospinal fluid (CSF) samples are a decision making criterion for managing intrathecal treatment (ITT). However, the latter can induce cell changes likely hampering FC diagnostic capacity during follow-up. The minimum number of acquired $CD45^+$ cell events allowing the best FC accuracy is undetermined. Among 1,131 body cavity fluids analyzed by four-color FC between 2002 and 2009, we selected CSF with (i) suspected or known HM at the time of withdrawal, (ii) cytomorphology (CM) performed on the same sample, and (iii) availability of follow-up findings for retrospective clinical assessment (RCA). FC and CM results [positive (for FC, at least 15 events consistent with HM phenotype) or negative for neoplastic cells] were compared to RCA. Inter-method comparisons were performed by means of

ROC curve analysis. One hundred twenty-three CSF submitted for suspected (3%) or disclosed HM {21% prior to treatment [differentiated non Hodgkin lymphomas (NHL)], and 76% during follow-up [44 differentiated NHL, 36 precursor NHL/leukemias, 13 acute myeloid leukemias]} were selected for analysis. During a median follow-up of 6.5 (interquartile range: 3.9-9.3) months, 32.5% of CSF were RCA-positive. Overall, 100% specificity was detected for both FC and CM; as compared to CM, FC retained significantly higher sensitivity (74.3% vs 63.3%) and negative predictive value (NPV)(90.2% vs 87.8%)(p=.014). FC displayed a better diagnostic value than CM in the analysis of samples submitted prior to any ITT (sensitivity 90% vs 62.5%; NPV 97% vs 91.2%)(p=.127) and after at least one ITT (sensitivity 63.6% vs 57.9%; NPV: 89.5% vs 84.6%, p=.061). When acquisition cut-offs were tested, 100% sensitivity was observed for FC by acquiring at least 50 total CD45$^+$ cell events, the correspondent sensitivity by CM being 75%. When fewer events could be acquired, the best sensitivity was instead obtained with CM, by considering positive also uncertain results (CM-U+): it was higher than that of FC when either <20 (50% vs 0%) or 20-50 (75% vs 50%) total CD45$^+$ cell events were acquired by FC. In the analysis of CSF, FC seems to be the most accurate method when at least 50 CD45$^+$ cell events can be acquired; in the other cases CM-U+ retains diagnostic advantage. In particular, the 20 events cutoff should be considered to distinguish true negative results from "quantity not sufficient" for FC analysis.

P043

IMMATURE PLATELET FRACTION IN HOSPITALIZED PATIENTS WITH NEUTROPHILIA AND SUSPECTED BACTERIAL INFECTION

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Objectives. Neutrophilia is frequently seen in hospitalized patients, so a screening test for infections would be very useful. The immature platelet fraction (IPF) is provided by the automated blood analyzer XE 2100 (Sysmex, Kobe, Japan) that could rapidly identify the aetiology of thrombocytopenia (IPF is increased in peripheral thrombocytopenia and reduced when there is bone marrow failure). This parameter could be used as a marker of increased bone marrow activity as in infectious disease.

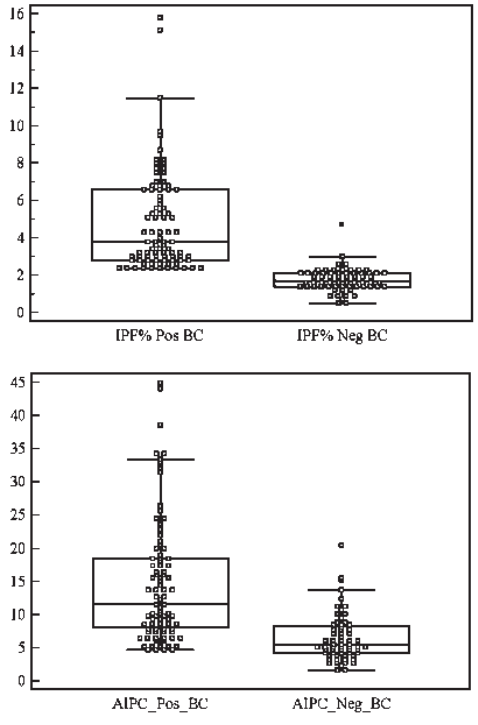

Figure 1. IPF in patients with suspected infection (IPF= immature platelet fraction; AIPC=absolute immature platelet count).

Material and Methods. We selected from routine cell blood counts 677 samples with neutrophilia (>8x10^9/L) and platelets >150x10^9/L (566 samples) to avoid bias due to physiological increase of IPF%). Their history were collected: 160 samples with clinically suspected infection (fever > 38°C) were submitted to blood cultures (BC) the same day of IPF% determination. *Results.* Eighty-nine samples (55,6%) had positive BC (75 Gram-positive agents, 14 Gram-negative). Using our IPF reference normal range [2.39% (0.8-5.1%)], we found a significantly (p<0.0001) higher level of IPF in samples with positive BC (mean 4.86±2.67%, median 3.8%, range 2.4-15.8%) than in BC negative samples (mean 1.90±0.84%, median 1.8%, range 0.5-6.0%). Conclusions: The increased IPF% shows a statistically significant correlation with BC positivity. This parameter could therefore be used in the daily laboratory routine as a low cost screening test for bacterial infection.

P044

SIMULTANEOUS DETECTION OF B-CLL GENOMIC REARRANGEMENTS BY MLPA AND CORRELATION WITH FISH DATA

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B-Chronic lymphocytic leukemia (B-CLL) is characterized by highly clinical and biological heterogeneity. Chromosomal changes have been detected in the majority of B-CLL samples by use of interphase FISH showing a clinical relevance of specific genetic abnormalities, such as 13q14, 11q22-q23 and 17p13 deletions and trisomy 12. Recently, a new method, named Multiplex Ligation-dependent Probe Amplification (MLPA) has been reported for the evaluation of DNA copy number. We performed MLPA assay in a panel of 33 B-CLL patients in early stage disease (Binet stage A) and compared the results with available FISH data. The MLPA assay was performed for all samples in two independent reactions, one for each probe mix (SALSA Probe-Mix P037 and P038) according to the manufacturer's recommendations (MRC-Holland). These mixes contain 55 target sequences specific for different chromosome regions: 17p13 (8 probes), 13q14 (12 probes), ATM 11q23 (7 probes), 10q23 (2 probes), 2p24 (3 probes), 8q24 (3 probes), 6q25–26 (3 probes), 9p21 (2 probes) chr. 12 (10 probes) and chr. 19 (5 probes). DNA from 10 healthy donors were used as controls. Data were analyzed with Coffalyser Software (MRC-Holland). FISH analyses were performed using a set of commercially available probes (Vysis, Downers Grove, IL) specific for del(11)(q22-23), +12, del(13)(q14) and del(17)(p13). A minimum of 100 interphase nuclei were evaluated for each sample. MLPA and FISH approaches detected 13q14 deletion in 20 (60%) patients, 4 of which showed biallelic deletion. Same results were obtained for 4 (12%) cases with trisomy 12. Deletions in TP53 gene were detected by MLPA in two of the three cases detected by FISH (3/33, 9%), whereas ATM deletions were found by MLPA in four of the five B-CLLs detected by FISH (5/33, 15%); in the two cases detected only by FISH, the low percentage of cells carrying the alterations (<30%) may hamper MLPA detection. Finally, in one additional case, FISH assay was not able to detect deletion at 11q22-23, instead identified by MLPA; this could be the result of a deletion too small for the FISH probe. Our study showed a good correlation between the MLPA and FISH results (91%). Moreover, MLPA and FISH showed a comparable sensitivity. These results strongly suggest that MLPA may represent a useful technique for the analysis of genomic alterations in B-CLL and proven to be rapid, cost effective and relatively easy performed, enabling the simultaneous analysis of many samples.

P045

EVALUATION OF ERYTHROPOIESIS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) GOOD RESPONDERS TO TREATMENT WITH ERYTHROPOIETIN,5-AZACITIDINE AND LENALIDOMIDE

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Introduction. Automated hematology analyzers allow us to obtain some erythrocyte and reticulocyte parameters that give information about dysplastic aspects of erythropoiesis; among these, Reticulocyte Mean Corpuscolar Volume (MCVr) is a parameter usually increased in dysplastic erythropoiesis (Bowen 1994, Ljung 2004) and it is not influenced by transfusion. The aim of this study was the evaluation of erythropoiesis in MDS patients good responders to treatment. *Materials and Methods.* 23 patients achieving good response to treatment, according to IWG response criteria in MDS, were divided into 3 groups. Group I: 10 patients treated with erythropoietin (EPO) (10000-80000u/week). Group II: 11 patients treated with 5-azacitidine (75 mg/m^2/day subcutaneous x 7 days every 28 days). Group III: 2 patients with 5q- syndrome treated with lenalidomide (10 mg/day reduced to 5 mg/day after 1 month because of haematological toxicity). Data were obtained at baseline and after 6 months of treatment. We evaluated Hemoglobin (Hb) and MCVr obtained with the hematology analyzer Siemens ADVIA®2120. We determined serum folates and serum holotranscobalamin in all patients after 6 months of therapy. We didn't performed a statistical approach because of the small number of patients enrolled in the study. *Results.* The data are shown in table. After treatment all patients showed an increased level of Hb and became transfusion independent. Serum folates and holotranscobalamin were normal. MCVr showed an increase in patients treated with EPO and 5-azacitidine while 2 patients with 5q- syndrome treated with lenalidomide showed a decrease in MCVr and 5q- metaphases (25% and 80% abnormal metaphases less than baseline, respectively). *Discussion*. The increase of the Hb values observed in 3 groups of patients demonstrated an increase in the efficiency of the erythropoiesis. MCVr values, higher than reference values at baseline, showed even higher values after treatment with EPO and 5-azacitidine, suggesting a possible increase in the efficiency of a dysplastic clone. The decrease of MCVr after treatment with lenalidomide, associated with the decrease of the clone 5q-, allows us to hypotize the involvement of normal clones in the increased efficiency of erythropoiesis after this therapy.

Table 1.

	Hb baseline means/range	*Hb after 6 months means/range*	*MCVr baseline means/range*	*MCVr after 6 months means/range*
Group 1 (Treatment with EPO)	9.0 (6.9-10.3)	11.5 (8.7-13.7)	119.7 (102.0-140.0)	126.4 (102.0- 145.0)
Group 2 (Treatment with 5-Azacitidine)	7.3 (6.8 - 8.0)	10.3 (8.4-12.7)	128.5 (120.0-140.0)	132.3 (123.0-145.0)
Group 3 (Treatment with Lenalidomide)	7.0	13.9 (13.6-14.1)	133.0 (130.0-136.0)	118.0 (116.0-119.0)

P046

FLOW CYTOMETRIC ANALYSIS OF RETICULOCYTE AND RED BLOOD CELL INDICES IN INFANTILE PYKNOCYTOSIS

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Infantile pyknocytosis is a rare transient haemoliytic anaemia of neonatal age, first described by Tuffy *et al.* in 1959. We report on the flow-cytometric analysis (Siemens ADVIA 2120® analyzer) of reticulocyte and red blood cell indices performed in a large number of patients affected by this disease, that were diagnosed and treated in our Department. In the last three years six newbors were admitted to the hospital for jaundice and/or anaemia, developing in the second or third week of life. Family history, clinical and haematological phenotype of parents were normal; all the patients were full-term babies, with a normal birth weight; direct antiglobulin test was negative in all cases. The diagnostic work up showed the presence in the peripheral blood of high percentage of irregularly contracted speculated erythrocytes morphological similar to acantocytes (pyknocytes). Renal and liver function tests, serum lipids and lipoprotein cholesterol pattern, osmotic fragility test, Hb electrophoresis, glucose-6-phoshate dehydrogenase and piruvate kinase levels were normal in all the patients. In the figure flow cytometric pattern of cell volume and Hb concentration of erythrocytes and reticulocytes of one patient is reported: it was peculiar and characterized by an increased percentage of hyperdense red blood cells (A) , with an asymmetry in the corpuscolar Hb concentration distribution curve (B) and a normal symmetry of the reticulocyte Hb concentration repartition curve (C), supporting the ethiologic role in infantile pyknocytosis of a still unknown extra-corpuscolar factor of peripheral blood. All the patients were treated with phototherapy, red blood cell transfusions and, in two cases, erythropoietin therapy, for persistent anaemia and pyknocytosis; they are now well at a minimun follow-up of eight months. So far the diagnosis of infantile pyknocytosis has been infrequent, requiring both the accurate evaluation of the peripheral smear and the esclusion of other neonatal haemolytic disorders. The flow cytometric pattern of reticolocyte and red blood cell, which is not found in other haemolytic disease such as hereditary spherocytosis etc., could support the diagnosis of this severe neonatal disease.

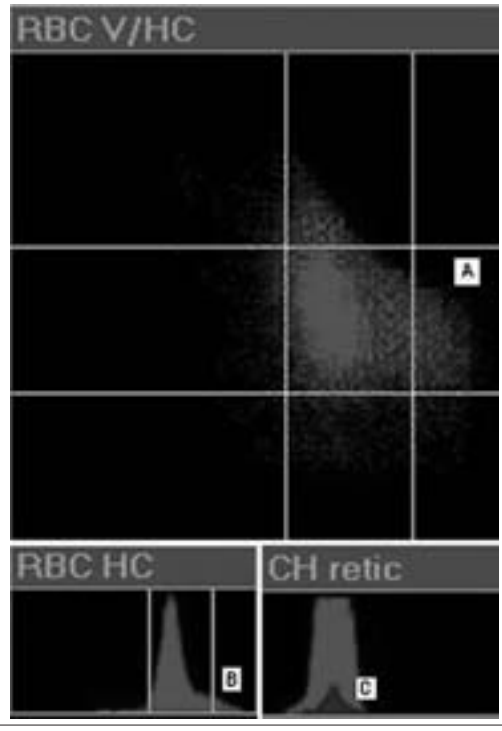

Figure 1.

P047

FIRST-LINE ASSESSMENT OF HAEMATOLOGICAL DISORDERS BY A SINGLE-TUBE CYTOFLUORIMETRIC EVALUATION

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Introduction. In the daily routine of laboratory haematology there are variable percentages of samples that show qualitative and quantitative abnormalities not clearly classified as haematologic neoplasm (light increases in leucocyte subpopopulations, immature-granulocytes or other morphological abnormalities) that have to be assessed by microscopic evaluation and/or deeper cytofluorimetric analysis, requiring skilled operators and further and time consuming laboratory tests. We used an automated cytofluorimetric test able to identify 16 leukocyte subpopulations , to evaluate the possibility to distinguish in a preliminary way

between haematological disorders that must be deeply investigated by specific and reference cytofluorimetric tests and leukocyte abnormalities by other origin. *Materials and Methods.* 16-population WBC differentials by flow cytometry (FCM) were obtained on haematological samples with a 6 markers, 5 colors CD36-FITC/CD2-PE/CRTH2-PE/CD19-ECD/CD16-Cy5/CD45-Cy7 cocktail by Coulter FC500. We examined a total of 139 abnormal samples, including 79 haematological disorders, with already known diagnosis according to the current criteria (13 Acute Myeloid Leukemia (AML), 1Acute Lymphoblastic Leukemia (ALL), 27 B Chronic Lymphocyte Leukemia (B-CLL), 4 Chronic Myeloid Leukemia (CML), 34 Myelodysplastic Syndromes (MDS) and 60 reactive disorders (19 lymphocitosis, 11 neutrophilias (>15000/mmc), 12 monocitosis (> 1000/mmc) and 18 leucopenias). All samples were previously evaluated with traditionally cytofluorimetric approach and revised at optical microscope according to NCCLS H20A protocol. *Results.* In all acute leukemias we clearly identified a blast population by the proposed gating strategy for CD45/CD16/CD19. All B-CLL were identified with CD19/CD2 and CML with CD45/CD16. The combination of SS and CD16 in the gating strategies identified 32/34 MDS except two refractory anaemia (RA) without leukocyte abnormalities. The leukocyte abnormalities by other origin could be clearly identified and separated from the haematological disorders by the gating strategies and the moAb combination. *Conclusion*. We conclude that the use of a single-tube analysis with a fixed gating panel could be a useful 1st-level screening tool to distinguish between samples with haematological disorders that must be deeply evaluated with the classical cytofluorimetric approach and samples with aspecific leukocyte abnormalities.

P048

NEW INSIGHTS INTO SUB-CELLULAR LOCALIZATION OF LEUKEMIC NUP98 FUSION PROTEINS

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Background. Translocations involving NUP98 have been reported in myeloid leukaemia (AML), myelodysplasia, chronic myeloid leukemia blast crisis, and T acute lymphoblastic leukemia (T-ALL). NUP98 belongs to the nucleoporin's family, a class of proteins which constitute the nuclear pore complex (NPC) and regulate the nucleo-cytoplasmic traffic. As alterations in the nucleo-cytoplasmic traffic might contribute to malignant transformation by oncoprotein mislocation, we investigated the cellular localization of leukaemia -associated NUP98 fusion proteins. *Aim.* To elucidate mechanisms underlying NUP98-X fusions we studied cellular localization and interactions with the nuclear cytopaslmic trafficking machinery of NUP98 and its homeobox (HHEX, HOXA9, PMX1) and non-homeobox (LOC348801) fusion partners. *Results and Comments.* EGFP-NUP98 was found in the nuclear rim and as small dots in nucleoplasm and nucleoli. Co-transfection experiments with DsRed-NPM1 showed NUP98 localized in the nucleolar fibrillar compartment. NUP98 and hCRM1 colocalized strongly in nucleoplasmic and nucleolar dots. LMB treatment, which impairs hCRM1 binding with nuclear export sequences, resulted in a diffuse nucleoplasmic staining pattern and disappearance of dots, confirming the NUP98-karyopherin interaction played a functional role in nucleo-cytoplasmic traffic. EGFP-HHEX diffusely stained nucleoplasm excluding nucleoli. EGFP-LOC348801 diffusely stained nucleoplasm and cytoplasm. NUP98 fusions: EGFP-NUP98/HHEX, gave a microspeckled pattern in nucleoplasm, sparing nucleoli. LMB treatment of EGFP-NUP98/HHEX mutants suggested the NUP98 N-terminal maintains its interaction with hCRM1 when fused to HHEX. NUP98/HOXA9 and NUP98/PMX1, yielded speckles like NUP98/HHEX. EGFP-NUP98/LOC348801(iso1) and EGFP-NUP98/LOC348801(iso2) showed speckles in nucleus, sparing nucleoli. Both isoforms were found in nucleoli when co-transfected with pDsRed-NPM1wt, a CRM1-binding protein. NPM1 co-transfection did not localize NUP98-HEX, NUP98-HOXA9, NUP98-PMX1 in nucleolus. *Conclusions.* Our observations suggest that the subcellular localization of NUP98 fusion proteins is influenced by both NUP98 and the partner in each type of leukemic fusion (HHEX; HOXA9; PMX1; LOC348801). We show that NUP98 interacts with the hCRM1 system through its N-terminal portion which is maintained in all fusions, and that the homeodomain of Homeobox genes partners prevents the localization of NUP98 in the nucleolus.

P049

ANALYSIS OF HFE, TFR2 AND FPN1 MUTATIONS IN PATIENTS WITH BIOCHEMICAL PARAMETERS OF IRON OVERLOAD.

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Introduction. Hereditary hemochromatosis is an autosomal recessive disorder of iron regulation that results in abnormal intestinal iron absorption with progressive iron overloading of parenchymal cells. The biochemical abnormalities of iron parameters include a transferrin saturation of greater than 45% and a serum ferritin concentration of greater than 300 micrograms/L in men and greater than 200 micrograms/L in women. The disease is primarily due to C282Y mutation in the HFE gene. The single point mutation 845A, changing cysteine at positon 282 to tyrosine (C282Y), in this gene has been identified as the main genetic basis of hereditary hemochromatosis. Two other mutations, 187G, a histidine to aspartate at amino acid (H63D), and 193T, a serine to cysteine at amino acid 65 (S65C), appear to be associated with milder forms of hereditary hemochromatosis. *Methods and Result.* From March, 2004 to April, 2009 we studied 162 patients admitted to our Molecular Biology Laboratory, referred for genetic testing due to familial links to this disorder; or due to chronic viral HCV/HBC; or to steatosis, etc. Samples were tested for 12 mutations of HFE gene, 4 mutations of TFR2 gene and 2 mutations of FPN1 gene. In the series studied, the overall C282Y allele frequency was 4.3% and that of the H63D and S65C was 20.2% and 1.23%, respectively. Here, we describe the case of a woman who had S65C/C282Y compound heterozygotes. None of the known TFR2 and FPN1 mutations were identified. When checked at a second examination, transferring saturation and serum ferritin was significantly higher in C282Y homozigotes, H63D/C282Y compound heterozygotes and H63D homozygotes as compared to wild type subjects ($p<0.05$). Interpretation and conclusion. Subjects with hemochromatosis-associated genotypes show a persistently higher mean saturation than do those with wild type genotypes. Our results confirm previous findings on C282Y and H63D mutations in southern Italy, showing a higher frequency of allele H63D with respect to C282Y. Furthermore, the homozygotes and compound heterozygotes for C282Y and H63D show heightened iron parameters in respect to wild type subjects.

P050

COPY NUMBER VARIATIONS (CNVS) IN SOLITARY BONE AND EXTRAMEDULLARY PLASMACYTOMA. A METAPHASE COMPARATIVE GENOMIC HYBRIDIZATION STUDY

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Background. B-cells are involved in solitary plasmacytoma (SP), a rare, poorly characterised, clonal malignant proliferation of plasma cells. A low mitotic index of terminally differentiated B-cells and difficulties in obtaining biological samples hinder cytogenetic analysis. *Aim.* To characterize genomic imbalances underlying pathogenetic mechanisms, detect new target regions and compare genomic pathways in SP and Multiple Myeloma (MM), metaphase comparative genomic hybridization (M-CGH) was applied to paraffin-embedded tissue sections from 12 patients with SP. *Materials and Methods.* Four patients had bone SP (2 vertebral, 1 ileus, 1 on the third rib); 6 patients had extramedullary SP (2 trachea, 1 pharynx, 1 larynx, 1 nasal fossa mucosa, 1 tonsils). SP localization was not reported in 2 patients. DNA from paraffin-embedded tissue sections was processed in M-CGH experiments as previously described. *Results.* All patients had multiple genomic imbalances. The most frequent gain (10/12 cases) was at chromosome 19/19p. Other recurrent gains involved chromosomal regions 17/17q (6 cases), 9/9q (4 cases plus 1 high-level amplification), 5/5q and whole chromosome 15 (4 cases each), 1q (2 cases plus 1 high-level amplification), 10/10p (3 cases), 2q, 3p, 3q, 6p, 11/11q and 21 (2 cases each). Other high-level ampli-

fications were found at chromosomes 4 and 15. The most frequent loss (9/12 cases) was at chromosome 13. Other recurrent losses involved 1p (5 cases), 4/4q, 6q, 8/8q, 11p and 14 (4 cases each), 4p, 12q, 18q (3 cases each), 8p and 11q (2 cases each). Other chromosomes were sporadically involved in gains and losses. Bone SP was associated with loss of chromosome 14 (3/4 cases versus 1/8 cases of other localizations) and 12q loss (3/4 cases) which was not observed in any other case ($p<0.05$). *Conclusions.* 19p gain and 13q loss, but not chromosome 8 gain, were confirmed as the most frequent CNVs in SP. Gains at 5q and 17q, and losses at 4q, 8q and 11p emerged as new recurrent CNVs. At least 1 patient with SP had gain at 1q, 3q, 5q, 7q, 9q, 11q, or 15q and loss at 6p, 13q, or 16q, which overlaps with CNVs in MM, indicating genomic pathways in SP and MM are elucidated by CNV studies. Distinctive CNVs characterised bone SP.

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P051

MITOGEN-STIMULATED DIRECT ANTIGLOBULIN TEST COMPARED WITH TRADITIONAL METHODS FOR THE DETECTION OF ANTI-RBC AUTOIMMUNITY

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The diagnosis of autoimmune haemolytic anaemia (AIHA) is based on the serologic evidence of anti-erythrocyte autoantibodies detected by the direct antiglobulin test (DAT), and its subsequent identification in the RBC eluate and serum. The aims of the study were 1) to compare different traditional (tube, microcolumn and solid phase) and new (mitogen-stimulated, MS) DAT methods in different diagnostic conditions: 54 consecutive cases of haemolytic anaemia of suspected autoimmune origin, 28 idiopathic AIHA in clinical remission, and 12 difficult cases of DAT-negative AIHA, and 2) to correlate results with hematologic and haemolytic parameters. DAT tube, microcolumn, solid phase, and eluates were performed by standard techniques. MS-DAT was performed by stimulating whole blood with PHA, PMA and PWM and antibodies were detected by competitive solid phase ELISA. Forty out of 54 consecutive cases of suspected AIHA were positive by one or more tests namely 14 DAT-tube, 19 microcolumn, 19 solid phase and 35 MS-DAT. Among the 14 DAT-tube positive cases, 11 were confirmed by all test, and 3 by one or more (1 microcolumn, 1 solid phase, and 1 MS-DAT), eluates were positive in 11, and the majority of patients (10/14) showed haemolytic anaemia. As regards the 26 DAT-tube negative cases, 7 were positive in microcolumn and solid-phase (eluates positive in 2/8, panreactive), and 16 in MS-DAT, although in both groups anaemia and haemolytic signs were less clear. Mitogen stimulation increased the amount of RBC-bound IgG in all groups, suggesting that MS-DAT could disclose a latent autoimmunity. Tube-negative/other methods positive cases included patients with B-CLL, myelodysplasia/aplasia, and thalassemia intermedia, in which autoimmune phenomena are more frequently observed than overt clinical autoimmune diseases. MS-DAT failed to detect anti-erythrocyte antibodies in half cases AIHA in clinical remission which still were tube-positive. Finally, MS-DAT was the only positive test in 10 cases of AIHA of difficult diagnosis, and mitogen stimulation allowed the identification of autoantibody specificity in culture supernatants of 2 cases which gave weak positive results in microcolumn/solid phase only. We conclude that a battery of tests rather than a single test is recommended for the diagnosis of AIHA, including MS-DAT as an additional test for selected cases, although results have to be cautiously interpreted in the whole clinical context.

P052

DIFFERENCE OF ERYTHROCYTE AND RETICULOCYTE INDICES IN MYELODYSPLASTIC SYNDROMES WITH OR WITHOUT RINGED SIDEROBLASTS (RS)

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Introduction. Myelodysplastic syndromes are frequently associated with erythrocyte and reticulocyte morphological abnormalities. Automated haematology flow cytometers allow us to obtain many erythrocyte and reticulocyte parameters that are influenced by the presence of dysplasia. MDS with ringed sideroblasts showed marked aspects of dysplasia including an increased percentage of hypocromic red cells (%Hypo) and macrocytic red cells (%Macro) (Bowen 1996, Ljung 2004).The aim of this study was designed to assess whether some erythrocyte and reticulocyte indices are useful in the evaluation of the erythropoiesis and to distinguish between MDS with and without RS. *Materials and Methods*. We analyzed 68 patients: 26 with MDS with RS and 42 with MDS without RS. The following erythrocyte and reticulocyte indices were obtained by the flow cytometer Siemens ADVIA® 2120: mean corpuscolar volume of erythrocytes (MCV)and reticulocytes (MCVr), cell volume distribution width of erythrocytes(RDW)and reticulocytes (RDWr), haemoglobin distribution width of erythrocytes (HDW)and reticulocytes (HDWr), cellular haemoglobin distribution width of erythrocytes (CHDW)and reticulocytes (CHDWr), %Hypo and %Macro. We evaluated also the scattergram volume/haemoglobin (Hb) concentration. Statistic test of Mann-Withney was utilized to evaluated differences. *Results.* Data obtained are shown in Table. All the parameters regarding distribution width of erythrocyte and reticulocyte volume, Hemoglobin content and Haemoglobin concentration (RDW,RDWr,HDW,HDWr,CHDW, CHDWr) were higher in MDS patients with RS than in MDS without RS. MCV, %Hypo and %Macro were equally higher in MDS with RS. The scattergram volume/haemoglobin concentration showed a wide scattering of the erythrocytes (double population macrocytic normocromic and hypocromic microcytic) related to the numeric parameters previously mentioned. *Conclusion.* Erythrocytes and reticulocytes analysis in flow cytometry showed a larger distribution width of cell volume, haemoglobin content and haemoglobin concentration in MDS with RS than in MDS without RS. We found also higher values of %Hypo and %Macro; this suggest a substantial heterogeneity of the erythropoieis with a double population of erythrocytes (macrocytic and hypocromic microcytic). Scattergrams showing the erythrocyte population could represent a diagnostic marker of MDS with RS because of its morphological pattern.

Table.

Parameters	*RARS*	*OTHER MDS*	*p*
	Median / Range	*Median / Range*	
CHDW	8.49 (6.76-11.79)	5.95 (3.36-8.64)	<0.0001
CHDWr	8.64 (6.81-11.97)	6.03 (3.26-9.41)	<0.0001
HDW	3.57 (2.97-4.85)	3.32 (2.50-4.33)	0.0017
HDWr	4.25 (3.04-4.24)	3.68 (2.54-5.08)	0.0004
RDW	23.75 (19.90-32.80)	18.75 (13.60-18.75)	<0.0001
RDWr	17.70 (12.60-25.20)	12.80 (8.60-27.80)	<0.0001
MCV	104.75 (89.3-123.1)	95.40 (78.80-123.90)	0.0019
MCVr	125.70 (96.10-145.20)	126.00 (95.00-147.00)	0.7429
% Hypo	11.25 (4.10-26.10)	4.70 (0.30-36.00)	0.0055
% Macro	23.00 (5.90-55.50)	9.7 (0.20-54.50)	0.0001

P053

PREDICTIVE INFORMATIONS OF SOME COULTER LH 780 HEMATOLOGY ANALYZER IN APLASTIC PATIENTS IN CD34 RECRUITMENT

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Our unit of clinical and laboratory analysis is always active for the specific treatment of patients treated with chemotherapeutic agents, cytostatic drugs and growth factors or patients involved in bone marrow

transplant or stem cell infusion. We are evaluating Coulter LH 780 hematology analyser that provides automated CBC parameters, 5-part leukocyte differential, NRBC and reticulocyte counting. It uses VCS (Volume, Conductivity and Light scatter) technology for the simultaneous evaluation of size, nuclear/cytoplasm ratio and granularity of leukocytes and new-blue-methilene stained reticulocytes. For each sample a 3-dimensional scatterplot is generated together with cell population data (CPD) in terms of mean and standard deviation of the VCS measurements (24 parameters for differential and 12 for retics). In this report we describe two findings that could be useful in stem cell transplant. Coulter LH 780 provide Immature Reticulocyte Fraction (IRF) parameter that express the young/mature retic ratio in RBC maturation (normal values: 0 – 0.3). We followed daily 3 patients that underwent to aplasia (close to 0,1 x 103 cells/mcl) until WBC recovery. As reported in the literature we investigated the role of IRF as a marker of bone marrow RBC production. We found that IRF values remain lower than 0.3 during aplasia and then follows ANC and WBC recovery, raising his value and then stabilysing to a normal value. Moreover, expecially in one patient, we observed that IRF change occurs before WBC and ANC raise. We also investigated on the values of Monocyte and Neutrophil CPD as they represent morphological characteristics of the cells and the change of their values is associated with the presence of abnormal cells in the peripheral blood. During the patient follow up we discovered that monocyte and neutrophil mean volume and its standard deviation raise day by day until they reach the highest value when CD34 stem cells are at the highest concentration in PB. This happens in less than 2 days. We think that probably the stem cells are recognised by VCS technology with the same morphological features of monoblast or myeloid blast and included in the monocyte or neutrophil cluster. The variation of MO and NE mean volume and standard deviation could be correlated with CD34 count as semiquantitative determination of stem cells. These data are very preliminary but they deserve to be deeply investigated. These parameters are generated in a CBC/Diff/Ret test in the laboratory and could be useful as screening tool during patient follow-up.

P054

GENOMIC DELETIONS OF THE CDKN2A LOCUS IN BCR-ABL1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) DEREGULATE G1/S CONTROL AND CONTRIBUTE TO PROGRESSION OF DISEASE

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Background. 9p21 is a major target in the pathogenesis of a number of human tumors. The locus harbors the CDKN2A/ARF tumor suppressor gene, which encodes two cell cycle regulatory proteins cyclin dependent kinase 2A (p16INK4a) and alternate reading frame (p14ARF). *Patients and Methods.* In order to assess whether and how it is inactivated in ALL, we studied 25 adult BCR-ABL1-positive ALL patients at the diagnosis and 5 only at the time of relapse following tyrosine kinase inhibitor treatments. Paired relapse samples were available for 4 patients. Affymetrix Single nucleotide polymorphism (SNP) Genome Wide SNP6.0 array was used to identify at the high resolution copy number changes on 9p21. FISH analysis was performed in order to confirm SNP results. PCR amplification and mutation screening of all exons by cloning and subsequent sequencing were performed. *Results*. SNP array analysis revealed CDKN2A genomic alterations in 28% of diagnosed patients and in 56% relapsed samples. Deletions were in the majority of cases biallelic and had a mean size of 101.5 kb (range, 27 kb-286 kb), ranging from 21823529 to 22122076. In two cases deletions of CDKN2A locus was due to losses of chromosome 9 involving the cytobands from 9p21.3 to 9p13.1 or to 9p13.2. FISH analysis confirmed these deletions but failed to detect focal alterations since most of them were below the resolution of the commercial FISH probes. In order to assess whether CDKN2A loss is responsible for progression, in four patients SNP analysis was performed both at diagnosis and at relapse. At diagnosis one patient showed a small monoallelic deletion (28.5 kb) that was maintained at the relapse. It is interesting to note that in another patient we found a focal heterozygous deletion (107.1 kb) at the diagnosis that became monoallelic at the relapse. In the two remaining patients, the alterations of CDKN2A were found only at the relapse, suggesting that loss of this locus is involved in disease progression. Mutation screening of all exons showed that CDKN2A locus is not affected by point mutations, since we only identified the SNP rs11515 (C/G) in 96%. *Conclusions.* Inactivation of the tumor suppressor gene CDKN2A by genomic deletions is a frequent event in Ph^+ ALL and is involved in disease progression.

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P055

CHARACTERIZATION OF GENOME HYPOMETHYLATION AND EXPRESSION PROFILING OF REACTIVATED GENES IN HL60 CELLS FOLLOWING 5-AZA-2-DEOXYCYTIDINE TREATMENT

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Introduction. The therapeutic activity of hypomethylating agents in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) has been the focus of several studies, but the mechanism of action of these drugs is so far unclear, including not only epigenetic regulation, but also apoptosis induction. In this work, we studied the apoptotic effect, the hypomethylating activity and the gene expression profile of the myeloblastic HL60 cell line after 5-aza 2-deoxycytidine (DAC) treatment. *Methods*. Cytofluorimetric analysis using the Annexin-V-FLUOS Staining Kit (Roche) was used to evaluate both early and late apoptosis. We used the HG promoter 1.0 Tiling arrays and HG U133 Plus 2.0 expression arrays (Affymetrix) to analyze methylation (region length 350 base pair, 10 probes, p lower than 0.05) and expression profiles (p-value lower than 0.01 and 2-fold change), respectively. To further characterize the apoptotic process, we performed a RT2 Profiler Human Apoptosis PCR Array (SuperArray), which includes 84 selected genes, and semi-quantitative Real Time PCR. Statistical analysis of microarrays results were performed by Partek Genomic Suite Software. *Results.* Decitabine treatment (72 hours at 1 micromolar) induced 25.2% and 9.4% early and late apoptosis, respectively, in HL60 cells. MeDIP (Methylated DNA Immuno-precipitation) Gene Chip analysis showed that more than 1500 hypermethylated regions were lost after treatment. In this line, within approximately 39000 well characterized human genes, 1123 were overexpressed. On the other hand, 498 genes were downregulated, suggesting a wide effect of DAC treatment. Looking into apoptotic pathways by a Real-Time PCR array, 13 genes (BIK, BIRC3, BNIP3L, CD40, CD70, NOL3, LTA, BCL2L10, CASP5, CASP7, CASP9, DAPK1 and TNFSF10) were overexpressed and 3 (LTBR, BID and DFFA) were downregulated following DAC treatment. *Conclusion.* Confirming previous results, DAC induced apoptosis in HL60 cells, together with gene hypomethylation. This was not only associated to induction, but also to downregulation of several cellular pathways, pointing to additional mechanisms other than inhibition of DNA-methyl transferase (DNMT) activity.

P056

SOMATIC CNVS AND LOH IN ACUTE MYELOMONOCITIC LEUKEMIA

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In acute myeloid leukemias (AML) chromosomal aberrations, detectable by conventional cytogenetics or targeted molecular techniques, provide the basis for a classification with prognostic relevance. Recent advances in genome-wide analysis of submicroscopic DNA segment copy number variations (CNVs) may allow the identification of novel molecular tumor-associated abnormalities (somatic CNVs). However, CNVs are also present physiologically in the normal population (germline CNVs) (Redon *et al.,* 2006) and can represent potential predis-

position factors in disease.

Table 1.

	CNV or LOH per sample			
	Normal karyotype		Abnormal karyotype	
	median	range	median	range
CNV (diagnosis)	38	16-52	79	18-163
ratio gain/loss at diagnosis	7	6-7	15	4-22
CNV (remission)	24	16-32	49	20-167
ratio gain/loss at remission	1	1-2	9	1-20
germline CNV	18	14-21	33	7-77
somatic CNV	16	2-30	51	3-86
LOH (diagnosis)	299	285-314	316	307-317
LOH (remission)	291	285-297	295	282-318
germline LOH	283	278-289	287	287-308
somatic LOH	16	12-19	20	8-30

Indeed, CNVs can have dramatic phenotypic consequences as a result of altering gene dosage, disrupting coding sequences, or perturbing long-range gene regulation. In the present communication we report preliminary results of a study aimed to test the ability of the last generation of Affymetrix single nucleotide polymorphism (SNP)/CNV platform (SNP Array 6.0), containing probes for the detection of CNVs and SNPs, to distinguish tumor-associated somatic CNVs and LOHs from germ-line ones. Until now, 8 M4-M5 FAB subtype AML patients have been studied comparing bone marrow samples from the same AML patients at diagnosis (>90% blasts) and at the remission phases to a predefined reference model file, obtained from 270 healthy individuals (HapMap collection). Results obtained are reported in Table 1. We found 13 somatic gains not in overlap with known CNVs deposited in the Toronto Database of Genomic Variants. The only recurrent somatic CNV (2/8 patients) was a gain of 109kb in 7q22.1, where genes MGC57359 and GATS map. Five recurrent germline CNVs have been detected, both at diagnosis and remission samples, which could represent regions determining susceptibility to AML. A trisomy 13 case showed a whole chromosome somatic LOH at chromosome 21. 3/8 patients had an interstitial somatic LOH in 19q13.12 in correspondence with adhesion molecules genes (CEACAM1, MEGF8, PSG 1-6-7, ZNF 526). Finally, we detected an interstitial germline LOH, common to all samples, in 16q22.1, where CBFB (core binding factor beta) gene maps, involved in FAB subtypes evaluated in this study. Although this is an ongoing study, with preliminary results, such genome-wide characterization of sub-microscopic DNA alterations might contribute to the discovery of new markers and target genes, with diagnostic, prognostic or therapeutic relevance.

P057

BCR/ABL FUSION GENE (M-BCR) QUANTITATIVE ANALYSIS USING *IN VITRO* DIAGNOSTIC (IVD) MARKED TESTS: NANOGEN VERSUS IPSOGEN

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Background. In the management of Chronic Myeloid Leukemia (CML) real time quantitative Reverse Transcription PCR (qRT-PCR) has become the standard method for detecting BCR/ABL fusion gene level. Quantitative real time PCR standardization has been implemented according to European Against Cancer Program (Gabert, Leukemia 2003). Currently, high quality tests, In Vitro Diagnostic (IVD) marked, are commercially available as Nanogen and Ipsogen methods. *Aim*. The aim of this study was to understand how our previously achieved results using Nanogen method could be in agreement with Ispsogen ones, since the latter method includes ABL standard curve currently used in the Italian CML Network quality controls. The main difference between Nanogen and Ipsogen tests concerns the ABL standard curve. *Methods*. We analyzed 40 patient samples with both methods using Applied Biosystem Platform 7500 Fast. Sample preparation and quantitative analysis were performed according to the Italian CML Network Protocol and following manufacturer's indications. We compared the results from the two IDV tests in terms of M-BCR copies, ABL copies and the relative M-BCR/ABL ratio. *Results.* In order to verify the fitting of the two series of tests, results were plotted and data analysis were performed. Nanogen vs Ipsogen data (in terms of median Nanogen/Ipsogen ratio) highlighted the following preliminar statistical results: 1. M-BCR copies showed a median ratio value of 1.16+0,570 (SD). 2. ABL copies showed a median ratio value of 0.629+0.205 (SD). 3. M-BCR/ABL ratio showed a median ratio value of 1.93+0.861 (SD). *Conclusion.* Taking into consideration the small size samples of this analysis we could, however, hazard some considerations. We detected in most patients a satisfactory correspondence between Nanogen and Ipsogen data. Interestingly, we observed the best fitting between M-BCR levels while a less evident agreement was found between ABL levels, leading to a final M-BCR/ABL ratio displacement (due to the higher ABL levels usually detected by Ipsogen method). Therefore, after the introduction of a new method in monitoring CML patients biologists and haematologists should be aware of quantitative system analysis differences.

P058

THE AUTOMATED COUNTS OF THE IMMATURE GRANULOCYTES

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The immature cells of the myeloid series such as metamyelocytes, myelocytes, and promyelocytes are immature granulocytes (IG). The IGs, normally absent from peripheral blood, are clinically useful to recognize many conditions such as bacterial infections, acute inflammatory diseases, surgical and orthopedic trauma, sepsis, acute transplant rejection, cancer (particularly with marrow metastasis), tissue necrosis, myeloproliferative diseases, steroid use and pregnancy. Microscopic IG counts have limits of imprecision and lack clinical sensitivity because these components are usually found in low concentrations (lower than 10 per cent). Conventional blood cell counting instruments can enumerated only the WBCs. The Extended Differential Count (EDC) is the counting of other cell types in addition to the 5 leukocyte populations normally present in peripheral blood, a possibility offered by analysers. Currently, the cell types included in the EDC are immature or atypical cells such as blasts, IGs, atypical lymphocytes, haematopoietic progenitor cells (HPCs), and NRBCs. The principal aims of the EDC are to further reduce the need for microscopic revision, to obtain more precise and accurate counts for rare populations with respect to microscopic count, and to allow for differential counts on material with a more complex cell composition, such as marrow blood. Our study aims to assess the new technologies that count the IGs, also considering their dysplastic features. Imprecision and accuracy between the different methodologies in comparison with the manual morphology count were evaluated. At the IG cut-off of over 3 per cent, the counting of immature granulocytes showed a good specificity for all analyzers (from 88 to 100 per cent) while sensitivity was very different, depending on the technology and software used for the analysis of events (from 12 to 82 per cent). Previous studies agree that IG counts have a high specificity for infectious conditions but are accompanied by low sensitivity. This low sensitivity does not allow to use the automated IG counting for the purpose of screening or early infection detection, while the clinical use in the evaluation of therapeutic response (e.g. to antibiotic therapy) seems more appropriate. At present, IG counting should not appear in the haematological report and must be used as a benchmark instrument-specific decision-making rules for reviewing or to be used in interpretative reporting.

P059

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) POLYMORPHISMS IN PATIENTS TREATED WITH INTERMEDIATE-HIGH DOSE OF METHOTREXATE

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Association with MTHFR polymorphisms and toxicity or outcome of disease was investigated in haematological patients treated with methotrexate, with no univocal results. We retrospectively studied 12 patients (M: 8/ F: 4, mediane age 45 yrs, range 31-60), affected by Non Hodgkin Lymphoma (3 Mantle cells, 3 Burkitt and 3 DLBC with CNS localization, 1 Anaplastic T cells, and 1 Lymphoblastic T Lymphoma) and one Acute Lymphoblastic Leukaemia, submitted to different chemother-

apeutic regimens, all containing intermediate-high dose of MTX for a total of 29 courses. We divided patients in two groups:group A submitted to intermediate dose of MTX (1-2 gr/m^2); group B with high dose of MTX (3-5 gr/m^2). In all patients, MTX were subministered in 24 hours infusion and folinic acid rescue were performed at standard dosage. We considered in particularly hepatic toxicity and incidence of mucositis, in association with delayed elimination of MTX. All patients were tested for MTHFR polymorphisms C677T and A1289. Group A: 5 patients, for a total of 13 courses. In 10/13 courses (76%), we have a delay in MTX elimination (>72h); hepatic toxicity of grade II-III were recorded in 4/13 courses (31%), mucositis in 7/13 (54%), 5 of grade I, 1 of grade II, 1 of grade III. Two patients showed a very high delay in MTX elimination (>120 h), with mucositis of grade II without hepatic toxicity. Four patients, who received an association with ARA-C, showed grade III-IV of haematological toxicity. Among these 5 patients 3 were mutated for C677T (2 eterozigosis, 1 omozigosis) and 2 were non mutated for both polymorphisms. The two patients with higher toxicity were not mutated. Group B: 7 patients for a total of 16 course. A delay of MTX elimination was seen in 14/16 (87%), hepatic toxicity in 5/16 (31%), 1 of grade III, 1 of grade II, 3 of grade I, mucositis in 9/16 (56%), 6 of grade I, 1 of grade II, 2 of grade III. In 6 out of 14 delayed MTX courses we recorded a very high delay (>120h) with hepatic toxicity in 3/6 and mucositis in 6/6 courses. Among these 7 patients, 2 showed C677T eterozigosis, and 1 A1289 omozigosis and 4 showed both mutations in eterozigosis. We were not able to find any correlation between toxicity and MTHFR polymorphisms in this subset of patients. In 6 out of 7 patients grade III-IV of haematological toxicity was seen: among these 6 patients, 5 were treated with an association with ARA-C, while one patient received only MTX.

P060

ERYTHROBLASTS: ANALYTICAL PERFORMANCES AND CLINICAL RELEVANCE

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Detection and count of erythroblasts (NRBC) is still an open question for automated haematology. NRBCs interference can affect blood cell counts as well as leukocyte differential counts. The presence or the persistence of NRBCs in peripheral blood of adults is a remarkable indicator of pathology. NRBC counting can provide relevant diagnostic and prognostic informations for clinicians and laboratory in many clinical conditions, and in diagnostic areas such as critical care, paediatrics, oncology and haematology. The improvements in blood cell counters technology has produced more accurate and precise counts giving to professionals new diagnostic tools. At present time several analytical methods for NRBCs detection and counting are available, based on fluorescence, scatter, nuclear density, conductivity and cytochemistry staining. An evaluation, promoted in 2008 by Haematology Study Group (GdS-E) of Italian Society of Laboratory Medicine (SIMeL), enrolled a large number of patients with known pathology and a wide range of NRBC concentration. All samples have been processed with five top performance haematology analyzers (Abbott Cell Dyn Sapphire, ABX Pentra 120 DX, Beckman Coulter LH 750, Siemens Advia 2120 and Sysmex XE 2100). The aim was to evaluate clinical sensitivity and analytical performances in samples with a wide range of erythroblasts concentrations and known pathology. Blood smears were obtained from each sample and 800 cells have been counted by 4 experienced morphologists. Positivity threshold was set at 1 erythroblast per 800 cells (0.125%). Preliminary results indicate that erythroblast counting performs better than NRBC flag alone, which has more sensitivity than specificity. At extremely high and low NRBC concentrations technology limits become evident and therefore, flag sensitivity set by manufacturers hardly affects sensitivity and specificity performances. Fluorescence based methods show a better correlation among them than non-fluorescence based methods. Future evolution of automated haematology devices should move toward a routine integrated NRBC count to provide positive outcomes for both clinician and patients, acceptable and a further reduction in the need of smear review rate.

P061

CLINICAL VALUE OF INSTRUMENTAL SEPSIS HAEMATOLOGICAL PARAMETERS

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Sepsis presents several different symptoms and signs for cellular events and humoral factors. Changes, activity, expression, release depend on variables as pathogenic agents, genetic susceptibility, coexisting conditions. Few innovative diagnostic markers are found to provide sensitive and specific tests in useful time, so inadequate treatment for delayed diagnosis can affect this status. CBC and leukocyte differential are useful to reach real time clinical goals in sepsis. The diagnostic value of old and new haematological parameters, and their clinical and timely correct contribute to managing in septic patients are here discussed. 44 CBC K2EDTA samples from 31 in-patients, 18 male, 13 female, 32-88 years aged (mean 70), with medical, surgical, different stage/degree sepsis, from Intensive Care Units are tested. For 8 patients a 2 weeks follow up, intra-day and day by day, was scheduled and, among the other tests, new full-automated haematological parameters from 5 top counter using different analytical principles were evaluated in sepsis condition. Results: a) good CBC performance and correlation between flow cytometric and counter integrated CD64; b) usefulness of IG per cent and amount, better performing if fluorescence supplied; c) interesting flags and functional parameters immaturity/dysplasia sepsis related; d) still poor monocytes automation performance if microscopy referred; monocytes counting and function are more accurate by flow cytometry; e) contribute in platelet count and MPV accuracy. In the very common sepsis low counts, with several cellular fragments and microparticles different from platelet circulating, performing CD 61-41 can be a real added value. New functional IPF (Immature Platelet Fraction RNA enriched) could measure in real time the new marrow platelet production without invasive problematic action; Mean Platelet Mass (MPM) gets same value. f) NRBC released in peripheral blood, a negative prognostic sign, and their automatic counts are useful and reliable, over all if fluorescence supplied. According to literature, reported data show today contribute of automated haematology as analytical accuracy, new parameters, technological syncretisms, positive time consuming. Information about IG and NRBC all together in the same sample are also an added value, useful in septic patient's clinical evaluation and managing. CBC is still crucial in clinical and laboratory pathways built upon cell morphology and functional evaluation.

P062

MONOCYTES BETWEEN REACTIVITY AND DYSPLASIA

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Monocytes represent 5-10 per cent of peripheral blood leukocytes. They originate from a myeloid precursor in the bone marrow, are released in the circulation and then enter tissues. The half-life of monocytes in blood is about 3 days, then they may continuously repopulate macrophage or dendritic cell (DC) populations to maintain homeostasis. Circulating monocytes are divided in two subsets on the basis of the expression of CD14 and CD16. The *classical* CD14hiCD16- monocytes are large, near 18 micrometer in diameter, and represent about 80-90 per cent of circulating monocytes. In contrast the 'nonclassical' CD14low-CD16+ are smaller, 14 micrometer in diameter, and constitute about 10 per cent of circulating monocytes. Additional, albeit smaller, monocytes subsets can also be distinguished by surface molecule expression. A population of CD14, CD16 and CD64 has been reported. These cells seem to combine characteristics of monocytes and DCs. Compared with $CD14^{hi}CD16^{-}$ classic monocytes (which are also $CD64^{+}$); these $CD14^{+}CD16^{+}CD64^{+}$ cells have a similarly high phagocytic activity. Although the origins of this subset are not known, it could be an immunoregulatory monocyte phenotype or an intermediate phenotype between monocytes and DCs. Another small subset, constituting about 1-2 per cent of mononuclear cells, expresses CD56, a neural cell adhesion molecule isoform. The frequency of CD $16^{+}CD56^{+}$ monocytes is increased in patients with inflammatory bowel disease. A considerable increase in the number of the $CD14^{+}CD16^{+}$ monocytes had been described for a variety of systemic, infectious agents in humans, bacterial sepsis. A diagnosis of CMML requires persistent peripheral monocytosis (more than $1.0x10^{9}$/L) and myelodysplasia or, if dysplasia is not evident, cytogenetic abnormalities or exclusion of other causes of persistent monocytosis (equal or grater than 3 months). In our tests some discrepancies between instruments in monocyte counts were observed; these may indicate that the different technologies do not always recognize the same type of cell and it may be dependent on the difficulty in identifying monocytes consequently their heterogeneity (size, nuclear morphology, granularity, and functionality). The reference method for monocyte count could be improved by using a flow cytometry. The reliable distinction of dysplasia from reactive monocytosis can be challenging. Additional diagnostic tools to aid in this differential diagnosis would be highly desirable.

Myeloma I

P063

BORTEZOMIB, HIGH-DOSE DEXAMETHASONE AND LOW-DOSE ORAL CYCLOPHOSPHAMIDE FOR RELAPSED-REFRACTORY MULTIPLE MYELOMA IN "VERY" ELDERLY PATIENTS: EFFICACY AND TOXICITY

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Introduction and Aims. MP-THAL represents the reference treatment for previously untreated elderly patients with multiple myeloma (MM). Unfortunately, the combination chemotherapy-thalidomide induces neutropenia and thrombocytopenia, and infections represent an important complication; grade 3 to 4 infections are reported in 16% patients. We have examined the anti-MM activity and toxicity profiles of the combination bortezomib (V), dexamethasone (D) and cyclophosphamide (E) in a small cohort of "very" elderly patients with relapsed or refractory MM. *Patients and Methods.* This study has been performed with the informed consent of patients. Eighteen very old patients (median age 76 years [range 73-84]), 10 female and 8 male, with relapsed-refractory MM after a median of 2 therapies (range 1-4) were enrolled to receive six-eight four-week treatment cycles with bortezomib, high-dose dexamethasone and low-dose oral cyclophosphamide. Bortezomib was given according to conventional scheduled treatment (1,3 mg/m^2 on days 1, 4, 8, 11); dexamethasone 20 mg was given on days 1 4 and 15 18; cyclophosphamide 50 mg PO was given daily for 21 days. A maximum of 6-8 cycles was planned. Every new cycle was allowed if the neutrophil count was $>1x10^{9}$/L and platelet count $>50x10^{9}$/L. In addition, all patients received prophylaxis antiviral, trimethoprim-sulfamethoxazole and oral non-absorbable antifungal medication. Response was defined according to IMWG criteria. *Results.* All eighteen patients (100%) completed at least two cycles of therapy and were evaluable for response. Three patients (17%) interrupted prematurely the treatment for WHO grade 4 neuropathy (peripheral sensory and motor neuropathy). Fifteen patients (83%) completed the planned 6-8 cycles. No patient died during treatment.

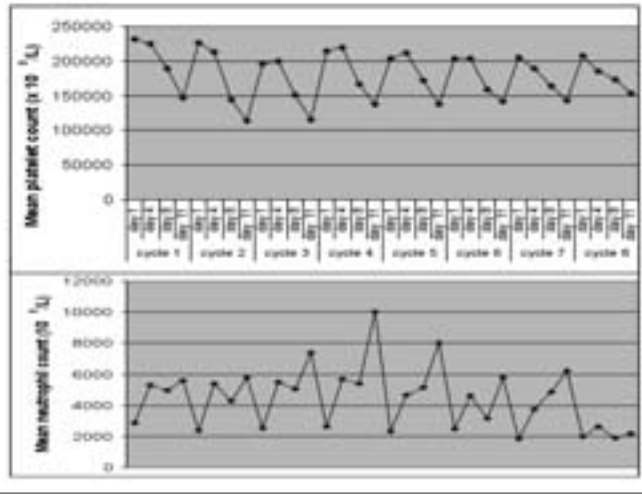

Figure 1.

Overall toxicity was acceptable and predominantly non-haematologic. The mean neutrophil count at the start of each cycle was $3.0x10^{9}$/L (range 1.7-5.4); neutropenia $<1x10^{9}$/L was not documented. The mean platelet count at the start of each cycle was $215x10^{9}$/L (range 69-522). WHO grade 2 thrombocytopenia was observed in 3 patients (17%); thrombocytopenia appeared prevalently on days 8 and 11; platelet count did not require a delay of the treatment or dose reduction of drugs; the transient reduction of platelet count returned to baseline during the recovery period of each cycle. The dose of dexamethasone was reduced in 3 patients (17%) for personality changes. The dose of bortezomib was reduced in 3 patients (17%) for the occurrence of WHO grade 2 neuropathy. Only one patient underwent a schedule modification to weekly administration of bortezomib because of severe orthostatic hypoten-

sion and two episodes of syncope. WHO grade 1-2 diarrhoea was observed in 5 patients (28%). Less common side-effects, which did not require any interruption of treatment or dose reduction, included hyperglycaemia, oedema of the lower limb, insomnia. Not a single episode of infections was seen during the study. In our experience VDE produced a rapid reduction of M protein within the first/second month. ORR (> PR) was 83% (15/18 patients); namely, 2 patients (11%) achieved a CR, 11 (61%) a VGPR, 2 (11%) a PR and 3 patients (17%) were NR. Responses were independent of β2microglobuline, albumine, disease stage according to Durie and Salmon, age, and number of prior therapies. Six out of 15 patients relapsed. Median time to progression was 9 months (range 3-31). *Conclusions*. The anti-myeloma activity (ORR 83%) and the favourable toxicity profile similar to that seen in the total population suggest that this combination approach is active and tolerable also in "very" elderly patients with refractory/relapsed MM and that "very" old patients might benefit most from bortezomib-based regimens, too.

P064

ESTABLISHMENT OF AN INTERLEUKIN-6 INDEPENDENT VARIANT (CMA-03/06) OF THE HUMAN MYELOMA CELL LINE CMA-03: BIOLOGICAL AND MOLECULAR CHARACTERIZATION BY A GENOMIC INTEGRATIVE ANALYSIS

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Background. Interleukin-6 (IL-6) has been identified as the most important growth and survival factor for multiple myeloma (MM) cells. The novel CMA-03/06 human myeloma cell line is an IL-6-independent variant of CMA-03/06, previously established in our laboratory. Aims and methods. To perform a biological and molecular characterization of the new cell line by means of standard procedures, and to provide insights into the signaling pathways and target genes involved in the growth and survival of CMA-03/06 using an integrative genomic analysis involving both gene expression and genome-wide profiling approaches. *Results.* The addition of IL-6 to the culture medium of CMA-03/06 cells or coculture with multipotent mesenchymal stromal cells didn't induce an increase in their proliferation. The immunophenotypic analysis revealed that CD45 expression was considerably reduced in CMA-03/06 compared with CMA-03 cells, whereas they were found positive for both chains of IL-6 receptor, CD126 and CD130, almost undetectable in CMA-03 cells. IL-6 could not be detected in the supernatants from either CMA-03 or CMA-03/06 cell lines within 48 h using a high sensitivity IL-6 specific ELISA. Nevertheless, western blot analysis revealed the IL-6 induced activation of signal transducer and activator of transcription 3 (STAT3) and STAT1 in both cell lines. Global gene expression profiling analysis of CMA-03/06 compared with CMA-03 cells allowed the identification of 21 upregulated and 47 downregulated genes, many of which particularly relevant for MM biology, that are mainly involved in cellular signaling, cell cycle, cell adhesion, cell development, regulation of transcription, immunologic, inflammatory or defense activity, and apoptosis. Comparison of genome-wide profiling analysis of CMA-03/06 and CMA-03 cells evidenced a different copy number in only 15 small chromosomal regions. None of the genes differentially expressed in CMA-03/06 compared with CMA-03 except one were positioned on these regions. Conclusions. Our data confirm the IL-6 independence of CMA-03/06 cell line and the absence of an autocrine IL-6 loop, even though the cells maintain the IL-6 signaling pathway responsiveness. The novel CMA03/06 cell line may represent a suitable model for studies investigating molecular mechanisms involved in clonal evolution towards IL-6 and/or stroma-independent growth and survival of myeloma cells.

P065

AGGRESSIVE THERAPEUTICS APPROACH INCLUDING IN HOME ASSISTANCE CHEMOTHERAPY IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is a B-cell neoplastic disease characterized by bone marrow infiltration by malignant plasma cells which secrete monoclonal immunoglobulin fragments. Although several parameters such as β-2-microglobulin, serum creatinine, haemoglobin, calcium levels or cytogenetics abnormalities have been considered as predictive factors of the outcome of patients (pts) with MM, the molecular features of this disease remain still unclear. Consequently therapy for MM remains empiric. New agents have improved the possibility of obtaining disease response and better quality and duration of responses. Although response to therapy traditionally has not been considered a good predictor of long-term outcome, most recent studies suggest that response is a surrogate of improvement in survival. Here, we want to show that, despite relapse and therapy-toxicity, pts with MM must be always treated after relapse or in presence of progressive disease, and if possible also in home assistance (HA) chemotherapy, especially elderly ones, because survival increases in presence of PR, VGPR or SD rather than in presence of PD. We report a retrospectively analysis of 181 pts (94 F, 87 M, median age 58 ys) diagnosed with MM in our institution from 1980 to 2007. Overall survival (OS) was influenced significantly by ISS (1 > 3 and 2 >3; and 1+2 >3), renal impairment (cr >1.5 micrograms/deciliters), β-2 microglobulin (>3.5 micrograms/deciliters), LCh-MM vs not LCh-MM, and haemoglobin <10 grams/deciliters, autologous or allogeneic transplamtation ; bone lesion or fractures, isotype kappa/lambda, precedent MGUS and the presence of Amiloid AL didn't influence survival. Pts who achieved PR or VGPR after first line therapy (47%) had an OS longer than pts in progressive disease (PD) (31.5%) or stable disease (SD) (21.5%), as pts with PR + VGPR vs PD; also after second and third line therapy. OS of pts in HA chemotherapy was 13 months from the moment of enrollment in home assistance chemotherapy. We treated 35 pts in HA chemotherapy and the median survival (ms) was 52.2 months vs 36 months of pts not in HA chemotherapy, matched for the same characteristics. 18 pts of these are still alive (ms 65.7 months vs pts not in HA chemotherapy ms 47.5) but our data are still limited and the comparison between the two groups doesn't achieve statistical evidence(p=0.07). We can only suggest that pts with PD or relapse must be treated also in HA chemotherapy, if elderly, to achieve at least a SD.

P066

IMMUNOHISTOCHEMICAL EVALUATION OF SARCOGLYCANS AND INTEGRIN IN GINGIVAL EPITHELIUM OF MULTIPLE MYELOMA PATIENTS WITH BISPHOSPHONATE-INDUCED OSTEONECROSIS OF THE JAW

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Osteonecrosis of the jaw (ONJ) is an adverse outcome associated to bisphosphonate (BPs) treatment. It is not known whether the ONJ lesion originates in the bone, or whether it may initiate in the oral mucosa. Samples of gingival epithelium were obtained from patients treated with zoledronate which no showed the ONJ and from patients treated with BPs which showed the ONJ. These samples were compared with gingival epithelium of control subjects which had undergone odontoiatric surgery for other reasons. Patients treated with BPs were affected by Multiple myeloma. Immunohistochemical study was made using confocal laser scanning microscopy. The triple immunofluorescence reac-

tion, performed between type IV collagen, integrin, and epsilon-sarcoglycan antibodies, in control subjects, showed a clear normal pattern of all proteins in basal lamina; besides, any vascular structure was detectable while the epithelial side was not detectable for these proteins. The fluorescence analysis of same proteins in samples of subjects treated with BPs that no showed lesions showed an almost absence of protein staining patterns in correspondence of basal lamina, but clearly detectable staining for all tested proteins in correspondence of vascular structures that are numerous. In ONJ subjects, we observed an increase of protein staining patterns on basal lamina and an increase of vessels below the basal lamina. Triple fluorescence reaction performed with laminin, α-sarcoglycan, and β-sarcoglycan in control subject showed a normal staining patterns of all proteins with a clear fluorescence on basal lamina. In BPs treated subjects that no showed lesions, basal lamina fluorescence was absent whereas the vessels were clearly detectable. In ONJ subjects it was possible to highlight newly an increase of staining patterns on basal lamina and a massive increase of vascular structures. Triple fluorescence reactions using vinculin, integrin, and gamma-sarcoglycan antibodies showed the same behaviours. Finally, in the samples treated with BPs showing lesion, it was detectable an increase of staining pattern both in the basal lamina and in vessels. The increase of these proteins in basal lamina, in concomitance with formation of the lesion, could indicate a compensative behaviour in the remodelling of the gingival mucosa in order to restore the epithelial architecture. The neoangiogenesis, unexpected feature during BPs treatment, could confirm this compensative role of tested proteins.

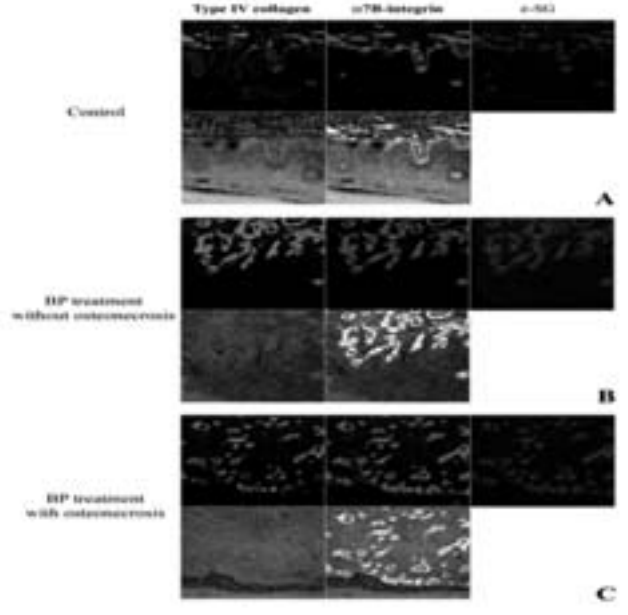

Figure 1. Compound panel showing immunohistochemical findings in human gengival epithelium immunolabeled by a triple fluorescence reaction, with type IV collagen (green channel), α7B-integrin (red channel) and β-sarcoglycan (blue channel). The gingival epithelium was analyzed in control subjects (A), in BPs treated subjects whitout ONJ (B) and in ONJ patients (C). For each reaction it is possible to analyze the single protein separately, the transmitted light and the merge beetween all channels.It was visible the almost complete absence of tested proteins on basal lamine in subjects without ONJ, and a new increase of the same proteins in ONJ subjects.

P067

CXCR4 AND CD43 CO-EXPRESSION IN PLASMA CELL MALIGNANCIES

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Bone marrow (BM) microenvironment has been envised as a "trap" for circulating multiple myeloma (MM) precursors, which expand and differentiate to malignant plasma cells within the BM, invading the peripheral blood only in the terminal stage of the disease, i.e. plasma cell leukemia (PCL). While several adhesion molecules have been identified that promote the binding of malignant plasma cells to BM accessory cells and extracellular matrix (i.e. CXCR4), little is known on molecules able to prevent such interactions, by eventually favoring the escape of tumor cells from the BM "trap". Previous studies have indicated that the CD43 sialoglycoprotein functions as an anti-adhesion molecule by providing a repulsive barrier around cells due to its extended conformation and negative charge of sialylated residues. We investigated CXCR4 expression and its association with the expression of the CD43 sialoglycoprotein on bone marrow plasma cells of MM and PCL patients. We have analyzed the bone marrow blood of 45 patients with MM and 8 with PCL. 29 out of 45 MM patients presented a IgG component, while the remaining 16 were IgA. On the basis of the staging criteria, 24/45 patients were in stage I-II and 21/45 in stage III. We evaluated CXCR4 and CD43 expression by flow cytometry. Mean fluorescence intensity ratios (MFIRs) were calculated by dividing the mean fluorescence intensity for CXCR4 and CD43 by the mean fluorescence of the respective nonspecific isotype control. 24/45 MM patients (stage I-II) showed high CXCR4 expression (median MFIR 15.2; range: 10.3-50.4) while 21/45 (stage III) showed lower CXCR4 expression (median MFIR 6.5; range: 5.1-9.5). All MM patients were CD43 negative. By contrast all PCL patients were CD43 positive (MFIR 7.8; range: 6.2-10.1), while CXCR4 expression was like MM stage III patients group (MFIR 6.2; range: 4.8-8.9). Our results that malignant plasma cells from MM lack surface CD43 as opposed to PCL in which CD43 expression was found at a high cellular density, indicate that CD43 expression may be related to the biological and clinical progression of MM. Moreover the possible prognostic role of CXCR4 in MM warrants further clinical investigation on a larger series of patients even on the basis of future therapeutic strategies.

P068

GLOBAL HYPOMETHYLATION OF REPETITIVE DNA ELEMENTS IS ASSOCIATED WITH TUMOR PROGRESSION IN MULTIPLE MYELOMA

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Multiple Myeloma (MM) is characterized by a wide spectrum of genetic changes. Global hypomethylation of repetitive genomic sequences such as long interspersed nuclear elements-1 (LINE-1), Alu and satellite α (SAT-α) sequences has been associated with chromosomal instability in cancer. Methylation status of repetitive elements in MM has never been investigated. In the present study we used a quantitative bisulfite-PCR pyrosequencing method to evaluate the methylation patterns of LINE-1, Alu and SAT-α in 23 human myeloma cell lines (HMCLs) and purified bone marrow plasma cells from 53 newly diagnosed MM patients representative of different molecular subtypes, 7 plasma cell leukemias (PCLs), and 11 healthy controls. MMs showed a decrease of Alu (median: 21.1% 5 mC), LINE-1 (70.0% 5 mC) and SAT-α (77.9%5 mC) methylation levels compared with controls (25.2%5 mC, 79.5% 5 mC and 89.5% 5 mC, respectively). Methylation levels were lower in PCLs and HMCLs (16.7 and 14.8% 5 mC for Alu; 45.5 and 42.4% 5 mC for LINE-1; and 33.3 and 43.3% 5 mC for SAT-α, respectively) compared with MMs. In addition, the LINE-1, Alu and SAT-α DNA methylation patterns were investigated in the context of the different clinical and molecular MM subtypes. Notably, LINE-1 and SAT-α methylation was significantly lower in nonhyperdiploid than hyperdiploid MMs (p=0.01 and 0.02, respectively according to Wilcoxon Rank test), whereas Alu and SAT-α methylation in MMs with t(4;14) was significantly lower than in patients without this lesion (p=0.02 and 0.004, respectively). Finally, we correlated methylation patterns with DNA methyltranferases (DNMTs) mRNA levels showing in particular a progressive and significant increase of DNMT1 expression from controls to MMs, PCLs and HMCLs (p<0.001). Our results indicate that global hypomethylation of repetitive elements observed using a quantitative method is significantly associated with tumor progression in MM and may contribute toward a more extensive stratification of the disease.

P069

A NOVEL FISH-BASED NORMALIZATION PROCEDURE FOR WHOLE GENOME MICROARRAYS DETECTING ACCURATE LOCAL COPY NUMBERS: APPLICATION TO MULTIPLE MYELOMA

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The introduction of mapping arrays have significantly contributed to understanding of genomic disorders, providing high-resolution profiles of DNA copy number (CN) aberrations. However, CN inference using available conventional analysis procedures lacks to identify the correct ploidy whenever the median CN value of the analyzed profile differs from normal ploidy, as in the case of marked aneuploidy found in most of human myeloma cell lines (HMCLs) and in a significant fraction of primary tumors (MM). In order to robustly normalize genomic profiles and correctly infer local CNs, we developed a FISH-based normalization (FBN) algorithm, which allows to estimate the scaling factor to normalize the raw CNs of the entire array to the exact nominal multiplicity values. We generated the profiles of 25 HMCLs and 45 MM samples (profiled on GeneChip Human Mapping 250K NspI and 50K XbaI arrays, respectively). For each sample, FISH data of 12 chromosomal loci critically involved in MM were collected; of these, complete and unambiguous information (i.e. FISH probe showing the same number of signals in >90% of highly purified plasma cells in the whole dataset) was found for 4 probes, namely mapped to 1p31.3, 4p16.3, 13q14.3 and 16q23.1-23.2. For each sample, using a k-means based algorithm, we determined all the clusters generated by the frequency distribution of the array values. Next, we linked them to the exact inferred local CNs based on ploidy assessed by FISH and subsequently normalized the entire profiles. Finally, for both HMCLs and MM datasets we determined accurate thresholds corresponding to different CN values. To validate the obtained FBN-corrected profiles, the entire normalization procedure was repeated using each of the four mentioned probes: as such, the most part of the available FISH data were correctly recognized (93.3% and 93,2% accuracy in HMCLs and MM datasets, respectively). Notably, the normalization allowed the identification of a significant fraction of MM samples (15%) showing marked aneuploidy (i.e. near-tetraploidy), not detectable by conventional normalization procedures. The novel FBN procedure for the detection of the real multiplicity of local CN alterations, robustly validated on MM and HMCLs datasets, may provide an important contribution to better define the genetic complexity of the disease and thus reinforce integrative genomics analyses for the identification of novel candidate tumor-associated genes in MM.

P070

GLOBAL MICRORNA EXPRESSION PROFILING IN MULTIPLE MYELOMA IDENTIFIES DEREGULATED PATTERNS ASSOCIATED WITH HIGH-RISK GENETIC GROUPS

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The recent discovery of microRNA (miRNA) genes, encoding for a class of small non-coding RNAs involved in the regulation of cell cycle, survival and differentiation programs, has added a further level of complexity to normal and cancer cell biology. To date only little evidence of miRNA expression/deregulation in multiple myeloma (MM) has been reported. To characterize miRNA expression profiling of MM plasma cells (PCs) and integrate miRNA expression data with other molecular features of MM patients, global miRNA expression profiles of PCs isolated from bone marrow biopsies of 38 newly diagnosed MM, 2 plasma cell leukemia patients, and 3 healthy donors were generated using the Agilent Human miRNA microarray V2 (representing 723 human mature miRNAs from the Sanger miRBase v10.1). All of the patients had previously been characterized by FISH for the main IGH translocations and other genetic abnormalities; they were also profiled for global gene expression by means of Affymetrix U133A arrays. An unsupervised analysis of the samples based on the most variably expressed miRNAs across the dataset grouped the PCs from healthy donors separately from MM PCs; among the pathological samples, the most striking finding was that the seven patients with t(4;14) (TC4) were tightly clustered, as were four out of the five samples with translocated MAF genes (TC5). A partial grouping of the TC2 cases (mostly hyperdiploid) was also observed, whereas the TC1 (showing t(11;14)) and TC3 (mostly expressing Cyclin D2) samples were dispersed along the dendrogram. A SAM multiclass supervised analysis of the miRNA expression between the members of the 5 TC groups highlighted specific miRNA signatures, in particular characterizing the TC4 and TC5 groups (upregulation of miR-34b*, miR-150, miR-1, miR-155, miR-133a, miR-133b, miR-155*). A less consistent miRNA signature was observed in t(11;14) cases and TC2 group, whereas we could not identify any TC3-specific miRNA signature. None of the differentially expressed miRNAs in patients with specific IGH translocations maps to the rearranged chromosomal regions. The expression of some miRNAs was validated by means of quantitative real time RT-PCR, using specific TaqMan® microRNA assays (Applied Biosystems). We are now integrating miRNA and gene expression data to identify experimentally-supported miRNA target genes and to reconstruct cellular networks which may provide insights into the role of miRNAs in MM.

P071

OSTEONECROSIS OF JAW PROPHYLAXIS IN MULTIPLE MYLOMA PATIENTS TREATED WITH ZOLEDRONIC ACID

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Bisphosphonate-associated osteonecrosis of the Jaw is frequently observed in multiple myeloma patients receiving treatment with potent bisphosphonates. Osteonecrosis can develop spontaneously, but appears more frequent after invasive dental procedures. Pathophysiology involves two mechanisms: antiangiogenic effect of bisphosphonates and accumulation of microdamage (observed in animal models) when bone mineralization increases. Between February 2005 and December 2008, a study was performed in our 42 patients (23 male and 19 female patients; median age 73 years, range 53-81) affected by MM, receiving zoledronic acid (4 mg i.v. every 28 days) in association to anti-myeloma therapy, to determine the frequency of occurrence of ONJ when proper prophylaxis is applicated. Every patient had received from a minimum 12 infusions to a maximum 24 infusions of zoledronic acid. All patients were informed of the potential risks and studied for dental evaluation with an accurate visit and orthopantomograms; dental treatment and other oral procedures were completed before initiating bisphosphonate therapy. Patients were instructed on maintaining of a good oral hygiene by chlorhexidine mouthwashes and subjected to frequent check-ups during and after bisphosphonate therapy. The management of ONJ focused on maximizing oral health, conservative actions with mouth rinses, antibiotics, drugs for control of pain and avoidance of unnecessary invasive dental procedures. The median follow-up was 36 months and no of these patients developed ONJ. The manegement of our patients, combined with the literature review, suggest that: 1) clinical dental examination and a panoramic jaw radiography should be performed before patients begin bisphosphonate therapy; 2) dental treatment and other oral procedures should be completed before initiating bisphosphonate therapy; 3) for patients who develop ONJ, conservative, non-surgical treatment is strongly recommended; 4) patients should be informed and instructed on the importance of maintaining good oral hygiene and having regular dental assessment; and 5) the medical community needs to be aware of the association between bisphosphonate usage and ONJ so that unnecessary and harmful surgical procedures can be avoided. The association of the therapeutic use of bisphosphonates and the development of jaw necrosis has to be studied in further investigations. However, the only effective treatment is so far dental prevention before starting treatment.

P072

BORTEZOMIB-BASED THERAPY AS INDUCTION REGIMEN OF AUTOGRAFT INCLUDING PROGRAM IN MULTIPLE MYELOMA (MM) WITH END STAGE RENAL DISEASE

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Renal failure is a common complication of MM. Up to 50% of newly diagnosed pts have renal failure and 9% require dialysis. Pts requiring dialysis have a poor prognosis with decreased response, shorter survival, and early mortality. Autologous stem cell transplant (ASCT) is still the gold standard for young MM *de novo* pts and dialysis should not represent an exclusion criteria, although a reduction of melphalan dose is suggested. There is some evidence that Bortezomib can be safely used in relapsed pts with renal impairment. We evaluated the efficacy and feasibility of Bor-Dex as induction regimen of an autograft program in 5 dialysis pts with newly diagnosed MM. The patient eligibility criteria included a newly diagnosis of symptomatic MM, end stage renal disease, age under 65 years, a WHO performance status 0-3, the absence of cardiac or hepatic dysfunction. BJ proteinuria was available in all pts. Treatment schedule included 4 Bor-Dex cycles, stem cells mobilization and ASCT. Bor was given at dose of 1.3 mg/m^2 on days 1, 4, 8 and 11 of a 21-day cycle, Dex was given at dose of 20 mg on the day of Bor and the day after. Stem cells mobilization was performed by CTX 3 gr/sq.m and G-CSF priming. Autograft was conditioned with melphalan 100 mg/sq.m. The IMWG criteria were used for definition of response; toxicity was graded according to NCI-CTC criteria. At time of this report all pts were available for response to Bor-Dex cycles, 4 have collected peripheral stem cells, 1 have performed ASCT. After the Bor-Dex cycles all pts achieved a major response (2 CR and 3 VGPR). Toxicity was mild and mainly consisted in fatigue (all cases), GI symptoms (2 cases), grade 1-2 PN (3 cases). Four pts mobilized adequate numbers of stem cells; one patient required a second course of mobilization to collect a sufficient number of PBSCs, obtaining a pront bone marrow recovery (ANC >500/microL at day 13 and platelet >20000/microL at day 18) and manageable toxicities, after ASCT. Our experience suggests that a Bortezomib-based therapy is well-tolerated and an effective option as preparatory regimen before ASCT for early MM pts in end stage renal disease. ASCT with low dose of melphalan is an attractive alternative for this subgroup of pts, although further studies are warranted to establish a comprehensive safety and efficacy profile.

P073

STEM CELL MOBILIZATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS AFTER LENALIDOMIDE INDUCTION THERAPY

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Background. Lenalidomide has raised concerns regarding its potential negative impact on the ability to collect stem cells. *Aim.* In the prospective randomized trial RV-MM-PI-209, Lenalidomide and Dexamethasone (RD) were used as induction and Cyclophosphamide (CY) was used to mobilize stem cells. The effectiveness of this approach was determined and compared with an historical control of patients treated at diagnosis with Vincristine-Doxorubicin-Dexamethasone (VAD) and mobilized with the same CY+G-CSF schema. *Methods*. Eighty newly diagnosed myeloma patients received Lenalidomide (25 mg/d for 21 days followed by a 7 days rest period) 28-days cycles in combination with Dexamethasone (40 mg days 1, 8, 15 and 22). Stem cells were mobilized with CY 4 g/m^2 /day, i.v. on day 1 and G-CSF 10 ug/kg/day from day 5 until the end of stem cell collection. A second CY+G-CSF mobilization course was performed in patients failing to collect the minimum of 4 x10^6/kg CD 34$^+$ cells. In the historical control 134 patients received VAD induction and were mobilized with the same CY+G-CSF schema used in the RD trial. The inclusion criteria for patients entering the RD or VAD trial were identical. Results: Baseline patient's characteristics were similar in the 2 groups. Median time from CY to leukapheresis was 12 days (range 8-28) in the RD group and 11 days (range 7-15) in the DAV group (p=.0002). The median days of leukapheresis was slighty superior in the RD patients, 3 (range 1-5) versus 2 days (range 1-3). A second mobilization was performed in 18% of patients in the RD and in 15% in the VAD groups. After the first mobilization course the number of patients who did not collect at least 2 x 106/kg CD34 was 9/80 (11%) in the RD and 22/134 (15%) in the VAD group. At the completion of the mobilization phase 4 patient (5%) in the RD group and 10 patients (7%) in the VAD group did not collect a minimum of 4x10^6/kg CD34 and therefore could not receive the planned autologous stem cell transplant. The median yield of CD34$^+$ cells collected was lower in the RD group (RD: median 10x10^6/kg, range 0-26; VAD: median 14x10^6/kg, range 0-54, p=.00004). *Conclusion*. Induction regimen with RD allowed to collect an adequate number of stem cells to support autologous transplantation in 95% of patients. The median number of CD34$^+$ cells harvested was 10x10^6/kg after RD and 14x10^6/kg after VAD. An update of the first 100 patients enrolled in the RD trial will be presented at the meeting.

P074

MULTIPLE MYELOMA POST-TRANSCRIPTIONAL REGULATORY NETWORK RECONSTRUCTED BY INTEGRATED ANALYSIS OF MICRORNA AND GENE EXPRESSION PROFILES

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Impaired expression of microRNAs (miRNAs) has been shown in both solid and hematological tumours. To date only little evidence of miRNA expression/deregulation in multiple myeloma (MM) has been reported. We have recently investigated global miRNA expression profiles in myeloma plasma cells (PCs) from 40 MM samples representative of the different genetic subtypes using the Agilent Human miRNA microarray V2 (representing 723 human mature miRNAs from the Sanger miRBase v10.1). All the samples have also been profiled with the U133A Affymetrix gene expression chip. Here we present a computational framework combining predictions with miRNA and mRNA expression profiles in MM, under the assumption that, since miRNAs tend to downregulate their targets, expression profiles of miRNAs and real targets are expected to be anti-correlated. The integrated analysis of miRNAs and genes expression profiles was used to infer, from the panel of potential regulatory relations, the network of interactions more probably functional in MM. Application of MiRanda algorithm to an updated database of miRBase miRNA sequences and human ENSEMBL transcripts, predicted 1,524,070 targeting relations, with about 2,000 predicted target genes per miRNA, in average. For each miRNA-gene pair, the Pearson correlation coefficient of respective expression vectors in MM samples was calculated and used as estimator to select functional interactions: by considering genuine miRNA targets only genes included within the top 3% of all anti-correlated pair, 23,123 regulatory relations were scored as trustable and used to reconstruct a miRNAs-genes regulatory network in MM. This bipartite directed network has two types of nodes (690 miRNAs and 6,289 target genes) connected by directed edges, each representing a probably functional regulatory effect of a miRNA to a target gene; the number of target genes per miRNA, ranging from 1 to 431, has

a mean value of 33.5, whereas the average number of miRNAs per gene is 3.7. From the general, huge, MM miRNAs-genes network, three different sub-networks were derived according to the specific patterns of miRNA expression found to be associated with distinct genetic subtypes, in particular patients with t(4;14), t(11;14) or translocated MAF genes.

P075

SURVIVAL AND FORMATION OF OSTEOCLASTS FROM MULTIPLE MYELOMA BONE DISEASE PATIENTS: ROLE OF DECOY RECEPTOR 3 (DCR3)

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Decoy Receptor 3 (DcR3), a member of the TNF receptor superfamily, is known to be involved in cell survival and osteoclast (OC) formation. In this study, we show that malignant plasma cells and T lymphocytes from multiple myeloma (MM) bone disease patients, as well as Karpas 909, a human myeloma cell line, directly produce DcR3. By interacting with FasL this molecule could inhibit OC apoptosis. In fact, the use of a neutralizing anti-DcR3 antibody induces a reduction of cell viability with a consequent increase of apoptotic cell number, the activation of caspase-8 and -3, and DNA fragmentation. Furthermore, we show that DcR3 supports OC formation in samples from MM patients through the upregulation of RANKL and TNF-α by T lymphocytes and only TNF-α by $CD14^+$ cells. In conclusion, our data provide the first evidence of the expression of DcR3 in MM, and the involvement of this molecule in supporting the survival and formation of OCs from MM bone disease patients. The production of DcR3 by T lymphocytes confers these cells a role in the pathogenesis of bone disease associated with MM.

P076

PERIPHERAL NEUROPATHY IN BORTEZOMIB, MELPHALAN, PREDNISONE AND THALIDOMIDE (VMPT) VERSUS BORTEZOMIB, MELPHALAN AND PREDNISONE (VMP): IMPACT OF LOW-DOSE THALIDOMIDE AND WEEKLY INFUSION OF BORTEZOMIB

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Background. Peripheral neuropathy (PN) is the major non-hematologic side effect in patients (pts) treated with VMP in the VISTA trial. According to these data, in 2007, the GIMEMA protocol, VMPT versus VMP in newly diagnosed myeloma pts, was amended: V schedule was modified from twice weekly to weekly administration. *Aims.* To determine the incidence, the characteristics and the risk factors of bortezomib-associated PN from this 2 different V schedules. Methods: Pts (N=511) older than 65 years were randomly assigned to receive VMPT or VMP. Initially, pts were treated with nine 6-week cycles of VMPT (V 1.3 mg/m^2 days 1,4,8,11,22,25,29,32 in cycles 1-4 and days 1,8,22,29 in cycles 5-9; M 9 mg/m^2 days 1-4; P 60 mg/m^2 days 1-4 and T 50 mg days 1-42, followed by V 1.3 mg/m^2 every 15 days and T 50 mg/day as maintenance) or VMP (the same doses and schedules previously described without maintenance). In 2007, the protocol was amended: both VMPT and VMP schedules were changed to nine 5-week cycles and V schedule was modified to weekly administration (1.3 mg/m^2 days 1,8,15,22 in cycles 1-9). *Results*. 221 pts for VMPT and 229 pts for VMP were evaluated. The incidence of PN was similar in the VMPT pts and in the VMP pts: the overall incidence was 47% and 40% (p=0.1), the grade ≥2 was 28% and 25% (p=0.4) and the grade ≥3 was 13% and 13% (p=0.9), respectively. Median cumulative dose to first onset of any-grade PN was 32 mg/m^2 in the VMPT pts and 35 mg/m^2 in the VMP pts (p=0.11). In pts who received weekly infusion of bortezomib, the incidence of all grade PN was 37% in the VMPT pts and 29% in the VMP pts, including 6% grade ≥3 in the VMPT pts and 7% in the VMP pts. The rates of discontinuation and of dose-reduction due to all grade PN were 5% and 21% in the VMPT pts and 7% and 19% in the VMP pts, respectively. In pts who received weekly infusion of bortezomib, the discontinuation and the dose-reduction rates were 3% and 11% in the VMPT pts and 4% and 13% in the VMP pts. The weekly infusion of bortezomib was the only predictive factor of lower incidence of PN (p<0.0001). Thalidomide, diabetes, cardiopathies, age, gender, ISS and Karnofsky status did not affect PN rate. *Conclusion*. The incidence of PN and its relationship with cumulative bortezomib dose were similar to those observed in the VISTA trial. The addition of thalidomide to VMP did not increase the incidence or the severity of PN. The weekly infusion of bortezomib significantly decreased the incidence of PN, the discontinuation and the dose-reduction rate due to PN.

P077

THREE OR FOUR DRUGS FOR TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM): COMPARISON BETWEEN THADD AND THADD-V

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Although bortezomib, bortezomib-doxil and lenalidomide-dexamethasone are currently the standard treatments for relapsed-refractory MM, they produce a median TTP ranging from 6.5 to 11 months. We previously showed that ThaDD produce promising results in terms of good quality response and TTP (Offidani *et al.*, Haematologica 2006). To improve these results, we added bortezomib to ThaDD combination and we compared these 2 regimens in terms of response, TTP and toxicity. Sixty two patients treated with thalidomide 100 mg/day continuously, dexamethasone 40 mg per os on days 1-4 and 9-12 and Doxil 40 mg/sm iv on day 1 every 28 days (ThaDD) were compared with 42 patients receiving the same dose of thalidomide plus Doxil 30 mg/sm iv on day 4, dexamethasone 20 mg on days 1-2, 4-5, 8-9, 11-12 and bortezomib 1.3 mg/sm days 1, 4, 8, 11 every 28 days (ThaDD-V). Due to a high incidence of severe neuropathy in the first 20 patients, the regimen was amended (thalidomide 50 mg/day, bortezomib 1.3 mg/sm days 1, 4, 11). Patients were matched for age, PS, β-2-microglobulin, ISS stage, CRP, creatinine, cytogenetics and refractory disease. In contrast with patients receiving ThaDD-V, those treated with ThaDD had not received prior thalidomide although they had been given more prior lines of therapy. According to the modified EBMT criteria, at least PR was achieved by 71.5% and 86% (p=0.088), VGPR by 36% and 71% (p=0.001) and CRIF- by 36% and 27% (p=0.041) of patients treated with ThaDD and ThaDD-V, respectively. Thirteen percent and 12% of patients progressed while none and 1.5% died early, respectively. After a median follow-up of 32 months, median and 3-years TTP were 17 vs 28 months and 21% vs 41% (p=0.031) in the ThaDD and ThaDD-V group, respectively. Grade 3-4 thrombocytopenia (17% vs 6%; p= 0.091) and neuropathy (2% vs 12%; p=0.026) were more common in the ThaDD-V protocol whereas neutropenia (22% vs 5%; p=0.015), infections (25% vs 12%:

p=0.090) and DVT (14% vs 5%; *p*=0.118) were more frequent in the ThaDD protocol. Drop-out from protocol and bortezomib or thalidomide interruption due to toxicity were similar in the two treatment groups (25% vs 26% and 9.5% vs 8.5% in ThaDD and ThaDD-V, respectively). In conclusion, despite more neuropathy but significantly decreased after protocol amendement, ThaDD-V further improves the good performance of ThaDD in terms of good quality of response and TTP. Moreover, ThaDD-V seems to be much better than current standard therapies.

P078

BORTEZOMIB , PEGYLATED LIPOSOMAL DOXORUBICIN AND DEXAMETHASONE (B-PLD-D) AS THERAPY FOR ELDERLY PATIENTS WITH RELAPSED REFRACTORY MULTIPLE MYELOMA: A WEEKLY BORTEZOMIB INFUSION SCHEDULE REDUCES TOXICITIES MANTAINING THE SAME EFFICACY

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Background. There is a synergism between the proteasome inhibitor bortezomib and anthracyclines. In addition, several *in vivo* data show synergic-additive effect of bortezomib and pegylated liposomal doxorubicin (PLD). Recently these findings were confirmed by the results of a large phase III study. However the majority of patients treated in this study were younger then 65 years old. Nobody so far described the feasibility of this combination regimen in an oldest group of patients. *Methods.* Twenty five patients (F:M 12:13) have been treated: median age 74 years, (range 71-82) with a disease resistant to or relapsed after high or conventional dose chemotherapy. Twenty patients had IgG k/L (15/5); 3 had IgA k/l (2/1), 2 had a Bence Jones k. Treatment started at a median of 21 months from diagnosis (range 2-108). Patient distribution according to disease status and previous therapy was as follows: 9 patients were refractory to the first regimen (primary refractory ,PR); 7 patients had an untreated relapse (UR); 9 a refractory relapse (RR). A median of 2 lines of previous chemotherapy were received (range 1-5), with 10 patients with more then 3 lines of CHT. 3 patients were relapsing after autologous stem cell transplantation (ASCT). 3 patients had a disease previously refractory to antracyclines (VAD therapy), and 8 patients had an extramedullary localization of myeloma. Bortezomib was given 1.3 mg/m^2 as a bolus IV injection on days 1,4,8 and 11 every 3 weeks. After two cycles bortezomib was given weekly on days 1,7, 14,21 with a 10 days rest period until a plateau phase was reached. PegLD was given IV at a dose of 30 mg/m^2 on day 4 every 3 weeks for the first two cycles and then was given on day 7 every 31 days cycle. Desamethasone was given IV 40 mg on days 1-4 every 3 weeks, for the first two cycles and then 20 mg IV on days 1,7,14,21 every 31 days. *Results.* Patients received a median of 4 cycles (range 3-6). B-PLD-D therapy resulted in 20/25 objective responses for an overall response rate (ORR) of 80% . In particular we observed 8CR (33%); 8 VGPR (33%) and 1PR (1%), 3MR (13%), 5 SD (20%). Four patients had bortezomib dose reduction to 1mg/m^2. Median overall survival (OS) was not reached. Median duration of response (PFS) was 8 months (range 1-36). 11/16 (68%) patients with ≥VGPR still maintain a response at median of 12 months (range 3-27) vs 4 months of pts with < VGPR (PFS, OS *p*=0.0001).3/3 VAD refractory obtained a response. 5/8 patients with extramedullary disease responded. Toxicities were mild to moderate in most of the patients and manageable. Grade 3 thrombocytopenia occurred in 3/25 (12%) patients and grade 3 neutropenia occurred in 3/25 patients (12%) but none developed febrile neutropenia. 5 patients (20%) complained grade 1-2 paresthesias, 3 patients (12%) had grade 3 paresthesias. 5 patients (20%) had HSV reactivation. *Conclusion.* B-PLD-D combination is safe and highly effective in resistant-relapsing MM patients over 75 years old. This weekly adapted schedule of Bortezomib can reduce hematological and extrahematological toxicities thus increasing the dose intensity given to patients. Best and more durable responses are seen in patients achieving at least a VGPR. Responses are fast with acceptable and manageable toxicities.

P079

BETA-2 MICROGLOBULIN IS AN INDEPENDENT PREDICTOR OF PROGRESSION IN ASYMPTOMATIC MULTIPLE MYELOMA

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Background. The rationale of the study stems from three considerations: i) to date, few clinical predictors of asymptomatic multiple myeloma (MM) progression are available; ii) no study has assessed the role of β-2-microglobulin (B2M) on risk of asymptomatic-MM progression; iii) B2M is a serum marker of tumor burden/disease kinetics and represents a key variable of the ISS staging system for symptomatic-MM. *Aim.* To assess the impact of B2M on risk of asymptomatic-MM progression. Methods. The study was based on a consecutive series of 148 asymptomatic-MM diagnosed according to the IMWG. Cumulative probability of progression to symptomatic-MM was calculated from diagnosis of asymptomatic-MM to progression to symptomatic-MM according to IMWG. The best cut-off for B2M, percentage of bone marrow plasma cells (BMPC%), serum monoclonal component (sMC) and urinary monoclonal component (uMC) were selected according to Youden's index using progression as state variable. Survival analysis was performed by Kaplan-Meier method using log-rank to test for associations. Cox proportional hazard regression was used to build a multivariate model.

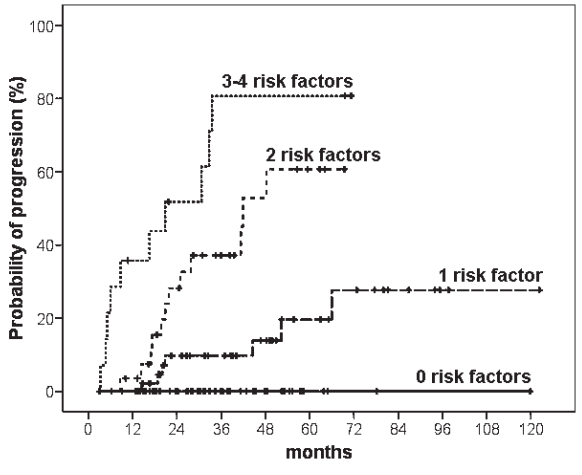

Figure 1.

Results. Clinical features at asymptomatic-MM diagnosis were as follows: median age 67 years, male:female ratio 1.02, previous MGUS 32/148 (21.6%), IgG MC 113/148 (76.4%), IgA MC 32/148 (21.6%), light chain MC 3/148 (2.0%), median sMC 1.1 g/dL, positive urinary immunofixation 27/148 (18.2%), median uMC 98 mg/24h, median BMPC% 15%, median B2M 2.0 mg/L, median albumin 4.3 g/dL, median C reactive protein 0.3 mg/L. After a median follow-up of 36 months, 24/148 asymptomatic-MM progressed to symptomatic-MM, accounting for a 30.5% 5-year probability of progression. According to Youden's index, best cut-off values were 2.5 mg/L for B2M, 1.5 g/dL for sMC, 500 mg/24h for uMC, and 20% for BMPC%. Univariate analysis identified B2M>2.5 mg/L (5-year risk: 64.5%; HR=3.85; *p*<0.001), sMC>1.5 g/dL (5-year risk: 49.1%; HR=5.76; *p*<0.001), uMC>500 mg/24h (5-year risk: 68.7%; HR=6.60; *p*<0.001) and BMPC%>20% (5-year risk: 50.2%; HR=5.77; *p*<0.001) as predictors of progression to symptomatic-MM. Clinical variables not associated with progression to symptomatic-MM (*p*>0.01 in all cases) were age, sex, sMC type, polyclonal Ig reduction, albumin, C reactive protein, Hb, calcium, creatinine, and previous MGUS. Multivariate analysis identified B2M>2.5 mg/L (HR=3.57; *p*=.001) as an independent predictor of progression to symptomatic-MM, along with sMC>1.5 g/dL (HR=4.07; *p*=.003), uMC>500 mg/24h (HR=3.66; *p*=.015) and BMPC%>20% (HR=3.20; *p*=.007). We next combined B2M, sMC, uMC, and BMPC% into a model for predicting progression to symptomatic-MM. This model stratified patients into four

risk groups: very low risk (0 risk factors: 5-year risk 0%), low-intermediate risk (1 risk factor: 5-year risk 19.6%), high-intermediate risk (2 risk factors 5-year risk: 60.7%), and high risk (3 or 4 risk factors 5-year risk 80.7%) (Figure 1). *Conclusions*. The implications of our results are twofold: i) B2M predicts progression of asymptomatic-MM to symptomatic-MM independent of conventional risk factors (sMC, BMPC%, light chain burden); ii) B2M refines the conventional model for predicting progression to symptomatic-MM by allowing the identification of a very low risk group of patients who never progress and a high risk group of patients who are virtually all projected to progress.

P080

SEQUENTIAL THERAPY WITH VAD, BORTEZOMIB AS INDUCTION THERAPY FOR MULTIPLE MYELOMA: A SEQUENTIAL PHARMACOLOGIC COMBINATION TO ACHIEVE COMPLETE REMISSION BEFORE STEM CELL MOBILIZATION

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Background. Multiple Myeloma remains an incurable disease despite intensive therapy such as high-dose Melphalan and autologous stem cell transplantation (ASCT), that remains the gold standard therapy. In multivariate analysis achieving complete remission (CR) before ASCT is a significant, independent variable for Overall Survival and Time to Progression. *Aim.* Following this assertion, in a preliminary and feasibility study, we tried to obtain the best response as soon as possible with a combinated and sequential therapy with VCD and Bortezomib. In fact using VAD or Bortezomib based regimen alone we obtained Complete Remission (CR/nCR) in 15% and 35% respectively ; instead in our opinion their sequential use could permit a better tumor mass reduction and the exploitation of synergic role of Bortezomib in front of chemotherapy. Material and methods From June 06 to April 08 we enrolled 9 patients with untreated MM; median age was 53 years (34-63), M/F was 2/7; the M-protein type was IgG in 3 pts, IgA in 3 patients, while 3 pts presented a Micromolecular Myeloma; the stage was IIA, IIIA and III B in 1, 7 and 1 patients respectively. Cytogenetic data were unfavourable in 3/9 patients. Patients underwent to VCD regimen (Vincristine 1,4 mg/m^2 and Pegylated Liposomal Doxorubicin 35 mg/m^2 on day 1 and Dexametasone 40 mg day 1-4) for 3 cycles (day 1-21); 15 days after last therapy, pts started Bortezomib regimen (1.3 mg/m^2 on day 1,4,8,11) for 4 cycles and 30 days after last dose pts received Cyclophosphamide 4 g/m^2 and G-CSF for stem cell harvesting. Results All patients achieved the partial remission after VCD scheme, while CR was achieved in 8/9 patients post Bortezomib. All pts mobilized stem cells achieving target of CD34$^+$ (10x10^6/kg). Toxicity was low and mainly consisted of neutropenia (WHO grade I-II in 15 % of pts) and mild peripheral neuropathy (WHO ≥ grade II in 33% of pts). *Conclusion.* Since our experience was a preliminary and feasibility study, we think that the sequential therapy with combination of VCD and Bortezomib could be highly effective as up-front therapy in patients with MM; moreover it is associated with low toxicity and results in an excellent stem cell harvesting. After ASCT 8/9 patients maintain CR/nCR, while 1 patient is in PR (median follow-up 16 months, range 2-24 months).

P081

BORTEZOMIB IS AN EFFICTIVE TREATMENT FOR PATHOLOGICAL FRACTURES AND EXTRAMEDULLARY RELAPSE OF MYELOMA AFTER AUTOLOGOUS AND MYELOABLATIVE ALLOGENIC TRANSPLANTATION IN PATIENT TREATED WITH HAEMODIALYSIS

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Bortezomib (B) is a proteasome inhibitor that has an extensive tissue penetration of extramedullary plasmacitoma and it is known to stimulate bone formation in multiple myeloma. We report a case of a 36-year-old man that was diagnosed with stage IIIB IgGk Multiple Myeloma in 2000. He was treated with chemotherapy, bisphosphonates (BPs) and autologous HSCT, followed by Thalidomide and myeloablative allogenic HSCT from his HLA identical sister in 2002. A CR was obtained and 100% of marrow cells were of donor origin. In 2006 the pt complained of dizziness. The cerebral RMI showed a large plasmacitoma extending both sides of the skull vault at the level of the left frontal region. It was completely removed. The chronic renal failure worsened because of cocaine abuse and he started treatment with haemodialysis. Serum paraprotein was detected and marrow biopsy revealed 40% of plasmacells with only 50% of cells being of donor origin. The pt refused any therapy. In January 2008, the pt complained of jaw pain. The CT showed a pathological fracture of right mandible with bone loss. Radiologist executed the embolization of maxillary artery because of continuing of bleeding from the vessel. The skeletal survey showed numerous and extensive lytic lesions. The pt refused the treatment with B and he was treated with thalidomide for 6 months without remission. In August 2008, he started treatment with B at the dose of 1, 3 g/m^2 on days 1,4,8 and 11 after dialysis and desametasone at the dose of 20 mg on days 1-4 and 8-11 every 21 days. From the fourth cycle B was infused on days 1 and 8 for haematologic toxicity (thrombocytopenia grade 4). He did not receive administration of BPs. At the beginning of the first cycle, the pt complained of pelvis pain. The CT scan with 3 dimensional imaging (Figure 1) revealed multiple pathological fractures of the left hip bone with join involvement and bilateral lytic lesions (7x5 cm) with soft-tissue involvement. He underwent urgent radiotherapy to extra osseous involvement from the disease and he started again treatment with B. No other toxicity was reported. At the end of fifth cycle the CT (Figure 2) showed reduction of pathological tissue with fracture consolidation. Serum paraprotein reduced of 70%. This good partial remission has persisted up to now. This report suggests that B is a feasible and safe treatment in pt undergoing haemodialysis and it is an effective approach to consolidate fractures and to reduce extramedullary disease

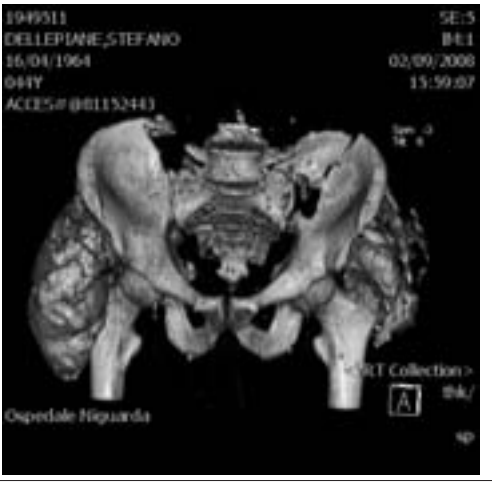

Figure 1.

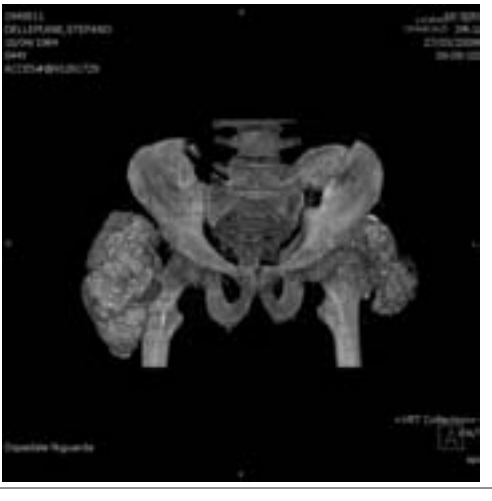

Figure 2.

P082

THE MTOR PATHWAY ACTIVATION PREDICTS SHORT TIME TO PROGRESSION AND OVERALL SURVIVAL IN RELAPSED MULTIPLE MYELOMA PATIENTS

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Purpose. To investigate the prognostic and predictive significance of the phosphorylation status of the mTOR pathway by immunohistochemistry on bone marrow biopsies of symptomatic multiple myeloma (MM) patients. *Methods.* Immunohistochemical analysis with p-AKT, p-mTOR, p-P70S6K and p-4E-BP1 was performed on bone marrow sections of 92 symptomatic MM patients (64 newly diagnosed and 28 relapsed). A value designed HSCORE was obtained for each case multiplying the intensity (0, no staining ; 1,weak staining; 2, moderate staining; and 3, strong staining) with the corresponding percentage of positive plasmacells [HSCORE = (1XPC)]. The median value for each antibody was calculated and considered as the cut-off value. The phosphorylation status of the mTor pathway was correlated with clinical parameters, cytogenetic data and clinical outcome separately in the two groups. *Results.* Twenty-seven out 64 (42.1%) newly diagnosed (group A) and 14 of 28 (50%) relapsed (group B) MM patients demonstrated high p-mTOR staining. In group A, 30 (46.8%), 30 (46.8%) and 28 (43.7%) cases also demonstrated high p-AKT, p-P706SK and p-4E-BP1 expression levels, respectively. A similar pattern was found in group B with 14 (50%), 13 (46.4%) and 15 (53.5%) cases demonstrating high p-AKT, p-P706SK and p-4E-BP1 expression levels, respectively. High p-mTOR staining significantly correlated with its upstream high p-AKT and downstream high p-P70S6K and p-4E-BP1 staining. No difference was found between patients with high and low p-mTOR expression level in both group A and group B with respect to presenting clinical and cytogenetic characteristics. In group B, relapsed myeloma patients with high p-mTOR staining (14 out 28) had a significantly shorter Time To Progression (TTP) (median time 6 vs 14 months p=0.001, HR=5.86) and Overall Survival (OS) (median time 9 vs 16.5 months p=0.03, HR=3.1) when compared with patients who did not. Multivariate Cox regression analysis adjusted for β2-microglobulin serum levels 3.5 mg/L, del 13, del 17 and t(4;14) performed by FISH analysis, confirmed that high p-mTOR expression level independently predicts short TTP in group B both as dicotomic (HR=7.87) and continuous variable (HR=6.03). *Conclusion.* We characterize a subset of MM patients with AKT/mTOR/ P70S6K/4E-BP1 pathway activation. In relapsed myeloma patients, high phospho-mTOR expression level is closely associated with a short TTP that may be related to chemo-resistance.

P083

DASATINIB ENHANCED APOPTOSIS OF RAPAMYCIN IN MULTIPLE MYELOMA CELLS THROUGH DOWN-REGULATION OF THE MTOR PATHWAY BY ABROGATING SIGNALING VIA AKT AND ERK

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Background. Previous studies have shown the in vitro and *in vivo* activity of the mTor inhibitor rapamycin and of the tyrosine kinase inhibitor dasatinib in Multiple Myeloma (MM). Aims.To evaluated the anti-myeloma activity of dasatinib in combination with rapamycin in MM cell lines and in primary human MM cells and investigated the critical role of the Src activity in the deregulation of mTor signalling pathway. *Methods*. MM cell lines (KMS18 and RPMI8226) and bone marrow samples of 10 MM patients subjected to CD138 immunomagnetic purification were used. Rapamycin was used at the fixed dose of 100 nM in combination with increasing doses of dasatinib. Apoptosis was assayed at 24 and 48h after treatment by flow cytometry evaluating annexin V marker. Src expression was reduced in cell lines by stable expression of a plasmid encoding small interfering RNA to Src. Pharmacological down-regulation of Src expression was obtained with the selective Src inhibitor PP2 (10 M). Western blot analysis was performed to assess the effects of the compounds and of the disruption of Src kinase activity on the phosphorylation status of AKT, mTOR, P706SK, Src and Erk. *Results*. Single agents rapamycin and dasatinib resulted in 20% and 32% (p<0.05) annexin V staining in KMS18 cells at 24 and 48h, respectively. The combination of rapamycin 100 nM and dasatinib 250 nM resulted in the highest level of apoptosis (47% at 48h, p<0.05). The RPMI8226 cells were less sensitive, values ranging between 15-25% for annexin V staining to the combination treatment. Rapamycin/dasatinib combination also enhanced apoptosis at 48h in MM cells of four out five mTor positive patients (38-75% annexin staining, p<0.05) while were ineffective in all MM cells of the five mTor negative patients. In KMS18 cells the drugs combination resulted in a robust dephosphorylation of AKT (90%), mTor (88%), P706SK (85%) and Erk (70%). A similar, but less evident pattern was expressed by the RPMI8226 cells studied in the same conditions. Pharmacological (PP2) and molecular (siRNA) down-regulation of Src and Dasatinib treatment strongly dephosphorylate mTor, P706SK, Src, AKT and Erk (24 and 48h). *Conclusions*. The rapamycin/dasatinib combination induces significant cell death in MM cell lines and in a subset of MM patients with complete down-regulation of p-AKT. The synergism of the combination resulted in the down-regulation of the mTor signalling mediated by Src by abrogating signalling via Akt and Erk.

P084

STUDIES ON C-KIT PROTEIN EXPRESSION AND C-KIT GENE MUTATION IN MULTIPLE MYELOMA

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Aims. To evaluate c-KIT (CD117) protein expression and its clinical implication in MM patients. To study c-KIT gene mutation and amplification in MM cell lines and MM patients and to evaluate drug sensitivity to tyrosine kinase inhibitors (Imatinib, Dasatinib). Methods. Immunohistochemistry with an anti-c-KIT (CD117) polyclonal antibody were performed on a series of 90 symptomatic MM patients. Fisher exact test was used to compare CD117 expression with clinical data of all patients (age, bone lesions, isotype, β2-microglobulin, haemoglobin, creatinine and albumin serum levels). Plasmacells isolated from 10 c-KIT positive MM samples and 4 MM cell lines (KMS18, AMN77, RPMI8226, U266) were Polimerase Chain Reaction amplified for the detection of c-KIT gene mutations or truncated form and sequenced directly. c-KIT gene copy numbers were also evaluated by Fluorescence In Situ Hybridization analysis with Chromosome 4 α Satellite/FITC probe and bacterial artificial chromosome (BAC) bA74L18 and bA586A2 probes labelled with Cy3 by nick translation for c-KIT gene on 25 MM cases CD117 positive. The anti-myeloma activity of dasatinib in MM cell lines KMS18, AMN77, RPMI8226, U226 and in primary plasmacells of 5 CD117 positive MM patients has been assayed by flow cytometry evaluating annexin V marker. *Results.* Expression of c-KIT protein occurred in 37 (41.2%) MM samples without clinical implications. FISH analysis demonstrated lack of amplification of the c-KIT gene in all the 25 samples examined. We found no evidence of mutations in exons 9, 11, 13 and 17. All 10 patients and all 4 MM cell lines expressed both the c-KIT isoforms GNNK- and GNNK$^+$ with predominance of the GNNK- isoform. Dasatinib (250 nM) induced significant apoptosis at 48h in KMS18 AND AMN77 cell lines and in three out five CD117 positive patients while was less effective in the RPMI8226 and the U226 cells. Conclusions. CD117 expression is not a prognostic marker in MM patients. c-KIT gene mutation or amplification do not characterize MM patients CD117 positive. Quantitative Polimerase Chain Reaction may be further use to better quantify c-KiT isoforms and to identify the molecular mechanisms responsible for drug sensitivity or resistance

P085

EXTRAMEDULLARY PROGRESSION DESPITE MEDULLARY RESPONSE IN MULTIPLE MYELOMA DURING BORTEZOMIB THERAPY: REPORT OF THREE CASES

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Extramedullary (EM) involvement in Multiple Myeloma (MM) has been reported in 15-20% of the patients at the time of diagnosis and in 15% during the course of the disease. EM MM is often very aggressive and a short survival period is reached with conventional chemotherapy.

Among novel agents firstly thalidomide has been used in EM disease. Since plasmacytomas are highly vascularised they are therefore hypothetical good responders to anti-angiogenic agents. However thalidomide has been shown to be ineffective in some reports in this setting. Bortezomib has shown a good response in some patients relapsing with EM plasmacytomas. Up to date no significant reports have been published concerning lenalidomide. We describe three elderly patients with EM MM involvement at diagnosis and during treatment with bortezomib. *Patient 1.* A 76 year-old man was diagnosed with stage II B, ISS 3 k chain MM. He had hyperdiploid karyotype (53 chromosomes) and deletion of 13. The lumbar-sacral MRI revealed the presence of a 5x4.5 cm solid mass expanding in the spinal canal. He started MPV chemotherapy (melphalan,prednisone,bortezomib) and sacral radiotherapy (Total dose 14 Gy), obtaining a reduction of the mass. After 7 MPV cycles he achieved a medullary CR and >90% reduction in urinary monoclonal component (MC). Despite this the expansion of the sacral mass occurred again. *Patient 2.* A 66 year-old man was diagnosed with IgA lambda MM stage IIIB ISS 2. The karyotype analysis showed: 49, XY, +3,+9,+14. Because of left omerus and right collarbone fractures radiotherapy was started (20 Gy and 14 Gy, respectively) together with MPV. After 4 cycle both medullary infiltration and serum CM disappeared. However he presented a 9x5.5 cm subclavear solid mass; histologically positive for high grade plasmocytoma. The patient underwent radiotherapy, with a good response of the mass size, and four more MPV cycles were administered. Despite the persistent complete medullary and serum remission he developed two other extramedullary lesions on left side of the spine and behind his bladder, the latter 16 cm in size. A cyclophosphamide, pegylated liposomal doxorubicin, dexamethasone based regimen has just been started. *Patient 3.* After 6 years of MGUS history a 67 year-old man developed symptomatic IgG lambda Bence Jones positive stage IIA ISS2 MM. The karyotype showed the presence of numerous polyploidies, not present previously. The skull radiography and CT scan showed the presence of bilateral parietal myeloma-derived masses (about 3 cm), confirmed at histological biopsy. He started MPV chemotherapy and after the second cycle they both completely disappeared. Unfortunately, at the end of the third MPV cycle, a reappearance and rapid increase in size of the right parietal mass (7x5 cm) occurred. Despite this, there was no evidence of MC. He underwent radiotherapy on his skull with good response. During bortezomib therapy in MM patients we observed progression of EM disease, despite achieving a good response resulting with the disappearance of plasmacellular infiltration in bone marrow and serum and urine reduction of MC. All three patients showed a hyperdiploid karyotype, which is indicated by some authors as a recurrent genetic feature of extramedullary plasmocytomas. The response to radiotherapy was only temporary in the three patients. This discordant behaviour has already been reported in patients during thalidomide therapy. It was hypothesized that even if plasmocytomas have high angiogenic activity, thalidomide needs bone marrow microenvironment to exhibit its drug effect. EM MM plasmacells may acquire the potential to grow regardless of bone marrow microenvironment and to escape the antiangiogenic and immunomodulatory effect of thalidomide. The role of lenalidomide in this sub-set of patients needs to be investigated. Bortezomib acts as a proteasome inhibitor and affects myeloma cell growth by NF-kB blockade, downregulation of adhesion molecules and inhibition of angiogenesis and of DNA repair mechanisms. In some reports its efficacy was described also in EM MM cases. Our experience seems to suggest that its role in this setting still needs to be clearly investigated to select a subset of patients who can benefit from this drug. Tumor biology of EM MM may be different from medullary disease and the discordant response to therapy may reflect tumor cell homing in different tissues.

P086

HOXB7 IN A KEY GENE INVOLVED IN MYELOMA CELL GROWTH AND MYELOMA-INDUCED ANGIOGENESIS IN MULTIPLE MYELOMA PATIENTS

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Bone marrow (BM) neo-angiogenesis has a critical role in multiple myeloma (MM) progression. However the molecular mechanisms at the basis of the angiogenic process in MM are currently under investigation. The deregulation of the homeobox genes has been previously associated to tumor progression and neoangiogenesis. Particularly, overexpression of the homeobox HOXB7 is critical in tumor-associated angiogenic switch in solid tumors as breast cancer. Actually the potential role of HOXB7 in MM-induced angiogenesis is not known. In this study, firstly, by microarray analysis in a large database of MM patients (n°=132) we found that HOXB7 was overexpressed by MM cells in about 10% of patients as compared to healthy donors and MGUS subjects. On the other hand HOXB7 mRNA was overexpressed in 18 out of 23 human myeloma cell lines tested as compared to normal plasmacells. In order to investigate the potential role of HOXB7 in the angiogenic process we enforced HOXB7 expression by lentivirus vectors in MM cells to obtain a stable transduced cell line. By Gene chips U133 plus 2.0 (Affymetrix) we evaluated the gene expression profiling finding that pro-angiogenic cytokines, metalloproteinases and chemokines including VEGF, bFGF, MMP-2 were significantly induced in HOXB7-transduced MM cells as compared to control cells. Data were validated either by real time PCR or by western blot and by an angiogenesis antibody array. These observations were confirmed by silencing HOXB7 by siRNA in MM cell line overexpressing HOXB7. Consistently, we found that conditioned media of HOXB7-transduced MM cells significantly stimulated vessel formation as compared to controls using an in vitro angiogenic model. Finally we show that in an *in vivo* mouse model that HOXB7 overexpressing MM cells grow more as compared to MM cells transfected with the empty vector. The mechanism involved in the increase of MM cell growth by HOXB7 involved the upregulation of Wnt5 and consequently the Wnt signaling. In conclusion our data suggest the HOXB7 regulates the angiogenic switch MM cell growth and could be a potential therapeutic target in MM-induced angiogenesis.

P087

PROGNOSTIC SCORING SYSTEMS FOR IGG AND IGA MONOCLONAL GAMMOPATHIES OF UNDETERMINED SIGNIFICANCE

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In a population of 1283 patients (pts) affected by IgG and IgA monoclonal gammopathies of undetermined significance (MGUS), we have analyzed the clinico-haematological variables correlated with the frequency of evolution into multiple myeloma (MM). Considering IgG MGUS, we analyzed two different series of pts: a training sample (553 pts) and a test sample (378 pts). The IgA MGUS population consisted of 352 pts. Forty-seven of the 553 training group pts developed MM after a median follow-up of 6.7 years; among the IgG test group 22/378 pts developed MM. after a median follow up of 3.6 years. Multivariate

analysis showed that three variables defined a prognostically favourable subset of pts with IgG MGUS: serum monoclonal component (MC) levels < or equal to 1.5 g/dl, the absence of light chain proteinuria (LCP) and normal serum polyclonal immunoglobulin (Ig) levels. Using these simple variables, we stratified pts into three groups at different 10-year risk of evolution: low (274 pts), intermediate (199 pts) and high risk groups (80 pts) (HR 1.0, 5.04, 11.2 respectively; $p<0.001$). This scoring system was validated in the test sample. Considering the IgA populations, thirty of the 352 pts developed MM after a median follow-up of 4.8 years. At multivariate analysis hemoglobin (Hb) levels of <12.5 g/dL and reduced serum polyclonal Ig correlated with progression. We could also confirmed the validity of Mayo Clinic risk model pooling IgG and IgA MGUS series, confirming the prognostic role of an unbalanced light chain production at urinary level or at serum level and of MC isotype. The Mayo Clinic model was also validated in our IgG MGUS subsets using a two-variable system (serum MC levels and LCP) that therefore identified three risk levels. However, the high risk group accounted for only 3.8% of the training sample and 2.1% of the test sample, instead of the 8% and 11% selected by our own proposed score Furthermore, our score performed better than that of the Mayo Clinic in the validation sample (C-Harrell: 73% vs 64%). In conclusion, using simple variables, we validated a prognostic model for IgG MGUS. Among the IgA cases, the possible prognostic role of Hb emerged in addition to a decrease in normal Ig levels.

P088

PRELIMINARY RESULTS OF TOPICAL MENTHOL FOR BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY

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Bortezomib has a remarkable activity against multiple myeloma (MM) through inhibition of the ubiquitin-proteasome pathway. However, its efficacy is hampered by the induction of peripheral neuropathy (PN) in a significant proportion of patients. Grade 1 and 2 PN are observed in 33%-75% of patients, whereas grade 3 and 4 neurotoxicity may affect up to 30% of patients. The current knowledge of the mechanism of bortezomib-induced PN is very limited. Probably, a neurotrophin disregulation and the activation of the mitochondrial-mediated apoptotic pathway in dorsal root ganglia neurons produce a damage of small fibers, which translates into a painful sensory distal neuropathy. Many drugs of different classes have been used to treat this complication, generally with disappointing results. The recent observation that a topical 0.5% menthol cream relieved the symptoms in a case of severe bortezomib-induced PN prompted us to evaluate this therapy in our patients. Starting from October 2008 we treated 11 MM patients with a 0.5% menthol in zinc oxide cream, applied twice a day to the lower limb area of pain or sensory disturbance and to the skin over the lumbosacral region. The patient median age was 66 years (range 49-82), the median number of lines of therapy was 2 (1-5). Nine patients had also received thalidomide in their previous therapies. In all cases PN was related to bortezomib. When the menthol cream was started, 5 patients had a grade I, 3 a grade II, and 3 a grade III sensory PN. Neuropatic pain was observed only in patients with more than grade I sensory PN. Three patients had grade I, 2 a grade II neuropatic pain. Ten out 11 patients were receiving systemic agents, mainly pregabalin (median dosage 450 mg per day, range 100-450 mg), and clonazepam. Five patients reported a significant benefit from menthol cream. In these patients on the Visual Analog Scale of pain a median improvement from 10 to 5 (range 9-2) was measured. Patients reported a particular benefit on paraesthesiae, quality of sleep and daily activities. The clinical benefits were observed in the first 2 weeks of treatment. The median duration of treatment with menthol cream was 8 weeks (range 1-25+). Four out of 5 responders had a grade I and one a grade II sensory PN. No side effects were observed. In conclusion our very preliminary experience suggests that menthol cream may improve mild forms of bortezomib-induced PN. Prospective studies are needed in order to confirm this observation.

Chronic Myeloid Leukemia

P089

A FULLY INTEGRATED AND AUTOMATED SYSTEM FOR THE DETECTION OF P210 (B2-A2 AND B3-A3 ISOFORMS) WITH NESTED DUPLEX RT-REAL TIME PCR; EVALUATION OF DIFFERENT SAMPLES: PERIPHERAL BLOOD, BONE MARROW AND CRYOPRESERVED CELLS

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Introduction. M- BCR-ABL transcript is an established marker in monitoring minimal residual disease in patients with chronic myeloid leukemia. However, different molecular testing methodology can lead to irreproducible results among different labs, for this purpose, an automated system for measuring BCR-ABL could overcome these differences between laboratories, providing fast and reliable results. GeneXpert is a new fully integrated system that automates the processes for real time PCR based molecular testing: sample preparation, RNA extraction, reverse-transcription , amplification and detection. This system with a limited hand-on time (10 min) can perform a duplex nested RT- Real time PCR for both ABL and BCR-ABL transcripts in a single closed microfluidic cartridge, providing quantitative results in just 2 h. *Aim.* To evaluate the performance of the automated closed GeneXpert system (1) in the detection and in the quantification of M-BCR-ABL transcript in monitoring CML patients; (2) to analyze different staring materials from CML patients, peripheral blood, bone marrow and cryopreserved mononuclear dried cells; (3) to investigate the advantages of an automated system in the early identification of patients at risk of drug resistance and disease relapse. *Materials and Methods*. We have analyzed 213 cases,: 6 CML at diagnosis, 192 CML at follow up, 6 AML at diagnosis, 9 patients in follow up after bone marrow transplant and 29 control patients, not expressing BCR-ABL transcript. Amoung these samples we tested 159 peripheral blood (PB), 50 bone marrow and 10 samples of cryopreserved cells from peripheral blood. We analyzed in the GeneXpert system 200 µL of whole peripheral blood, 20 µL bone marrow (diluted 1:20 in PBS), or 200 µL of cryopreserved cells. All the fresh samples were used within 48 h from the arrival of the material, cryopreserved cells were analyzed within 5 days from the preparation . GeneXpert system requires minimal hands-on time, the sample is lysed by a solution containing proteinase K, SDS Tween, guanidina HCl and then the sample is inserted in the self contained cartridge. In the cartridge RNA is bound to the solid-phase purification matrix, RNA is eluted from the matrix and mixed with 1-step RT-PCR beads. cDNA is made and 1st PCR reaction takes place, as endogenous control ABL was used. Aliquot of reaction mixture is transferred to reaction tube where the nested PCR reaction is performed, ΔCt is reported at end of run and the quantification of % BCR-ABL/ABL is calculated by the software based on a K562 RNA standard curve. (validation criteria ABL Ct range 12-18, endpoint fluorescence >200; BCR-ABL Ct range 12-32, endpoint fluorescence >40). *Results and conclusions*. Excellent and valuable results were obtained from PB and cryopreserved cells in all samples analyzed. 10/50 bone marrow sample diluited 1:20 aborted the analysis due to solid-phase purification matrix overload 100% valutability was obtained with bone marrow diluted 1:40 in PBS. Optimum results were also obtained with cryopreserved cells. We believe GeneXpert is an easy, fast and highly sensitive system that can lead to the standardization of molecular testing methods to monitor BCR-ABL transcripts.

P090

CONJUNCTIVAL HEMORRHAGIC EVENTS ASSOCIATED WITH IMATINIB MESYLATE

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Introduction. Imatinib mesylate (IM) is used in the targeted therapy of chronic myeloid leukemia (CML) and gastrointestinal stromal tumours

(GISTs). It is well tolerated and leads to no higher incidence of hemorrhagic events than other therapies. *Materials and Methods* 108 consecutive patients (66 M and 42 F, M/F ratio 1.6; median age 53 years, range: 15-77) diagnosed as having CML between 1992 and 2008, were followed up for a median of 58 months (range: 4-245) and were treated with modulated IM doses for a minimum of three months. At diagnosis, the CML clinical risk scores were: Sokal high risk (16 pts; 15%), intermediate risk (38 pts; 35%), low risk (36 pts; 33%), not evaluated (18 pts; 17%); Hasford score high risk (11 pts; 10%), intermediate risk (41 pts; 38%), low risk (38 pts; 35%), not evaluated (18 pts; 17%). When they started IM, 96 patients (89%) were in chronic phase, 2 pts (2%) in accelerated phase, and 10 pts (9%) in blastic phase on the basis of standard criteria. At the time of data collection, 16 pts (15%) had died because of disease progression and 1 had been lost to follow-up. *Results.* 21 pts (19%) developed unilateral or bilateral conjunctival hemorrhage (CH), occasionally associated with cutaneous hematoma or epistaxis: these included 13 M and 8 F (M/F ratio 1.6) with a median age of 60 years (range: 15-77), a median follow-up of 32 months (range: 8-206), Karnofsky performance scale (KPS) 100%, and Eastern Cooperative Oncology Group (ECOG) performance status 0. All were in chronic phase (20 in complete cytogenetic response and 1 in partial cytogenetic response). During the follow-up, no other hemorrhagic events were observed except for the recurrence of CH in 14 cases (13%). At the time of CH onset, the patients' hemoglobin, white blood cell and platelet (PLT) values were normal, as were the results of coagulation tests and PLT function analysis: the results of aggregation and secretion studies were normal, and no α or delta storage pool deficiency were detected. All of the patients underwent an ophthalmological examination: 7 presented mild periorbital edema, but none showed ephiphora. No local ocular causes were detected. There was no documented comorbidity that could be seen as playing a clear role in the etiology of the CH. No other side effects of IM were observed, except for 2 episodes of generalized erythematous maculopapular and pruritic skin rash. The CH spontaneously resolved within 7-14 days. *Conclusion*. As there was no other obvious reason for such a high CH incidence of unclear pathogenesis, we hypothesise drug hypersensitivity or ocular irritation induced by IM. Otherwise, 21 patients treated with Dasatinib for more than 6 months showed no CH.

P091

INCIDENCE OF CYTOGENETIC AND MOLECULAR RESPONSES IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS RECEIVING LOW DOSE OF IMATINIB FOR INTOLERANCE TO STANDARD DOSE

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Imatinib mesylate is the gold standard treatment of chronic myeloid leukaemia (CML) and 400 mg/day is considered the standard dose. Although it is generally well tolerated, some patients require temporary drug discontinuation followed by permanent dose reduction owing to haematological or non-hematological toxicities. Whether or not reduced doses are effective as the standard dose in inducing and/or maintaining complete cytogenetic and molecular response is not clear. We report the outcome of 45 CML patients in early (17) and late (28) chronic phase (CP) who, among a series of 250 patients treated with imatinib, reduced the dose of the drug after experiencing adverse events. Forty-three patients reduced the dose to 300 mg/day and 2 patients to 200 mg/day. There were 19 males and 26 females, median age was 56.8 years (range 30-78). Causes of imatinib reduction consisted of haematologic toxicity of grade 3 in 24 patients and non-haematologic toxicity in 21 patients, including 3 patients with liver parameters alterations. All patients experienced at least 2 or 3 episodes of toxicity before imatinib dose reduction. Median time interval between the start of therapy and dose reduction was 58 days, whereas median administered dose after the reduction was 300 mg/day. At 6 months after imatinib dose reduction, major cytogenetic responses (MCR) were observed in 67% of patients, with 58% being complete (CCR), and complete molecular response (CMR) were obtained in 18% of patients. At 12 months, all patients who had obtained MCR reached CCR. CMR and major molecular response (MMR) were detected in 20% and 22% of patients, respectively. CCR frequency significantly correlated with Sokal risk, with higher rate of responses being observed in low risk (87%) versus intermediate risk (66%) and high-risk (46%, p=0.005) patients. A significant difference in the rate of CCR was also observed in patients in late CP (53.5%) as compared to patients in early CP (82%, p=0.0001). Low dose imatinib appears effective in patients with poor compliance to standard dose, even though long-term effects remain to be established. Determination of imatinib plasma concentrations might be of help to interpret these results.

P092

IDENTIFICATION OF PROGNOSTIC FACTORS THAT INFLUENCE SURVIVAL AND CYTOGENETIC RESPONSE IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH SECOND GENERATION TKI AFTER IMATINIB FAILURE

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For CML patients in chronic phase (CP) who failed imatinib, second generation TKIs have displaced the allogeneic stem cell transplant option. Nevertheless, a proportion of patients also fail to respond to second line treatment. We report here on the outcome of 48 CML patients treated with nilotinib (22), dasatinib (23) or both (3) after imatinib failure, and present univariate analysis results for the identification of prognostic factors that can individuate patients who will benefit of these drugs. Median patient age was 46 years, with prevalence of males; 20 patients had primary resistance to imatinib (2 hematologic and 18 cytogenetic) and 22 patients had secondary resistance (all cytogenetic), while 6 patients were intolerant. Eight patients developed kinase mutations during imatinib. Thirty patients received high doses of imatinib before second generation TKI: 6 had reached the maximum dose of 800 mg. Median follow-up was 2.5 years. With second line TKI the cumulative incidence of complete cytogenetic response (CCR) was 50% and of major molecular response (MMR) 20%. Eight patients reduced the dose of the drug during therapy for toxicity: of these, 6 patients were on dasatinib. Overall survival at 2 years of the entire patient cohort was 94%. Six patients underwent allogeneic transplant and 1 patient died for disease progression. The univariate analysis identified predictive factors for CCR: age under 50 years (p=0.02), low Sokal risk (p=0.03), previous cytogenetic response to imatinib (p=0.002), time from failure to imatinib to start of second line TKI over 6 months (p=0.001). We applied the score proposed by Milojkovic et al at the ASH meeting 2008 (one point when each of the following features was present: intermediate or high Sokal risk, need of G-CSF support during imatinib therapy, institution of second line TKI more than 18 months after imatinib failure, and failure to achieve a cytogenetic response on imatinib) and identified a cumulative incidence of CCR of 80% for 0-1 point, 36.8% for patients with 2 points and 28% for patients with 3-4 points (p=0.001). In conclusion, it is possible to individuate prognostic factors before starting second generation TKIs useful for predicting cytogenetic response and optimizing therapeutic strategies in imatinib failing patients.

P093

INCIDENCE OF INFECTIVE EPISODES DURING IMATINIB TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE

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Several evidences exist that imatinib can impair various cellular functions involved in the immune response. We report here on the incidence of infective episodes observed during imatinib treatment in CML patients in early or late chronic phase (CP). From January 2000 to September 2006 we treated with imatinib 250 Ph^+ CML patients out of clinical trials: 150 of these subjects were in late CP and had received prior therapy with Interferon (IFN) for a median of 24 months, whereas 100 patients received imatinib soon after diagnosis (early CP). Physical examination and complete blood cell count were performed weekly for the first month, then monthly. During treatment, all side effects and all possibly related infective episodes were recorded. In the series of 100

patients who received imatinib as first line therapy, we observed 16 episodes (16%) of fever associated to infections, which required antibiotical or antiviral therapy and discontinuation of imatinib. In particular, in 4 patients a pneumonia requiring systemic antibiotical therapy was diagnosed: all patients were leukopenic without severe neutropenia, at the time of infection. One out of these 4 patients had two additional episodes of fever and radiological pneumonia, and then had a progression of disease; during progression to accelerated phase, a tubercolisis infection of the lung was diagnosed. One patient had a bilateral orchitis, one patient had a bacterial pleuro/pericarditis which required hospitalization and systemic antibiotical therapy and other 3 patients experienced a urinary infection with fever, nausea and chills (2 patients with E. Coli and 1 patient with Klebsiella Pneumoniae) in the absence of neutropenia. In seven patients a reactivation of herpes zoster was diagnosed after development of painful, multiple and distinct dermatomal distribution of tender blisters, which required high doses of Acyclovir. In all these patients a reduction of lymphocyte count was revealed at the time of viral infection. In the series of 150 patients treated with imatinib in late CP, we recorded 19 infective episodes (13%), with 3 pneumonias occurring during neutropenia phase and 2 patients experiencing urinary infections (E. Coli) without neutropenia. Fourteen late CP patients developed a viral infection during treatment with imatinib: also these patients had a significant reduction of lymphocyte count at the moment of viral infections. In conclusion, the occurrence of infections during imatinib treatment in CP-CML is infrequent and is similar between late and early CP patients.

P094

IMATINIB FIRST LINE IN CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE: A REPORT OF 145 PATIENTS WITH A 48 MONTH FOLLOW-UP

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Imatinib mesylate is now the first line therapy for CML: it acts by competitively binding the inactive form of BCR-ABL, preventing a switch to the active form and partially blocking the enzyme ATP binding site. To date, only authors from the Hammersmith Hospital, in addition to what reported by IRIS study, referred on the experience of a single institution in treating patients in early chronic phase, in a setting where all events were recorded. We describe here our experience on 145 early CP patients with a median follow-up of 4 years, who received imatinib as first line. We evaluated haematological, cytogenetic, molecular responses, progression free survival (PFS) and overall survival (OS). At 4 years, only 3 patients (2%) had failed to achieve complete haematological remission (CHR); complete cytogenetic response (CCR) was obtained by 81% of patients: of these, 34% achieved a major molecular response (MMR) and 25% a complete molecular response (CMR) as per international scale (IS). After 4 years, 17% of patients discontinued the drug for lack of efficacy and/or intolerance. During follow-up, 18 patients (12%) lost their CCR and were treated with second generation TKI. Median estimated OS and PFS were 96% and 95%, respectively. At 3 months, 113 patients were assessable for cytogenetic response: 86 (76%) were in CCR, 13 (11%) in major cytogenetic response (MCR), 6 (5%) in minor (MinCR) and 8 (7%) had not responded. Considering patients by response at 3 months, the 4-year cumulative rate of CCR was 92% for patients in CCR, 78% for patients in MCR and 10% for patients in Min CR. Considering patients by response at 6 months, the 4-year cumulative rate of CCR was 92% for patients who had achieved CCR, 100% for patients in MCR and 10% for patients in MinCR. Patients who obtained MMR or CMR in our experience did not loose CCR after 4 year of follow-up, whereas 11 patients out of 32 who did not achieve MMR/CMR lost CCR (p<0.001). In conclusion, imatinib is an highly effective drug as first line therapy for CP CML patients: achieving CCR or MCR at 3 and 6 months correlates with better survival; in our experience, patients who obtain major molecular responses do not experience events during follow-up.

P095

BCR-ABL LEVEL REDUCTION AT 6, 12 AND 18 MONTHS CORRELATES WITH PROGNOSIS AND OUTCOME AT 48 MONTHS IN CHRONIC PHASE CML PATIENTS TREATED WITH IMATINIB

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Imatinib is an effective drug in treating chronic myeloid leukaemia (CML) patients in chronic phase (CP). To date, most of the molecular available data come from IRIS study, which has demonstrated that reduction of BCR-ABL transcript levels in the first months of therapy significantly correlates with long-term outcomes. We report our experience in a total of 322 patients treated with imatinib at standard dose: among these, 183 were treated in late CP and 139 soon after diagnosis. Major molecular response (MMR) with a BCR-ABL/ABL ratio <0.1%, was observed in 40% of samples available at 6 months, in 64% of samples at 12 months and in 66% of samples at 18 months. Clinical features at presentation of patients who achieved MMR at 6 months and of patients who achieved MMR at 12 months did not differ, except for median time from diagnosis to imatinib start, which was shorter for the former group (p=0.02). None of the patients who achieved MMR at 6 months lost complete cytogenetic response (CCR) during the follow-up, whereas 5 patients who achieved MMR at 12-18 months lost CCR due to secondary resistance and acquisition of a mutation (3 patients). In the series of patients who did not achieve MMR, 22 patients lost CCR during 48 months of follow-up (p=0.002). Patients who reached a BCR-ABL/ABL ratio <0.1 at 6 months had an event free-survival (EFS) and a progression free-survival (PFS) of 100% at 48 months, respectively, whereas patients with a ratio >1-10% had an EFS and a PFS at 48 months of 75% (p=0.001). Also patients with ratio <0.1 % at 12 months could be statistically distinguished from patients with ratio > 1-10%: in patients who achieved MMR, PFS and EFS remained stably 100% at 48 months, compared to EFS of 60% and PFS of 80% in patients with ratio >1-10%. In conclusion, in patients treated with imatinib first line or after IFN failure, MMR rates increase over time; at 6 and 12 months a ratio < 1% identifies patients with excellent long-term outcome.

P096

OUTCOME OF CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE WITH SUBOPTIMAL RESPONSE OR FAILURE AT 3, 6, 12 AND 18 MONTHS ACCORDING TO EUROPEAN LEUKEMIANET CRITERIA (ELN)

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In 2006 the European LeukemiaNet published recommendations designed to help identifying patients responding poorly to imatinib. Two major categories were identified at 3, 6, 12 and 18 months: failure and suboptimal responders at imatinib as first line therapy. We analysed the outcome of 145 chronic myeloid leukemia (CML) patients in early chronic phase (CP) treated at a single institution, to validate the criteria used for this classification. Median follow-up was 48 months. At 3 months we identified only 1 patient with suboptimal response and 3 patients with failure: median survival was 65 and 36 months (p=0.001), PFS was 100% and 30% (p<0.001) and probability to achieve complete cytogenetic response (CCR) was 100% and 0% (p<0.001), respectively. At 6 months, 5 patients were classified as failure and 6 patients as suboptimal response: median survival was 20 months and 21 months (p=NS), PFS was 60% and 70% (p=NS), probability to achieve CCR was 10% and 0%, respectively. At 12 months, we classified 12 patients as failure and 7 patients as suboptimal response (all patients were treated with dose escalation): median OS was 26 and 38 months, PFS was 80 and 86% and probability of CCR was 0% and 77%, respectively. At 18 months, 1 patient was considered as failure (median OS 25 months, PFS 50% and probability of CCR 0%) and 31 patients did not achieve major molecular response and were considered as suboptimal responders (median OS 42 months, PFS 100% and probability of CCR 100%). In our experience, the criteria used for defining suboptimal responses both at 6 and 12 months identified patients with very poor prognosis, similar to that

of patients considered as failure. Criteria for suboptimal response at 18 months, as previously suggested also by Marin et al in the Hammersmith experience, was less useful and failed to identify patients with worse prognosis. In conclusion, in some instances suboptimal response and failure criteria of the 2006 European LeukemiaNet, should be used to identify patients who need early therapeutic intervention

P097

IMATINIB DOSE ESCALATION IN CHRONIC MYELOID LEUKAEMIA PATIENTS AFTER FAILURE OR SUBOPTIMAL RESPONSES TO STANDARD DOSE

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Imatinib mesylate (IM) given at a daily dose of 400 mg currently represents the gold standard of care for patients with chronic myeloid leukemia (CML) in chronic phase (CP). European LeukemiaNet (ELN) guidelines propose IM dose escalation to rescue those CML patients who are either in suboptimal disease response or after failure. We report on the long-term efficacy of IM dose escalation in 74 patients with CP-CML after suboptimal response or failure to IM conventional dose. Patient's median age was 50 years (range 19-85), they were 52 males and 22 females. Thirteen patients were classified as hematologic failure (10 primary and 3 secondary resistance) and 57 patients had cytogenetic resistance (24 primary and 33 acquired resistance). All of them received IM dose escalation from 400 to 600 mg (50 patients) or to 800 mg (20 patients). After a median follow-up of 36 months, complete cytogenetic responses (CCR) were overall achieved in 37% of the total CML patients (38% of the haematological failure patients, 27% of the primary cytogenetic resistant, and 50% of the acquired cytogenetic resistant patients). Three patients escalated the dose for cytogenetic suboptimal response and obtained CCR and complete molecular response (CMR), whereas one patient escalated the dose for molecular suboptimal response at 18 months and did not obtain a CMR. After 3 years of follow-up all patients have sustained CCR. The estimated 2 years PFS and OS is 87% and 85% respectively. Sixteen patients (21.6%) experienced toxicities and had temporarily IM interruption. In conclusion, IM dose escalation appears to induce sustained responses in CML patients with cytogenetic resistance, in particular in those with acquired resistance. In haematological failure patients a rapid switch to second generation TKI is recommended.

P098

TKIS IN CML: THE APPEARANCE OF ABNORMAL CLONES IN PH- CELLS

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Introduction. Tyrosine kinase inhibitors (TKIs) have changed the approach to the management of chronic myeloid leukemia (CML). Imatinib and dasatinib are potent TKIs to which most patients (pts) show a major (MCR) or complete cytogenetic response (CCR). There have been reports of clonal changes in the Ph- cells of CML pts treated with TKIs, the most common of which are trisomy chr.8 and monosomy chr.7. *Materials and Methods* Before and during TKI treatment, cytogenetic and FISH analyses using a dual fusion double colour probe were made of 101 pts (84 treated with imatinib, and 17 with dasatinib) diagnosed as having CML between 1999 and 2008. *Results.* Eight pts developed a total of nine chromosomal abnormalities, six of which occurred during imatinib and three during dasatinib treatment (Table). The (20q) deletions in the pts on imatinib were observed 43 and 54 months after starting therapy, 7 and 42 months after they had achieved CCR; the -y anomalies were observed 27 and 31 months after starting therapy, 24 and 25 months after CCR. The -y anomaly in the patient treated with dasatinib developed 45 months after starting therapy, six months after CCR. It is worth noting that one of the two pts with trisomy chr.8 in MCR on imatinib subsequently developed trisomy chr.9 (20 months after switching to dasatinib, 11 months after CCR): the trisomy chr.8 is still present. All of the abnormalities arising during MCR and not CCR are trisomy chr.8.*Conclusions.* All of the pts are still in MCR/CCR and no clinical progression or myelodisplastic disease has been documented during follow-up. Whether the TKIs played a role in the occurrence of the abnormalities remains to be determined as they could be related to CML clonal instability and/or drug effects. The prognostic implications need to be verified in a larger CML group over a longer follow-up.

Table 1.

Imatinib	+ 8	2 pts in MCR
	del(20q)	2 pts in CCR
	-y	2 pts in CCR
Dasatinib	+8	1 pt in MCR
	-y	1 pt in CCR
	+9	1 pt in CCR

P099

PERSISTENT TELOMERIC LOSS AND IMPAIRED FUNCTIONAL PERFORMANCES IN PH-NEGATIVE HEMATOPOIESIS EMERGING AFTER SUCCESSFUL TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA

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Background. Most chronic myelogenous leukemia (CML) patients (pts) restore non-neoplastic hematopoiesis following therapy with tyrosine kinase (TK) inhibitors. However little is presently known on the functional and genetic integrity of Ph-negative hematopoietic cells (HC) repopulating bone marrow (BM) after successful treatment. Indeed, the frequent detection of cytogenetic abnormalities (CA) suggests the potential presence of functional and genetic defects. These issues have been addressed using cultures assays and telomere length (TL) analysis, which is considered a reliable marker of proliferative and oxidative damage.

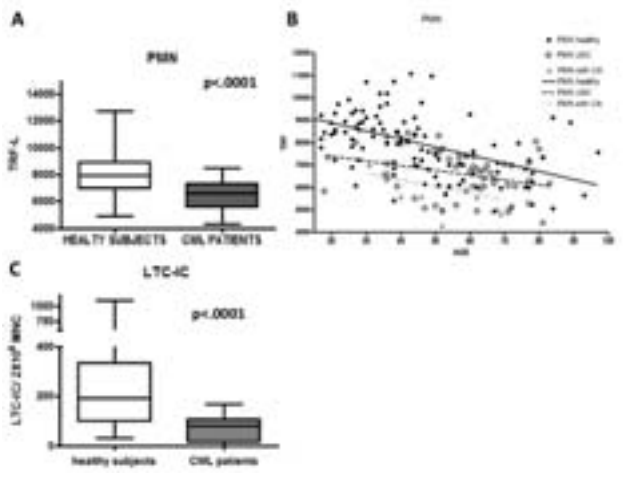

Figure 1.

Aims and methods. We investigated 77 CML pts in stable complete cytogenetic remission (CCR) from at least one year. Median time from diagnosis and CCR were 53 (7-915), and 39 months (12-150). Ten pts showed evidence of acquired CA in Ph-negative HC. TL analysis was performed

by Southern Blotting, both on polymorphonucleates (PMN) and on monocyte-depleted PBMC (MD-PBMC) to monitor the myeloid and lymphoid compartment. CFU-GM, BFU-E and CFU-Mix along with LTC-ICs have been performed on 30 pts, using BM mononuclear cells. A control database of 96 healthy subjects has been used for comparison. Results. PMN from CML pts showed a striking erosion of their TL (Figure 1A). On the contrary MD-PBMC showed a not significant telolmeric loss (in PMN 1555 pb $p<0.001$; in MD-PBMC: 225 pb, $p=0.2$) We found no correlation between TL and any clinical parameters. Telomeric erosion is more severe in younger CML pts, resulting in loss of the association between TL and age, typically seen in healthy subjects (Figure 1B). TL shortening was observed regardless of the use of TK inhibitors. In a multivariate analysis the presence of CML resulted a stronger predictor of TL damage compared to age. TL erosion show no evidence of recovery on 46 follow-up samples taken after a median time of 10 months (range 6-15). Moreover Ph-negative HC of CML pts were functionally impaired compared to controls with reduced numbers of CFU-Mix(median 2,62 vs 4, $p=0.01$), CFU-GM (median 99.5 vs 181, $p<0.0001$) and particularly of LTC-IC(median 88 vs 198, $p<0.0001$)(Figure 1C). *Conclusion*. Ph-negative HC repopulating BM after successful therapy display severe TL erosion and major defects in their functional performances. These findings underline the need of additional investigations and careful clinical monitoring of the Ph-negative haemopoietic compartment.

P100

COMORBIDITIES INFLUENCE COMPLIANCE AND DEVELOPMENT OF PLEURAL EFFUSIONS IN ELDERLY CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH DASATINIB

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The Charlson comorbidities index (CCI) comprises a list of 19 conditions (including cardiologic, pulmonary diseases, diabetes, etc) with a weight assigned from 1 to 6, derived from relative risk estimates of a proportional hazard regression model using clinical data. We retrospectively evaluated the weight of this index in a cohort of 83 elderly CP-CML patients receiving dasatinib after imatinib resistance or intolerance. Score point 0 was assigned to 59 patients, whereas 41 had a score point >1 (18 patients =1, 10 patients= 2; 2 patients= 3; 1 patient= 5). Forty-nine patients received 70 mg twice daily and 43 patients received 100 mg once daily, in accordance with the results of the phase III trial, whereas 7 patients received less than 100 mg. During dasatinib treatment 57% of score 0 patients experienced a reduction of the dose compared to 67% of patients with score 1, 80% of patients with score 2 and 100% of patients with score 3 and 5 ($p=0.02$). Of the 59 patients with score 0, 52% and 54% suspended the drug for haematologic and for non-hematologic toxicity, respectively, compared to 66% of patients with score 1 (33% for hematologic and 61% for non-hematologic toxicity), 70% of patients with score 2 (40% for hematologic and non-haematological toxicity) and 100% of patients with score 3 and 5 (all patients for both types of toxicity) ($p=0.003$). Despite interruptions and dose modifications of the drug, CCI score seemed not to influence the rate of cytogenetic and molecular response. Twenty-three patients (23%) experienced pleural effusion during treatment: of these, 15 patients (65%) had score = 1 and 8 had score > 1. Seven patients experienced grade 3 and 16 patients grade 1-2 effusions. In patients with high score, the effusions were repeated and associated to persistent skin toxicity. No pleural effusions were observed in patients with score 0 ($p=0.002$). In conclusion, in elderly CML patients treated with dasatinib the compliance and incidence of pleural effusions are associated with the presence of comorbidities, mostly due to pre-existing cardiologic or pulmonary diseases, with high scores according to Charlson index.

P101

BCR-ABL B2A2 VARIANT AND RESPONSE TO IMATINIB MASYLATE THERAPY

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In the majority of cases, Chronic Myeloid Leukemia (CML) is characterized by the expression of BCR-ABL onco-protein that plays a causal role in the disease. The fusion of BCR and ABL genes, as a result of the reciprocal translocation t(9;22)(q34;q11), produces a tyrosin kinase fusion protein continuosly activated by the juxtaposed BCR sequence. Several rearrangements of BCR-ABL have been previously described, although in CML there are two major forms: the b2a2 (BCR exon 13-ABL exon 2) and the b3a2 (BCR exon 14-ABL exon 2) fusion genes, both encoding for a p210. While the functional ABL sequence is constantly represented, in fact the breakpoint usually interests exon 2, rarely exon 3, different BCR exons may be included. As suggested by clinical evidence, the detection of BCR-ABL transcript by real time PCR (RT-PCR) is a rapid and specific method to confirm CML diagnosis. However, this technique does not allow detection of BCR-ABL transcripts even if slightly different from the most common form. We here present a CML case, firstly resulting BCR-ABL negative by RT-PCR but Ph-positive in FISH, that showed by sequencing an in frame BCR-ABL b2a2 variant. Breakpoint on BCR was inside exon 13 (nucleotide 866 of the coding sequence), excluding 51 nucleotides in the fusion gene and 17 aminoacids in the fusion protein. In the lost sequence, there was the site of annealing of the forward primer employed in BCR-ABL detection by RT-PCR (Gabert et al 2003). This type of b2a2 rearrangement is the only one detected in 134 new CML cases analysed since 2005, when we introduced RT-PCR as analyses technique for BCR/ABL detection. After BCR-ABL detection, the patient started imatinib therapy (400 mg/die). To exploit the possibility in monitoring the therapy response by RT-PCR, specific primers were synthesized. At 3 months, peripheral blood control revealed BCR-ABL transcript undetectable and FISH analyses detected 3,4% Ph-positive nuclei. At 6 months, cytogenetic analyses control showed a cariotype 46,XY in 20 metaphases, BCR-ABL transcript still undetectable on bone marrow (BCR-ABL/ABL=0/25100), while FISH analyses detected 0,1% Ph-positive nuclei. The preliminary data on response to therapy indicates that this b2a2 variant form is sensitive to imatinib mesylate.

P102

TREATMENT WITH IMATINIB IS EFFECTIVE AND SAFE ALSO IN VERY ELDERLY (> 75 YEARS) CHRONIC MYELOGENOUS LEUKEMIA PATIENTS

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Median age of patients with Chronic Myelogenous Leukemia (CML) is about 65 years, according to SEER 2000-2005 data. However, treatment with Imatinib (IM) in elderly patients has been only seldom specifically addressed: in particular, there is a lack of data regarding very elderly CML patients. To highlight peculiar aspects of toxicity and efficacy of IM in this subset which accounts for at least 10-15% of all CML cases, we retrospectively revised 75 CML patients in chronic phase treated with IM when aged >75 years from 14 haematological Institutions in Italy; there were 42 males and 33 females, median age at IM start was

78.6 years (IR 75.7-81.7), Sokal Risk at diagnosis was low in 2 patients, intermediate in 41, high in 21 and not evaluable in 11. One or more concomitant diseases requiring specific treatments were present in 69/75 patients (92%), with 41 patients (54.6%) assuming 3 or more concomitant drugs. Twenty-four patients (32%) were pretreated ≥6 months (23 with HU and 1 with IFN) before starting IM; on the whole, median time from diagnosis to IM was 1.8 months (IR 0.7-21.4). Starting dose of IM was 400 mg/day in 59 patients (78.6%) and 300 mg/day in 16 patients (21.4%); overall, 34 patients (45.3%) (29/59 at 400 mg starting dose and 5/16 at 300 mg starting dose) needed a dose reduction and 11 (14.6%) discontinued IM for toxicity (early toxicity in 8 and late toxicity in 3). Excluding the 8 patients who discontinued IM due to early toxicity, maximum tolerated daily dose during treatment was 400 mg in 27 patients, 300 mg in 26 patients and < 300 mg in 14 patients. According to CTC-AE, grade 3-4 haematological and extra-haematological toxicities were observed in 19 (25.3%) and 23 (30.6%) patients, respectively; 3 patients (4%) presented a pleural effusion during IM treatment. After a median treatment period of 31.6 months (IR 12.0-31.6), 4 patients (5.3%) are still too early (<6 months of treatment), 8 patients (10.7%) discontinued IM due to early toxicity and 63 (84%) achieved a complete haematological response (CHR). Among these 63 patients in CHR, 8 refused any other karyotipic or molecular evaluation (5 are still alive in CHR, 3 died in CHR from unrelated causes) and 47/55 (62.6% of all 75 patients) achieved a cytogenetic response (CyR), which was major in 6 patients and complete (CCyR) in 41 patients. In addition, among the 41 patients in CCyR, 29 (38.6% of all 75 patients) achieved a molecular response (major molecular response in 10 patients and complete molecular response with an undetectable BCR/ABL hybrid gene at qualitative nested PCR in 19 patients). At the last follow-up, 18 patients have died (2 from disease progression and 16 from unrelated causes), 1 patient was lost to follow-up and 56 are still alive. In conclusion, IM seems a relatively safe and quite effective treatment for very elderly CML

P103

BONE MARROW TRANSPLANTATION AFTER SECOND GENERATION TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA RESISTANT TO IMATINIB

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In Ph+ chronic myeloid leukemia (CML), resistance to imatinib is an increasingly recognized problem and second generation tyrosine kinase inhibitors (TKI), such as dasatinib and nilotinib, have been designed to overcome this phenomenon. Only limited data exist on the effects that treatment with these latter drugs may have on hematopoietic stem cell transplantation (HSCT) outcome. For this pourpose, the outcome of 12 patients with chronic phase (CP)-CML resistant to imatinib, who received dasatinib (5) or nilotinib (6) or both (1 patient) before HSCT, was retrospectively analyzed, in particular as to engraftment and transplant-related complications. Median time of treatment with second generation TKIs was 8 months (range 1-17). Best responses obtained were a major cytogenetic response (MCyR) in 6 patients, with 3 of them achieving a complete cytogenetic response (CCR), a major molecular response (MMR) in 1 case, a complete hematologic remission (CHR) in 4 patients and no response in 1 patient. Four patients underwent an HSCT from an HLA-matched related donor and 8 patients from an unrelated donor (MUD). The stem cell source was peripheral blood in 6 patients, bone marrow in 5 and cord blood in a MUD transplant. All patients engrafted successfully: median time to neutrophil and platelet recovery was 21 days (range 11-41) and 22 days (range 14-86), respectively. Bacterial infections occurred in 8 patients during the HSCT period, with an apparently not increased frequency compared to that observed prior to the second generation TKI era. Acute and chronic graft-versus-host disease (GVHD) occurred in 3 patients. Chimerism studies at day 100 following HSCT showed 100% of donor type in 10/11 patients. Best response obtained after HSCT was a complete molecular remission (CMR) in 9/12 patients, a MMR in 1 patient and a CCR in 2 patients. The median overall survival was 16.5 months. At the last observation, 9 patients were still in CMR and 1 patient was in MMR, while the 2 patients who had obtained a CCR died due to disease progression. In our experience, treatment with second generation TKIs prior to HSCT does not increase the transplant-related toxicity and can serve as a useful bridge towards this procedure in patients with imatinib-resistant disease.

P104

IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA AND ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS, IMATINIB-RESISTANT BCR-ABL MUTATIONS ARE ALREADY DETECTABLE AT LOW LEVELS BUT DO NOT CORRELATE WITH SUBSEQUENT CLINICAL OUTCOME – A STUDY BY THE GIMEMA-ALL AND GIMEMA-CML WORKING PARTIES

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Bcr-Abl kinase domain (KD) mutations may cause resistance to tyrosine kinase inhibitor (TKI) therapy in Ph+ acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML). Mutations in rare Ph+ cells have been detected in some imatinib-naïve advanced-phase CML patients (pts), but it is unclear whether low level mutations may already be present at diagnosis in chronic-phase (CP) CML as well as in Ph+ ALL, and whether their detection predicts for subsequent treatment failure. We analyzed cDNA samples from 24 newly diagnosed pts with Ph+ ALL (n=13) or CP-CML (n=11) who subsequently received TKI therapy. Screening for low level mutations was performed by cloning the Bcr-Abl KD (a.a. 206-524) in a bacterial vector and sequencing 200 clones for each pt. All pts had evidence of aberrant KD sequences. Three to twelve different mutations were detected in each pt. A total of 115 mutations (41 silent, 5 nonsense, 69 missense mutations) were observed. The majority (107/115, 93%) have never been reported in association with TKI resistance and are likely not to confer any advantage under TKI selective pressure. Interestingly, 103/115 (90%) mutations were transitions: G>A (n=30), A>G (n=25), C>T (n=25), T>C (n=23). One of the eleven CP-CML pt received hydroxyurea for 6 months before starting imatinib therapy. In this pt, high-sensitivity mutation screening was performed again immediately before imatinib start and showed further accumulation of mutations. Eight Ph+ ALL pts and three CML pts subsequently relapsed with mutations, but only two with a mutation (T315I) already detectable at diagnosis. The remaining thirteen pts are in persistent remission (follow-up, 12-52 months), although four of them were harbouring known imatinib-(H396P, D276G, E355G) or dasatinib-(F317L) resistant mutations at low levels. We can conclude that: a) mutations can probably be found at diagnosis in all CP-CML and Ph+ ALL pts; b) mutations seem to arise randomly and most of them are silent/not conferring any growth advantage; c) generation of mutations seems to be linked to Bcr-Abl-driven genetic instability; d) TKI-resistant mutations present at low levels at diagnosis do not always outgrow and lead to relapse, probably because some of them arise in cell clones with limited self-renewal capacity. This warns against high-sensitivity mutation screening of all pts before the start of therapy. *Supported by European LeukemiaNet, PRIN, AIL, AIRC and Fondazione del Monte di Bologna e Ravenna.*

P105

LONG-TERM MUTATION FOLLOW-UP OF PHILADELPHIA-CHROMOSOME POSITIVE LEUKEMIA PATIENTS TREATED WITH SECOND-GENERATION TYROSINE KINASE INHIBITORS AFTER IMATINIB FAILURE SHOWS THAT NEWLY ACQUIRED BCR-ABL KINASE DOMAIN MUTATIONS LEADING TO RELAPSE MAINLY ARISE DURING THE FIRST YEAR

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Dasatinib and nilotinib are tyrosine kinase inhibitors (TKIs) active against many imatinib-resistant Abl mutations. However, both have

been shown to retain Achilles heels. We monitored Abl mutation status in 121 patients (pts) who received dasatinib (n= 78) or nilotinib (n=43) as 2nd TKI after imatinib failure. Fifty-eight (48%) pts had chronic phase chronic myeloid leukemia (CML), 63 pts (52%) had accelerated phase/blast crisis (BC) CML or Philadelphia-positive (Ph^+) acute lymphoid leukemia (ALL). Median age was 55 years (range, 18-76); median time from diagnosis was 49 months (range, 4-181); median time on imatinib was 32 months (range, 4-66). Median follow-up of all pts is 7 months (range, 1-38). Median follow-up of pts still on 2nd TKI is 32 months (range, 28-38). Relapses after an initial response have so far been observed in 46/121 pts. Thirty-eight of 46 pts had AP/BC CML/Ph+ ALL at the time 2nd TKI was started. Forty-one of 121 (34%) pts experienced relapse after an initial response during the first 12 months of 2nd TKI treatment (median time to relapse, 6,5 months; range 4-12), while only five of the 45 (11%) pts who were still on 2nd TKI treatment after >12 months have relapsed (at 13, 15, 18, 20, 33 months, respectively). Interestingly, 0/5 pts had never achieved more than a minor cytogenetic response (CgR), and 4/5 pts were receiving a reduced TKI dose because of toxicity. In 36/46 (78%) pts relapse was associated with newly acquired Abl mutations. In particular 26/30 (87%) pts who relapsed on dasatinib and 10/16 (63%) pts who relapsed on nilotinib had evidence of a newly acquired KD mutation responsible for treatment failure (dasatinib: T315I, F317L, T315A, V299L, F317I; nilotinib: E255K, E255V, Y253H, T315I, F359V, F359C). We conclude that: a) newly acquired mutations leading to relapse in pts receiving 2nd TKI usually arise rapidly; the likelihood of mutation selection consistently decreases over time, and seems mainly confined to advanced phase pts and to pts with no/minor CgR; b) almost all (87%) cases developing resistance to dasatinib had newly acquired KD mutations - suggesting that the higher potency with respect to imatinib can overcome Bcr-Abl amplification and that Src kinase inhibition may turn off Bcr-Abl-independent resistance mechanisms; c) a lower incidence (63%) of newly acquired KD mutations was observed in pts who developed resistance to nilotinib. Supported by European LeukemiaNet, PRIN, AIL, AIRC and Fondazione del Monte.

P106

SECOND- AND THIRD-LINE TYROSINE KINASE INHIBITOR THERAPY IN IMATINIB-RESISTANT PHILADELPHIA-POSITIVE LEUKEMIAS: PATIENTS WHO ALREADY HARBOUR BCR-ABL KINASE DOMAIN MUTATIONS HAVE A HIGHER LIKELIHOOD OF DEVELOPING FURTHER MUTATIONS LEADING TO RELAPSE AFTER AN INITIAL RESPONSE

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Dasatinib and nilotinib are novel tyrosine kinase inhibitors (TKIs) developed to overcome imatinib resistance due to Bcr-Abl mutations in Philadelphia-positive leukemias. To assess how mutation status evolves during sequential therapy with these TKIs and which mutations may further develop and impair their efficacy, we monitored 95 imatinib-resistant patients before and during treatment with dasatinib and/or nilotinib as 2nd or 3rd TKI.. Thirty-eight pts had CML in chronic phase; 46 pts had CML in accelerated phase/blast crisis; 11 pts had Ph^+ ALL. At the time of IM failure, 51/95 (54%) pts had KD mutations. After switching to a 2nd TKI (n=95 pts), 23/51 (45%) pts with baseline mutations as against 8/44 (18%; p=0.01) pts without baseline mutations subsequently relapsed with newly acquired mutations. Median time to relapse was 9 months (range, 1-20). Twenty-six of the 43 patients who did not respond or relapsed on 2nd TKI switched to a 3rd TKI. Twenty out of 26 patients had Bcr-Abl KD mutations. With a median duration of 3rd TKI therapy of 4 months (range, 2-9), 13/20 mutated patients as against 1/6 non-mutated patients achieved a response and then relapsed again with newly-acquired mutations. Median time to relapse was 3 months (range, 2-6). Interestingly, all patients with mutations at baseline who lost response to 2nd or 3rd TKI developed new mutations (36/36). In contrast, none of the patients who achieved and maintained a response was found to develop new mutations. Also, none of the patients who never achieved a response to 2nd or 3rd TKI was found to develop new mutations, irrespective of their baseline mutation status. The likelihood of developing further mutations in patients already harbouring baseline mutations was higher in the subset of advanced-phase CML and Ph^+ ALL (p=.005). *Conclusions*. a) in IM-resistant pts treated with 2nd/3rd TKIs, Abl KD mutations are often the mechanism by which the Ph^+ clone tries to escape from inhibition (90% of failures associated with presence/emergence of mutations). However, the spectrum of "critical" mutants is very limited as compared to that of IM. b) Pts already harbouring mutations, especially those with CML in AP/BC or with Ph^+ ALL have a higher likelihood of developing further mutations under the selective pressure of novel TKIs. Supported by European LeukemiaNet, AIL, AIRC, FIRB, COFIN, Fondazione del Monte di Bologna e Ravenna.

P107

ADDITIONAL CHROMOSOMAL ABNORMALITIES IN IMATINIB-TREATED CHRONIC MYELOID LEUKEMIA PATIENTS: FREQUENCY AND IMPACT ON TIME-TO-CYTOGENETIC RESPONSE IN A SINGLE-CENTRE COHORT

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Introduction. The clinical significance of Additional Chromosomal Abnormalities (ACA) and of variant Philadelphia (Ph) chromosome (chrom.) is still debated. In the pre TKIs era, occurrence of these cytogenetic anomalies tended to be associated with development of resistance and deemed a worse prognosis. Aim of this retrospective analysis was to assess frequency and possible prognostic significance of ACA and variant Ph chrom. in CP-CML front-line Imatinib-treated pts. *Patients and Methods.* A total of 106 pts were diagnosed with CP-CML at our Institution between April 2000 and July 2008. The 60 pts with newly diagnosed CP-CML treated with front-line Imatinib 400 mg/die comprise our study population. Cytogenetic analysis was performed - using standard G-banding techniques - at diagnosis and during treatment as recommended by the European Leukemia Net expert panel (Baccarani et al, Blood 2006). At least 20 metaphases were analysed; chrom. aberrations were considered as clonal when present in 10% or more metaphases. *Results*. Chrom. abnormalities were detected in 6/60 (10%) of pts at diagnosis: 3 ACA [chrom. 8 trisomy, deletion of chromosome Y and t(11;11)] and 3 variant Ph^+ [t(X;9;22), t(9;10;22) and t(8;9;22)]. As a group, 55/60 pts (91.7%) achieved CCyR; all pts with chromosomal anomalies reached CCyR. Median time-to-CCyR was 6 mos (range 3-56 mos); in the one pt with t(11;11), achievement of CCyR took longer than 18 months (i.e. 19 mos). *Discussion*. Occurrence of chromosomal anomalies, either ACA or variant chrom. Ph, is in line with published reports. In our study group, presence of such anomalies did not negatively impact on either achievement of CCyR or time-to-CCyR. Possible impact on overall prognosis needs to be evaluated in larger cohorts of pts and for longer follow-up.

Allogeneic Transplantation I

P108

FEASIBILITY AND OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS OLDER THAN 60 YEARS WITH POOR RISK ACUTE MYELOID LEUKEMIA

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Introduction. Older age is considered one of the most important adverse prognostic factors in Acute Myeloid Leukemia (AML). Allogeneic stem cell transplantation (allo-SCT) is one of the most recommended approach in poor prognosis AML but there are limited data about this procedure in elderly AML patients (pts). We report our experience about the feasibility and outcome of allo-SCT in poor risk AML patients aged 60 years or older. *Patients and results.* Between 2003 and 2008, 30 consecutive AML pts aged 60 years or older (median age 63; range 60-70 years) received an allo-SCT at our Center and all cases were high risk at disease onset. The median time from diagnosis to transplant was 7 months. At the time of transplant 12/30 cases (40%) had an advanced disease (relapsed or refractory), while 18/30 (60%) were in complete remission (CR). The hematopoietic cell transplantation specific comorbidity index (HCT-CI) was two or less in 15/30 cases (50%) and three or more in 15/30 cases (50%). Donor were MUD in 12 (40%) and sibling in 18 (60%) of 30 cases. Twenty-six of 30 pts (87%) received a reduced intensity conditioning regimen (RIC). All patients engrafted. Acute GvHD was observed in 17/30 cases (57%) with 13 having grades I-II and 4 having grades III-IV. Data on chronic GvHD was available for 22/30 pts (73%); of those 2/22 (9%) developed extensive chronic GvHD and 9/22 (41%) limited chronic GVHD. One year Nonrelapse mortality rate (NRM) was 20% (6/30 cases). At the time of analysis, after a median follow-up of 16 months (range 1-64), 17/30 pts (57%) were alive and in CR while 13/30 (43%) have died (leukemia refractory or relapse 7/13 and NRM 6/13). One year probability of Overall Survival (OS) of the whole population was 57%. The OS did not differ between unrelated and related donors. The pts transplanted in CR have a significantly better DFS and OS compared to those with refractory or relapsed AML (log rank 0.02). The patients with a HCT-CI 2 or less at transplant have a significantly lower NRM compared to those with HCT-CI 3 or more (6% vs 33%). *Conclusions.* These data indicate that allo-SCT is a feasible treatment option in selected poor prognosis AML pts older than 60 years. In this experience NRM rate is 20% and OS rate (1 year 57%) is promising taking into account the poorer outcome of elderly AML pts. Favourable outcome was observed expecially in pts with low HCT-CI (2 or less) and in those transplanted while in complete remission. We confirm that for older AML patients lacking a family donor MUD can provide a suitable alternative option.

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MONITORING OF MINIMAL RESIDUAL DISEASE BY QUANTITATIVE WT1 GENE EXPRESSION AFTER REDUCE INTENSITY CONDITIONING ALLOGENEIC SCT IN ACUTE MYELOID LEUKEMIA

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Introduction. WT1 is a panleukemic marker that was expressed in 90% of acute myeloid leukemias (AML). Quantification of WT1 in bone marrow samples may be useful as a marker of minimal residual disease (MRD). We evaluated the validity of this AML-MRD marker after Reduce Intensity Conditioning (RIC) allogeneic SCT. *Methods and Results.* The quantitative assessment of WT1 expression by Real-Time Quantitative PCR (RQ-PCR) was measured in 25 AML pts at diagnosis, at the time of transplant and after RIC-SCT (at precise time points). All cases showed high WT1 levels at diagnosis with a mean of 5130 (SD 4829) and a median of 2486 (range 454-10292) copies WT1/104 Abl. At transplant 19/25 pts (72%) were in complete cytologic remission (CcR) and 6/25 (28%) had refractory/relapsed AML. Bone marrow samples from pts transplanted in CcR showed at SCT significantly lower WT1 expression levels compared to the samples from pts with a relapsed or refractory AML (p=0.002). Median follow up after RIC-SCT was 17 months (range 1-49). Of the 19 pts transplanted in CcR, those (18/19) who maintained CcR after RIC -SCT displayed WT1 copy numbers persistently low during all the follow-up period, as shown in Figure 1A. In the four patients who received transplant with active disease attaining a sustained CcR after SCT, WT1 levels decreased to normal range (less than 100 copies WT1/104 Abl) in the first two months after RIC-SCT and remained low trough the entire study period (Figure 1B). All 3/25 pts who relapsed after RIC-SCT had high WT1 copy number before the cytological disease recurrence. In these 3 cases increase of WT1 were seen that preceded the decrease of molecular chimerism. *Conclusions.* In our experience, there was a complete concordance between WT1 expression levels (measured by RT-PCR at precise and sequential time points) and status of AML before and after RIC-SCT and we found a complete concordance between WT1 expression levels and other molecular and/or phenotypic disease markers (when available). Our data confirm that sequential and quantitative analysis of WT1 may be useful as a leukemia marker for monitoring MRD after RIC-SCT and as a predictor of AML cytologic relapse.

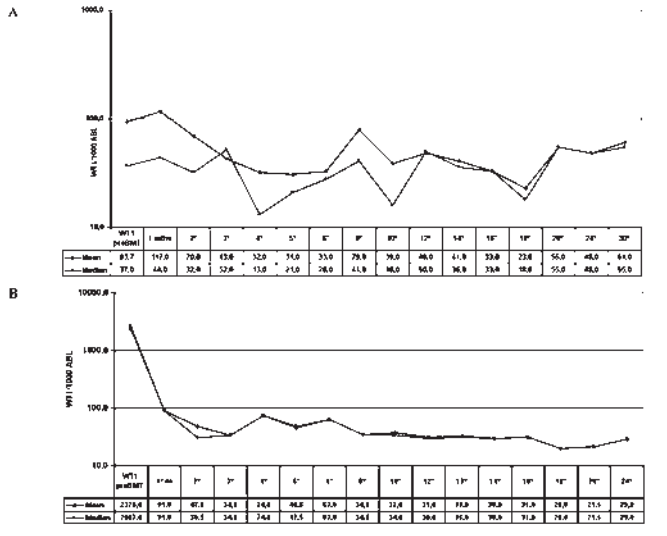

Figure 1.

P110

LESS EXTENSIVE CHRONIC GVHD WITH PBSC AND ATG FOR SIBLING TRANSPLANTS WITHOUT INCREASE IN TRM OR RELAPSE

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PBSC transplants are associated with a high risk of severe cGVHD and no convincing evidence of a survival advantage, especially for early leukemia. For these reasons some have advocated the return to bone marrow as the preferred source of stem cells. The aim of the study is to evaluate the role of the source of HSC in HLA-identical sibling transplants. We previously observed a high incidence of cGVHD, mainly the extensive type, after peripheral sibling allogeneic SCT, then, starting in 1999, we have added policlonal antibodies, (ATG-F) in the preparative regimen of PBSC transplants with the scope to reduce cGVHD. We analyze three groups of patients (245) transplanted here for hematological malignancies from 1996 to 2007: group I: PBSC no ATG, 146 pts; group II: PBSC and ATG 47 pts; group III: BM no ATG 52 pts. The three groups are comparable for age (groups I,II and III, 41yr, 43yr, 39yrs, respectively), conditioning regimens (myeloablative/ RIC, 84% and 26% group I, 75% and 25% group II, 82% and 18% group III), phase at transplant (early disease 38%, 38% and 42%), interval diagnosis-transplant (13, 15, 12 mos). With regard to diagnosis, group II had a slightly higher propor-

tion of CML compared to groups I and III. ATG-Fresenius (Bad Homburg, D) was added to the preparative regimens, at a dose of 3 or 6 mg/kg/day for five days (from days -6 to -2, total dose 15 or 30 mg/kg). The prophylaxis of GVHD consisted of the association of CsA and short MTX. aGVHD: incidence of grade II-IV was 29.5% in group I, 21% in group II and 28% in group III. Overall incidence of cGVHD was 60% in group I, 49% in the group II and 54% in group III. Extensive cGVHD represented 71% of all patients suffering from cGVHD and 43% of all evaluable patients in group I; 52% and 25.5% in group II; 55% and 30 % in group III, respectively. No significant differences among the three groups were observed for TRM, relapse and OS, both for the early and advanced phases. The addition of a low dose of ATG-F to PBSC transplants resulted in a slightly lower incidence of aGVHD and significant reduction of extensive cGHVD, compared to PBSC alone, without impairment of transplant outcome; also the incidence of GVHD was diminished compared to bone marrow. We conclude that the advantages of PBSC transplants can be retained by adding ATG-F to the preparative regimen; by doing so, PBSC should remain the preferred source of hemopoietic stem cells for HLA-identical sibling transplants.

P111

ALLOGENIC TRANSPLANTATION IN ADULT AML 1 ST CR: BETTER RESULTS WITH UNRELATED DONORS COMPARED TO HLA MATCHED SIBLING TRANSPLANTS

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The role of allogeneic transplantation in 1 st CR of adult AML, whether from HLA identical siblings or volounteer unrelated donors, is subject to debate. We present here ours results on sixty one patients-37 HLA-identical siblings and 24 unrelated- transplanted between 1998 and 2008. Their main demographic chararcteristic were: median age 42 yrs (HLA identilcal sibling) vs 39 yrs (VUD). 14 unrelated transplants were fully matched (10/10 at high resolution), with the remaining patients being 9/10 or 8/10. The source of stem cells was predominantly PBSC in the sibling group (83%) while it was bone marrow in the VUD group (84%). The preparative regimens were myeloablative in nearly 90% of the cases: they were based on Busulfan and Cyclophosphamide in 76% of the sibling group vs 50% in the unrelated group, while TBI/Cyclophosphamide was used only in 5% of the sibling and 35% of the VUD groups. Low dose ATG (Fresenius, Bad Homburg, D) was used in all the VUD and 21% of the sibling transplants, from days -6 to day -2, at a total dose of 15-30 mg/kg. Prophylaxis of GVHD consisted of CsA and short term, four doses MTX. Supportive measures consisted of protective isolation, HEPA air filtration, fluconazole, acyclovir and cotrimoxazole/trimethoprim prophylaxis, weekly CMV monitoring with preemptive intervention. All patients engrafted and no rejections or graft failures occurred. The incidence of aGVHD, grade III-IV was 8% in both groups; the incidence of overall cGVHD was 43% and 29% in the sibling vs unrelated transplants, respectively and of extensive cGVHD 27% vs 12.5%. The 5 year actuarial probability of TRM was 23% in the siblings and 17% in the unrelated transplants. The 4 yr actuarial relapse risk was 25% in the sibling group and 6% in the VUD group. With a median follow-up of 56 months, OS is 61% for the siblings vs 77% of the VUDs. Within the VUD group, the patients matched at 10/10 fared better in term of TRM, relapse and OS compared to those who were matched at a lesser degree (8 or 9/ 10); the overall survival of the 10/10 is 92% at 6 years. These results show a better outcome of the VUD vs siblings, following a myeloablative regimen, because of slightly less TRM and much less relapse in the VUD population, despite ATG-F pre-transplant; thus allogenic transplantation remains a strong indication as post remission therapy in adult AML.

P112

EFFECT OF HLA DISPARITY ON THE OUTCOME OF ALLOGENEIC UNRELATED TRANSPLANTS IN AML

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The aim of the study was to evaluate the role of HLA matching on the outcome of unrelated transplant for AML. 60 adult pts where transplanted from 1998 to 2008. Median age was 39 yrs (range 16- 56). 24 were in 1st CR , 8 in 2nd CR and 28 in more advanced phase. Patients and donors were typed for HLA-A, B, C, DR, DQ at the molecular level: 23 patients were fully matched, at 10/10 loci (group I); 10 had one or two allelic mismatches and 27 had antigenic mismatches and/or more then 2 allelic mismatches (group II). Of the 23 fully matched pts 14 were in 1st CR vs 10 of 37 of the mismatched transplants. The source of stem cells was predominantly bone marrow (50 pts). The preparative regimen was myeloablative in 90% of the cases, being TBI-based in 21 patients and Busulfan-based in 30. In all cases, ATG-Fresenius was added to the preparative regimens, usually at a dose of 3-6 mg/kg /day for five days (from days -6 to -2, total dose 15-30 mg/kg). The prophylaxis of GVHD was based on the association of CsA and MTX (four doses). Engraftment: all patients but two engrafted. aGVHD: overall incidence of grade II was 12%, grade III 6% and grade IV 6%. cGVHD: overall incidence of limited was 18%, extensive 10%. The incidence of grade III-IV aGVHD and cGVHD was 4% and 22% in group I, and 19% and 32% in group II, respectively. TRM and relapse were mainly related to the phase of the disease, with the worst results observed in decreasing order from the advanced phases to 2nd CR to 1st CR. However, even within each category of disease phase, the degree of matching had an impact. 1st CR: 1yr TRM 7% in the fully matched group vs 30% in the remaining patients; 1yr relapse 0% in the 10/10 group vs 14% for the others. Overall Survival: 92% vs 60 % at 4 years respectively. Advanced Disease: 4 yr TRM 45% for the fully matched transplants vs 46% for the mismatched; 4yr relapse 14% in the fully matched group vs 77% in the others. Overall Survival at 4 years was 52% for the 10/10 pairs vs 10% in the others. Results of myeloablative unrelated transplants in AML are very successful when a 10/10 match is used, both in 1 st CR and advanced disease. It is likely that a stronger GVL effect occurs with VUD transplants, not evidently related to an increase in GVHD.

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THE ASSOCIATION OF A SALVAGE TREATMENT CONTAINING NOVEL AGENTS FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING REGIMEN IS A FEASIBLE AND EFFECTIVE PROCEDURE IN MULTIPLE MYELOMA PATIENTS FAILING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Salvage treatments containing thalidomide, lenalidomide or bortezomib can induce a response rate ranging from 30 to 60% and a median response duration between 6 and 12 months in multiple myeloma (MM) patients (pts) relapsing after single or tandem autologous transplantation (auto-SCT): however, long-term disease control or cure cannot be commonly achieved. Allogeneic stem cell transplantation (allo-SCT) employing reduced intensity conditioning (RIC) has been increasingly performed in MM, but its efficacy in this clinical setting is still a matter of debate. *Aims*. We investigated the role of RIC allo-SCT in MM pts who had relapsed after auto-SCT and had been treated with a salvage therapy based on novel agents. Our study was structured similarly to an intention to treat analysis and included only those pts undergoing a HLA-typing immediately after the failure of auto-SCT. The cohort of pts having a donor (donor group) was compared with the one not having a suitable donor (no donor group). *Patients and Methods.* Sixty-eight

pts were retrospectively evaluated. Twenty-eight found a donor and 26 (93%) underwent an allo-SCT: 8 identical sibling (34%) , 18 MUD (66%). Conditioning regimens were fludarabine , melphalan ±thiotepa in 13 patients (50%) and fludarabine + 2 Gy TBI in 13 cases (50%). Two pts having a donor did not receive allo-SCT for progressive disease. Median age of donor group was significantly younger than no donor group . No differences were detected between the donor and no donor group with regard of median time between auto-SCT and relapse , treatment of first relapse (thalidomide-based 65% vs 54%, bortezomib-based 23% vs 37%, lenalidomide 12% vs 9%), median duration of salvage treatment and percentage of responsive patients after treatment of first relapse. *Results.* The median follow-up was 16 months. The cumulative TRM was 23% for the donor group. Acute GVHD grade II-IV occurred in 7 patients (33%) and chronic GVHD in 8 patients (38%). The 2-year overall survival (OS) and time to progression (TTP) were significantly better in the donor group compared to the no donor group (OS 75% vs 45%, *p* 0.0006; TTP 70% vs 25%, *p* 0.0002). *Conclusions*. We conclude that the association of a salvage treatment containing novel agents consolidated by RIC allo-SCT seems a feasible strategy with a rather low mortality and an encouraging TTP and OS . We are enrolling more patients in our study to corroborate our results.

Table.

	Donor group	*No donor group*	*p*
N° pts	21	42	
Age at ASCT	54 (42-63)	59 (33-70)	0.0129
Diagnosis year			0.07
≤2000	6 (28%)	12 (29%)	
2001-2003	10 (49%)	11 (26%)	
≥2004	5 (23%)	19 (45%)	
Time diagnosis-ASCT (months)	10 (5-48)	9.5 (2-74)	0.8387
ASCT year			0.5
≤2000	4 (19%)	8 (19%)	
2001-2003	7 (33%)	13 (31%)	
≥2004	10 (48%)	21 (50%)	
Tandem ASCT	8 (38%)	30 (71%)	0,03
13 deletion on karyotype at diagnosis	5/11 (45%)	6/17 (38%)	0.2
Time ASCT-relapse (months)	15 (2-62)	18 (2-87)	0.2069
Time relapse-treatment (months)	0 (0-2)	1 (0-51)	0.1151
Treatment of 1°relapse			0.09
Thalidomide-based	14 (65%)	23 (54%)	
Bortezomib-based	5 (23%)	15 (37%)	
Lenalidomide-based	2 (12%)	4 (9%)	
Treatment duration (months)	5 (2-15)	5 (1-55)	0,3229
Response to 1° relapse treatment			
CR+VGPR	5 (24%)	6 (17%)	0.1
PR	9 (43%)	15 (36%)	
resistance	7 (33%)	20 (47%)	

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INTRAVENOUS BUSULFAN (BUS) AND TWO DIFFERENT DOSAGES OF CYCLOPHOSPHAMIDE (CY): PHARMACOKINETICS, TOXICITY AND OUTCOME AFTER ALLOGENEIC HSCT IN ADULTS WITH HEMATOLOGICAL MALIGNANCIES

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Several studies have shown that the substitution of oral Bus with i.v. Bus appears to provide a better efficacy/tolerability ratio due to predictable linear kinetics with the i.v form. We report the clinical and pharmacokinetics results of a myeloablative conditioning based on the association of iv Bus and Cy in adults with hematological malignancies undergoing allogeneic HSCT. 28 patients, with a median age of 41 yrs had AML (18),ALL (2),CML (5), MDS (1),MM (2).14 were considered as standard risk (1st CR of AL, 1st CP of CML, MDS) and 14 high risk (all the others). Donors were HLA identical siblings (13), unrelated(14) and syngeneic (1); the source of HSC was BM(14) or PB(14).The preparative regimen consisted of i.v. Bus, followed, after a 24 hrs rest, by Cy 200 mg/kg(15) or 120 mg/kg(11) or Melphalan 140 mg/m^2. Unrelated transplants and sibling males with female donors received also ATG-F,15-30 mg/kg. Bus was administered 4 times daily x 4 days at 0.8mg/Kg of ideal body weight (IBW), for those with a BMI < 30; for higher BMI dosing was calculated on the adjusted IBW (AIBW); if actual body weight was less than IBW, the first was used. PK studies, with dense sampling, were conducted after dose 1, 9 and 13. No seizure prophylaxis with phenitoin was given. At a median follow-up of 35 months,TRM for the standard and high risk group were 7% and 29%, respectively, while the relapse risk was 15% and 38%. OS was 86% and 43% at 3 years. AGVHD II-IV occurred in 25% while cGVHD in 43% of cases. With regard to toxicity, severe mucositis occured in 36% of cases; the mean of peak bilirubin levels was 7.8 mg/dL in those receiving the 200 mg/kg Cy and 5.7 in those receiving 120 (*p*=0.007); statistically significant differences between the two groups were also observed for alkaline phosphatase and ALT levels; only one case of reversible VOD occurred. Pharmacokinetics: The median value of AUC after dose1 (AUC1) and dose 9 (AUC3) were 1063 and 1174 microM/L/min; interpatient and intrapatient CV were 30% and 13.8%, respectively. No correlations were found between AUC and toxicity, engraftment, GVHD and relapse. Simulation analysis was performed according to the most common anthropometric measures: the targeting performance according to Adjusted IBW was 67%. This study shows for the first time the toxicity and PK of the combination of i.v. Bus and Cy 200 mg/kg, which resulted feasible but with a greater but reversible hepatic toxicity than with 120 mg/kg. Finally, the study confirms a good PK profile without dose monitoring but an higher interpatient variability than that published. We suggest to use adjusted IBW also in non obese-patients.

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KERATOCONJUNCTIVITIS TREATMENT WITH AUTOLOGOUS SERUM EYE IN PATIENTS WITH SEVERE CHRONIC GVHD

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Introduction. Chronic graft-versus-host-disease (cGVHD) is a major complication after allogeneic stem cell transplantation, iparing the quality of life of transplant recipients. Dry eye is one of the major symptoms of cGVHD. Although several therapies have been used to minimaze this symptom, an effective treatment has not been established. We investigated the efficacy and safety of autologous serum eye in severe dry eye as manifestation of cGVHD. *Methods*. A total of 5 patients (3 males and 2 females; median age 33 years 28-54) with severe dry eye associated with cGVHD were enrolled in the study. All patients were refractory to treatment with conventional artificial tears. Autologous serum eye drops, a solution made of 20% autologous serum sterile saline, were applied 10 times per eye per day. The patients were evaluated every 4 weeks according to visual acuity, corneal sensitivity, vital staining of the ocular surface, tear dynamics, and subjective assessments of symptoms complaints scores). *Results*. The median follow-up period was 12 months (4-16 months). After 4 weeks of treatment, significant improvement was observed in both complaint scores (from 30.7±10.3 to 22.6±9.4 points; *p*=0.02) and fluorescein scores (6.0±2.1 to 2.6±1.0 points; *p*=0.03). Significant improvements were observed also in rose-bengal staining and tear break-up time. In 4 of the 5 patients the response were maintained for 6-16 months, while 1 patient died for progressive disease and was not valuable. Not serious adverse events were observed. *Conclusion*. Autologous serum eye drops are safe and effevtive for treating severe dry eye associated with cGVHD and more efficient control of dry eye may be achieved by the combined use of autologous serum eye drops with punctual plugs.

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HOMOZYGOSITY FOR KIR-HAPLOTYPE A INCREASES THE RISK OF ACUTE GVHD IN UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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NK cells exert their activity through interaction of Killer cell immunoglobulin-like receptors (KIRs) with specific ligands on the surface of target cells. Many of the KIRs recognize human leukocyte antigen (HLA) class I molecules. Two broad haplotypes of KIR genes have been defined. The A haplotype is characterised by the presence of a single activating KIR gene (2DS4), whereas the B haplotype is characterised by two or more activating KIR genes (2DS1, 2DS2, 2DS3, 2DS5 e 3DS1). The combination of these haplotypes generates three major phenotypes: AA, AB and BB. Numerous studies have highlighted the impact of NK cells on the outcome of hematopoietic stem cell transplantation (HSCT). This prompted us to analyze the KIR genotype in 78 thalassemia patients transplanted from an unrelated donor which, to our knowledge, represents the largest cohort of such cases worldwide. The conditioning regimen was the same in all patients. All donor/recipient pairs were identical at molecular level for the HLA Class I and Class II loci. The KIR genes were typed using KIR-gene-specific primers. Out of 78 transplanted patients, 62 are alive and well (disease-free survival 79.5%), 8 rejected and 8 died. Twenty-five patients (25/78-32%) developed acute graft-versus-host disease (aGvHD). In 8 of these patients, aGvHD was Grade III-IV. Patients transplanted from donors who were homozygous for KIR haplotype A had a higher risk of developing aGvHD (RR = 4.5; CI: 1.39 - 40.34 p=.004;). In particular, the AA phenotype increased the risk for severe grade III-IV aGvHD (RR= 17.5; 95% CI: 2.0-151.6; p=.002). Conversely, donors and recipients carrying other phenotypes had a much lower risk for developing aGvHD (RR= 0.052; 95% CI: 1.05-342.6; p=.005). Overall, our findings suggest that homozygosity for the A haplotype is a predictive factor that requires careful consideration when evaluating the risk for GvHD.

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ARTIFICIAL NEURAL NETWORK AND LOGISTIC REGRESSION IN PREDICTING ACUTE GRAFT-VERSUS-HOST DISEASE FOLLOWING UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction. A relatively high number of immune-biological variables have shown to play a role in aGVHD following unrelated hematopoietic stem cell transplantation (HSCT) and research of a predictive algorithm is still ongoing. We compared the prognostic performance of an artificial neural network (ANN) with that of standard logistic regression (LR), in predicting aGVHD in patients undergoing unrelated BMT. *Methods*. Because of their homogenous immune-biological features, 78 β-thalassemia major patients (median age 11.4 years), transplanted from an unrelated donor were included in the study. 24 different variables were considered: donor/recipient sex and age, Pesaro risk class, CMV serology, HCV-RNA, conditioning regimen, CD34 dose infused, HLA-DPB1 disparity, donor/patient KIR ligands C1/C2, patient KIR ligand/donor activatory or inhibitory KIR, patient and donor HLA G 14-basepair polymorphism, donor haplotype AA. Patients were randomly assigned to five consecutive random learning data sets (n=68) and test data sets (n=10). After training with learning sets, LR analysis and three-layer ANN were used to predict aGVHD outcome. *Results*. Three-year Kaplan-Meier estimates for the 78 patients studied were 91% for survival, 79.5% for thalassemia-free survival, 11.5% for the cumulative incidence of rejection and 9% for TRM. 24 patients (30.8%) developed grade II-IV aGVHD. 9 patients rejected the allograft and 7 died of TRM. In multivariate analysis, donor KIR AA haplotypes were independently significantly correlated to aGVHD (p=0.37). In test data sets, the mean specificity of LR was 80.5% compared to 90.1% of ANN (capability of predicting the absence of acute GVHD in patients who did not experience acute GVHD; p=NS). The mean sensitivity of LR was 21.7% compared to 83.3% of ANN (capability of predicting acute GVHD in patients who developed acute GVHD after HSCT; p<0.001). *Conclusion*. This is the first report of a possible role for ANNs in predicting onset of aGVHD. The advantage of ANNs over LR may be explained by their ability to recognize complex relationships that exist between variables. By contrast, ANNs are unable to calculate the weight of a single variable, while LR determines a relative risk for each variable. A combination of the two approaches has the potential to significantly improve the clinical decision-making process and the overall outcome of HSCT.

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RECIPIENT CTLA-4 GENOTYPE IS A PROGNOSTIC FACTOR FOR ACUTE GVHD IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a member of the immunoglobulin superfamily and encodes a protein which transmits an inhibitory signal to T cells that down-regulates T-cell activation. Polymorphisms of this gene have been associated with autoimmune diseases and it has recently been observed that donor genotypes correlate with the clinical outcome of allogeneic stem cell transplantation in leukemia patients. In an attempt to replicate this result in unrelated HSCT of thalassemia patients, we compared the genotypical distribution of 3 polymorphisms of the CTLA-4 gene in 71 thalassemia patients and their unrelated donors with the clinical outcomes of the transplantation procedure. A significant association was observed for the CTLA-4/CT60-AA genotype in recipients and the development of Grade II-IV acute graft versus host disease (aGVHD) (48% vs 15%; p=0.0058, OR=5.3) and Grade III-IV aGVHD (60% vs 20%; p=0.02; OR=6.1). In the donors, this analysis did not reach statistical significance (36% vs 25%; p=0.3). Logistic regression demonstrated that the association observed in the patients did not depend upon other known or putative risk factors for Grade II-IV or Grade III-IV aGVHD. In our patient sampling, none of the 3 polymorphisms analyzed were associated with rejection. Overall, the data obtained in this study confirm that the genetic variability of CTLA-4 is an important prognostic factor for the development of aGVHD after HSCT. More specifically, our findings suggest that the recipient and not the donor genotype is the main risk determinant. Other studies on larger case reports will be necessary to confirm this association and to clarify its functional aspects.

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RITUXIMAB AND DONOR LYMPHOCYTE INFUSIONS: AN EFFECTIVE SALVAGE TREATMENT FOR LYMPHOMA PATIENTS RELAPSED AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION

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Lymphoma patients relapsed after allogeneic stem cell transplantation (alloSCT) have a dismal prognosis. Best salvage treatment and the role of donor lymphocyte infusions (DLI) and Rituximab are to be defined yet. This study aimed to assess whether salvage chemotherapy plus DLI with or without Rituximab may improve progression free and overall survival (PFS, OS) and reduce relapse incidence compared to

chemotherapy alone. Seventy-eight consecutive lymphoma patients were allografted at our institution between 2000 and 2007 with a reduced-intensity conditioning (RIC, thiotepa-fludarabine-cyclophosphamide) from HLA-matched siblings (49, 63%) or matched unrelated donors (29, 37%). Thirty-five patients relapsed after alloSCT (45%), their median age was 45 (19-63), 11 (31%) had indolent, 16 (46%) aggressive non Hodgkin's lymphoma, 8 (23%) Hodgkin's lymphoma. Seventeen patients (49%) received DLI (median 2, range 1-4) after salvage chemotherapy, 18 (51%) received chemotherapy alone. Nine patients did not receive DLI because of GVHD. Fourteen patients (40%) received Rituximab in the salvage regimen, 11 (31%) received DLI after Rituximab-including chemotherapy. OS and PFS were estimated by Kaplan-Meier method, relapse by cumulative incidence (CI) method with competing risks. Multivariate analysis was done with Cox models. Median follow-up after relapse was 15 months (1-104). One- and 2-year PFS was 34% and 31%. One- and 2-year OS was 54% and 45%. Relapse CI was 38% at 1 and 2 years. DLI significantly improved PFS (p=0.04, HR=0.44 [CI95%=0.1-1.0]). Rituximab and DLI improved PFS (p=0.007, HR=0.25 [CI95%=0.08-0.75]) and OS (p=0.001, HR=0.07 [CI95%=0.01-0.5]), and reduced CI of relapse (p=0.03) compared to other salvage treatments. After DLI 7 patients out of 17 (41%) developed aGVHD (5 grade>=2), 5 (29%) cGVHD (1 extensive). GVHD after DLI did not significantly affect PFS and OS. Among DLI patients Rituximab improved PFS (p=0.008, HR=0.20 [CI95%=0.05-0.74]) and OS (p<0.001, HR=0.06 [CI95%=0.01-0.52]) compared to DLI alone. Multivariate analysis considering salvage treatment (Rituximab+DLI vs other) and histology showed that Rituximab and DLI improve PFS (p=0.03, HR=0.28 [CI95%=0.09-0.88]) and OS (p=0.01, HR=0.06 [CI95%=0.01-0.54]) regardless of lymphoma subtype. In conclusion DLI improve PFS and Rituximab before DLI improves PFS and OS of lymphoma patients relapsed after RIC alloSCT, suggesting that this may be an optimal salvage strategy in this setting.

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HUMAN CYTOMEGALOVIRUS DNAEMIA CUT-OFF FOR RISK-ADAPTED PRE-EMPTIVE THERAPY IN ADULT ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Pre-emptive therapy is a well-established strategy for human cytomegalovirus (HCMV) disease prevention in allogeneic haematopoietic stem cell transplant (HSCT) recipients. Direct detection of HCMV matrix protein pp65 (pp65 Ag) by immune-fluorescence assay in peripheral blood leukocytes still represents the gold standard for monitoring HCMV reactivation. More recently, quantitative determination of the viral genome (DNAemia) by real-time polymerase chain reaction (rt-PCR) has been employed. To investigate whether a DNAemia cut-off corresponding to clinically safe antigenaemia cut-offs could be identified to guide pre-emptive therapy in low and high-risk patients, we performed a double HCMV screening program using both pp65 Ag and rt-PCR. During a two years period (April 2006-April 2008), 43 patients who underwent allogeneic HSCT for haematological malignancy were analyzed. Thirty-three patients were monitored for HCMV infection from engraftment to at least day +100, 10 patients were studied during steroid treatment for chronic graft-versus-host disease (GVHD). Overall, 489 HCMV determinations by both pp65 Ag and rt-PCR were performed. Therapy was given on an antigenaemia risk-adapted strategy. High-risk patients, nominally those with acute or chronic GVHD on steroids, were given ganciclovir upon first positive pp65 Ag. Low-risk patients were treated after detection of a single high level (> 3 cells) or repeated low levels (≤3 cells) of pp65 Ag. No cases of HCMV disease were recorded during the observation period. Our analysis shows a significant association between DNAemia and therapy needs (Wald's test: p<.05). In high-risk group, the receiver operating characteristics (ROC) analysis recognized 1,200 genome copies per ml as optimal cut-off for therapy needs (AUC 0.829), with 71.4% sensitivity, 80.0% specificity, 83.3% positive-predictive value (PPV) and 66.7% negative predictive value (NPV) (Figure 1a). For low-risk patients, DNAemia cut-off was set at 2,500 genome copies per ml (AUC 0.923), corresponding to 100.0% sensitivity, 92.3% specificity, 83.3% PPV and 100.0% NPV (Figure 1b). The odds of therapy passing from below to above the cut-point value in high and low-risk patients increased by 2.300% and 3.545%, respectively. HCMV DNAemia assay is a reproducible and less time consuming test. With the identification of clinically validated cut-off, it could become the first choice assay for monitoring and guiding pre-emptive therapy in adult allogeneic HSCT recipients.

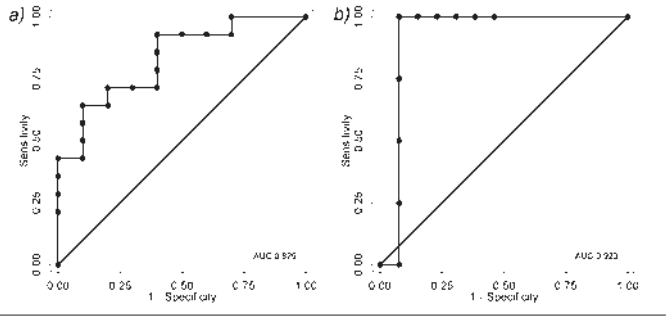

Figure 1.

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CONTRAST ENHANCED ULTRASOUND SONOGRAPHY IN INTESTINAL ACUTE GRAFT VERSUS HOST DISEASE

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Three patients with diagnosis of ALL (pt1), MCL (pt2) and AML (pt3) received a matched unrelated donor allogenic transplant, fully myeloablative in two and reduced intensity in one. Conditioning was with BU/Cy, Cy/TBI and Thiotepa, Flu and MEL. GVHD prophylaxis was with cyclosporine (CSA) and short course MTX in all. All three patients developed biopsy proven intestinal GVHD. Two were steroid refractory (pt1 and pt2). All three patients were scanned with transabdominal ultrasonography (US) and with contrast enhanced ultrasound sonography (CEUS) using a linear phased-array 7.5-MHz transducer. A sulphur hexafluoride-based with a phospholipid shell microbubble contrast agent (SonoVue®, Bracco) was injected i.v. as a bolus (2.4 mL) followed by 5 mL saline flush allowing real-time imaging of microcirculation. US in pt1, pt2 and pt3 revealed mucosal oedema and thickening of the terminal ileum (5.1, 5.8 and 7.9 mm) and the ascending colon (5.8, 6 and 8.4 mm), in pt3 thickening was up to proximal descending colon (6 mm). CEUS showed: in pt1 and 3 an arterial phase complete enhancement of the entire wall section from the mucosal to the serosal layer (terminal ileum), and in pt2 there was absence of enhancement only in the outer border of the muscolaris propria. Such enhancement pattern has been previously described in active Crohn disease. In pt1 CEUS showed still activity (enhancement) after 2 dosed of Infliximab despite improvement of symptoms suggesting further treatment and showed enhancement of descending colon when she flared 3 months later. In pt3 a 2nd CEUS performed 5 days after steroid was initiated showed a reduced wall enhancement and few days later diarrhoea resolved. In pt2 a 2nd CEUS performed at 7 days showed no enhancement reduction and the patient died three days later. In conclusion: CEUS showed 1)initial intestinal microcirculation wall enhancement in all three patients; 2)residual GVHD activity despite the improved clinical symptoms in pt1 suggesting further treatment with eventually complete remission, 3) good concordance with a GVHD flare 3 months later, 4)improvement in intestinal wall microcirculation in pt3 (steroid-responding) in agreement with clinical symptoms improvement, 5)no improvement of enhancement of microcirculation of intestinal wall (despite identical US wall thickening) in the steroid refractory pt2 who died few days later. Further prospective studies are needed to evaluate usefulness of CEUS in monitoring intestinal GVHD.

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ANALYSIS OF OUTCOME PREDICTORS IN 157 PATIENTS TREATED WITH REDUCED INTENSITY CONDITIONINIG REGIMENS AND ALLOGENEIC STEM CELL TRANSPLANTATION

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Objectives. In order to identify prognostic factors, we analyzed the outcome of 157 patients with haematological malignancies who underwent allogeneic SCT with reduced-intensity conditioninig (RIC) in our Centre between January 2002 and December 2008. P*atients and methods*. Median age was 56 years (range 19-69). Haematological diseases were: acute leukemia 28 %, aggressive non-Hodgkin lymphoma 21%, indolent lymphoma 15%, Hodgkin lymphomas 14%, multiple myeloma 17%, idiopathic myelofibrosis 5%. Pretreatment included autologous SCT (38%) or ≥3 previous chemotherapy regimens (42%). Disease status before SCT was : complete remission or very good partial remission (37%), partial response (21%) , refractory disease (42%). HCT-Comorbidity index (CI) was ≥1 in 54% of all patients. Conditioning regimens were mainly based on thiotepa plus cyclophosphamide±fludarabine. Stem cells came from sibling donors (42%) or from unrelated donors (58%). One-hundred-thirty-four patients (84%) received peripheral blood stem cells (PBSC). GVHD prophylaxis was based on cyclosporine and short-term methotrexate, with the addiction of ATG in unrelated transplants. Fluconazole was administered as antifungal prophylaxis. We analyzed the influence of pre-transplant and post-transplant variables on TRM, EFS and OS by means of the univariate and multivariate analysis. *Results.* The cumulative incidence of engraftment was 93%. The estimated 1- and 3-year TRM were 25% and 33% respectively. The estimated 3 year- OS and disease-free-survival (DFS) were 46% and 36%. Grade II-IV acute GVHD and chronic GVHD developed in 37% and 49% of the patients, respectively. Thirty-six (23%) presented a proven or probable mycotic infection after SCT. In multivariate analysis, prognostic factors that significantly increased TRM were age at SCT and the use ATG as GVHD prophylaxis . Refractory disease before transplant and development of mycotic infections were significative predictors of shortened EFS and OS. Moreover, HCT-CI ≥1 was significantly associated with poorer OS. *Conclusions.* Our experience demonstrated that, among pre-transplant factors, recipient older age, higher HCT-CI, refractory disease and GVHD prophylaxis with ATG negatively affected outcome after SCT influencing TRM or OS. Among post-transplant features, development of mycotic infections was the most powerful unfavourable prognostic factor.

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INCIDENCE, RISK FACTORS AND OUTCOME OF EPSTEIN-BARR VIRUS REACTIVATION AND ASSOCIATED LYMPHOPROLIFERATIVE DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objectives. This retrospective study aims to evaluate incidence, risk factors, outcome and management of Epstein-Barr virus (EBV) reactivation and EBV-associated post-transplant lymphoproliferative disease (PTLD) in 59 patients with haematologic malignancies who underwent allogeneic hematopoietic stem cell transplantation (HSCT). *Patients and Methods.* Since January 2007, 59 consecutive transplanted patients had been monitored for EBV-DNA load on at least 4 samples (median number for each patient 6, range 4-13). Table 1 summarizes patients' main characteristics. Peripheral blood samples were collected on days +14, +21 and +28 from HSCT and then at 2-4 week intervals, depending on the clinical course. A viral load exceeding 1000 EBV-DNA copies/ml (confirmed in 2 consecutive samples) was defined as EBV reactivation. *Results.* The median time of observation was 70 days (range 30-365). Twelve patients (20%) experienced EBV reactivation after a median time of 43 days from transplantation (range 20-110). EBV reactivation was significantly more frequent in patients transplanted from mismatched unrelated donors than from matched unrelated or sibling donors (p=0.04) and in patients who received T-cell depletion with antithymocyte globulin ATG at high doses in comparison to low doses (p=0.05). At the time of diagnosis of EBV reactivation, 6 patients (10%) had no symptoms or signs of lymphoproliferative disease: three of them received pre-emptive therapy with 2 weekly administrations of rituximab and the other 3 underwent careful monitoring and reduction of immunosuppression. All these 6 patients achieved EBV-DNA normalization, none of them developed PTLD. In the other 6 patients (10%) the EBV reactivation was diagnosed with concomitant signs of PTLD. Four out 6 patients with PTLD achieved long-term remission after 4 weekly rituximab administrations associated with 2-4 vincristine infusions (2 patients). *Conclusions.* In our study the incidence of EBV reactivation among recipients after HSCT was 20%, with 10% incidence of EBV-associated PTLD. We highlighted as risk factors for EBV reactivation the use of unrelated mismatched donors and *in vivo* T-cell depletion with high-dose ATG. Rituximab was effective in the treatment of both EBV reactivation and EBV-associated PTLD.

Table 1. Patient's main characteristics.

Number of patients		59
sex	M	34
	F	25
median age, years (range)		48 (22-67)
diagnosis	acute leukemias	28 (48%)
	lymphomas	19 (32%)
	other	12 (20%)
number of previous therapy lines	0-1	18 (31%)
	2 o >2	41 (69%)
disease status at TMO	remission (PR + CR)	36 (61%)
	no remission	23 (39%)
graft type	related donor	
	mached	8 (14%)
	haploidentical	3 (5%)
	unrelated donor	
	mached	21 (35%)
	mismached	27 (46%)
conditioning regimen	conventional	16 (27%)
	all others	43 (73%)
ATG	NO	11 (19%)
	ATG-Genzyme 7 mg/kg	22 (37%)
	ATG-Fresenius 10mg/kg	13 (22%)
	ATG-Fresenius 30mg/kg	13 (22%)
source of stem cells	BM	13 (22%)
	PB	45 (76%)
	CBU	1 (2%)
aGvHD	grade 0-I	29 (49%)
	grade II - IV	30 (51%)
cGvHD	not valuable	14
	limitate	38 (84%)
	extensive	7 (16%)

Cell Therapy

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TELOMERASE KINETICS AND TELOMERASE ACTIVITY IN MYELOID MALIGNANCIES: PRELIMINARY RESULTS

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Telomeres are regions of highly repetitive GC-rich DNA sequence (TTAGGG in human) at the end of a eukaryotic chromosome that act to protect loss of vital genetic information due to incomplete DNA replication at the ends of chromosomes in late S phase. Telomeres are progressively shortened with cell divisions, and trigger cellular senescence in somatic cells. However, most stem cells and cancer cells are able to proliferate indefinitely, as they have a telomere maintenance mechanism, such as activation of telomerase, a ribonucleoprotein that synthesizes telomeric DNA sequences and almost universally provides the molecular basis for unlimited proliferative potential. Loss of telomerase function produces short telomeres, potentially resulting in chromosome recombination, end-to-end fusion and recognition as damaged DNA. We evaluated telomerase enzymatic activity (TA) and telomeres length in mononuclear cell (MNC) from bone marrow of 20 patients with Acute Myeloid Leukemia (AML, n = 9) and Myelodysplastic Syndrome (MDS, n = 11). Compared with the control (normal donor bone marrow, n = 10), telomerase activity (TA) was increased 2 fold in 36% of low risk MDS patients, 4 fold in 50% of high risk MDS patients and 4- to 10-fold in 55% of AML patients. On the other hand, telomeres length was similar in patients with MDS and AML. The Telomere Restriction Fragment length (TRF) was 6.65 kb±0.9 kb in MDS patients and 6.45 kb±1.45 kb in AL patients, whereas normal bone marrows showed TRF of 8.1 kb±0.5 kb and control cells expressing high telomerase activity showed TRF of 9.5 kb±0.6 kb. Our data suggests that in MDS and AML patients TA is higher than the normal donor. In particular high risk MDS and AML patients showed TA higher than low risk MDS patients. To increase our knowledge upon telomere maintenance mechanism and correlation to the clinical features in MDS and AL patients, we are investigating the expression of hTERT and transcription factors involved in hTERT regulation such as wt1, c-myc, mad-1 and p53.

P125

PURINERGIC SIGNALING MODULATES HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS FUNCTION

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Nucleotides triphosphates are extracellular messengers binding to specific plasma membrane receptors (P2Rs) that modulate a wide variety of biological responses in several cell types. Little and controversial information is available on the role of extracellular nucleotides in human mesenchymal stem cells (hMSCs). In this study, we assessed whether P2Rs were expressed and functional in bone marrow-derived hMSCs. We show that bone marrow-derived hMSCs expressed the mRNA for the following P2R subtypes: P2Y1R, P2Y2R, P2Y4R, P2Y6R, P2Y11R, P2Y12R, P2Y13R, P2Y14R, P2X1R, P2X3R, P2X4R, P2X5R, P2X6R and P2X7R. P2Y1R, P2Y2R, P2Y11R, P2X1R, P2X4R, and P2X7R, were also detected by Western blot. hMSCs were very resistant to the cytotoxic effects of high concentrations of extracellular ATP, as demonstrated by the lack of morphological and mitochondrial changes or release of intracellular markers of cell death. Gene expression profiling revealed that hMSCs stimulated with ATP underwent a down-regulation of genes involved in cell proliferation, whereas those involved in cell migration were strongly up-regulated by the nucleotide. Functional studies confirmed the inhibitory activity of ATP on proliferation of hMSCs and clonogenic stromal progenitors. Furthermore, ATP potentiated the chemotactic response of hMSCs to the chemokine CXCL12, and increased their spontaneous migration. Moreover ATP increased production of the pro-inflammatory cytokines IL-2, IFN-gamma, and IL-12p70, while decreased secretion of the anti-inflammatory cytokine IL-10. Besides, preliminary data suggest that ATP modulates the hMSC ability to differentiate into various cell types including osteocytes and adipocytes. Thus, our data show that purinergic signaling modulates hMSCs functions and highlight a role for extracellular nucleotides in hMSCs biology.

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HEMATOPOIETIC STEM CELLS ARE EFFECTIVE IN REDUCING FIBROSIS IN A NEW ANIMAL MODEL OF CHRONIC LIVER DISEASE

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Chronic fibroproliferative diseases of the liver and its complications are major causes of morbidity and mortality worldwide. Recently, we set up an efficient and well-tolerated protocol to induce cirrhosis and capable to progress to ascitc stage in mouse. We demonstrated that C57BL/6N mice receiving CCl4 (2 L/min) by short inhalation cycle (2 min each) trice/week for thirteen weeks developed significantly more ascites at sacrifice than those receiving CCl4 subcutaneously or intraperitoneally. Furthermore, no extra-hepatic damage could be detected in mice inhalating CCl4. Because experimental evidence suggests that human hematopoietic stem cells (huHSCs)may contribute to liver regeneration after damage, we investigated the effects of the transplantation of immunomagnetically highly purified human $CD34^+$ and $CD133^+$ huHSCs on liver fibrosis/regeneration of mice with chronic liver injury. HSCs were collected by leukapheresis from the peripheral blood of cirrhotic patients treated with G-CSF (15 g/Kg/day). C57BL/6N mice were divided into four groups: healthy controls; CCl4-treated cirrhotic mice not receiving huHSCs; cirrhotic mice transplanted with $1x10^6$ $CD133^+$ or $CD34^+$ huHSCs. To prevent huHSC rejection in this xenotransplant model, a daily dose of 25 mg/Kg of cyclosporin A was administered to all mice by oral gavages, starting from the day before the transplantation. Transplantation of huHSCs was performed by a single intravenous injection (tail vein). After four weeks, all mice were sacrificed to perform histological and morphological analysis. Samples from liver, lungs, kidney and spleen were fixed, embedded in paraffin and stained with haematoxylin-eosin. Liver fibrosis was evaluated by Sirius red staining technique. Xenotransplantation of huHSCs was safe for immunosuppressed animals. Cirrhotic mice, with or without cell transplantation, showed no morphological alterations of lung, kidney and spleen. Sirius Red staining revealed clear differences in fibrotic tissue between experimental groups: mice treated with CCl4 presented cirrhosis because Sirius Red staining showed the presence of fibrotic septa and regenerative nodules. By contrast, mice which received $CD133^+$ or $CD34^+$ huHSCs showed the significant reduction of the number and the extension of the fibrotic septa. These preliminary data suggest a possible role of huHSCs in the recovery of liver fibrosis in a novel animal model of decompensated cirrhosis.

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THE IMMUNOREGULATORY ENZYME INDOLEAMINE 2,3-DIOXYGENASE (IDO) IS EXPRESSED BY NATURAL KILLER (NK) CELLS AND ITS EXPRESSION IS REGULATED BY INTERFERON (IFN)-GAMMA

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NK cells are crucial mediators of the immune response against tumour cells and pathogens through the modulation of adaptive and innate immune response. Indoleamine 2,3-dioxygenase (IDO) is an enzyme catalyzing the degradation of the essential amino acid L-tryptophan into kyurenines. Several cell types, including dendritic cells, have been shown to express IDO, which acts as a potent immunosuppressive agent. However, little is known about the expression of IDO in NK cells. To this end,

highly purified $CD3^-CD56^+$NK cells from healthy donors were treated with IL-2 (100 U/mL) for 12h, 40h, 64h and 88h. At the end of each IL-2 stimulation, NK cells were collected and IFN-gamma production was measured by colorimetric assay. IDO expression in NK cells was evaluated both by RT-real time PCR and Western blotting. Our data show that, during IL-2-mediated activation, NK cells markedly upregulate IDO expression both at the molecular and the protein level. This effect is maximum after an overnight incubation and it decreases at later time points. IDO expression is clearly correlated with IFN-gamma production by NK cells whereas IFN-gamma receptor expression is not affected. To test whether IDO expression may be regulated by IFN-gamma signalling, IL-2-activated NK cells were cultured in the presence and absence of a blocking anti-IFN-gamma antibody. Our results demonstrate that IL-2-mediated up-regulation of IDO expression is significantly impaired by anti IFN-gamma antibody, thus suggesting that IDO expression in NK cells may be regulated through IFN-gamma-signalling. Interestingly, IDO mRNA upregulation seems to be associated with the downregulation of DAP12 gene, which has been showed to negatively regulate IDO expression in different cell subsets such as dendritic cells. In conclusion, our results demonstrate that: 1) NK cells upregulate IDO expression during cytokine-mediated activation and 2) IDO expression is regulated by IFN-gamma. These data provide novel insights in NK cell biology which may have some implications for their clinical use in cell therapy strategies.

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INTERFERON-α-ENGINEERED MULTIPOTENT MESENCHYMAL STROMAL CELLS FOR THE TREATMENT OF MYELOMA

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Bone marrow mesenchymal stromal cells (BM-MSCs) are non-hematopoietic progenitor cells with multilineage differentiation potential. Exogenously administered BM-MSCs have been shown to survive and proliferate in the presence of malignant cells, becoming stromal cells and supporting tumor growth. Thus, BM-MSCs are attractive candidates to deliver biologically active molecules in the tumor environment *in vivo* and to enhance specific immune responses. Interferon-α (IFN-α) has been used for years for the maintenance treatment of multiple myeloma (MM), but its administration is limited by the temporary efficacy and the toxicity. We analyzed the *in vivo* effects of mouse BM-MSCs transduced with IFN-α cDNA in the Sp6 plasmocytoma mouse model. BM-MSCs were transduced with a lentiviral vector containing EGFP cDNA or murine IFN-α cDNA (efficiency = 70%). Two months-old Balb/c mice (Balb/cByJIco, Charles River Italia, Calco, LC, Italy) (H-2d), were injected subcutaneously (s.c.) with the tumorigenic dose of $0.5x10^6$ Sp6 cells (H-2d). The same mice were then weekly injected with $0.5x10^6$ BM-MSCs/IFN-α (1, 4 or 8 doses), in the same anatomical quarter. Some mice was injected s.c. with Sp6 and with BM-MSCs/EGFP s.c. or intravenously (i.v.) to test *in vivo* homing. Tumor immunohistochemistry was performed with anti-von Willebrand factor, anti- α -smooth muscle actin, anti-CD4, anti-CD8, anti-asialo GM1, anti-CD45, anti-CD90, anti-murine IFN-α. BM-MSCs were capable of homing into Sp6 tumor, forming clusters of cells. Treatment with BM-MSCs/IFN-α resulted in a significant delay in the onset of palpable tumors (event free survival, EFS, of 50% at day +17 for 1 dose, +20 for 4 doses and +64, for 8 doses, whereas Sp6 alone or coinjected with BM-MSCs showed tumor incidence of 100% 10-13 days after injection). Weekly delivery of BM-MSCs/IFN-α induced a significant decrease of kinetics tumor growth and an increment of overall survival (OS) (median OS in controls: 19 days, animals receiving BM-MSCs: 17 days, mice treated with 1 and 4 doses of BM-MSCs/IFN-α: 30-31 days, mice treated with 8 doses:77 days). The antitumor effect is associated with tumor necrosis, reduction in microvessel density, and NK cell infiltration. These findings indicate that transduced BM-MSCs could be useful to deliver anti-cancer molecules in the microenvironment of myeloma and become a promising tool for specific, low-toxic, and long-lasting anti-myeloma therapy.

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PHASE I-II TRIAL OF ADOPTIVE IMMUNOTHERAPY WITH HAPLOIDENTICAL KIR LIGAND-MISMATCHED NATURAL KILLER CELLS IN HIGH RISK ACUTE MYELOBLASTIC LEUKEMIA PATIENTS

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The effector function of natural killer (NK) cells is regulated by activating and inhibitory receptors, termed killer immunoglobulin receptors (KIRs). In haploidentical T-cell depleted transplantation the donor/recipient KIR mismatch significantly impacts on NK-mediated tumor cell killing in acute myeloid leukemia (AML). Forty-two high risk AML patients, based on poor prognostic features at diagnosis, advanced age or resistant/relapsed disease, entered a phase I-II study of adoptive NK-cell based immunotherapy and were screened for the avalilability of one haploidentical KIR ligand mismatched donor. Eighteen of them had one suitable donor. NK cells were enriched from steady-state leukaphereses by using an immunomagnetic separation system. So far, 13 patients (1 partial remission, 4 progressive disease, 2 molecular relapse and 6 complete remissions) received NK cell infusion after immunosuppressive chemotherapy (fludarabine and cyclophosphamide) and followed by interleukin-2 injections. The median number of reinfused NK cells was $2.74x10^6$/Kg and contaminating $CD3^+$ T cells were always less than $1x10^5$/Kg. No significant toxicity, including GVHD, related to NK cell infusion was observed. One patient in partial remission obtained a complete remission, which lasted for 6 months. Among the 4 patients with progressive disease, 3 individuals showed the persistence of disease and one patient died during the aplastic phase. Three/6 patients in morphologic complete remission are stable after 22, 19 and 6 months, whereas 3 patients relapsed early after NK cell infusion. Among the 2 patients in molecular relapse, one patient achieved a molecular CR maintained after 4 months while the other patient achieved molecular CR but then relapsed after 9 months. Biological studies demonstrated the presence of alloreactive NK cell clones in 10 donors and in 11 patients. Donor NK cells were demonstrated in all evaluable patients with a peak at day 10 after NK therapy. A mixed chimerism was shown only in patients achieving CR or in CR and this finding was supported by in vitro studies indicating that AML cells induce apoptosis of NK cells in a dose-dependent manner. In summary, a two-step enrichment of CD56+/CD3- cells allows the collection of large numbers of NK cells suitable for adoptive immunotherapy in AML patients. Infusion of NK cells is feasible and safe and adoptively transferred NK cells can be detected in the peripheral blood after infusion.

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INDOLEAMINE 2,3-DIOXYGENASE IS DIFFERENTIALLY EXPRESSED AND FUNCTIONALLY ACTIVE IN HUMAN CD34+-DERIVED DENDRITIC CELL SUBSETS

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Indoleamine 2,3-dioxigenase (IDO) is the rate-limiting enzyme in tryptophan catabolism along the kynurenine pathway. IDO expression by different cell subsets inhibits T-cell activation, proliferation and survival and induces regulatory T cells (Tregs). Although human monocyte-derived dendritic cells (DCs) have been shown to express IDO, little is known about its expression in other subsets of human DCs. In particular, no data are currently available for IDO expression in $CD34^+$-derived DC subsets, such as dermal DCs and epidermal Langherans cells (LCs). In the present study, we tested IDO expression and function by DCs, generated from purified $CD34^+$ cells after 7 days of culture with GM-CSF and TNF-α. In some experiments, day 7-DCs were sorted into different

subsets and tested for IDO expression and function. Alternatively, day 7-DCs were cultured with GM-CSF and IL-4 and then matured with IL-1β, TNF-α, IL-6 and PGE2. $CD34^+$ cells did not express IDO mRNA, without significant differences among different progenitor cell sources (cord blood, mobilized peripheral blood, bone marrow). During DC differentiation, IDO mRNA expression was observed at day 7, but not at day 14. At day 14, maturation stimuli induced a marked up-regulation of IDO mRNA and protein, which resulted in increased kynurenine production and inhibition of T-cell proliferation. FACS analysis of day-7 cells revealed a double population, which comprised $CD14^+CD1a^-$ dermal DCs and $CD14^-CD1a^+$ LCs. Interestingly, IDO mRNA and protein were observed only in dermal DCs, but not in LCs. IDO expression by dermal DCs resulted in increased production of kynurenine and in reduced allostimulatory capacity of T-cell proliferation. Dermal DCs were shown to induce a population of $CD4^+CD25^+Foxp3^+$ which acted as Tregs by inhibiting allogeneic T-cell proliferation. This effect was abrogated by the addition of the IDO inhibitor 1-methyl tryptophan. In conclusion, DC differentiation of $CD34^+$ cells results in the expression of a functionally active IDO protein in dermal DCs, but not in LCs. Given the role of IDO in regulating immune tolerance, our data may suggest that within the complex skin microenvironment dermal DCs are intrinsically committed to function as regulatory DCs, whereas LCs are devoted to act as activating DCs. These data have implications for a better understanding of the development of the immune response during inflammation/infection.

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THE EXPRESSION OF INDOLEAMINE 2,3-DIOXYGENASE BY DENDRITIC CELLS AFFECTS THEIR FUNCTION IN VIEW OF IMMUNOTHERAPY CLINICAL TRIALS

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Indoleamine 2,3-dioxigenase (IDO), the immunosuppressive enzyme involved in the catabolism of tryptophan, contributes to tolerance in a number of biological settings. IDO is expressed by different cell subsets, including human dendritic cells (DCs) and it is upregulated in DCs upon maturation. DCs are largely used in vaccination against tumors. PGE2 plays a pivotal role in maturation of DCs for clinical use, because it facilitates migration to lymph nodes. However, PGE2 is a strong IDO inducer, that can limit anti-tumor activity of DC-based immunotherapies. Here, we characterized the expression and function of IDO in human dendritic cells after generation and maturation with different stimuli. DCs were generated from purified $CD14^+$ monocytes after culture with GM-CSF and IL-4 and then matured with CD40L, LPS alone, LPS plus IFN-gamma and a combination of IL-1β, TNF-α, IL-6 with or without PGE2. After culture, DCs were analyzed for IDO expression and function by real-time PCR, western immunoblot, kynurenine production and Tregs induction. Moreover, we tested DCs capacity of inducing an allogeneic response and an antigen specific autologous response. Our results demonstrate that, during maturation, PGE2 associated with the cytokine cocktail was the most effective stimulus in up-regulating IDO, both at mRNA and protein level. In this condition, we measured the highest kynurenine production and the highest generation of $CD4^+CD25^+FOXP3^+$ Tregs from normal $CD3^+$ T cells. In contrast, CD40L was the least effective stimulus in inducing IDO expression. Tregs generated by PGE2 matured DCs highly suppressed allogeneic T-cell proliferation, whereas Tregs generated by CD40L matured DCs were less effective. PGE2-matured DCs and CD40L-matured DCs induced similar allogeneic response and antigen specific autologous response. However, when the IDO inhibitor 1-methyl tryptophan (1-MT) was added, PGE2-matured DCs showed higher immunogenic activity compared to CD40L-matured DCs. These data suggest that the strong IDO up-regulation by PGE2 can limit the effectiveness of PGE2 in clinical practice. Actually, CD40L might be preferred as maturation stimulus rather than PGE2, since PGE2-matured DCs induce Tregs. Alternatively, to improve the efficacy of a DCs-based vaccine, PGE2-matured DCs should be combined with molecules which inhibit IDO activity.

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ATP RELEASED DURING INFLAMMATION AND TUMOR CELL GROWTH MODULATES CD4+ CELL FUNCTIONS

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Adenosine triphosphate (ATP) is emerging as extracellular signalling molecule playing pivotal roles in several cellular processes. ATP modulates subcellular functions through specific cell membrane purinergic P2 receptors (P2Rs). Recent studies show that ATP is released during inflammation and tumor cell growth. In the present study, we characterize the effects of ATP on $CD4^+$ cells in resting, activated or regulatory conditions. We show the differential expression of P2X and P2Y receptor subtypes. Specifically, P2X1 and P2X7 receptors, involved in the induction of apoptosis, were down-regulated in regulatory $CD4^+$ whereas their expression was increased in resting and activated $CD4^+$ cells. Conversely, resting T cells expressed less P2X5 than regulatory and activated cells. Of interest, Tregs cells did not express P2X6 and P2Y2 while P2Y1, P2Y4, P2Y11 and P2Y14 were up-regulated on activated $CD4^+$ cells. P2Rs activation by critical concentrations of ATP induced intracellular Ca^{2+} concentration changes, plasma membrane depolarization and permeabilization. At the functional level, high ATP concentrations induced to apoptosis $CD4^+$ during activation but not Tregs, whereas resting $CD4^+$ cells showed an intermediate sensitivity. Apoptosis did not seem to be mediated by the receptor P2X7, because the use of the inhibitor KN-62 had no effect. The same ATP concentrations that induced apoptosis in $CD4^+$ cells during activation, stimulated proliferation in activated and regulatory cells. The use of apyrase during incubation of $CD4^+$ with ATP, partially inhibited the induction of proliferation, demonstrating that is triphosphate to induce proliferation. Conversely, during activation, $CD4^+$ cells proliferation was enhanced by low doses of extracellular ATP. High dose of ATP acted as chemokine for activated and regulatory $CD4^+$ and increased IL-8 and IL-12 production by resting and regulatory $CD4^+$ respectively. High dose of ATP inhibited IL-8 release by activated $CD4^+$. Low dose of ATP induced the release of IL-12 by resting, IL-2 and IFN-gamma by activated, and TNF-α by regulatory $CD4^+$. Overall, extracellular ATP, which is released in "damage situations", modulates human T-cell functions according to the differential expression of P2Rs which depends on their activation status. Thus, the inflammatory microenvironment influences T-cell responses through purinergic signalling.

P133

TARGETING OF A THERAPEUTIC SUICIDE GENE TO HUMAN MINOR HISTOCOMPATIBILITY ANTIGEN-SPECIFIC T LYMPHOCYTES WITH STEM-CELL FEATURES REQUIRES IL-7 AND IL-15

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In a phase I-II non-randomized clinical trial investigating the prophylactic infusion of suicide gene-modified donor T cells after haploidentical hemopoietic cell transplantation, we observed a rapid and effective immune reconstitution (Ciceri, Bonini *et al*., Lancet Oncology, 2009). After activation with anti-CD3 antibodies, T cells were modified with a retroviral vector (RV) encoding for the Herpes Simplex thymidine kinase (TK). TK^+ cells displayed an effector memory (EM) phenotype ($CD45RA^-CD62L^-$, $CD28^{\pm}CD27^+$, IL-$2^{\pm}$IFN-γ^+). When needed, graft-versus-host disease (GvHD) was controlled upon administration of the prodrug ganciclovir (GCV). The graft-versus-leukemia (GvL) effect was substantial in patients transplanted in remission, but failed to cure patients in relapse. Genetic modification with RV is limited to memory T cells. EM TK+ cells have a reduced alloreactivity. Central memory (CM) T cells ($CD45RA^-CD62L^+$, $CD28^+CD27^+$, IL-2^+IFN-$\gamma^{\pm}$) share many characteristics with stem cells, namely the ability to self-renew and to differentiate into effector cells. Recently, it has been proposed that alloreactivity may be confined to memory T cells with stem-cell features. Since alloreactivity is the common ground of both graft-versus-host dis-

ease (GvHD) and the GvL effect, crucial to the success of the strategy is the suicide gene-modification of cells with such properties. We found that addition of CD28 costimulation on cell-sized beads and the use of homeostatic cytokines, such as IL-7 and IL-15, generates central memory (CM) TK^+ cells. CM TK^+ cells were highly alloreactive, both in vitro and *in vivo* in a humanized animal model of GvHD based on the grafting of human skin onto NOD/scid mice. GCV administration abrogated GvHD (Kaneko *et al.*, Blood, 2009). Stimulation of CM, but not of EM TK+ cells with autologous dendritic cells pulsed with HLA2-restricted peptides from the minor histocompatibility alloantigen (mHag) HA-1 or H-Y efficiently induced mHag-specific T cells that lysed natural ligand expressing HLA^-A2^+ targets. A fraction of mHag-specific TK^+ cells expressed IL-7Ra. Only IL-$7Ra^+$ mHag-specific TK^+ cells could self-renew and differentiate into effector cells. When infused in NOD/scid mice harboring human $mHag^+HLA^-A2^+$ leukemia, TK^+ mHag-specific T cells significantly delayed disease progression ($p<0.001$). Altogether, these data suggest that targeting of a suicide gene to human mHag-specific T lymphocytes with stem-cell features requires IL-7 and IL-15 and warrant their use in the clinic for a safe and powerful GvL effect.

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TRANSGENIC T CELLS REDIRECTED TO PREFERENTIALLY EXPRESSED ANTIGEN OF MELANOMA (PRAME) TARGETING CHRONIC MYELOGENOUS LEUKEMIA (CML) CELLS

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Adoptive transfer of tumor antigen-specific T-cell lines may help to eradicate neoplastic cells spared by chemotherapy in hematological malignancies. The main drawback of this approach is the difficulty to produce a sufficient amount of adoptive antigen-specific T-cells. Transfer of tumor antigen (TAA)-specific T-cell receptor (TCR) genes into recipient T cells has been recently indicated as an alternative approach (Morgan, Science 2006). The TAA PRAME is over-expressed by many solid tumors and hematological malignancies, including CML. In this study, we isolate multiple T cell clones reactive against PRAME-derived peptide (ALY) from a bulky T cell population obtained after in vitro expansion of ALY-specific T cells from HLA-A*02 healthy donor. To this aim, first we took advantage of an artificial antigen presenting cells (K562 cell line genetically modified to stably express HLA-A*02, CD80 and CD40L) loaded with ALY-peptide, as recently shown by us (Quintarelli, Blood 2008), to generate polyclonal PRAME T cell lines. Then, 32 individual T-cell clones were isolated by tetramer+ FACS sorting, expanded and finally characterized according to their ability to produce IFN-g in response to ALY-peptide (range of 200-0.02 nM). The and -chains of the TCR were isolated from the most affine ALY-specific T cell clone and used to construct a single, bicystronic retroviral vector using IRES sequence. Gene transfer efficiency, expressed as % of ALY-specific TCR molecules on transduced lymphocytes, was monitored by ALY-tetramer staining. We were able to exceeded a 20% gene transfer efficiency after codon-optimization and cysteine substitution in the constant region of the cloned ab-TCR chains. The transgenic T-cells maintained a very high affinity for the PRAME-ALY epitope. Indeed, we challenged these cell clones by pulsing them with decreasing concentration of peptide, and measured significant amounts of IFNg SCF/105 (range 70-120) after exposure to low peptide concentration (0.002nM) by ELISpot assay. Finally, we proved a high functional activity of transgenic PRAME-abTCR T cells on exposure to PRAME+ CML blasts from HLA-A*02 patients (448±69 IFNg SFC/105 cells) and their specific cytotoxic activity on PHA blasts loaded with the ALY-peptide (42%±7% at 20:1 E:T ratio). In conclusion, our data show that it is possible to generate highly functional T cells expressing a transgenic abTCR targeting PRAME antigen. These cells may be of value for patients with CML disease.

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THE WASHOUTS OF DISCARDED BONE MARROW COLLECTION BAGS AND FILTERS ARE A VERY ABUNDANT SOURCE OF HMSC

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Background. Human Multipotent Mesenchymal Stromal Cells (hMSCs) are considered good candidates for a growing spectrum of cell therapies. We have validated, in two different Cell Factories which operate in strict GMP compliance, a protocol for the production of very large numbers of hMSCs for therapeutic use which makes use of the washouts of normally discarded collection sets left over at the end of the filtration of the bone marrow explants performed for hematopoietic stem cell (HSC) transplantation. This protocol reduces manipulation to a minimum and limits the use of animal and non clinical-grade reagents. *Methods*. Total nucleated cells (TNC) were isolated both from unmanipulated bone marrow aspirates and from washouts of discarded bags and filters, left over after the filtration of whole bone marrow explants which is routinely performed before infusion to patients. Cells were seeded without any manipulation in 5% human platelet lysate (hPL) supplemented α-MEM. After 48 hours nonadherent cells were removed and the adherent cells were expanded for 7-14 days with periodic feeding. The cells were then harvested and seeded at low density and allowed to expand for additional 10-20 days. Finally the cells were harvested and frozen. *Results*. In a median of 26 days, 14 bags for adult patients and 9 bags for pediatric patients for the standard dose of $1x10^6$ hMSCs/kg body weight could be prepared from the expansion of a fraction of the cells recovered from 7 independent washouts. Moreover, 151 vials could be frozen from the remaining cells. The theoretical full expansion of all the frozen vials (validated by the expansion of 2 independent vials) could have allowed the production of 173 bags for adults and 348 bags for pediatric patients. Clinical scale expanded hMSCs identity and purity were assessed by testing viability and expression of CD14, CD34, CD45, CD73, CD90 and CD105. Cytogenetic analysis and clonogenic assay in methylcellulose were performed and no chromosomal alteration or tumorigenic trasformation has been revealed. Bacterial, fungal, mycoplasma and endotoxin contamination were tested by validated tests according to European Pharmacopea guidelines and always found negative. *Conclusions.* The washouts of discarded bags and filters left over at the end of the routine bone marrow explants filtration are a very abundant source of hMSCs precursors which can be easily utilised for clinical application.

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THE SUSCEPTIBILITY OF INDOLENT NON-HODGKIN LYMPHOMA CELLS TO UNDERGO AN IMMUNOGENIC DEATH PREDICTS THE CLINCAL RESPONSES TO OUR DC-BASED VACCINATION

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We recently reported that vaccination using dendritic cells (DCs) loaded with killed autologous tumor in relapsed indolent NHL patients with measurable disease induced clinical responses that strongly correlated with multifaceted immunologic responses. Since the only patient characteristic predicting the outcome was disease extension before vaccination, we investigated whether the observed clinical benefits depended on a different immunogenic profile between vaccines administered to responders and those administered to non-responders. To this aim, we evaluated the susceptibility of tumor cells of enrolled patients to undergo an immunogenic death when apoptotic and necrotic tumor cell bodies were induced by heat-shock, gamma-irradiation and UVC to generate the antigen source for our DC-based vaccine. Translocation of calreticulin (CRT) and exposure of heat shock proteins (HSP) on dying cells as well as the release of high mobility binding protein 1 (HMGB1), a ligand of TLR4, are among the most consolidated features to define cell death as immunogenic. Flow cytometry was

used to analyze the surface expression of CRT, HSP-70 and HSP-90 in apoptotic and necrotic cell bodies of patient tumors and ELISA assay to test the amount of released HMGB1 in their culture supernatants. We found an increased exposure of CRT and HSP90 on dying tumor cells of responders compared to those of non-responders. However, no statistically significant differences were observed in HSP-70 expression or HMGB1 release by killed tumor cells between the two groups of patients. To further assess the impact of an immunogenic tumor cell death in predicting the outcome of vaccinated patients, we investigated in our series the frequency and the correlation with responses of TLR4 loss of function A896G polymorphism, known to impair antigen cross-presentation by DCs. Unexpectedly, only two patients that completely responded to vaccination showed to carry such polymorphism. These results demonstrate that the translocation of the endoplasmic reticulum-associated proteins CRT and HSP-90 on killed tumor cell surface is crucial for the immunogenicity and *in vivo* efficacy of DC-based vaccination exploiting them as source of tumor antigens. The evidence that two patients, showing an impaired HMGB1-TLR4 circuit but high exposure of these molecules, could still achieve a complete response further strengthens our findings.

Quality of Life and Support Therapy I

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FERTILITY PRESERVATION IN YOUNG WOMEN UNDERGONE HODGKIN'S LYMPHOMA TREATMENT

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Chemotherapy, radiotherapy and haematopoietic stem cell transplant (HSCT) may cause infertility in young women with Hodgkin Lymphoma (HL) because of the massive depletion of the ovarian follicle reserve resulting in POF. Factors affecting the risk of POF include the age at the time of therapy, the types of drug used and the intensity of treatment. Without any ovarian protection, the expected rate of POF in postpubertal women is approximately 10-20%, 70% and 90-100% following the use of alkylating-free regimens (ABVD/ABVD-like), alkylating-containing regimens (MOPP/ABV, COPP/ABVD, BEACOPP) and autologous HSCT, respectively. We evaluate the incidence of POF and infertility after chemoradiotherapy in prepubertal girls and in postpubertal women who received a Gonadotropin Releasing Hormone analogue (GnRHa) to prevent ovarian damage during HL treatment. From January 1991 to December 2008, 115 untreated female patients (pts) aged 8-40 years (median 24) with HL have been treated in our institution. Before HL, 4 pts are prepubertal girls and 111 pts are postpubertal women. To protect ovarian function during chemo-radiotherapy, 111 postpubertal pts received GnRHa monthly, while 4 prepubertal girls not received ovarian protection. HL treatment included 4 to 6 courses of chemotherapy plus radiotherapy for CS I-IIA and 6 courses of chemotherapy plus radiotherapy to residual masses for CS IIB-IV. Overall, 90 pts received alkylating-free chemotherapy and 25 pts received alkylating-containing regimens (MOPPEBVCAD, COPPEBVCAD, COPP/ABV, BEACOPP) as first-line (20 pts) or salvage treatment (5 pts). Four of the 81 irradiated pts received subdiaphragmatic RT. Ten relapsed/refractory pts received salvage treatment including autologous HSCT in 7 cases and allogeneic HSCT in 1. Today, after a median follow-up of 148 months (range 9-208) 3 pts died of HL, 112 are alive and 108 are evaluable for treatment-related gonadotoxicity. All 4 pts treated during prepubertal phase have today normal menses. Considering the group of postpubertal women treated with GnRHa, we recorded POF in 7 pts, irreversible in 6 cases (6%) and transient in one, while 102 pts (94%) recovered a normal ovarian function. After treatment, 28 pts attempted pregnancy and conceived. Twenty-six healthy babies were delivered and 4 pregnancies are ongoing. We analyzed risk factors and found that the salvage treatment had a very negative impact on incidence of POF ($p<0.0001$). Age > 30 years correlates with POF only in pts who received salvage treatment ($p=0.05$), while first-line treatment with alkylating drugs ($p=0.2$) and advanced stages of disease ($p=0.18$) were not significant risk factors. Our data confirm that prepubertal status may protect the ovaries from toxicity of chemoradiotherapy. In agreement with this concept, we think that in postpubertal women who received GnRHa during HL treatment, the low incidence of POF following first-line therapy is due to the reversible induction of a prepubertal hormonal milieu. Unfortunately, in relapsed/refractory pts GnRHa does not seem to be very effective, and further experimental approaches are required for ovarian protection and fertility preservation. In this regard ovarian tissue cryopreservation represents a promising technology that may restore both complete ovarian function and fertility.

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INCIDENCE AND RISK FACTORS OF PROVEN OR PROBABLE INVASIVE FUNGAL INFECTIONS (IFI) IN 286 PATIENTS UNDERGOING RELATED OR UNRELATED ALLOGENEIC BONE MARROW TRANSPLANTATION.

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Introduction. Allogeneic bone marrow transplantation (BMT) is increasingly used to treat hematologic diseases. Invasive fungal infections (IFI) remain an important cause of morbidity and mortality in this setting. Recent reports have indicate that the incidence of IFI (expecially aspergillosis) has increased in BMT recipients, particulary after engraftment. *Patients and Results.* To evaluate the epidemiology, outcome and

risk factors of proven or probable IFI in allogeneic BMT recipients, we retrospectively reviewed the medical records of 286 consecutive adult patients (pts) who underwent allogeneic BMT (170 from related and 116 from unrelated donor) at our Department. 213/286 (74%) pts received a myeloablative conditioning regimen and 73/286 (26%) a nonmyeloablative one. The median age of patients was 43 years (range 19-68). We identified 36 cases of proven or probable IFI (Aspergillus sp. 25, Candida sp. 5, Fusarium 2, Mucor 5) with an overall incidence of 13%. The incidence after related BMT (RD-BMT) was 9% while it was 16% after unrelated BMT (UD-BMT) ($p<0.05$). The incidence was the same in the myeloablative and non myeloablative setting. IFI occurred after a median of 38 days from BMT (range 5-1440); 16/36 (44%) cases occurred during pre-engraftment phase while 20/36 (56%) occurred after engraftment (with 12/20 cases after day 100). The sites of infection were: lung only 22/36 (61%), CNS 6/36 (17%), multiple sites 8/36 (22%). Advanced hematologic disease (relapsed or refractory) at time of transplant, history of pre-transplant IFI, presence of acute or chronic graft-versus-host-disease (GVHD), but not neutropenia, were significant risk factors ($p<0.05$). In the UD-BMT setting the incidence of IFI was significantly higher in patients who received a combination of immunosuppressive agents in the conditioning regimens (ATG +/- Fludarabine +/- Campath). Overall Survival after 100 days from diagnosis of IFI was only 20% and in these cases 64% of deaths were directly IFI related. *Conclusions*. 1) IFI is a significant cause of non-relapse mortality following RD and UD-BMT. 2) Aspergillus sp. remain the most important aetiologic agent. 3) Incidence of IFI in UD-BMT is significantly higher than in RD-BMT probably as a result of more intensive immunosuppressive conditioning regimen in this setting. 4) IFI can develop late after engraftment (after day 100 from transplant) and without neutropenia. 5)Status of hematologic disease (relapsed or refractory) at transplant, history of pre-transplant IFI and presence of GVHD, are important predisposing factors. 6)Retrospective studies, like this one, can be useful in order to identify high-risk BMT patients for which targeted and more effective diagnostic and therapeutic strategies should be used to prevent and treat IFI.

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CLINICAL MANAGEMENT OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA REFERRED TO THE A.I.L. HEMATOLOGY HOME CARE SERVICE IN MODENA

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Despite recent advances in therapeutics, acute myeloid leukemia (AML) in the elderly is still characterized by bad prognosis and poor quality of life. A palliative approach based on home care (HC) is able to guarantee regular follow-up appointments, to avoid discomfort due to driving distances, waiting times and hospital admissions and to optimize outpatient and inpatient healthcare resources. In our department a domiciliary program of palliative and supportive care, run by the fundraising organization A.I.L., is active in order to manage fragile hematology patients outside the standard in-hospital assistance. Eligibility criteria include poor performance status, appropriate home logistics, caregiver availability, reasonable distance from hospital. HC team is composed by a hematologist and a specialist nurse fully dedicated to at-home activities. Among 450 patients followed in the period 1999-2009, 64 (15%) were AML patients aged more than 60 years (29 male, 35 female). Median age was 76 years (range 60-95). 18 patients had previously undergone curative chemotherapy. The time interval between diagnosis and the beginning of HC was on average 111 days (range 4-672). Median duration, corresponding to the remaining lifetime, was 87 days (range 10-386). Home transfusion support was provided in 58 patients for a total of 418 units of packed red blood cells and 363 of platelet concentrates. When needed, cytoreductive agents and antimicrobials, in all formulations, were administered. Emergency accesses to hospital regarded 39 patients for a total of 46 admissions (mostly due to severe infections, caregivers' burn-out and end-of-life care). The days of hospitalization were 522, compared with 5191 cumulative days of HC. Home was the place of decease in 42% of cases. Our model of HC for elderly patients with AML is feasible, sustainable and safe. Approximately half of patients live more than expected, without presenting uncontrolled symptoms nor inappropriate hospital admissions. The proportion of patients deceased at home is higher than local historical data. The relationship among HC team, general practitioners, community nurses and families is very collaborative. Several issues remain unsolved, such as the validation of palliative prognostic tools to predict life expectancy, the conception of guidelines for the management of terminally ill hematology patients and the urging necessity for the public health systems to sustain and develop the home care setting in hematology.

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HEART FUNCTION IN MULTITRANSFUSED PATIENTS: MEANING OF N-TERMINAL PRO B-TYPE NATRIURETIC PEPTIDE

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The Authors measured NT-proBNP in multitransfused patients, comparing data with those of healthy controls, evaluating pre and post-tranfusional variations, and relating hormone concentrations to the number of transfusions and to the levels of serum ferritin. The patients were 26 subjects suffering from chronic haematological disorders. Controls were 21 donors free from known heart disease and with age and gender similar to those of the patients. For both populations descriptive statistics of NT-proBNP values (pg/mL) were calculated giving the following results (patients/controls): MEAN: 1723.78 (mean of the means)/117.3; MEDIAN: 917/95.8; MINIMUM: 99/27.7; MAXIMUM: 10263/296.5; STANDARD DEVIATION: 2220.8/73.1. The Mann-Whitney U test was applied in order to analyse the differences between cases and donors: these resulted always highly significant ($p<0.00001$). Lastly cases were subdivided both on the basis of the number of transfusions (less or more than 100) and with regard to serum ferritin levels (less or more than 1000 ng/mL). Mean NT-proBNP concentrations are reported in Table 1. Such groups resulted too small to allow the use of statistical tests for significance. In multitransfused patients both hypooxigenation and iron overload could be responsible for the impairment of myocardial function. The results of the present study actually show mean NT-proBNP levels absolutely higher than those of the controls, while there is no evidence for an incremental trend in the short-term (6 months) and not even for changes between pre and post-transfusional values attributable to volume overload. Anyway patients with more than 100 transfusions have increased concentrations of the marker as a consequence of the fact that heart function gets substantially worse in the long run. The overlap between mean values in the subgroups with ferritin higher or lower than 1000 ng/mL suggests a non fundamental role of iron overload on myocardial function, and this observation is in accordance with other Authors. Finally, the precision of the intra-series data for the studied cases and the fact that the values of healthy controls are well comparable with those reported by literature account for a good reliability of the method and confirm NT-proBNP as a sensitive and potentially useful marker of cardiac disease also in chronic haematological disorders.

Table 1. NT-proBNP concentrations in patients' subgroups defined by ferritin levels and number of transfusions.

Variable	*Cut-off*	*Number of cases*	*Mean NT-proBNP (pg/mL)*
Serum ferritin (ng/mL)	< 1000	9	1805.7
Serum ferritin (ng/mL)	> 1000	13	1578.5
Number of transfusions	< 100	9	615.3
Number of transfusions	> 100	17	2290.8

P141

ROLE OF RITUXIMAB FOR TREATING PATIENTS WITH IDIOPATHIC TTP/HUS WITH OR WITHOUT ADAMTS-13 ANTIBODIES

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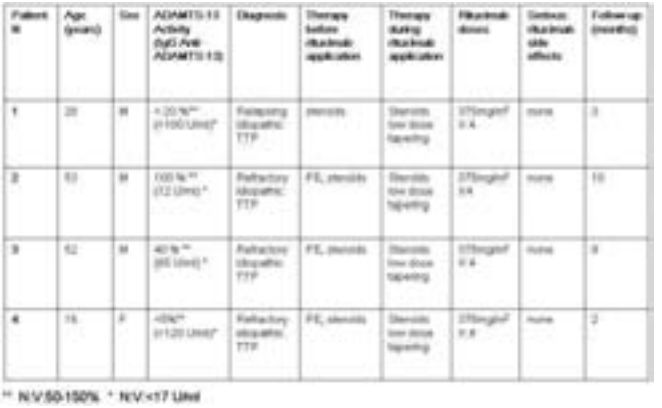
Background. Idiopathic TTP/HUS (Thrombotic Thrombocytopenic Purpura-Hemolytic Uremic Syndrome) namely defined as absence of congenital deficiency of ADAMTS-13 is a rare disease usually respon-

sive to plasma exchange (PE). Because of the high rate of relapses, a useful strategy is that to select high-risk patients such as those with low level of ADAMTS-13 activity for alternative therapy. Conventional immunosuppressive therapy (cyclophosphamide, cyclosporine, azathioprine and intravenous immunoglobulins) have been shown to successfully treat patients with relapsed/refractory TTP/HUS; few evidences are currently available about therapy with rituximab. *Aims*. We tested the role of anti-CD 20 drug for treating patients with relapsed/refractory TTP/HUS. *Methods*. Rituximab was administered to 4 patients with relapse/refractory idiopathic TTP/HUS, with or without deficient ADAMTS-13 activity. Characteristics of patients are reported in the table. Complete remission was defined if presence of PLT >100x10^9/L and normal laboratory values (LDH, serum bilirubin, creatinine, hemoglobin). Three cases were due to ADAMTS-13 deficiency antibody-mediated; among them, one relapsed 39 months after complete remission, obtained by PE, while the remaining 2 patients were refractory to PE. The fourth patient did not have ADAMTS-13 deficiency and he was refractory to PE. All 4 patients received rituximab at the dose of 375 mg/m^2, one shot a week for one month; no other therapy were given except steroids low dose tapering. *Results*. Complete remission was achieved in all patients just after the first two infusions of rituximab. In patients with ADAMTS-13 antibodies, these were not longer detectable after all courses of rituximab therapy. Remission was maintained for a median period of 6 months (range 2 to 10 months). *Conclusions*. The role of rituximab in patients with idiopathic refractory/relapsed TTP/HUS is still unclear in patients with or without ADAMTS-13 antibodies. In our experience, rituximab has been proven to be effective in producing a durable complete remission; this happened in 3 patients with deficient ADAMTS-13 activity and in the fourth patient with refractory TTP/HUS without deficient ADAMTS-13 activity. Properly designed studies are required to confirm safety and efficacy of such approach.

Table. Patients' characteristics.



P142

PALLIATIVE CHEMOTHERAPY IN HAEMATOLOGIC PATIENTS: HOME CARE SERVICE EXPERIENCE OF NIGUARDA CA' GRANDA HOSPITAL

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Introduction. Chemotherapy in advanced haematologic malignancies is often considered as highly toxic and little effective, therefore its application in palliative medicine is still debated. Primary goal of palliative chemotherapy (PC) is the relief of symptoms due to the disease progression: expected benefits must be greater than side effects. Several factors need to be considered before starting a PC: haematological diagnosis, chemosensitivity of tumor, age, performance status, life expectancy, and patient's will. This is a retrospective observational study performed on the clinical records of patients assisted by the Home Care Service of the Niguarda Hospital (Milan, Italy). *Materials and Methods.* We reviewed the clinical record of 32 patients who received home care for the terminal phase of illness, median age was 71 years(range 32-91). Diagnosis was distributed as follows: 3 acute lymphoblastic leukemia (ALL), 14 acute myeloid leukemia (AML), 1 chronic lymphocytic leukemia (CLL), 8 non Hodgkin lymphoma (NHL), 1 chronic myeloproliferative disorder (CMD), 1 myelodysplastic syndrome (MDS), 3 multiple myeloma (MM), 1 Waldenström disease (MW). *Results*. Of the 32 patients that were retrospectively analyzed, 14 (44%) underwent palliative chemotherapy with the aim of reliefing symptoms as: fever (2 pts), dispnea due to pulmonary leukostasis (2 pts), bone pain (3 pts), metabolic acidosis due to hyperleukocytosis(1 pts), neoplastic ascities (1 pt), fatigue (5 pts). In 11 pts chemotherapy was administered orally (6-thioguanine, hydrossiurea, methotrexate, 6-mercaptopurine, etoposide, thalidomide), 2 pts were treated both orally (thioguanine) and intravenously (i.v.) (cytarabine), 1 pt was treated only with intravevous cyclophosfamide. Therapy was well tolerated in all 14 patients: we registered both a low general toxicity (WHO<2) and a low hematologic toxicity (transfusions rate and growth factors rate were unchanged under PC). In 10 pts (71%) the relief of symptoms was achieved. PC was well tolerated clinically and psychologically. *Conclusion*. A large group of patients mantained a partial chemosensivity and obtained a temporary relief of symptoms. PC was well tolerated in all cases, even when administered intravenously. The home care setting allowed a better acceptance of chemotherapy, even in the terminal phase of disease. Palliative chemotherapy in home care seems to be an useful resource to improve patient's quality of life.

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HIGH DOSE OF LIPOSOMAL AMPHOTHERICINE B (HDL-AMB) IN THE PROPHYLAXIS OF INVASIVE ASPERGILLOSIS (IA) IN ADULT ACUTE LEUKEMIA (AL) PATIENTS UNDERGOING INTENSIVE CHEMOTHERAPY.

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The outcome of AL can be significantly influenced by the IA and the attributable mortality rate (AMR) which are reported between 8-10 and 40-60%, respectively. The best approach to prevent IA is a still unresolved issue. HDL-Amb activity is related both to an improved tissue penetration and to the drug bioactivity in lung, brain, liver and spleen. In the attempt to overcome the risk of IA in AL patients during induction phase, in November 2004 we designed a still open single centre prospective study to evaluate the feasibility and efficacy of HDL-Amb(15 mg/kg) as IA prophylaxis. The aims are a) the incidence (I) and AMR of proven IA according to EORTC criteria during and up to 4 weeks after prophylaxis; b) the drug-related severe adverse events(SAE); c) the L-Amb pharmacokinetic (PK) plasma profile. Between November 2004 and March 2009, 41 adult (M/F 28/13),median age 58 y (range32-78) AL undergoing intensive induction chemotherapy with an expected severe(<500 ANC/L) neutropenia >10d, were enrolled. Of these,38 were AML, 2 ALL, and 1 Biclonal; in all cases, before induction, chest CT scan, cultures, mannano and galattomannan assays were negative.The 1st HDL-Amb administration is given the day after the end of induction, a 2nd one is planned if severe neutropenia persists; PK samples are collected at 0,7 and 14 d. Of the 41 enrolled pts, 27(69%) achieved CR, 4 were PR, and 10 died during induction (ID). Median time of neutropenia was 14d(range 11-29); all received the 1st prophylactic dose, and six a 2nd one; in none of them >2 SAE were recorded. In 39 (95%) pts there was not evidence of IA during and up 4 weeks after the end of prophylaxis, but 3 of 10 ID died because of infection. At autopsy, the diagnosis of IA was done, since, when pts were alive, workup for IA did not met EORTC criteria. These pts were elderly (>60y) with comorbidities, and have had prolonged bacterial infections before induction start. In this prospective study we were able to lower the I of IA with the respect to our previous experience (7.3vs10%), but AMR remained the same (100 and100%); this events didn't correlate to L-Amb PK. Since 95% of pts didn't develop IA, it might hypotize that the 14th d L-Amb clearance correlate directly with the drug complete diffusion in the tissues.Elderly AL pts with bacterial infections at onset and/or chronic comorbidities can have an increase risk to develop fatal IA; in these pts prophylaxis might be anticipate at beginning of induction.

P144

NEUROLOGICAL EFFECT OF RECOMBINANT HUMAN ERYTROPOIETIN (EPOETINA ALFA) IN FRIEDREICH'S ATAXIA: A CASE REPORT

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Introduction. Friedreich ataxia is the most frequent hereditary ataxia, with an estimated prevalence of 3-4 cases per 100,000 individuals. This autosomal-recessive neurodegenerative disease is characterized by progressive gait and limb ataxia, dysarthria, lower-limb areflexia, decreased vibration sense, muscular weakness in the legs, and a positive extensor plantar response. Non-neurological signs include hypertrophic cardiomyopathy and diabetes mellitus. Symptom onset typically occurs around puberty, and life expectancy is 40-50 years. Friedreich ataxia is usually caused by a large GAA-triplet-repeat expansion within the first intron of the frataxin (FXN) gene. FXN mutations cause deficiencies of the iron-sulfur cluster-containing subunits of the mitochondrial electron transport complexes I, II, and III, and of the iron-sulfur protein aconitase. Therapeutic trials in the next 5 years are expected to address amelioration of the effects of frataxin deficiency and methods for increasing frataxin expression. In a "proof-of-concept" study the authors demonstrated that recombinant human erythropoietin (rhuEPO) increases frataxin levels. Description of case A 36-year-old female patient affected by Friedreich's ataxia referred to our institution in December 2007; at that time the neurological symptoms were dramatically active and none of the prior therapies (baclofen intratecal, idebenone) showed positive effects; anaemia with martial depletion was present. The patient received 2.000 IU rhuEPO (epoetina alfa) thrice a week subcutaneously, and low -molecular-weight heparins (LMWH) for deep venous thrombosis prophylaxis was administrated. Clinical outcome measures included Ataxia Rating Scales, Frataxin levels and indicators for oxidative stress were assessed. Hematological parameters were monitored biweekly. Scores in Ataxia Rating Scales such as FARS (9-hole peg test, the timed 25-foot walk, PATA test, and low-contrast letter acuity) and SARA improved significantly in the subsequent 6 months. Frataxin levels increased, while indicators of oxidative stress such as urine 8-OHdG and peroxide levels decreased. The hematocrit increase was moderate and martial therapy was not required. Conclusion The use of recombinant human erythropoietin (rhuEPO) may have a role in the treatment of Friedreich's ataxia. These findings are promising and will serve as a benchmark for future clinical trials in this patient population.

P145

EMERGING RISK FACTORS FOR INVASIVE FUNGAL INFECTIONS AMONG HAEMATOLOGICAL PATIENTS: RESULTS OF A 60-MONTH SURVEILLANCE STUDY

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Background. Acute leukaemia (AL), particularly myeloid during first induction, is known to be the main predisposing factor for invasive fungal infections (IFI) outside allogeneic transplant. *Aims.* To evaluate the recent evolution of IFI epidemiology among haematological non-allotransplant patients. Methods. From April '04 to March '09, all admitted patients were prospectively observed for febrile/infectious episodes. Patients with >7-days expected neutropenia received antifungal prophylaxis with itraconazole 400 mg/day p.o. and nebulized amphotericin-B. Infections were considered "microbiologically proven" (MPI) when microrganisms were isolated from infection sites. IFI were defined as probable/proven according to EORTC/MSG criteria. Results. Among 1288 fever/infections recorded, 554 were MPIs. Forty-four were probable/proven IFI, i.e. 8.1% of MPI. Forty-seven fungal pathogens were isolated (2 mixed). Aspergillus spp was responsible for 28 (63.6%) cases (13 A. fumigatus, 1 A. niger, 1 A. terreus, 1 A. flavus, 12 A. not specified), Candida spp for 11 (25%) (5 C. parapsilosis, 2 C. albicans, 1 C. giullermondii, 1 C. famata, 1 C. kefyr, 1 C. tropicalis), Mucor spp for 2, and Cryptococcus spp, P. boydii, P. jirovecii and Fusarium spp for 1 case each. Only 27.3% of IFI showed AL as underlying haematological disease, whereas 50% of IFI occurred in patients with lymphoma/CLL. IFI occurred more in refractory/relapsed patients (59.1% of cases), than at diagnosis (31.8%), during postchemotherapy aplasia in AL (20%), or in patients without evidence of disease (9.1%). In 63.6% of cases patients developed IFI while on corticosteroids; neutropenia was present in 52.3% of cases. By univariate analysis a diagnosis of lymphoma/CLL and active underlying disease were significant risk factors among patients with MPIs, both for the presence of IFI (12.7% vs 5.8%, $p<0.01$; 11.7% vs 1.9%, $p<0.01$, respectively) and for aspergillosis (8.1% vs 3.7%, $p<0.05$; 7.6% vs 0.9%, $p<0.01$ respectively), whereas IFI turned to be less frequent in pts with neutropenia (6.3% vs 11.2%, $p<0.05$), as well as IFI and aspergillosis in patients with AL/MDS (4.5% vs 11.7%, $p<0.01$; 3.1% vs 7.2%, $p<0.05$, respectively). *Conclusions.* Among patients admitted at our Institution, IFI and aspergillosis developed predominantly in those with active haematological disease, particularly lymphoma/ CLL in advanced stage, whereas they did not significantly impact on patients with AL, even during induction treatment.

P146

TWO YEARS MICROBIOLOGICAL SURVEY WITH MOLECULAR TYPING METHODS IN HAEMATOLOGIC AND HSCT PATIENTS

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Molecular epidemiological survey is an important tool to understand the nosocomial pattern of each hospital and to identify outbreaks and possible transmission routes. To evaluate this risk in haematologic and in HSCT patients (pts), from May 2006 to December 2008, we conducted a prospective investigations using a control system (VIGI@act) and a real time AFLP (amplified length fragment polymorphism). During this period 375 hematologic and 193 HSCT pts were monitored. In hematologic pts, documented infections (DI) were recorded in 51%: bloodstream infections (BSI) in 37% and microbiologically infections (MDI) without bacteremia in 14%. Out of 138 pathogens responsible for BSI, gram positive infections were 94 (68%): CNS 65%, enterococci 32%, S.aureus 3%; 40 gram negative infections (29%): E.coli 35%, P.aeruginosa 30%, S.malthophilia 17%, KES 18%; 4 fungi (3%). Of enterococci, 13% were VRE and 50% HLAR. In May-September 2008, an unexpected high incidence of VRE was documented: from 4 blood cultures and from 2 rectal surveillance culture. AFLP documented that 2 isolates had a genetic relationship, the other 4 a different but similar genetic pattern among themselves, suggesting a possible person to person transmission. After corrective measures no other case of VRE was documented. In HSCT pts, DI were recorded in 32%: BSI in 21%, MDI without bacteremia in 11%. Out of 39 pathogens responsible for BSI , gram positive infections were 20 (49%): CNS 75%, enterococci 20%, streptococci 5% and 19 gram negative infections (51%): P.aeruginosa 53%, E.coli 32%, KES 10%, S.malthophilia 5%. The AFLP analysis showed 2 outbreaks of P.aeruginosa in HSCT patients. The first episode occurred in May-October 2006 involving 4 patients allocated consecutively in the same room. AFLP showed that the isolates from the patients and an isolate from Irgasan soap had a significant molecular similarity (dice index >0.93). In spite of corrective measures, the same P.aeruginosa clone was isolated in May-June 2007 in 3 infected patients and in a shower tap sample. After additional measures, consisting in hydraulic works, up until now no other outbreak of P.aeruginosa was observed. Our results showed a new increase of gram negative infections and a progressive antimicrobial resistance. Moreover these data suggest that environment is an important source of infections. The AFLP method was fast enough to allow a 'real-time' monitoring of the outbreak, permitting additional preventive measures.

P147

BKV-RELATED HAEMORRHAGIC CYSTITIS IN ALLOGENIC BONE MARROW TRANSPLANT RECIPIENTS: PROGNOSTIC IMPACT AND RISK FACTOR STRATIFICATION

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The post-transplant incidence of BKV-related haemorrhagic cystitis is about 15-20% and correlates in most cases, but not all, with viral load as detected by PCR and urinary symptoms. Early diagnosis and treatment may ensure good outcomes. In our Transplant Centre 50 recipients of allogeneic bone marrow transplant between November 2007 and April 2009 (males 11, females 39; age range 18 - 58 years; median age 39 years) were monitored for urinary symptoms of BKV and viral load using PCR. Twenty patients (40%) were positive for BKV at least one PCR analysis and 15 (30%) developed haemorrhagic cystitis (grade II-III-IV according to the clinical classification).Ten of these 15 patients (60%) received Cidofovir at dosages ranging from 1 to 5 mg/Kg once a week until symptoms cleared. Therapy was successful in 6 patients but failed in 3 who died of unrelated causes (1 VOD, 3 fungal pneumonia). Another patient died of BKV- related pyelonephritis. Haemorrhagic cystitis developed within 30 days post- transplant in 6 patients (probably due to transplant-related toxicity), within 30-180 days post-transplant in 6 (probably due to opportunistic infection) and after 180 days post-transplant in 4 (probably of autoimmune origin). Half of the patients who developed BKV-related haemorrhagic cystitis in the early and late post-transplant periods were cured as were all 6 patients with onset between 30 and 180 days post-transplant. In this series risk factors which impacted negatively upon prognosis and outcome were, in order of importance, presence of GvHD, concomitant viral infection, apparent autoimmune origin, and high viral load. Morbidity and mortality rates were highest in patients with late onset BKV-related haemorrhagic cystitis who responded poorly to therapy probably because of the autoimmune origin. In conclusion many factors concur in the origin of BKV infection which is prevalently linked to immunodepression and autoimmune disease.

P148

REACTIVATION OF TUBERCULOSIS AFTER TREATMENT WITH FLUDARABINE, ALEMTUZUMAB (CAMPATH-1H) AND CYCLOPHOSPHAMIDE (FCC) REGIMEN IN A CASE OF RELAPSED B-CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Infections are a significant cause of morbidity and mortality in patients with CLL. The epidemiology of infections has changed in CLL through use of agents with potent immunosuppressive effect. Although bacterial still remains the most common type of infection, more fungal and virus infections were diagnosed in the last years. We report the case of a 62-year old woman with CLL admitted at our division because of fever, fatigue and poor general condition lasting more than two months. Thirteen years before for a Binet stage A B-cell CLL she received chlorambucil and prednisone for six months followed by four courses of R-CHOP regimen achieving a CR lasting several years. On July 2008 for relapse of disease six courses of FCC (Fludarabine 25 milligrams per square meter, Cyclophosphamide 250 milligrams per square meter, and Alemtuzumab 10 milligrams per square meter all given IV on days 1-3) regimen were planned achieving a CR according to NCI criteria. During and up to three months after the end of treatment prophylaxis with trimethoprim-sulfamethoxazole and valaciclovir was delivered. Two months after the end of therapy the patients presented fever up to 38.5 °C and asthenia. At admission physical examination, Rx-chest, tuberculin skin test, PCR-CMV-DNA analysis and blood cultures were negative while $CD4^+$ cells were less than 100/microLiter. Disappearance of fever occurred after 10 days of broad spectrum antibiotic, antifungal and antiviral therapy. After 15 days from admission, headache and a severe mental status alteration arose and fever returned. Cerebrospinal fluid (CSF) examination showed 300 cells/microLiter and high levels of proteins (2.33 grams/Liter). Mycobacterium tuberculosis PCR assay on CSF was positive; Brain MRI confirmed meningitis with hydrocephalus. An accurate clinical history revealed an ovarian tubercular infection in childhood. Therapy with Isoniazide, Ethanbutol, Rifampicin and Pyrazinamide was started and patients was discharged after 30 days in good general clinical status without neurological deficits. Conclusion: the case stressed the need to obtain before starting treatment an accurate remote medical history and to perform targeted microbiological testing to prevent potential endogenous reactivation in patients undergoing to heavily immunosuppressive therapy.

P149

CASPOFUNGIN AS SECONDARY ANTIFUNGAL PROPHYLAXIS IN BONE MARROW TRANSPLANTATION PATIENTS WITH PRIOR, PROBABLE OR PROVED FUNGAL INFECTION

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Introduction. Several therapeutic strategies were employed to reduce the risk of Invasive Fungal Infections (IFI). Of them, the secondary antifungal prophylaxis is an approach reserved for patients with high risk of fungal reactivation. Study Design. We designed a prospective study in which caspofungin is administered in 10 high risk patients (pts) undergoing Bone Marrow Transplantation (BMT) without sign of active pre-transplant IFI. Caspofungin was administered using 70 mg at first day 1 (1 day before starting the conditioning regimen) and 50 mg from 2nd day until resolution of neutropenia. The aim was to evaluate the efficacy of secondary prophylaxis to prevent reactivation or new IFI. Patients and *Methods.* From January 2005 until April 2007, 10 selected pts were enrolled: 4 Autologous BMT (A-BMT), 2 haplotipical BMT (HaploBMT) and 4 Allogeneic Conventional BMT (AC-BMT) (Table 1).

Table 1.

Patients	10
Sex	
Male	6
Female	4
Age	
Mean (range)	48 (35-46)
Disease	
Acute Myeloid Leukemia	6
Lymphoma non Hodgkin	3
Acute Lymphoblastic Leukemia	1
Transplant Type	
Autologous	4
Conventional Allogeneic	4
Haplotipical T	2
Source of Cell	
Marrow	3
Peripheral Blood Stem Cell	7
Disease Status at Transplant	
Complete Remission	7
2nd Complete Remission	2
Resistant	1

The fungal history prior transplant showed: 2 proven by candida albicans esophagitis and 6 probable infections with lung mycetoma, all treated with antifungal drugs. 3/6 patients showed galactomannan-positive (Platelia Aspergillus), detected during the previous chemotherapies. 2 HaploBMT were considered at high risk for the type of transplant. During the prophylaxis was planned weekly galactomannan detection. Nasal, oral and rectal swabs were performed to research any fungal colonization. Results. Febrile episodes were documented in according EORTC criteria: 4 bacteremias and 6 Fever Unknown Origin. 2/3 pts with previous galactomannan-positive showed reactivation confirmed by Aspergillus Q-PCR (Nanogen).The Caspofungin was replaced with Voriconazole. One pt (AC-BMT) died six day after engraftment for Acute Respiratory Distress Syndrome with TC lung iconography suggestive for Aspergillus infection (Halo Sign and Air Bronchogram) asso-

ciated with Cytomegalovirus and Herpes 6 reactivation, all documented on blood and sputum with PCR. The 2nd pt (A-BMT) showed a pulmonary lesion responsive to therapy with clearance of galactomannan and PCR copies. The 3rd pt HaploBMT died at engraftment, in relapse, for Multiple Organ Failure without evidence of galactomannan reactivation. Conclusion. No side effects were correlated with the Caspofungin. Unfortunately, in our experience, it did not show the same effectiveness in preventing the aspergillus relapse. Probably, Caspofungin dose escalation studies will be necessary to define more effective activities against Aspergillosis.

P150

OUTCOME ANALYSIS IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES FOLLOWED IN A SINGLE-CENTER OF ROMAGNA (1994-2008)

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Background. Survival of hematologic neoplasias is generally calculated on the basis of therapeutic protocols including selected patients, or on the basis of Tumor Registries structured without "high resolution encode" as defined in (1, 2, 3). *Patients and Methods.* All the patients (1733 cases) with a first diagnosis of an hematologic malignancy, living in the province of Ravenna, come to our observation between 1994 and 2008 were considered for overall survival, irrespectively of other comorbidities.

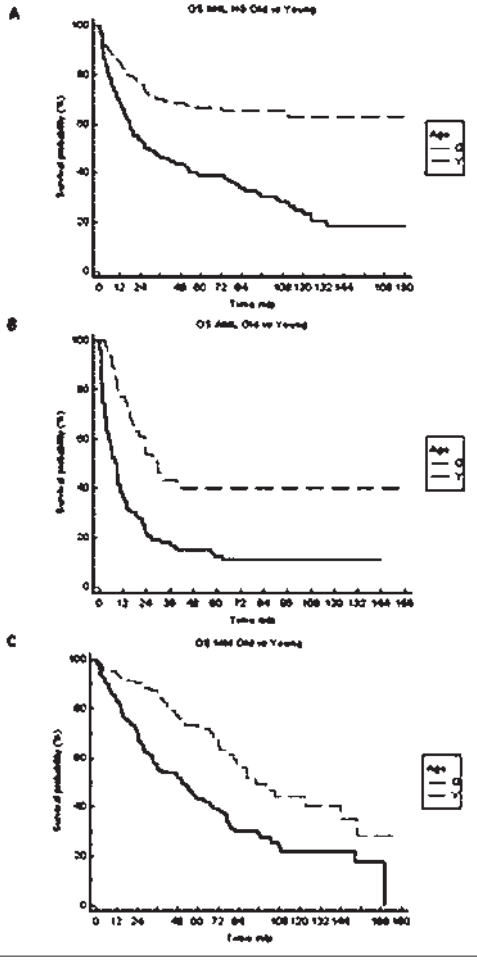

Figure 1.

Cut-off between young (>18 years) and old patients was 60 years for all the categories apart from MM (65 years). *Results.* One hundred six HD (23 old and 83 young) pts were observed. Old ones had a median survival of 54 months, while younger ones had an OS 87% at 96 months. Three hundred forty eight NHL LG (218 old and 130 young) pts.were observed. Median survival was 99 months for old pts. OS was 60% at 156 mos for the young pts. Three hundred fifty five NHL HG (212 old and 143 young) pts were observed. Median survival was 29 months for old ones and not reached at 180 months for younger ones (Figure 1-a). Two hundred sixty seven B-cell CLL (204 old and 63 young) pts were observed. Median survival was of 81 months for older pts. OS was 75% at 132 mos for the young pts. One hundred ninety two AML (145 old and 47 young) pts were observed. Median survival was 5 months for old and 18 months for young pts (Figure 1-b). Thirty four ALL (11 old and 23 young) were observed. Median survival was 5 mos for older and 18 for younger pts. The 343 MM (233 old and 110 young) pts showed a median survival of 51 months for older and 94 for younger pts. Part of them have never been treated. (Figure 1-c). *Conclusions*. This study will be useful in standardizing the inclusion of the patients into Tumour Registries according to high resolution encode criteria.

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P151

HEALTH-RELATED QUALITY OF LIFE OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES – A SYSTEMATIC REVIEW FROM 1980 TO 2008

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Health-related quality of life (HRQOL), symptom burden and other types of patient-reported outcomes (PROs) can provide precious information on the patient's perspective of disease symptoms and treatment-related effects. HRQOL in patients with myelodysplastic syndromes (MDS) may be compromised for several reasons including severe anemia, blood transfusions and the frequent occurrence of infections. Many MDS patients are elderly patients who present with co-morbidities from the time of diagnosis. Our approach was to start a systematic search of the literature in which prospective studies were identified and evaluated according to a pre-defined-coding scheme. Both HRQOL outcomes and traditional clinical reported outcomes were systematically analyzed. Overall, we found nine prospective studies, four of which evaluated HRQOL in a randomized controlled trial (RCT) setting. Interestingly, all these studies were published after the year 2001, possibly reflecting a recent interest in HRQOL of MDS patients. Although small sample size and missing data may cause some bias, HRQOL assessment has shown to be feasible in MDS patients and there are several reports demonstrating how this approach can provide additional key outcomes. A good example is offered by two recent RCTs that highlight the benefits obtained with azacitidine and decitabine compared to supportive care. HRQOL assessment provides us with valuable clinical data according to the patient's perspective. However, if HRQOL outcomes are to provide meaningful data, investigators will need to carefully address a number of methodological issues when preparing HRQOL trial-based study protocols. Major efforts should be made to help investigators become familiar with HRQOL research methods. The patient's perspective is unique and cannot be inferred by other indirect or proxy indicators. Patient fatigue cannot be evaluated simply by looking at hemoglobin levels but should always be measured using patient's self-reported fatigue tools. Considering that PRO assessment has the potential to provide valuable information in support of clinical decision-making, we strongly recommend its implementation in future studies of MDS patients.

P152

SINGLE DOSE PALONOSETRON TO PREVENT CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING (CINV) IN AGGRESSIVE NON HODGKIN'S LYMPHOMAS (NHL) PATIENTS WHO UNDERWENT MODERATELY EMETOGENIC CHEMOTHERAPY (MEC)

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Background. 5-HT3 receptor antagonists are the key agents recommended by antiemetic guidelines to prevent CINV in NHL. Palonosetron is a selective and potent serotonin antagonist with a distinct pharmacological profile, different structure and enhanced binding affinity. Clinical studies in solid tumours have demonstrated the advantage of palonosetron compared to older 5-HT3 receptor antagonists. The objective of this GISL (Gruppo Italiano Studio Linfomi) study was to evaluate the efficacy and safety of a single dose of Palonosetron in preventing CINV in aggressive NHL patients who underwent MEC. *Methods*. This is an open-label, multicenter phase II study assessing the efficacy of a single i.v. dose of palonosetron (0.25 milligrams) prior to the administration of chemotherapy in the first day of treatment. Drug activity was evaluated based upon a one-stage Fleming study design for determination of response rates based on a single treatment group. Complete Response (CR), defined as no vomiting and no rescue medication, during the overall phase (0-120hrs) was the primary endpoint. Relevant secondary endpoints included: Complete Control (CC) defined as CR and no more than mild nausea, no emesis and no nausea rates. Endpoints were evaluated during the acute (0-24 hrs), the delayed (24-120 hrs) and overall (0-120 hrs) phases. Adverse events were also recorded throughout the 5-day after chemotherapy administration. Nausea and vomiting were monitored by a 5 days diary. *Results*. In ten Italian centers eighty-six patients, median age 65 (range 20-87) affected by aggressive NHL, mostly undergoing CHOP±R (74.4%), were evaluated for the study. The primary endpoint was achieved with a CR rate of 86% (74/86 pts). The CR in the acute and delayed phases was 90.7% (78/86) and 88.4% (76/86) respectively. 89.5%, 84.9% and 82.6% of patients achieved a CC during the acute, delayed and overall phase. No emesis rates were 91.9% (0-24h), 89.5% (24-120h) and 88.4% (0-120h). Similarly no nausea rates were 84.9%, 75.6% and 74.4% in the acute, delayed and overall period respectively. No serious drug related adverse events were reported. Conclusions. A single dose Palonosetron o.25 mg i.v. alone given as antiemetic treatment is effective and safe in preventing CINV in patients with aggressive NHL underwent MEC.

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NEED AND FEASIBILITY OF A MOTOR REHABILITATION PROGRAM IN A HOME CARE HEMATOLOGICAL PATIENTS POPULATION.

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Introduction. Motor disability (MD) is defined as a reduction of ability to attempt activities of daily living (ADL) and is poor investigated in the setting of home care hematological patients (pts). Methods. We evaluated the incidence of MD and the feasibility of a rehabilitation program in selected patients followed at home for hematological diseases. MD, assessed monthly using Barthel Index (BI), was classified as: mild (BI>66), moderate (BI 33-66), severe (BI<33). Patients with a BI reduction and life expectancy of more than 3 months were evaluated for a rehabilitative home-based program. Exclusion criteria were: patient refusal, contraindications to mobilization, moderate-severe grade of respiratory impairment, mental deficit or fatigue. Rehabilitative program was tailored on patient target; feasibility of the program was evaluated by assessing the administered-intensity / planned-intensity ratio. *Results*. From April 2008 to February 2009, 136 patients entered the study. MD was observed in 122 patients (90%), mild, moderate and severe in 48, 46 and 28, respectively. 18 patients were evaluated for rehabilitation. 3 were considered not eligible, while 15 patients (9 male, 6 female) were so far enrolled: median age was 75 (range 37-92), diagnosis was MM in 5, MDS in 4, AML in 2, NHL in 2 and CLL in 2. Disease stage was advanced in 8, chronic in 4 and partial remission in 3 cases. Intensity of rehabilitation plan was 3/wks in 12, 2/ wks in 3. 2 pts were excluded after less than 2 weeks of treatment. Total sessions were 223. Among 13 pts, 3 received more than one rehabilitation cycle. Median duration and intensity of rehabilitative cycles were 3.4 wks (0.9-18) and 2.8 sessions/wk (1-4.7), respectively. Administered-intensity / planned-intensity ratio was 0.97 (0.35-1.56). Reason for reduction or interruption was complications or death in 10, different causes in 3 patients. *Discussion*. Need of motor rehabilitation is high in hematological home-care patients. Rehabilitation is feasible, although disturbed by several factors, including both disease and complication aspects. Effectiveness of rehabilitation program needs to be evaluated on a larger series. An increase in motor ability, autonomy and quality of life, as well as a decrease in complications incidence, caregivers work load and cost of patient management are expected.

Coagulation Disorders

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VENOUS THROMBOEMBOLIC COMPLICATIONS IN PATIENTS WITH SEVERE COAGULOPATHY UNDERGOING MAJOR SURGERY

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The risk management for venous thromboembolism (VTE) is still unsatisfactory in patients with severe coagulopathy undergoing major surgery that requires peri-operative replacement of lacking coagulation factor. In fact, information about the incidence of proven VTE is lacking in this setting. Some patients receive pharmacological antithrombotic prophylaxis but the efficacy and safety of such approach is doubtful; moreover, when applied, the dosage and duration of anticoagulant prophylaxis is controversial. The first step to evaluate whether patients with severe coagulopathy may need antithrombotic prophylaxis is to evaluate the incidence of post-operative VTE. For this purpose, we conducted, among four centres in Italy (Palermo, Milano, Castelfranco Veneto and Parma), a prospective investigation to objectively evaluate the occurrence of proximal deep vein thrombosis (DVT) and or pulmonary embolism (PE) in consecutive patients with severe coagulopathy planned for major surgery. During the period 2003-2007, Forty-seven patients with congenital coagulopathy who underwent major surgery were evaluated in the post-operative period for symptomatic DVT or PE and asymtpomatic DVT, detected by compression ultrasonography (C-US) of the lower limbs. C-US was performed 10±3 days after surgery (short-term follow-up); the clinical surveillance was conducted for the entire period of 3 months after surgery (long-term follow-up). None of the patients received pharmacological antithrombotic prophylaxis pre or post-operatively; mechanical prophylaxis (with compression graduated stocking) was applied in most of them (41/47, 87.2%) in the post-operative period. During the short-term followup (10±3 days) or during the long term clinical surveillance (3 months) no symptomatic or C-US detected thromboembolic events were registered (0/47, 0% 95 CI-0.7–0.7). In conclusion, in patients with severe coagulopathy undergoing major surgery requiring replacement therapy, the incidence of proven DVT in the immediate or delayed followup is low, less than 1%; in these patients, mechanical antithrombotic prophylaxis seems to be an effective and safe approach.

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PATIENTS REQUIRING INTERRUPTION OF LONG-TERM ORAL ANTICOAGULANT THERAPY BECAUSE OF INVASIVE PROCEDURES: THE ADVANTAGE OF FIXED SUB-THERAPEUTIC DOSES OF LOW-MOLECULAR WEIGHT HEPARIN

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Background. The optimal management of patients who require interruption of long-term Vitamin-K antagonist (VKA) therapy for surgery or other invasive procedures is still uncertain. We tested the hypothesis that fixed doses of Low-Molecular Weight Heparin (LMWH) are a safe and efficacious perioperative bridging therapy during major surgery or invasive procedures at increased risk for bleeding. Material and Methods. Patients, considered to be at low or at high-risk for thrombosis, discontinued VKA 5 + 1days prior to the procedure. In those considered at low-risk for thrombosis, LMWH at a dosage of 4.000 UI anti-FXa once daily was commenced the night before the procedure. In patients considered at high-risk for thrombosis, LMWH at fixed sub-theraputic doses (4.000 UI anti-FXa twice daily) was started pre-operatively and continued until the night before the procedure. In the post-operative period, LMWH was reinitiated 12 hours post-procedure at the some pre-operative dosage; VKA was restarted the day after procedure. Heparin was continued until a therapeutic INR value was reached. The primary efficacy endpoint was the incidence of thromboembolism from VKA cessation to 30+2 days post-procedure. The primary safety endpoint was the incidence of major haemorrhage from first dose of LMWH until 24 hours after the last dose. Results. Over a period of 5 years (2003-2008), a total 486 patients on chronic anticoagulation were considered because of the need for invasive procedures or surgery. Among them 134 were excluded, mainly because of severe comorbidities/poor performance status, planned dental procedures or minor surgery. In total, 352 patients were included in the study; of the enrolled patients, 24 did not receive study medication. In total, 328 patients received at least one dose of LMWH. Among them 182 (55.4%) belonged to low-risk group and 146 (44.6%) to high-risk group. In total, 103 (31.4%) patients underwent major surgery and 225 (68.6%) other invasive procedures requiring VKA suspension. Thromboembolic events occurred in 5 patients (1.5%); 4 belonging to high-risk and 1 to low-risk group. Major bleeding occurred in 7 patients (2.1%); 5 patients belonged to high-risk and 2 to low-risk group. Five out of 7 major haemorrhage occurred in patients undergoing major surgery; none was fatal, intracranial, retroperitoneal, intraocular or required re-surgery (Table). Three deaths occurred during the 30 days follow-up but none was related to major outcomes (recurrent thrombosis or major bleeding). We also had 11 (3.3%) adverse events, 5 (1.5%) protocol violation and 2 (0.6%) patients were lost to follow-up. Conclusions. LMWH given at fixed sub-therapeutic doses is a feasible and safe approach for bridging therapy in chronic anticoagulated patients.

Table. Thromboembolic and haemorragic events in low-risk, high-risk groups and total patients.

Patients n (%)	Low risk group 182 (55.4%)	High risk group 146 (44.6%)	Total 328 (100%)
Thromboembolic Events, total n (%) 95%CI	1 (0.54) -0.54 to 1.6	4 (2.7) 1.4 to 4	5 (1.5) 0.2 to 2.8
Arterial 95%CI	0 -	3 (2.0) -0.2 to 4.2	3 (0.9) -0.1 to 1.9
Venous 95%CI	1 (0.75) -0.45 to 1.95	1 (0.6) -0.6 to 1.8	2 (0.6) -0.2 to 1.4
Bleeding total n (%) 95%CI	7 (3.8) 2.4 to 5.2	12 (8.2) 3.8 to 12.6	19 (5.7) 3.2 to 8.2
Major 95%CI	1. (0.54) -0.52 to 1.6	6 (4.1) 0.9 to 7.3	7 (2.1) 0.6 to 3.6
Minor 95%CI	6 (3.3) 2.7 to 3.9	9 (6.1) 2.3 to 9.9	15 (4.5) 2.3 to 6.7

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RETROSPECTIVE EVALUATION OF DYSFIBRINOGENEMIC PATIENTS AT A SINGLE CENTER: CLINICAL FEATURES AND LABORATORY FINDINGS

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Background. Dysfibrinogenemia is a very rare bleeding disorder in which the clinical phenotype is unpredictable. The literature consists predominantly of collections of case reports. A relatively recent compilation of over 250 patients, revealed that 53% were asymptomatic, 26% had hemorrhages and 21% had thromboses, some of whom also had hemorrhages. *Aims*. To retrospectively investigate the clinical features and laboratory findings of our dysfibrinogenemic patients and to compare them with the literature data. *Methods*. Over the last 10 years, 27 dysfibrinogenemic patients (15 different families) were diagnosed at our center: 12M/15F; median age at diagnosis: 39.9 years (6.1-80.9). Reasons for admission: reduced fibrinogen activity in routine screening in 10 patients, pre-operative coagulation screening in 7, bleeding in 1, ischemic symptom in 1, familial study in 8. Laboratory findings are shown in the Table. *Results.* "Full" mutational screening of the 3 fibrinogen genes (FGA,

FGB, FGG) has, so far, led to a genetic diagnosis in 5/14 studied patients: 1 was heterozygous for the novel FGG Asp330Val (g.7641A>T) mutation, whereas the other 4 were heterozygous for previously reported mutations; 3 carried the FGA Arg16His (g.1203G>A) mutation and 1 the FGG Arg275Cys (g.7475C>T). Sixteen/27 patients experienced hemorrhagic symptoms, mostly mild: traumatic cutaneous bleeding in 9; gastro-intestinal bleeding in 3; epistaxis in 7; gum bleeding in 2; heavy menses in 7. One patient experienced cerebral ischemia (concomitant disease: acleisto-cardia). Twenty one patients underwent surgery and 15 dental extractions. Tranexamic acid and plasma infusions were used as prophylactic anti-hemorrhagic treatment before surgery in 2 and 1 cases, respectively. Only 1 patient bled after dental extraction. Eleven spontaneous deliveries and 8 cesarian sections were carried out without any prophylaxis treatment. No hemorrhagic or thrombotic complications were reported. No spontaneous abortions occurred. *Conclusions.* In our case series the prevalence of asymptomatic patients is inferior to literature data (37% vs 53%). Only 1 patient (4%) had a thrombotic event. Most hemorrhagic patients (59%) experienced only mild symptoms. None of them needed red blood cell transfusions. Anti-hemorrhagic prophylaxis of surgery with tranexamic acid and plasma was administered in other institutions, before diagnosis.

Table 1.

	Mean value	Median value	Range
Fibrinogen activity (n.v. 200-400 mg/dL)	37	38	0-112
Fibrinogen antigen (n.v. 200-400 mg/dL)	251	250	140-470
PT ratio (n.v. 0.90-1.14)	1.2	1.2	0.9-1.74
aPTT ratio (n.v. 0.92-1.16)	1.1	1.09	0.9-1.3

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DIFFERENT REGIMENS OF PROPHYLAXIS TREATMENT IN YOUNG SEVERE HEMOPHILIA A PATIENTS: COMPARISONS ON EFFICACY, FVIII CONSUMPTION AND THERAPY COMPLIANCE

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Background. Primary and secondary prophylaxis is the emerging standard treatment for severe hemophilia A patients. The routine administration of FVIII is effective in children as prophylaxis against hemophilic arthropathy. Nevertheless, the costs and the patient's compliance represent barriers to prophylaxis treatment and to date the most efficacious, cost-effective regimen has not yet been determined. *Aims*. We retrospectively evaluated our severe hemophilia A patients, aged ≤18 years, treated with different prophylaxis regimens to compare efficacy, FVIII consumption and patient/family's compliance. *Methods.* Nineteen patients (median age 13 years, range 3.6-17.8) with severe hemophilia A) on prophylaxis treatment were evaluated. Three regimens of therapy were implemented: administration of FVIII once a week in 1 patient, twice a week in 12, three times a week in 6. Prophylaxis was initiated because of increasing hemorrhages and presence of target joint. *Results.* Are shown in the Table. Before prophylaxis, 4 patients presented a low titer inhibitor that disappeared during treatment. All patients are HCV, HIV, HBsAg seronegative and are treated with rFVIII. In patients on the "twice a week regimen" treated with rFVIII at a dosage of 50 IU/kg, the level of FVIII before the administration was always between 0.5-1.5%. *Conclusions.* When the two most used prophylaxis regimens (twice and three times a week) were evaluated in young patients with severe hemophilia A, an important reduction of hemorrhagic episodes on prophylaxis versus on demand treatment was observed; however, no significant differences were recorded between the two prophylaxis regimens. There is no difference in the total amount of concentrates administered between the two prophylaxis regimens, but there is a very important increase in the consumption of FVIII concentrate during prophylaxis. There is no difference in the orthopedic score before (median 0; 0-2) and during prophylaxis (median 0.5; 0-2), probably due to the young age of patients. The twice a week prophylaxis regimen should be a good alternative treatment to the classic one, and preferable especially for young children because of the reduction in the number of venipunctures. The consequences are a better compliance and a greater adherence to treatment by patients and their families.

Table 1.

	Once a week	Twice a week	Thrice a week
Duration of prophylaxis (months)	9.6	Median 60 (18-111)	Median 113.5 (35.7-141.6)
rFVIII Dosage (IU/kg)	50	Median 42.5 (35-50)	Median 29 (25-30)
Number of hemorrhages in the year before prophylaxis	4	Median 9 (4-30)	Median 4 (0-65)
Number of hemorrhages/year during prophylaxis	0	Median 0 (0-1)	Median 1 (0-2)
Number of hemarthroses in the year before prophylaxis	1	Median 7 (1-18)	Median 5.5 (2-26)
Number of hemarthroses/year during prophylaxis	2	Median 0 (0-2)	Median 0.25 (0-2)
rFVIII units/kg/year before prophylaxis	300	Median 1200 (200-5760)	Median 850 (200-900)
rFVIII units/kg/year during prophylaxis	1800	Median 4320 (2900-5200)	Median 4320 (4032-4800)

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EFFICACY AND SAFETY OF PROTHROMBIN COMPLEX CONCENTRATE (UMAN COMPLEX) AND VITAMIN K IN A COHORT OF 56 PATIENTS WITH ANTICOAGULATION-RELATED ACUTE INTRACRANIAL HEMORRHAGE: CLINICAL FEATURES AND OUTCOMES AT THREE MONTHS.

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Central nervous bleeding emergencies are the most serious complication of Oral Anticoagulant Therapy (OAT) with an incidence of 1% per patient-year. From January 2004 through January 2009 we observed 59 episodes of anticoagulation-related Acute Intracranial Hemorrhage (AIH) in a cohort of 56 consecutive patients at our emergency department. All patients were treated with a systematic approach: single bolus of 25-30 IU per Kilogram of Prothrombin Complex Concentrate (PCC) Uman Complex® and endovenous administration of 10 mg of vitamin K1 within one hour after baseline CT scan of the head. Simultaneously all patients received urgent neurosurgical evaluation. We analyzed the following variables: age, sex, indication for OAT, INR at admission and after PCC bolus, site of intracranial bleeding, neurosurgery, previous history of hypertension and chronic cerebral arteriopathy, serum cholesterol level, traumatic events, drug interactions, mass effect of AIH and thrombotic complications. Functional outcomes at 90 days were assessed with the modified Rankin Scale (on which 0 indicates full recovery and 6 indicates death). Poor outcomes were considered scores from 4 to 6. Median age of our cohort was 80 years (range 38-91) with a male-female ratio of 1,16. Furthermore, indications for OAT were atrial fibrillation in 45 (80%), mechanical valves in 7 (13%), other indications in 4 (7%) cases. 16 patients (29%) underwent neurosurgical procedures and 28 (50%) patients registered a recent trauma. Acute reversal of OAT (INR<1.5) was obtained in 86% of cases. Among these 56 patients treated with PCC, we observed 4 thrombotic events, (2 pulmonary embolisms and 2 ischemic ictus). Sadly, in our cohort mortality rate at 90 days was 46%; this value is slightly greater as compared with that of spontaneous intracerebral hemorrhage known in literature. Moreover male sex and INR >3 vs INR< 3 correlate with an increased mortality rate

(respectively p=0.04 and p=0.004). Finally, although hypertension is a well-known risk factor of AIH, surprisingly in our cohort previous history of hypertension is associated with more favorable outcome (p<0.002), may be because of better control of blood pressure values at moment of AIH.

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ANTI-PLATELET STRATEGIES FOR PATIENTS WITH A RECENTLY IMPLANTED CORONARY DRUG-ELUTING STENT (DES) NEEDING URGENT MAJOR SURGERY: RESULTS OF A PHASE-TWO STUDY USING TIROFIBAN DURING TEMPORARY WITHDRAWAL OF CLOPIDOGREL

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Introduction. Coronary artery stenting has become the standard of care for the treatment of acute coronary syndromes (ACS). After DES implantation, patients (pts) must receive dual anti-platelet therapy with aspirin and clopidogrel for one year. Premature withdrawal of dual anti-platelet therapy is associated with a high risk of stent thrombosis which may result in myocardial infarction (MI) or death. On the other hand, impaired platelet aggregation induced by clopidogrel leads to an increased risk of bleeding unless the drug is discontinued at least 5 days before surgery. Surgery induces a pro-thrombotic state and resumption of oral therapies may be delayed. Coincidence in the age of peak incidences of coronary artery disease and of morbidities needing surgery, renders the event of a pt with a recently implanted DES who needs surgery more common. The present pilot study explores the use of the short-acting IIbIIIa antagonist tirofiban, instead of clopidogrel, in the peri-operative period in an attempt to overcome the dual problems of stent thrombosis and surgical bleeding. *Patients and Methods.* Following a Simon two-stage design, 29 pts with a recently implanted DES (median delay 4 mos, range 12 days to 12 mos) and high-risk characteristics for stent thrombosis underwent urgent major surgery. Clopidogrel was withdrawn 5 days prior to surgery. Tirofiban was started 24h after the last clopidogrel tablet, continued until 4h before surgery and resumed 2h after surgery until oral clopidogrel reinstitution. *Results.* The surgery was successfully completed in all pts; no death, MI, stent thrombosis or re-operation due to local bleeding events (1-tail 97.5% C.I. 0, 11.9%) were recorded. Two pts experienced GI post-operative bleeding, one resolved spontaneously (angiodysplasia), the other needed operative coloscopy (2 clips). *Discussion.* In high-risk pts needing urgent surgery early after DES implantation, a "bridge strategy" using intravenous tirofiban may allow anti-thrombotic protection during temporary withdrawal of oral clopidogrel, without increasing the risk of surgical bleeding. It is conceivable that locally generated platelet agonists and pro-coagulants (cell-derived ADP, thrombin, neutrophil-derived pro-coagulants) may have overcame the anti-aggregating effects of infused tirofiban leading to effective local hemostasis with no effect on distant sites thus preserving stent patentecy. This strategy deserves further, larger scale clinical testing.

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REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA: THE ROLE OF LAPAROSCOPIC SPLENECTOMY

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Background. The role and the popularity of splenectomy are currently under dispute. Nevertheless, surgical approach remains the best therapy after failure of the first-line treatments, but the surgical mortality, thromboembolic events and overwhelming sepsis may complicate the open splenectomy. Rituximab is able to induce an overall response in about 60% of patients with a median duration of remission of 8 weeks and a low rate of long-lasting remissions. Thrombopoietin receptor agonists may have a relevant role but their long-term safety and efficacy have not yet been established. Simultaneously, there has been an evolution in the surgical technique for splenectomy To date, the laparoscopic surgery is considered the standard approach. *Methods*. We analyzed 45 pts (22,3% of 202 pts) (33 F and 12 M; median age 38 years; range 6-71) with unresponsive ITP after one or two medical approaches (steroids, IgG HD, vincristine, rituximab) underwent laparoscopic splenectomy at our Institution from 1999 to date. *Results.* There was no short and long-term mortality. Immediate postoperative complications rate was 4,4%: we observed 2 cases of hemoperitoneum related to a trocar's tube and to an active bleeding, respectively, both resolved with new laparoscopic approach. The mean postoperative hospital stay was 4,5 days (range 4-8). Neither cases of bacterial sepsis, nor cases of splenic-portal vein thrombosis (SPVT) and no cases of neoplasms occurred. Hematologic response rate was 100%. After a median follow-up of 78 months (range 8-112 months), 37 pts (82.2%) are still in CR or PR with a platelet count more than 50x10^3/microL and 2 pts are taking ASA. Five pts (11.6%) relapsed, 3 of them with a platelet count less than 10x10^3/microL. *Conclusion.* Our experience clearly shows that laparoscopic splenectomy is an excellent approach to pts with refractory ITP in terms of safety, efficacy and costs. Moreover, the laparoscopy makes the splenectomy even safer and suitable for a larger number of pts including frail and older patients. For that reason we can not hide that in our hands laparoscopic splenectomy has proved a winning card to play as soon as possible and rituximab or new drugs should be reserved only in the cases in which splenectomy fails. Undoubtedly there is a great expectation for the new drugs and only prospective clinical trials will be able to say a final word and to challenge the role of splenectomy.

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TRANSIENT RITUXIMAB-INDUCED ACUTE THROMBOCYTOPENIA: A CASE REPORT

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Background. Rituximab, a chimeric anti-CD20 monoclonal antibody, is indicated for the treatment of patients with CD20 non-Hodgkin lymphoma (NHL). Rituximab side effects are restricted to first infusion-related fever, chills, rigors, nausea and vomiting, which are usually self-limited. Although some episode of late-onset neutropenia associated with rituximab therapy has been reported, acute thrombocytopenia is extremely rare. Only few cases are reported in literature, generally in patients with splenomegaly. We report transient acute thrombocytopenia after rituximab infusion in a patient with mantle cell lymphoma (MCL). *Case report.* A 55-year-old man was referred to our centre in February 2009 for lymphocytosis, splenomegaly (10 cm below the costal margin) and lymphadenopathy. Hematological findings were hemoglobin (Hb) 13.9 g/decilitre, platelets 181x10^9/L and white blood cell (WBC) 41.8x10^9/L with 80% lymphocytes. Bone marrow biopsy showed nodular infiltrate of lymphocytes with CD20, CD5 and cyclin D1 positivity. The diagnosis of MCL, stage IV, was made and was started R-CHOP therapy. The patient receiving rituximab 375 mg/m^2 on day 1; he developed a cytokine release syndrome requiring transient discontinuation of rituximab infusion. On day 2 his platelet count dropped from 181 to 22 x10^9/L, without change in Hb level and without signs of bleeding and he received CHOP at full dose. The patient remained asymptomatic and his platelet count spontaneously increased to 115x10^9/L three day after rituximab infusion. No other hematological toxicity were observed in the later days. Subsequently he continued the next cycles, including rituximab, at full dose. *Discussion.* Severe thrombocytopenia after rituximab is unexpected. The mechanism of thrombocytopenia remains unclear. Previous reports suggested the presence of CD20 antigen on the platelets themselves or that soluble CD20 antigen in the circulation may cause an antigen-antibody reaction and immune-mediated cell lysis. Others Authors hypothesize a unique syndrome characterized by significant infusion-related side effects, thrombocytopenia and rapid decrement of lymphocytosis in patients with high tumor mass, especially splenomegaly and high lymphocytosis. We suggest that blood counts should be performed 24 hours after rituximab infusion, specially in patients with marked splenomegaly. In our experience, however, this transient thrombocytopenia doesn't interferes with continuance of therapy.

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PLATELET RADIO-LABELING IN PATIENTS WITH IDIOPATHIC TROMBOCYTOPENIC PURPURA: LONG TERM FOLLOW UP OF SPLENECTOMY

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The role of platelet radio-labeling methods and destruction site determination in chronic idiopathic thrombocytopenic purpura (ITP) in order to consider splenectomy as treatment of choice it remains a controversial subject. To estimate the possibility of predicting treatment response in patients with ITP, between 1985 and 2005, 190 ITP patients underwent platelet kinetic study by means of autologous platelet labeling index with 111 In-oxinate. Of these, 96 (38 males and 58 females) aged from 10 to 76 years we have the clinical record with a median follow-up of 139 months (range 36-281). Before the kinetic 63 cases received steroids therapy and 33 were not yet treated, at the time of examination platelets count ranged from 1000 to 90.000x10^9/L. Platelets sequestration/destruction sites were the following: spleen 58 cases, liver 5 cases, spleen and liver 14 cases, diffuse pattern 19 cases. Splenectomy was performed in 38/96 cases (40%). In 25/38 (66%) of the splenectomized patients the site of platelets destruction was the spleen, in 8 (21%) liver and spleen, and in 5 (13%) the platelets had a diffuse pattern of destruction. In 7/38 (18%) splenectomized cases the ITP relapsed while 31/38 (82%) didn't relapse and have been considered cured. All the relapsed cases were treated with immunosuppressive therapy before splenectomy, and the platelet kinetic had shown a spleen destruction in 5/7, and a diffuse destruction in 2/7 of them. Three of the relapsed patients underwent a second platelets kinetic examination, and an accessory spleen was found to be the cause of relapse in 2 of them. Whilst the group of cases was small the long term observation/follow-up has confirmed that non-invasive method of platelets labeling and platelets sequestration/destruction site determination make easier the decision for the splenectomy when the spleen is at least one sequestration site of the labeled platelets. This favorable result appear to be stronger when splenectomy was performed at the onset of the disease, before immunosuppressive treatment.

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CD200 EXPRESSION IN PATIENTS WITH AUTOIMMUNE DISEASES

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Background. CD200 (OX-2), is a cell surface ligand which acts as an important molecule in controlling autoimmunity, inflammation and adaptive immune responses, thus suggesting that it is a key immunotolerant molecule. It is expressed on a variety of cells types, including myeloid cells such as macrophages, dendritic cells, endothelium, B cells, T cells, neurons. CD200 exerts its effect binding to its receptor CD200R which is expressed on myeloid leucocytes and a subset of T-lymphocytes. We investigated the surface expression of CD200 on PBMC, on B- and T-lymphocytes of 15 patients with autoimmune hemolytic anemia, 10 patients with systemic sclerosis, 7 patients with idiopathic thrombocytopenic purpura (ITP) and 26 healthy donors (HD) by flow cytometry. *Methods.* Peripheral blood were collected in EDTA. From each sample, 1x10^6 cells were simultaneously stained with 10 microlitre of the following monoclonal antibodies (MoAbs): CD3, CD19, CD45, CD200. After incubation for 20 minutes at room temperature, erythrocytes were lysed adding ammonium chloride and analyzed by flow cytometry. The expression of CD200 were evaluated on gated side light scatter (SSC)/CD45$^+$ for PBMC, while for B and T- lymphocytes on gate SSC/CD19$^+$ and SSC/CD3$^+$ respectively. *Results.* We observed a reduced expression of CD200 on total PBMC of patients with autoimmune hemolytic anemia (3.28±2.0%), systemic sclerosis (3.20±1.38%) and ITP (2,8±1.36 %) compared to HD (6.38±3.5%) (p=0.002; p=0.001 and p=0.07 respectively). In addition, expression of CD200 were significantly lower on T-cells of patients with autoimmune hemolytic anemia (3,34±1,57 %), systemic sclerosis (2,05±1,03 %) and ITP (2.57±1,77%) compared to HD (5.95±2.96%) (p= 0,003, p<0.0001 and p=0,003 respectively). No statistical difference were observed in expression of CD200 on B-cells of these patients vs HD: autoimmune hemolytic anemia (55.86±21.59%), systemic sclerosis (64.68±11.14%), ITP (58.50±1 5.90%), HD (65.67±11.64%). *Conclusion.* These results suggest that the decreased expression of CD200 on lymphocytes might play an important role in the immunopathophysiology of these autoimmune diseases. Therefore the CD200 may serve as ideal targets for pharmacological manipulation of immune response.

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HEPARIN-INDUCED THROMBOCYTOPENIA: IDENTIFICATION OF RISK FACTORS FOR THROMBOSIS

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HIT is a prothrombotic disorder caused by platelet-activating antibodies (HIT-Abs) raised against PF4-heparin complexes. The estimated prevalence of HIT ranges from 0.5-5% of patients, depending on heparin type (unfractionated, UFH, or fractionated, LMWH), patient type (medical or surgical) and possibly additional factors. Only about 10% of HIT patients develop thromboembolic events (TE). We studied a cohort of 124 pts. positive for HIT-Abs, aiming to 1. establish the role of heparin type in the occurrence of thrombosis; 2. correlate dosage/duration of heparin administration and thrombosis; 3. identify additional risk factors for thrombosis. 18 pts. had received UFH, 11 as prophylaxis (100-200 U/kg/d) and 7 at therapeutic dosage (350-400 U/kg/d). 61 pts. had received LMWH, 47 as prophylaxis (50-100 U/kg/d) and 14 at therapeutic dosage (200 U/kg/d). 45 pts. had received both heparins at both dosages. 46 pts were surgical (cardiovascular, abdominal, orthopaedic surgery), 22 had ischemic heart disease, 11 were septic, 16 were on dialysis for renal failure, 16 had neoplasia, 7 DVT/PE, 3 autoimmune disorders and 3 were polythaumatized. 14 subjects had also diabetes as comorbidity. Mean duration of heparin prophylaxis was 26 (+/-10) days for UFH and 14 (+/-2) days for LMWH. Statistical analysis among groups was carried out by the (2) test. Thrombosis occurred in 18 pts (14.4%); of these, 6(33%) were surgical patients, 6(33%) were diabetics, 2(11%) were septic, 2(11%) were polythraumatized, 2(11%) had ischemic heart disease. A significant correlation with thrombosis was found for diabetes (OR 0.29, p=0.009) and for surgery (OR 0.29, p=0.009). A trend was observed for neoplasia, polythrauma and renal failure although the small number of the sample did not allow significance. Even if 80% of thromboses occurred in pts. treated with prophylactic LMWH for more than 6 days, type and duration of exposure to heparin did not reach a significant degree of correlation with thrombosis. Our data confirm that in pts. with HIT-Abs the chance of developing thrombosis is higher in clinical settings characterized by a highly increased thrombotic risk.

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THROMBOTIC THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE

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Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disease with systemic formation of platelet thrombi in the microcirculation within all the organs. This results in thrombocytopenia, microangiopathic hemolytic anemia and symptoms of damage to different organs, especially the brain and kidneys. Almost 80 years after Eli Moschcowitz published the first description of the disease, most patients with TTP were found to have acquired autoantibody inhibitors of the ADAMTS13 metalloprotease. Plasma ADAMTS13 normally cleaves von Willebrand factor within nascent platelet-rich thrombi, and ADAMTS13 deficiency allows unchecked thrombus growth to cause microangiopathic hemolysis, thrombocytopenia, and tissue infarction. The modern era in the management of TTP began with a randomized trial, published in 1991, which established that plasma exchange is superior to plasma infusion for the treatment of TTP. During the decade before these results were reported, anecdotal experience indicated that plasma therapy could cure patients who otherwise had an expected survival of <10%. By demonstrating that plasma exchange increased survival to >80%. Approximately 20% of patients with idiopathic TTP still die during the first month of acute illness, and at least 30% experience one or more relapses within 2 years. We report the experience in 16 patients with TTP admitted to our hospital from January 2003 to December 2007. Data were retrospectively analyzed. Among 16 patient analyzed, 5 (31%) were male and 11(69%) were female. The mean age was 53 years old (male 57 years old, female 52 years old). A total of 20 admission for many episodes of idiopathic TTP (14 (88%) *de novo* and 6 (12%) relapses) were studied; the mean number of days of hospitalization was 8 days. All the patient presented with anemia, thrombocytopenia and LDH elevation. The most common clinical presentation was the central nervous system involvement. 10 (62.5%) patients accused at least one neurological symptoms, of which the most frequent was the coma (7 patients=44%). All patients were homogeneously treated with daily plasmapheresis and costicosteroids, of them 7 (44%) died. Finally, we observed that died patient have higher serum lactate dehydrogenas (p=0.022), lowest platelet count (p=0.026) and neurological symptoms (p=0.019). There isn't a statistically significant correlation with Hb level (p=0.97) and WBC level (p=0.110).

Chronic Lymphocytic Leukemia I

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RISK FACTORS, NEW MOLECULAR AND IMMUNOHISTOCHEMICAL MARKERS IN CHRONIC LYMPHOCYTIC C LEUKEMIA

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Introduction. The course of chronic lymphocytic leukemia(CLL) is variable. In aggressive disease, the CLL usually express an unmutated immunoglobulin heavy-chain variable-region gene (IgVH) and the 70kD zeta-associated protein (ZAP-70), whereas in indolent diseases, the CLL cells usually express mutated IgVH but lack expression of ZAP-70, chronic lymphocytic leukemia upregulated gene1 (CLLU1), (first LLC-specific gene) and predictive instrument of LLC risk was used. Multiple myeloma oncogene-1 (MUM1) is instead a recent prognostic factor in the LLC, the expression correlates with a favourable clinical course and long survival. TNF- , IL4,and IL10 have also been suggested to be important for B-cells growth and survival. *Aims of study*. In the present study, we examined purified CLL-B cells to characterize the molecular alterations and gene rearrangements, to find biological markers in specific disease stages and to estimate the patient prognosis on the risk categories (Binet stage B-C). Common polymorphisms in gene coding for cytokines implicated in the inflammatory response and TH1/Th2 balance might play a role in the development and prognosis of CLL. To test this hypothesis, we investigated 13 single nucleotide polymorphisms (SNPs) in nine of such genes. *Materials and Methods* We followed 23 patients with CLL between 2006-2008 from the Haematology "A. Businco" Cancer Hospital of Cagliari. Case age with a range from 40 to 75 years, and ratio M/W 2:1. Blood and bone marrow samples were collected from patients who satisfied diagnostic and immunophenotypic criteria for common B-cell CLL. All samples were assessed by flow cytometry analysis for CD5, CD79a and MUM1. RNA and DNA were isolated for molecular biology Real Time and sequence study. Besides 40 incident CLL cases and 113 population controls were available from case-control EPILYMPH study . A Taq man platform was exclusively used for genotyping. Sequence data and assay conditions for Taqman assay are available on the U.S. National Cancer Institute SNP500 project (http:/snp500cancer.nci. nih.gov). *Results*. 30% of the patients showed aggressive clinical course. The CD79b level was negative or weakly expressed in all cases (91%), only two cases were positive. 43% of the cases it were positive for CD38. Mutational status of immunoglobulin IgVH and CD38 correlation: 5% ZAP-70 +/ IgVH unmutated, 69% ZAP-70 -/ IgVH mutated, and absence of correlation in 26% of cases. Histopathology analysis has been performed with standard markers (CD5, CD79a, CD20, CD23) for eventually differentiation between classic CLL and its varying. The mutation status of IgVH was performed for VHL, VHFs, VHD specific families: IgVH extension molecular analysis showed 89% IgVH mutate. We examined a single IgVH subgroup, the most common of which was VH3 (52%), followed by VH4 (29%) and VH1(19%). ZAP-70 and CLLU1 were performed through Real-Time PCR and relative quantification expressed as (2^- CT). These data revealed that: 26% of patients was positive for ZAP-70 and was not presented CLLU1 and IgVH correlation. Polymorphisms in IL1B and IL6 genes, but not the other tested interleukin SNPs, were associated with CLL risk. Individuals with aplotype including both variant alleles showed a 5.3-fold increase in CLL risk and individuals with SNP IL1B variant allele were significantly protect against CLL. *Conclusions.* In the IgVH analysis: discordant data respect European guideline and absence of VH3-21 expression were obtained. The ZAP-70 values performed by molecular biology techniques remain the more important index regarding the IgVH mutation status. The MUM1 high expression has indicated patients with favourable clinical course and a longer survival. CLLU1, CD38, and ZAP-70 correlation has identified groups of patients to good and bad prognosis. Future research on prognostic indicators, such as clinical stage of disease, lymphocyte doubling time, CD38 and /or ZAP 70 expression levels, and IgVH mutational status, as related to the IL1B and IL6 gene polymorphisms and expression, would be important to provide molecular support to our findings. Poor statistical power is a major limitation in the present study, and caution is recommended in the interpretation of our findings.

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MULTIPLE MYELOMA ONCOGENE-1 (MUM1) AND CHRONIC LYMPHOCITIC LEUKEMIA UPREGULATED GENE 1 (CLLU1): MOLECULAR AND IMMUNOHISTOCHEMICAL MARKERS IN CHRONIC LYMPHOID LYMPHOCYTIC LEUKEMIA

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Introduction. The chronic lymphocytic leukaemia up regulated gene1 (CLLU1), first LLC-specific gene can be a predictive instrument of LLC risk investigation. Multiple myeloma oncogene-1 (MUM1) is instead a recent prognostic factor in the LLC, the expression correlates with a favourable clinical course and long survival. *Purpose*. To investigate on a novel molecular and immunoisthochemical marker as prognostic approach and as start therapy in untreated B-cell Chronic Lymphocytic leukemia (B-cell) patients. *Materials and Methods*. The study was performed between 2006 and 2008 at the "A. Businco" Cancer Hospital in Cagliari, Sardinia, (Italy) and department of pathology of Oncology Hospital "A.Businco"Asl 8 Cagliari; 23 cases with a CLL diagnosis were analyzed, case age, 42-88 years, W/M 39% /61% , ratio M/F 2:1. DNA and total RNA were extracted from cells obtained from bone marrow and peripheral blood. Measurement of prognostic indicators by real time Polymerase Chain reaction, and study of transcripts by sequence analysis were performed. CLL1 Kit by Iposogen was used. Instead biopsy of samples from CLL patients were analyzed for MUM1; DAKO reagent after separation from paraffin (clear solution) was used. The slides were incubated with specific antibody and show with Dako Real Detection System Alkaline/Phosphatase/Red Rabbit/Mouse and Fast red- and levamisole (Dako). *Results and conclusions.* In 23 cases enrolled, at exordium 85.7% were positive for MUM1 in immunohistochemical qualitative evaluation, (one was highly positive and 2 negative). All positive samples were with IgVH mutated. All CLL1 value as copies CLL1/B2M were not over espressed in CLL follow – up, range (0-1.07).The MUM1 high expression has indicated patients with favourable clinical course and a longer survival. MUM1 and CLLU1, correlation has identified groups of patients to good prognosis but have not indicate patients will be in need of therapy. But poor statistical power is a limitation in the present experience, and caution is recommended in the interpretation of our findings.

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ZAP-70 EXPRESSION AND IGVH MUTATIONAL STATUS AS MARKERS FOR GENE EXPRESSION SIGNATURE IN B-CLL PATIENTS FOR PROGNOSTIC CLASSIFICATION

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Background. Chronic lymphocytic leukemia (CLL) is a heterogeneous disease; ZAP-70 protein expression and IgVH mutational status have shown to be strong associated and to offer important prognostic information. *Aim.* Our aim was to determine gene expression profiles of 46 CLL patients divided into three classes: the first (n=26) with mutated IgVH and ZAP-70$^-$, the second (n=12) with unmutated IgVH and ZAP-70$^+$, and the third (n=8) included CLL patients with unmutated IgVH and ZAP-70$^-$, or mutated IgVH and ZAP-70$^+$ respectively. Finally, the purpose was to define prognostic biomarkers and the biological pathways related to B-CLL. *Methods.* We determined gene expression profiles using Affymetrix HG U133 Plus 2.0 in CD19$^+$ leukemic cells. Subjects were clustered in groups with similar expression signature using cluster analysis (K-means, Euclidean distance). *Results*. Statistical analysis revealed 154 differentially expressed probe-sets in the first (mutated IgVH and ZAP-70$^-$) vs the second (unmutated IgVH and ZAP-70$^+$) group, corresponding to 88 genes annotated in public databases. Interestingly, six genes were associated to the following biological pathways: MAPKsignaling (heat shock 70kD protein 8 HSPA8), B cell receptor signaling (ZAP-70, CKLF-like MARVEL transmembrane domain containing 3 CMTM3, dual adaptor of phosphotyrosine and 3-phosphoinositides DAPP1), Matrix Metalloproteinasis (transcription factor 20, TCF20), Apoptosis (X-linked inhibitor of apoptosis XIAP) and T cell receptor signaling (ZAP-70). In particular, ZAP-70, HSPA8, CMTM3 were significatively underexpressed while XIAP, TCF20 and DAPP1 were overexpressed in the first class of patients in comparison to the second class, respectively. Based on the expression of the 88 genes identified in the comparison between the first and the second class of patients, the 8 patients of the third class were divided in two clusters: 5 subjects were more similar to the first class, while 3 subjects appeared to be more similar to the second one. In particular, cluster analysis revealed that the 46 patients were better partitioned in two rather than in three classes, based on their expression profiles. *Conclusions.* Our preliminary data revealed that MAPK signaling, B cell receptor signaling, apoptosis and T cell receptor signaling may ultimately influence CLL biology. Gene expression analyses are in progress on larger series of CLL patients in order to assess the association of the molecular signature with respect to prognostic information.

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MOLECULAR APPROACH TO CHRONIC LYMPHOCYTIC LEUKEMIA THROUGH MLPA ANALYSIS

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Background. Chronic lymphocytic leukaemia (CLL) is the commonest form of adult leukaemia in western countries. From a prognostic point of view, it is characterized by an heterogeneous course. As a consequence the characterization of prognostic factors is very important in order to prefigure evolution of disease. Nowadays it is well known that FISH analysis provides the identification of independent prognostic factors predicting outcome in CLL. *Aim*. We applied MLPA method (multiplex ligation-dependent probe amplification) to check for copy number variation in genes not included in standard FISH panel. *Methods and Patients*. CLL patients, whose diagnosis was performed according to IWG-CLL criteria, were selected for this study. Selection criteria were the following: no treatment and a disease history lasting less than 2 years. In the mean time standard cytogenetic and FISH probe set for chromosomal regions 13q14, 11q22, 17p13, 6q23, 14q32 and chromosomal 12 were evaluated. Peripheral blood samples from 15 patients were collected and a genomic profiling using MLPA was performed. We used the SALSA P037/P038 kits. Samples analysis has been performed with sequence analyzer ABI PRISM 310 and peak area and height area were measured using Genescan analysis software. Loss or gain of targets was normalized using MLPA on DNA of healthy donors. Cut-off levels for loss or gain of relative copy numbers were set at 0.7 and 1.3, respectively. Values included between 0.4 and 0.7 indicate a deletion, while values between 1.3 and 1.6 indicate a duplication. Each result was validated in two experiment independently. *Results and Conclusions*. Data obtained by MLPA were compared with cytogenetic and standard FISH. MLPA results supported the outputs obtained by FISH, in addiction in some cases MLPA was able to detect more information about specific chromosomal regions not included in standard FISH panel. These results underline the importance to add more genetic markers in FISH panel in order to obtain better prognostic information. The following step, thereby, will be to continue this research in order to validate this method and to control the possible rearrangements of the patients' chromosomes during the course of disease.

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INCREASED ANGIOGENESIS INDUCED BY CHRONIC LYMPHOCYTIC LEUKEMIA B CELLS IS MEDIATED BY LEUKEMIA-DERIVED ANG2 AND VEGF

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Leukemic cells are able to disrupt the normal architectures of tissue microenvironment to generate a favourable soil for their survival and growth. As part of this process, the CLL capacity to modify the vascular structures of infiltrated tissues may be crucial to provide sufficient oxygen and metabolites, to allow dissemination throughout the body and to

establish positive cellular interactions. In our study, we investigated the in vitro and *in vivo* angiogenic capacity of CLL cells. We showed that CLL cells spontaneously express both VEGF and Ang2, and are able to secrete these pro-angiogenic factors in the surrounding microenvironment. Moreover, we demonstrated that CLL cells can enhance the secretion of VEGF and Ang2 protein when subjected to hypoxic condition. In CLL supernatants, significant increase of VEGF protein was observed at 24 and 48 hours of hypoxic exposure (respectively 2.7-fold and 7.5-fold above the control): a significant increase of secreted Ang2 was already detected at 4 hours of hypoxic treatment (9-fold above the control), reaching a 16-fold change at 48 hours. Highly variable levels of bone marrow (BM) vascularization were observed in CLL patients. Increased microvessel density was detected in CLL patients with unmutated Ig compared to mutated ones (p=0.012), implying that a precocious 'angiogenic switch' may contribute to progressive phenotype. Interestingly, we found that BM vascularization positively correlates with Ang2 mRNA expression (p=0.044). We finally demonstrated that supernatants derived from CLL cells significantly induce *in vitro* angiogenesis by HUVEC cells on Matrigel. In control samples consisting of HUVEC in endothelial-specific medium without growth factors, the tube formation was notably inhibited: some endothelial cells began to migrate and aligned themselves at 6 hours, however capillary tube structures didn't develop even after 10-12 hours. On the contrary, the addition of CLL-derived conditioned medium (CM) resulted in marked increase of HUVEC elongation and branching to form complex mesh-like structures just after 6 hours of culture. Furthermore, HUVEC were plated on Matrigel in presence of CLL supernatants previously incubated with antibody anti-hAng2, anti-hVEGF or both: the increased angiogenesis induced by CLL-derived CM was clearly affected by these neutralizing antibodies. The results demonstrated that both CLL-derived VEGF and Ang2 have a role in the enhanced angiogenesis induced by leukemic cells.

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PROGNOSTIC VALUE OF PLASMATIC LEVELS OF ANGIOPOIETIN 2 IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Patients affected by B-cell chronic lymphocytic leukemia (CLL) follow extremely variable clinical courses with overall survival times that range from months to decades. Clinical and biological factors with potential prognostic relevance have been searched in order to find markers that may help to refine outcome prediction for these individuals. Angiopoietin 2 (Ang2) is a pro-angiogenic factor which mRNA expression is increased in poor prognosis CLL patients. In order to better define the prognostic role of this molecule in CLL, we measured Ang2 plasmatic levels in 244 patients collected from several Italian centers, valuing associations with main established prognostic factors and clinical behavior. We observed variable plasmatic quantities of Ang2 (median value 1963 pg/ml, range 972-17281 pg/mL). We compared Ang2 amounts between different CLL prognostic subgroups and we found they were higher in cases with Binet stage B/C than A (p=0.002), in patients with unmutated Ig than mutated (p<0.0001) and in cases CD38 positive than negative (p=0.027). Moreover, we showed positive correlation between Ang2 plasmatic levels and β2 microglobulin (p<0.0001), LDH (p=0.029) and percentage of bone marrow lymphocytosis (p=0.030). Defining the best cut-off for Ang2 plasmatic levels in relation to time to first treatment (TTT), CLL patients were divided into Ang2 positive and Ang2 negative subset. Ang2 positive cases had shorter TTT than Ang2 negative (p=0.001): TTT was confirmed to be correlated with known established CLL prognostic factors. In bivariate analysis, Ang2 positive patients had shorter TTT than Ang-2 negative, even inside the subgroup with Binet stage B/C (p=0.005), with mutated Ig (p=0.010), CD38 negative (p=0.002), with low cytogenetic risk (p=0.005), with low CD49d (p<0.0001) and ZAP70 negative (p=0.006). In multivariate analysis with biological variables, plasmatic Ang2 resulted to be an independent prognostic factor for TTT as well as Ig mutational status and cytogenetic risk. In multivariate analysis with variables related to tumor burden, plasmatic Ang2 was an independent prognostic factor for TTT as well as Binet staging and β2 microglobulin. These data suggest that Ang2 plasmatic levels are associated with a progressive behavior of CLL and represent an independent factor predicting time to first treatment, allowing a more accurate prognostication and helping in a better managing of therapies for CLL patients.

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DETECTION OF CHROMOSOMAL 14Q32 ABNORMALITIES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Conventional cytogenetic and FISH studies with the standard panel (13q14, 11q22.3, 17p13, 12, 6q23) are used to detect chromosomal abnormalities of clinical significance in patients with chronic lymphocytic leukemia (B-CLL). Recently, translocations and 5'IGH deletions involving chromosome14q32 are associated with B-CLL. *Aim.* Our aim was to investigate the presence and the characteristics of chromosome 14q32 aberrations in a group of patients with B-CLL. *Methods.* A total of 58 patients with B-CLL were investigated by conventional cytogenetic, in addition the cultures were stimulated with CpG oligodeoxynucleotide plus IL2. FISH studies with 14q32/IGH break-apart probe designed to detect chromosomal breakage of the IGH (14q32) locus, were done for 59 patients. *Results*. Chromosomal abnormalities were detected in 60.3% of cases by cytogenetic. 14 patients showed complex cariotype while trisomy 12 was the most frequent anomaly found in 8 patients. Among the twelve other patients several chromosomal aberrations were noted: del(13)(q14), del(13)(q12q21), del(13)(q12q14), del(13)(q13q32), +21, t(11;13)(q23;q14), +12 t(1;4)(q31;p14), t(3;14)(p21;q22), del (11)(q12) +21, del(6)(q21), inv3(?p13q21), del(11)(q12). Abnormalities of chromosome 14 was found in 23.8% of patients. Deletion of 5'IGH, corresponding to the variable IGH segment, was the most frequent anomaly found in 8 patients. Interesting, a 3'IGH deletion was detected in two patients, while only one patient showed a complete deletion of chromosome 14 (47,XXY,add14q32). Three patients showed 14q32 translocations involving the IGH locus. *Summary/Conclusions*. Based on our findings, deletions of the variable region of the IGH gene (IGHv) and 14q32/IGH translocations are involved in B-CLL. As these preliminary data are based on small sample size, our goal will be to study the cytogenetic profile of a large number of CLL patients. Future studies will permit the identification of 14q32 translocations and the type and the frequency of IGH rearrangements. Finally, chromosome 14 abnormalities will be correlated to other cytogenetic and FISH abnormalities, and associations with known prognostic markers, such as IGVH mutation status and ZAP-70 expression, will be investigated.

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MULTIDRUG RESISTANCE-1 GENE POLYMORPHISMS ASSOCIATED WITH THE NEW MARKERS OF DISEASE PROGRESSION AND POOR PROGNOSIS IN B-CELL CHRONIC LIMPHATIC LEUKEMIA

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The clinical course of B-cell chronic lymphatic leukemia (CLL) is highly variable, and survival from the time of diagnosis can range from month to decades. Novel biological markers such as IgVh mutation, CD38, and ZAP-70 expression have shown to offer prognostic informations. P-glycoprotein (P-gp) is produced by expression of the multidrug resistance-1 gene (MDR1), and variations may occur due to genetic alterations,

such as single nucleotide polymorphisms (SNP), affecting drug-metabolizing enzymes and thus resulting in altered pharmacokinetics of drugs, and are likely to influence the response to therapeutic agents. Various SNP of MDR1 have been found, some of which appear to be associated with altered transporter functions, affecting the metabolism of drugs. Previous studies have identified 29 kinds of MDR1 SNP, including the G2677T SNP at exon 21, affecting the function of P-gp. Therefore an altered expression of the MDR1 may represent an additional prognostic marker. Multidrug resistance was in fact previously identified as an independent prognostic factor in AML and ALL patiens. In our study we evaluated MDR1 polymorphism in B-CLL patients and its relationship with other prognostic markers. 53 patients with B-CLL (25 men, 28 women), aged from 60 to 83 years (median 71) were included to the study. Each patient was described by following diagnostic tests: complete blood count, liver and renal function tests, immunoglobulins, LDH, 2microglobulin level, bone marrow aspiration, immunophenotyping of peripheral blood lymphocytes including CD38 positivity, IGVH gene analysis, determination of Zap-70 expression and sCD138 plasma levels. For MDR-1 genotyping, the genomic DNA was extracted from patients' peripheral blood using standard procedures. Two MDR-1 gene polymorphisms (G2677T in exon 21; G3435T in exon 26) were detected based on polymerase chain reaction (PCR), using primers amplifying a short fragment of DNA containing the polymorphic sites. In our patients the frequency of genotypes were as follows: for G2677T at exon 21, the GG genotype was detected in 11 patients (20.8%), GT in 21 (39.6%), TT in 21 (39.6%). For C3435T at exon 26, the CC genotype was detected in 10 patients (18.8%), CT in 30 (56.6%), and TT in 13 (24.6%). When comparing the prognostic patients' characteristics according to the MDR1 gene polymorphism, polymorphisms showed significant differences in the genotype distribution between the patients with high and low risk of progression.

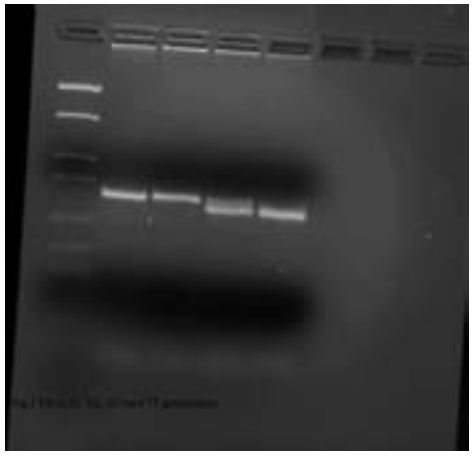

Figure.

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THE PROGNOSTIC POWER OF THE IN VITRO RESPONSE OF IGD LIGATION IN BINET STAGE A CRONIC LYMPHOCYTIC LEUKEMIA

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Background. sIgM cross-linking of CLL B-cells result primarily in cell apoptosis while cellular response to sIgD ligation induce mainly cell survival. However, there is no information whether response to BCR engagement of malignant B-cells has prognostic relevance. Aims. To investigate the *in vitro* response of CLL cells to sIgD cross-linking, whether this feature correlates with sIgD density and whether both characteristics have substantial clinical implications. *Methods.* Purified CLL B-cells from 106 untreated Binet A CLL patients were investigated. B-cells were exposed to goat antihuman delta-chain antibodies (adelta-Ab) at 10 µg/mL. Apoptosis levels induced by adelta-Ab were measured by annexin-V/propidium iodide staining and compared to levels of spontaneous apoptosis. Results. Based on the response to adelta -Ab, CLL cases could be subdivided into 3 groups. In the first group, we identified 65 cases (61.3%) with a null response (response to adelta -Ab ranged from <20% and >-20%, N); in the second, we found 33 cases (31.1%) in which B-cells were rescued from spontaneous apoptosis at least 20% above the spontaneous values (Inhibition, I); in the third, 8 (7.5%) cases showed an induction of apoptosis (Death, D) by adelta-Ab (response to apoptosis less than the 20% threshold). After a median follow-up of 4 years, the risk of treatment start was significantly higher for I and D cases (41 cases, H.R. 2.3, 95% C.I., 1.2-4.7, p=0.016) as compared with the remaining 65 N cases (H.R.=1). Of the 47 cases showing sIgD above the median value of 9, 24 responded in vitro to adelta-Ab, while conversely (14/56 of adelta-Ab responding cases had sIgD MFI<9. Clinically, those patients with sIgD MFI >9.0 showed a 2.6 folds higher risk (95% C.I. 1.3-5, p=0.008) to be treated compared with those showing a sIgD MFI<9 (H.R.=1). Response to adelta-Ab (I + D cases) still maintained their independent prognostic value in the multivariate model also in the presence of either CD38, ZAP-70, or IGHV. Similarly, sIgD MFI remained significant either in the presence of CD38, or of ZAP-70 expression, or of IGHV mutational status. *Conclusions.* Our data indicate that sIgD are capable of delivering signals to the cells in a large fraction of B-CLLs, a feature likely correlated with the sIgD density. These findings have substantial clinical implications, given the observation that samples responsive to surface IgD cross-linking *in vitro* display a tendency to progress more rapidly to stages requiring treatment.

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INTEGRATIVE GENOMIC APPROACH BASED ON SNP-ARRAY AND GENE EXPRESSION PROFILING REVEALS THE PRESENCE OF MOLECULARLY DISTINCT SUBGROUPS IN EARLY STAGE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL) is the most recurrent leukemia in the Western world, with a highly variable clinical course that reflects its heterogeneous genomic pattern. To characterize allelic imbalances and to define clusters of patients based on their genomic profiles, we applied SNP array technology (Affymetrix GeneChip® Human Mapping 250K Nsp) in a panel of 100 newly diagnosed, untreated B-CLL patients in early stage disease (Binet stage A). We performed an integrative approach between whole-genome and gene expression profiling data (Affymetrix GeneChip® HG-U133A) for 60 patients for whom RNA material was available. The non-negative matrix factorization (NMF) algorithm allowed the identification of four significant genomic clusters (correlation coefficient = 0.95), mainly driven by the major chromosomal alterations. Groups I and II, containing respectively 27 and 13 cases, were characterized by the presence of 13q14 deletion, stratified on the basis of the deletion size and the presence of biallelic deletion; group III consisted of all the 21 cases with trisomy 12; the remaining 39 patients, among which patients showing 11q (8 pts) and 17p (4 pts) deletions, were placed in group IV. A SAM multi-class analysis between the four groups identified sixty well-characterized differentially expressed genes, with a prevalent deregulation of genes located at 13q14 (8%) and on chromosome 12 (60%) (group I, II and III, respectively), whereas no specifically modulated genes were recognized in group IV. As regard the putative functional features of the deregulated genes, we found a significant involvement in transcription regulation (9/60=15%); regulation of apoptosis (6/60=10%), among which 3 genes mapping at chromosome 12 (DYRK2, TEGT and BTG1) and 1 at 13q14 (TPT1); glycolysis (3/60=5%) and negative regulation of cell cycle (3/60=5%), all located on chromosome 13 (TRIM13, RB1 and DLEU1) and already described by others as involved in B-CLL pathogenesis. The natural grouping of genome profiles reveals that the complex scenario of copy number alterations affecting B-CLL is mainly driven by the presence of deletion at 13q14 (groups I and II) and trisomy of chromosome 12 (group III). The prevalent deregulation of genes located in these regions suggests that the modulation of gene expression observed in the 4 groups could be mostly due to a gene dosage effect.

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GLOBAL SNP-BASED MAPPING OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA REVEALS THE ROLE OF 2P GAIN IN PREDICTING CLINICAL OUTCOME

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B-cell chronic lymphocytic leukemia (B-CLL) is a clinically heterogeneous disease characterized by recurrent common chromosomal lesions of prognostic significance. A panel of highly purified neoplastic cells from 100 untreated patients in Binet stage A was characterized by FISH for the most recurrent genomic aberrations and for the major prognostic markers. Trisomy 12 was identified in 21 cases; 13q14 deletion in 44 cases, 34 as the sole abnormality; 11q23, 17p13.1 and 6q23.3 deletions in 15, 7 and 2 cases, respectively. ZAP-70 and CD38 expression resulted positive in 42 and 46 cases, whereas IgVH genes were mutated in 45 cases. To provide insights into the genomic complexity of B-CLL, genome-wide profiling data were generated by means of Affymetrix GeneChip® Human Mapping 250K Nsp single nucleotide polymorphism (SNP) arrays. Copy number alterations (CNAs) were identified in all cases. A total of 782 aberrations (from 1 to 31 per sample, mean and median values 7.8 and 7, respectively) were detected; losses (365/782=46.7% loss; 194/782=24.8% biallelic deletion) were more frequent than gains (148/782=18.9% gain; 75/782=9.6% amplification). The most recurrent alterations detected by FISH were all confirmed by SNP array analysis. We identified a total of 18 minimally altered regions (MARs) larger than 100 kb with a frequency higher than 5%. Beside well known alterations, MARs included in particular gain of part if not the entire 2p arm (11 pts), and novel alterations such as gains of 4q35.2 (5 pts) and 11q25 (6 pts) and loss of 8q24.23 (6 pts). Deletions were shown at 14q32.33 (12 pts) and 22q11.2 (5 pts) involving the IgH and IgLlambda loci, respectively. In addition we found a high frequency of losses/gains at 14q11.2 (42 pts) and 15q11.2 (33 pts), two genomic regions reported to be affected by DNA copy number variations. The prognostic relevance of CNAs and MARs was evaluated as predictive of time to first treatment. Notably, 2p gain (HR 2.5, p=0.021) along with 17p loss (HR 3.4 p=0.03) was found to be an independent risk factor. Our data indicate that genetic abnormalities involving chromosomal CN changes are very common in early-stage B-CLL and further support the use of high resolution SNP arrays to investigate genomic changes in B-CLL. In addition we detected novel altered chromosomal regions that warrant future investigations to better define their pathogenetic role in B-CLL.

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QUANTITATIVE GLOBAL METHYLATION ANALYSIS OF DNA REPETITIVE SEQUENCES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA REVEALS A SIGNIFICANT HYPOMETHYLATION IN PATIENTS WITH 17P DELETION

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The identification of reliable prognostic factors useful in predicting patient outcome and planning therapeutic strategies is a crucial task to better define the clinical heterogeneity of B-cell chronic lymphocytic leukemia (B-CLL). Gene-specific hypermethylation and global hypomethylation have been previously reported in B-CLL. However, the relationship between aberrant global DNA methylation and clinical and biological risk factors remained unclear. Global hypomethylation of repetitive elements, such as long interspersed nuclear elements-1 (LINE-1), Alu and satellite α (SAT-α) DNA has been associated with chromosomal instability in cancer. We used a quantitative bisulfite-PCR pyrosequencing method to evaluate the methylation patterns of Alu, LINE-1, and SAT-α in highly purified (>90%) peripheral mononuclear $CD19^+$ cells from 7 healthy donors and 77 untreated B-CLLs in Binet stage A, and we correlated them with the major prognostic parameters and cytogenetic markers known to predict clinical outcome in B-CLL. Specifically, 33 B-CLLs carried the 13q14 deletion, 22 of which as the sole abnormality; biallelic 13q14 deletion was found in 6 cases. Both 11q23

and 17p13 deletions were detected in 12 patients, whereas trisomy 12 was found in 19 B-CLLs as a sole exclusive abnormality. ZAP-70 and CD38 expression resulted positive in 29 and 35 cases, respectively, whereas IgVH genes were found to be unmutated in 48 patients. B-CLLs showed a significant (p<0.001) methylation decrease of Alu (median 21.4% 5mC), LINE-1 (66.8 %5mC) and SAT-α (84 %5mC) compared with controls (25.9 %5mC, 85.7 %5mC, and 88.2 %5mC, respectively). Notably, Alu, LINE-1 and SAT-α methylation was significantly lower in 17p deleted B-CLLs (p<0.001). Additionally, a statistically significant association between the methylation levels of Alu and LINE-1 as well as between Alu and SAT-α and LINE-1 and SAT-α (Pearson's correlations coefficient rho=0.64, 0.78 and 0.66, respectively; p<0.001) was identified, while no statistical correlation between global DNA methylation level and IgVH mutation status, CD38 or ZAP-70 expression, was found. Our results extended previous evidence in global methylation in B-CLL and demonstrated a significant hypomethylation associated with high risk patients carrying the 17p deletion. Further studies on larger series would be useful to define whether global methylation levels may have a prognostic relevance in terms of B-CLL clinical outcome.

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INCIDENCE OF CYTOGENETIC ABNORMALITIES IN NEWLY DIAGNOSED BINET STAGE A B-CLL AND RELATIONSHIP WITH PROGNSOTIC BIOMARKERS: PRELIMINARY RESULTS ON 240 PATIENTS INCLUDED IN THE PROSPECTIVE, MULTICENTER O-CLL1 GISL STUDY

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CD38 and ZAP-70 expression, IgHV mutational status and genetic abnormalities have been integrated in clinical prognostic evaluation of B-CLL. We investigated the incidence of the known major cytogenetic alterations (+12 and 13q14, 17p13, 11q23 deletions) in a series of Binet A B-CLL patients included in the prospective multicenter O-CLL1 GISL trial. The study was performed by FISH in 240 out of 310 patients enrolled to date. At least one abnormality was found in 151/240 (62.9%) cases. The most frequent was del(13)(q14) (120/240, 50%), followed by +12 (30/240, 12.5%) (one case harboring 17p13 deletion), del(17)(p13) (5/240, 2%) and del(11)(q23) (11/240, 4%). 13q14 deletion was found as a sole abnormality in 110 patients; in the remaining cases, it was combined with +12 (2 pts) and 17p13 (2 pts) or 11q23 deletions (6 pts) deletion. Among patients with 13q14 deletions, 80 were monoallelic, 10 biallelic and 30 showed a combination of the two patterns. Biomarkers data were available in all of the patients. CD38 percentages were (mean value±sem) 8.2±1.7, 16.8±2.2, 55.9±6.3, 37.2±10.4, 31.1±10.6 for del(13)(q14), normal karyotype, +12, del(11)(q23) and del(17)(p13) alterations, respectively (p<0.0001). The percentages of IgVH mutations significantly correlated with cytogenetic alterations; namely, 5.6±0.3 for cases with del(13)(q14), 4.7±0.4 for normal karyotype, 2.1±0.6 in +12, 0.2±0.1 in del(11)(q23), and 2.1±1.3 in del(17)(p13) cases (p<0.0001). Similarly, a significant correlation was found for ZAP-70 expression: namely 30±1.9 for cases with del(13)(q14), 38.8±2.7 for normal karyotype, 50.2±4.8 for +12, 59.4±9.1 for del(11)(q22) and 35.7±7.5 for del(17)(p13) (p<0.0001). Finally, cytogenetic abnormalities were clustered in 3 risk groups [i.e. low del(13)(q14) and normal; intermediate (+12); and high risk del(11)(q23) and del(17)(p13)] and correlated with a scoring system in which cases were stratified in 4 different groups according to the absence (group 0) or presence of 1 (group 1), 2 (group 2) or 3 (group 3) biomarkers (Morabito et al., BJH, 2009, in press). Interestingly, 117/121 cases scoring 0, gathered in the low FISH group, whereas 12/15 high FISH risk cases clustered in scoring 2-3. Our results indicate that cytogenetic abnormalities predicting unfavorable prognosis show a relatively low incidence in newly diagnosed Binet stage A B-CLL patients and are significantly associated with negative prognostic biomarkers predictive of disease progression.

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DIAGNOSTIC POTENTIAL OF CD38 COMBINED WITH ZAP70 EXPRESSION IN PREDICTING MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION IN CHRONIC LYMPHOCYTIC LEUKEMIA: PRELIMINARY RESULTS OF A PROSPECTIVE, MULTICENTER O-CLL1- GISL STUDY

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Background. Ten years ago, two different groups reported the prognostic impact of the IGHV status in CLL. CD38 and ZAP70 were proposed as surrogate markers of this parameter; subsequent studies failed to confirm these data. Aims. To assess the diagnostic power of CD38 and ZAP70 expression alone or in combination in predicting IGHV status in Binet stage A patients included in the prospective multicenter O-CLL1 GISL study. *Methods.* 290 CLL patients were characterized for ZAP70 by western blot (ZAP70wb: ZAP70strong, ZAP70weak and ZAP70neg) and by flow cytometry (ZAP70flow), CD38 expression and IGHV gene status. *Results.* The AUC of ROC curves of CD38, ZAP70wb and ZAP70flow to predict IGHV status were significantly higher (p<0.0001)

than that of diagnostic indifference (0.82 for CD38, 0.83 for ZAP70wb and 0.85 for ZAP70flow). The diagnostic potential of CD38 (categorised according to the best cut-off=10%), ZAP70wb [categorized in negative (negative and weak expression) and positive (strong expression)] and ZAP70flow (best cut-off=35%) to predict IGHV status was as follows: sensitivity (85%, 81% and 72%, respectively), specificity (77%, 81% and 84%), positive predictive value (90%, 91% and 91%), negative predictive value (69%, 65% and 56%), accuracy (83%, 81% and 76%). Kappa statistic revealed that the agreement between IGHV status and CD38, ZAP70wb and ZAP70flow was of moderate degree (K=0.6, p<0.001; K=0.58, p<0.001; and K=0.49, p<0.001, respectively). We designed two models (CD38/ZAP70wb and CD38/ZAPflow), clustering cases into Group1 (CD38neg/ZAP70neg), Group2 (CD38pos/ZAP70neg), Group3 (CD38neg/ZAP70pos) and Group4 (CD38pos/ZAP70pos). Logistic regression analysis showed that the risk (odds ratios, OR) of being IGHV germline was equally high for both models in patients in Groups 2 and 3 (CD38/ZAP70flow, OR: 8.9 and 6.6, respectively; CD38/ZAP70wb: OR 8.0 and 8.5, respectively) achieving the highest value in patients in the Group4 (CD38/ZAP70flow, OR=112, P for trend <0.0001; CD38/ZAP70wb, OR=166, P for trend <0.0001). According to ZAP70wb, 143/150 (95%) cases belonging to Group1 were IGHVmutated, while 57/64 (89%) cases of Group4 were unmutated. Similarly, using ZAP70flow, 128/134 (95%) Group1 cases were mutated, while 58/69 (84%) Group4 cases were unmutated. Conclusions. The combination of CD38 and ZAP70 is of high diagnostic potential for distinguishing between IGHV mutated and unmutated patients.

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CLINICO-BIOLOGICAL CHARACTERIZATION OF A SUBSET OF VARIANT B-CLL, DEFINED ACCORDING TO A COMBINED CYTOFLUORIMETRIC/FISH DIAGNOSTIC APPROACH.

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Sixty three cases of variant B chronic lymphocytic leukemia (v-B-CLL), characterized by a intermediate CLL/mantle cell lymphoma immunophenotype, atypical cytomorphology in absence of t(11;14)(q13;q32) in FISH analysis were compared with a series of 130 B-CLL. The v-B-CLL were significantly different from the B-CLL in terms of: age <70 yrs (p<0.001), lymphocytosis <20x10^9/ (p<0.001), lymphocyte doubling time £ 12 months (p=0.02), high serum β2-microglobulin levels (p<0.001), and splenomegaly (p=0.002). CD38 and CD49d expression was significantly different between v-B-CLL and B-CLL (p<0.001) whereas, no statistical difference was observed for ZAP-70 reactivity. There were more patients mutated in the v-B-CLL group (80.0%) than in the B-CLL group (50%) (p=0.001). FISH analysis demonstrated that trisomy 12 was more frequent in v-B-CLL (p<0.001), while del13q14, considered as a single alteration, was more frequent in B-CLL (p=0.008). Gene expression profiling of a panel of 9 v-B-CLL compared with 60 B-CLL samples indicated that the variant group is characterized by an up-regulation of different genes involved in oncogenesis (TPD52, AFF1, GMPS, PICALM, JUN, REL, RAC2), regulation of apoptosis (IL-7, HSP90B1, NOTCH2, BECN1, ANXA4, MCL1) and involved in the I-kB kinase/NF-kB cascade of the canonical NF-kB signaling pathway (TRIM38, EEF1D, CASP1, MALT1, RHOH0). Among the genes found differentially expressed, CD1c, OSBPL3 and ITGA4 were upregulated. After a median follow-up of 55 months (range 4-196) and 60 months (range 6-180), 25/42 (59%) v-CLL and 55/93 (59 %) CLL pts were treated. Time to treatment was significant different between 2 groups when the IgVH mutational status was considered (p=0.006). Median OS of v-CLL subset was 112 months vs 171 months of CLL subset. When the IgVH mutational status was considered, mutated cases showed a worse OS even if a statistical difference was not observed (p=0.062). In conclusion, our study identifies a form of B cell leukemia that shows peculiar biological and clinical features and should not be misdiagnosed as a B-CLL. The inclusion of this form in B-CLL study could alter the interpretation of results, especially related to biological markers.

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AUTOIMMUNE HEMOLYTIC ANEMIA (AHA) IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) IN THE NEW PROGNOSTIC FACTORS ERA.

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Background. Autoimmune complications occur in up to a quarter of B-CLL patients and in this context AHA represents the most frequent manifestation. Aim of this study was to revise in the light of the new prognostic factors – mutational status of immunoglobulin heavy-chain variable region genes (IGVH), chromosome analysis by FISH and expression of CD38 and ZAP70 on leukemic cells- the risk of developing both a positive direct antiglobulin test (DAT) and AHA. Materials And Methods. In 104 B-CLL patients, 62 males with a mean age of 63 (SD ±11), 41 in Rai stage 0, 47 I/II and 16 III/IV, 60 in Binet stage A, 32 B and 12 C, the DAT test together with IgVH mutational status, CD38 and ZAP70 expression on leukemic cells were performed. FISH analysis was limited to 61 (58,6%) of the cases. Unfavourable prognostic markers were considered the expression on leukemic cells of CD38>30%, ZAP70 strong at Western Blot or > 30% at cytofluorimetric analyses, IgVH unmutated configuration and detection at FISH analysis of 17p- and 11q. Trisomy of chromosome 12 was evaluated at intermediate prognosis. High risk patients were considered those showing > 2 unfavourable factors. *Results.* Five out of 104 patients showed DAT positivity and 3 of them developed an overt AHA, chacterized by an unexpected deep fall in haemoglobin associated to a raised reticulocyte count and increase of unconiugated bilirubin and LDH levels. About the two patients with DAT positivity not developing AHA, both were in clinical stage A/0 and the DAT positivity was detected at diagnosis. Regarding the biological profile, one was classified at high risk because three worse prognostic factors – unmutated IgVH configuration, CD38 and ZAP70 positivity, the other at low risk because one adverse factor, although 17p-. However, none of them has developed AHA at moment, after a follow-up of 16 and 48 months respectively. About the three patients who underwent overt AHA, two were in stage A/0 and the other in II/B at diagnosis. At the beginning DAT was positive in patient in stage II/B and negative in both A/0. In one of A/0 cases DAT positivity and AHA occurred after 37 months from diagnosis in stable disease, while the other showed DAT positivity in progressive disease after 24 months from diagnosis. However, whether in this latter case or in patient in II/B stage the overt AHA developed during the treatment with Chlorambucil. On respect of biologic factors, all the three patients showed a mutated IgVH configuration, absence of worse cytogenetic alterations and only in one case the presence of two adverse risk factors, showing, after all, a favourable biologic profile. *Conclusion.* These data seem to suggest that the probability to develop AHA could concern patients with a low biological risk profile.

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POSITIVE DIRECT ANTIGLOBULIN TEST AND PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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The clinical course of pts with B-cell chronic lymphocytic leukemia (CLL) is often complicated by autoimmune phenomena which mainly target the blood cells. Autoimmune hemolytic anemia (AIHA) is the most common form, occurring in 10% to 25% of pts during disease course. The direct antiglobulin test (DAT) may be positive at some time during the course of the disease in up to 35% of cases, but overt AIHA occurs less frequently. Although AIHA may occur in asymptomatic untreated CLL, it is more common in pts with advanced-stage disease. So far it is unknown if also the biological characteristics of CLL have any influence on the risk of developing autoimmune phenomena. Recently an exceedingly high prevalence of un-mutated IgVH, with an excess of VH1 and VH3 families, has been described in pts with autoimmune thrombocytopenia. The aim of this retrospective study was to investigate the relation between incidence of positive DAT test and biological features of CLL pts. In our institution 143 pts with CLL were studied

with DAT at some time during the disease course. Characteristics of pts are summarized in the Table. DAT resulted positive in 39 pts (27%), overt AIHA occurred in 23% of them. Univariate analysis by Fisher's exact test was performed to establish the association between DAT result and the following variables: age, sex, Binet stage, LDH and β2 microglobulin (β2m) at diagnosis, IgVH status, VH families, ZAP70, CD38, FISH and overall survival (OS). A DAT-positive test was associated to higher B2m, higher LDH, IgVH un-mutated status, ZAP70 positivity and 6q21 monosomy at FISH analysis. In conclusion in our series test positivity correlated with poor risk biological features, however no difference was detected in terms of OS between pts with positive or negative test. Furthermore we could speculate that pts with un-mutated IgVH status are characterized by a peculiar antibody reactivity, resulting in a major failure controlling the emergence of auto-antibodies from normal B cells. Although the results of this study warrant further confirmation from larger series, to our knowledge this is the first report showing a correlation between DAT positivity, assuming a correlation of an increased risk of AIHA development, to biological features in CLL patients

Table.

	Positive DAT	*Negative DAT*
N° of pts	39	104
Age y median	67	67
M/F %	64/36	64/36
Binet stage %		
A	54	70
B	33	22
C	10	6
High LDH %	23	8
High B2m %	56	37
IgVH un-mutated %	51	22
ZAP70+ %	49	22
CD38+ %	36	35
FISH %		
17p13	23	25
6q21	18	4
11q22	31	16

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ZAP-70 MEAN FLUORESCENCE INTENSITY T/B RATIO BY FLOW CYTOMETRY IS A STRONG PREDICTOR OF CLINICAL OUTCOME IN B-CELL CHRONIC LYMPHOCITYC LEUKEMIA

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Background. ZAP-70 protein tyrosine kinase has been clearly demonstrated (Crespo, 2003; Del Principe, 2006) to be an independent prognostic factor in B-cell chronic lymphocytic leukemia (B-CLL), but a standardization of the cytometric protocols is still lacking. In clinical settings, analyses are usually performed evaluating the percentages of ZAP-70+ B-CLL cells either compared to isotypic control or to autologous T-cells (T-method). Since both methods suffer of an operator dependent variability, we suggest to evaluate ZAP-70 by using mean fluorescence intensity (MFI) ratio between gated T and B-CLL cells (T/B ratio method). *Aims.* The primary aims were: 1) to determine progression free survival (PFS) and overall survival (OS) upon ZAP-70 expression using T-method and T/B ratio method and 2) to evaluate the independent prognostic impact of ZAP-70, determined with both methods. P*atients and Methods.* We investigated 341 patients (pts), median age 65 years, 189 males and 152 females. With regard to modified Rai stages, 105 pts had a low stage, 227 an intermediate stage and 9 a high stage. ZAP-70 was quantified by multicolor flow cytometry using a cut-off value of 20% for T-method and a value of 3.0 for T/B ratio method. *Results.* ZAP-70$^+$ pts were 138/341 (40.5%) with T/B ratio method and 172/341 (50.4%) with T-method. Fifty (14.6%) pts were discordant: 42 pts were T-method positive and T/B ratio negative and 8 pts T-method negative and T/B ratio positive. There was a close correlation between ZAP-70 determined by T- or T/B method and Ig V gene mutational status (p<0.00001) in 229 examined CLL pts. With regard to clinical outcome, a shorter PFS was observed in ZAP-70$^+$ pts both with T-method or T/B ratio (13% vs 55% and 8% vs 54% at 12 years, respectively; p<0.00001). Equally, ZAP-70$^+$ pts either with T-method or T/B ratio showed a significant shorter OS (61% vs 94% and 58% vs 95% at 14 years, respectively; p<0.00001). The discordant pts showed an intermediate outcome (Figure). In a multivariate analysis of PFS, ZAP-70 resulted an independent risk factor, apart from the method employed for its evaluation; however ZAP-70 prognostic impact was higher when T/B ratio method was applied (hazard ratio = 8.5 vs 3.8 with T-method). *Conclusions.* We confirm the independent prognostic impact of ZAP-70 expression by flow cytometry in a large series of B-CLL pts suggesting to use MFI and T/B ratio method because of its operator and laboratory independent variability.

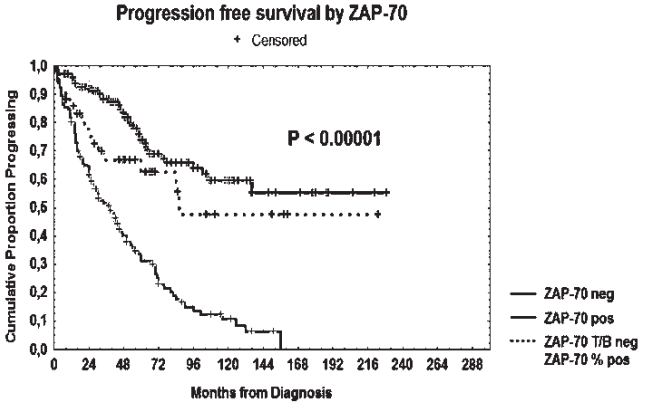

Figure.

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RELATIONSHIP BETWEEN EXPRESSION OF TLRS AND DISEASE ACTIVITY IN B-CLL

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Toll-like receptors (TLRs) are major agents of innate immunity and initiators of adaptive immunity, involved in the activation of normal B-lymphocytes. TLRs directly activate the immune system recognizing viral and microbial agents; moreover, they are involved in the self-antigen recognition and could play a role in autoimmune phenomena. It is well known that B-chronic lymphocytic leukemia (B-CLL) is characterized by an increased incidence of autoimmune phenomena and immunodeficiency, which can greatly influence the disease outcome leading to a variable clinical course. We evaluated the correlation between the gene expression of TLRs in 25 patients with B-CLL (mean age±SD 72±12 years, range 42-90, 10 female and 15 male), the clinical course of the disease and the expression of prognostic factors (mutational status of IgVH region, CD38 and ZAP70 expression, and cytogenetic alterations). The gene expression of TLR4, TLR9, and TLR10 was studied in B cell from B-CLL patients and controls. Total RNA was extracted from B-CLL patients and controls (pool of 10 healthy donors), cDNA was synthesized, and real-time PCR was performed. For each reaction 50 ng of cDNA were mixed with 1.25 microl of TaqMan primer/probe set and 10.25 microl of TaqMan Universal Master Mix. The TLR4, TLR9, and TLR10 expression was normalized according to GAPDH as an internal control gene and it was expressed as percentage of control. The results showed that TLR4 gene expression was lower in B-CLL patients compared to controls (23%±4.5%, mean±SE) while TLR9, and TLR10 gene expression was higher (4467%±773% and 2705%±387%, respectively). Moreover, considering "progressive" B-CLL patients (n=15), TLR4 gene expression was significantly lower compared with "indolent" B-CLL ones (n=10) (9.5%±2.7% vs 38.8%±7.9%, p<0.001). The 2 patients with autoimmune diseases (hemolytic anemia and Evan's syndrome) showed

no characteristic TLRs gene expression pattern. At present no clear relationship was found between TLRs expression and CD38/ZAP70 levels. Our findings showed that the reduced TLR4 gene expression, even more evident in "progressive" B-CLL patients, is consistent with the reduced ability to a proper immune response to Gram-negative bacterial lipopolysaccarides; the clinical significance (number, severity and outcome of infectious episodes and autoimmune phenomena) as well as the relationship with standard prognostic markers is under investigation in a larger number of patients.

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SINGLE-CELL PROFILES OF B-CELL RECEPTOR PHOSPHO-PROTEIN NETWORKS ARE ASSOCIATED WITH PROGNOSIS AND PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL) patients exhibit a variable clinical course. Several biological parameters have been shown to be associated with clinical outcome in CLL. Among them, the most reliable markers are represented by the absence of somatic mutations within the immunoglobulin variable heavy chain genes (IGHV), the expression of CD38 antigen, the presence of the ZAP-70 tyrosine kinase. These parameters of poor clinical outcome are structurally and/or functionally linked to B-cell Receptor (BCR) expressed by CLL cells, thereby strengthen the hypothesis that antigenic stimulation mediated by the BCR represents a driving event in the onset and progression of the malignant B cells. To investigate whether different BCR signaling networks may distinguish clinical-biological groups of CLL patients, we applied a "network level" view of BCR signaling by analyzing single-cell profiles of phospho-protein networks by flow cytometry. We evaluated the response to BCR engagement in primary cells isolated from 27 CLL patients by analyzing the phosphorylation states of 5 phospho-proteins on the route of BCR signaling, which included p-Syk, p-NF-kappaB, p-Erk1/2, p-p38 and p-JNK. BCR was cross-linked by incubating cells with anti-IgM antibodies. The unsupervised clustering analysis distinguished BCR response profiles of phospho-proteins that differentiated cases of CLL with mutated IGHV from those with unmutated IGHV (p=0.0003), cases with low levels of CD38 from those with high levels (p=0.0004) and cases with low levels of ZAP-70 from those with high levels (p=0.001). Furthermore, the same BCR response profiles were also associated with time to progression (p=0,0014) and with overall survival (p=0,049), as assessed by Kaplan–Meier curves and the log-rank test. This study shows that single-cell profiles of BCR phospho-protein networks are associated with prognostic parameters and disease progression in CLL.

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VALIDATION OF A NEW PROGNOSTIC INDEX IN PATIENTS WITH EARLY CHRONIC LYMPHOCYTIC LEUKEMIA: THE GIMEMA EXPERIENCE

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Oon behalf of the GIMEMA CLL Study Group

An observational chronic lymphocytic leukemia (CLL) database of the GIMEMA, including 310 patients with newly diagnosed Binet stage A cases who were observed at different primary Italian hematological centers during the period 1991 – 2000, has been used to evaluate the validity and reproducibility of a new prognostic index recently proposed by investigators at the M.D. Anderson Cancer Center. In this cohort, consisting of patients with early disease, only two risk categories could be identified using the prognostic index. The estimated median time to first treatment (TFT) was not reached by low-risk group patients, while it was 111 months for patients belonging to the intermediate risk category (p=0.003). The prognostic index remained a strong predictor of TFT when the analysis was limited to Rai stage 0 (p<0.0001). The original prognostic index was derived from a database of patients observed at a reference academic center; thus, the patient population was characterized by more advanced disease status (p<0.0001) in comparison to ours. With this in mind, we used an optimal cut-off search to determine how to best dissect Binet stage A patients in different prognostic groups. According to recursive partitioning (RPART), a classification tree was built that identified three subsets of patients who scored respectively: 0-2 (low risk); 3-4 (intermediate risk); 5-7 (high risk). The probability of remaining free from therapy was 100% at 5-years in the low risk group, 81.2% in the intermediate risk group and 61.3% in the high risk group (p<0.0001). The results of this study extend the utility of a prognostic index in a cohort of non–selected patients in early stage CLL with horizontal long- term observational follow-up from first diagnosis that is representative of the natural course of the disease. Furthermore, we demonstrated that the score retains its prognostic value when applied to Rai stage 0 patients and is effective to predict TFT.

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THE SURVIVAL OF TUMOR CELLS IN IGVH UNMUTATED CHRONIC LYMPHOCYTIC LEUKEMIA IS HIGHLY DEPENDENT ON EXOGENOUS SIGNALS DELIVERED BY THE TUMOR MICROENVIRONMENT

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Chronic lymphocytic leukemia (CLL) is a clinically heterogenous disease. A very reliable prognosticator is the mutational status of the tumor immunoglobulin heavy chain variable region (IgVH): patients with unmutated (UM) IgVH have a worse prognosis than patients with mutated (M) IgVH. It has already been reported that soluble factors (i.e. IL-4 and CD40L) and cellular components of the local microenvironment [i.e. bone marrow stromal cells (BMSC) and peripheral blood T cells (PBT)] are important survival factors for CLL B cells. It is currently unknown to what extent UM and M CLL cells depend on the local microenvironment for their survival. We have evaluated the spontaneous apoptotic rate of tumor cells isolated from UM and M CLL patients. Leukemic cells purified from the peripheral blood of UM CLL patients showed a significantly higher apoptotic rate than leukemic cells of M patients. Both M and UM CLL cells showed high level of expression of Bcl-2 and NF-kB soon after purification. In vitro spontaneous apoptosis of UM B-CLL cells was associated with a progressive downregulation of the intracellular expression of Bcl2 and with a complete loss of the active nuclear form of NF-kB. On the contrary, the higher long term viability of M CLL cells was paralleled by the maintenance of Bcl2 and NF-kB expression. We next investigated whether the enhanced pro-apoptotic tendency of UM CLL cells could be reverted by extrinsic survival factors. We found that IL-4 and CD40L, used alone or in combination, as well as murine and human BMSC were capable of rescuing UM tumor cells from apoptosis. The pro-survival effect of these stimuli was exerted through the upregulation of Bcl-2 and was totally independent from the recovery of NF-kB expression. A pro-survival effect on UM CLL cells was also exerted by autologous T cells. Indeed, the cocolture of B and T cells at different ratios showed that the presence of sufficient numbers of viable T cells could prevent UM CLL cells apoptotic death by restoring the expression of the nuclear form of NF-kB and of Bcl2. These data indicate that the survival of UM tumor cells is dependent on the local microenvironment. Conversly, M tumor cells are intrinsically more resistant to apoptosis and minimally influenced by the local microenviroment. The higher dependency of UM CLL cells from extrinsic signals might be exploited to develop new therapies targeting the tumor microenvironment and to improve the outcome of more aggressive CLL.

Myelodysplastic Syndromes

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JAK2 V617F/G1849T MUTATION AND BCR/ABL REARRANGEMENT IN PATIENTS WITH SUSPECTED MYELOPROLIFERATIVE DISORDER: MUTASCREEN AND REARRANGEMENT ANALISYS WITH ALLELIC DISCRIMINATION STUDY

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Introduction. Myeloproliferative disorder is clonal disorder of haematopoietic progenitors. Mutation in the JAK2 gene encoding a cytoplasm Tyrosine Kinase in most common disease (polycythaemia Vera, essential thrombocythaemia or primary myelofibrosis). Specific tumor sequences as mutations are utilized in diagnosis, prognosis and follow-up. In positive V617F/G1849T case with homozygosis status were reported a short overall and increase of disease. *Objective.* JAK2 V617F/G1849T molecular mutation and allelic discrimination were studied in Myeloproliferative Patients suspected disorder for diagnosis and prognosis. BCR/ABL rearrangements were determinate for chronic myeloid leukemia exclusion. The understanding of pathology process in all myeloproliferative disease can open the way at development of new target therapy. *Materials and Methods*. We have analyzed 213 cases; of Myeloproliferative Patients suspected from Cancer Hospital "A.Businco" Asl 8 Cagliari. This study included 4 (2%) patients with suspected polycythemia Vera, 2 (0.9%) patients with suspected Thrombocythemia and 1 (0.5%) with idiopathic myelofibrosis and 1 case with polycythemia Vera in pre BMT status for the next follow-up study. Other 174 (81.6%) case were at first diagnosis for suspected myeloproliferative disease. Individual (n= 213) who had been for clinical suspected or Myeloproliferative diagnosed disorder to the haematology department were enrolled in we study. All cases BCR/ABL rearrangements were evaluated with RT-PCR procedure. JAK2 V617F/G1849T molecular mutation was performed with RT-PCR procedure and quantitative JAK2 V617F/G1849T determination by taq-man. Only on JAK2 mutation positive case was determined allelic discrimination by ABI Prism System with standardized procedure. *Results and conclusions*. In 213 cases enrolled, 3 were positive for BCR/ABL rearrangements. 74 (25%) of the 213 cases observed were positive for JAK2 V617F/G1849T in RT-PCR procedure and quantitative determination of JAK2 V617F/G1849T by taq-man. Five (1.7%) cases were homozygosis positive with allelic discrimination assay and 69 (23.5%) cases were heterozygosis positive Both, specific RT-PCR procedure and new diagnostic method by taq-man in allelic discrimination by ABI prism System may provide correct evaluation of JAK2 V617F/G1849T derivates. Molecular mutation for diagnosis, follow –up will be utilized in the next develop of new target therapy. This study will be continued with search of others specific mutation in negative JAK2 V617F/G1849T case.

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COMORBIDITIES AT DIAGNOSIS INFLUENCE OUTCOME IN MYELODYSPLASTIC SYNDROMES

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Recent findings suggest that proper assessment of comorbidities is useful to predict the outcome of MDS patients receiving allogeneic transplantation. However, the results obtained in this highly selected subset of patients cannot be applied to the whole MDS population. We evaluated the impact of comorbidities in 418 consecutive patients diagnosed at our Institute from 1992 to 2005. All patients were classified according to WHO criteria and all patients received only conservative and supportive treatment. One or more comorbidities were evaluated in 390 patients (93%) at the time of diagnosis: we found a higher prevalence of comorbidities in older patients. Cardiac diseases were the most frequent comorbidities (30%) and diabetes and correlated adverse events were the second cause of comorbidity (20%). We applied 3 comorbidity prognostic scores (CCI, HCT-CI and a score proposed by Della Porta et al). According to CCI score, 253 patients had a score 0, 111 patients had a score 1 and 54 patients had a score> 2. According to HCT-CI, 209 patients had a score 0, 105 patients a score 1 and 106 patients a score > 2. With Della Porta et al score, 288 patients had a score 0 and 129 patients a score > 1. We found a significant correlation between survival and stratification according to CCI and Della Porta et al scores (respectively, p=0.01 and 0.02), but not according to HCT-CI score. Development of RBC transfusion-dependency was directly correlated to more comorbidities stratified according to CCI and was associated to a significantly higher risk of death, not related to leukemic evolution (HR=2.12 p=<.001). The development of transfusional requirement according to HCT-CI and Della Porta et al scores did not correlate with a significantly higher risk of non-leukemic death (respectively p=0.3 and 0.43). As suggested by Della Porta et al, also in our experience the onset of cardiac, liver, renal, pulmonary diseases and solid tumors was found to independently affect the risk of death in a multivariable Cox regression (p values from <0.01 to 0.004). In conclusion, the assessment of comorbidities at diagnosis in MDS patients may improve the ability of therapeutic decisions.

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EFFICACY OF DEFERASIROX (EXJADE) IN MYELODYSPLASTIC SYNDROMES AND PRIMARY MYELOFIBROSIS PATIENTS: REPORT OF 5 CASES WITH HEMATOLOGICAL IMPROVEMENT

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Many patients with myelodysplastic syndromes (MDS) and primary myelofibrosis (PMF) are susceptible to iron overload from ongoing blood transfusions. Deferasirox is a once-daily oral iron chelator, which has shown efficacy in maintaining or reducing body iron assessed by serum ferritin (SF) in both types of patients. We here describe a single institution experience on 24 patients treated with Deferasirox at the dose of 10-30 mg/kg/day according to Consensus Guidelines on Iron Chelation Therapy (ICT) of 2007. SF was assessed monthly and safety assessment included monitoring of adverse events during treatment and of liver and renal parameters. Twenty-three patients (17 with MDS and 7 with PMF), with median SF of 2,438 ng/dl at baseline were treated with Deferasirox. Of the 17 patients with MDS, 2 had RAEB-1, 1 had CMML, 12 had RA and 2 del (5q) syndrome. Median transfusion duration period was 3.2 years, with a median transfusional requirement of 3 RBC units/month and a median of RBC units received of 48; all patients were pre-treated with deferoxamine. At a median follow-up of 9 months of treatment, there was a significant reduction in median SF the value being 1.600 ng/dL (p=0.001). Overall, 20 % of the patients suspended the drug for adverse events. Most common drug-related adverse events were diarrhoea (4 patients, 80%), nausea (2 patients, 40%). Three patients had increased serum creatinine values > 33% above baseline, but there were no progressive increases. We observed haematological improvement with increased haemoglobin level and decreased transfusional requirement in 5 patients (2 RA, 1 RAEB and 2 PMF). In these patients mean Hb value increased from 8.5 gr/dl to 10.5 gr/dl, with a mean Hb improvement of 2 gr/dl (p=0.02); mean transfusional requirement decreased from 5 RBC units/month to 1 RBC unit/month. The degree of the haematological response was not affected by median SF level decrease (from 3010 ng/dL to 2899 ng/dL): median SF was stable in 1 patient, decreased in 2 patients and increased in the other 2 patients. In conclusion, a possible effect of the iron chelator on the neoplastic clone or on bone marrow microenvironment has to be considered in patients who improve their Hb level during therapy. Future large trials to prove this effect are warranted.

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HIGH RATE OF MAJOR ERYTHROID RESPONSES IN MYELODYSPLASTIC SYNDROMES TREATED WITH WEEKLY EPOETIN β

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Once weekly dosing of epoetin β in patients with myelodysplastic syndromes has not been investigated and ASCO/ASH recommenda-

tions are based on randomized controlled trials with epoetin α. We describe here the use of weekly epoetin β in newly diagnosed MDS patients. Seventy consecutive patients with MDS were treated with epoetin β given subcutaneously at the dosage of 30.000 IU/week for a minimum period of 3 months. Median age was 76 years (range 51-83): there were 39 males and 31 females. According to WHO criteria, 22 patients were classified as pure refractory anemia (RA) whereas 17 patients received appropriate classification as refractory cytopenia with multilineage dysplasia (RCMD). Seven patients were classified as having refractory anemia with ringed sideroblasts (RARS) and 2 as refractory cytopenia with multilineage dysplasia with ringed sideroblast (RCMD-RS); 21 patients were classified as refractory anemia with excess of blasts type 1 (RAEB-I). According to IPSS stratification, 39 patients were low risk, 26 intermediate-1 and 5 patients intermediate-2 risk. Clinically significant responses were seen in 51/70 (72.8%) patients, with 35 major and 16 minor erythroid responses. All the 15 patients with transfusional requirement who obtained major response did eliminate the transfusion need. Treatment was generally well tolerated and no side effects were recorded. From univariate analysis, significant prognostic factors associated to response were female sex, category of pure refractory anemia according to WHO classification, low median duration of MDS phase before starting EPO, low risk according to IPSS stratification. Our preliminary data provide encouraging results regarding the benefits of once weekly epoetin β in low risk MDS patients.

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NEGATIVE PROGNOSTIC FACTORS FOR SURVIVAL IN TRILINEAGE DYSPLASIA MYELODYSPLASTIC SYNDROMES ACCORDING TO WHO CRITERIA

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In 1999, the WHO proposed the first revised classification of MDS: low risk MDS were defined according to the presence of uni- versus multilineage dysplasia; however the exact role of trilinear dysplasia remained to be fully clarified. We retrospectively analyzed the outcome of 358 patients with trilineage dysplasia diagnosed from 1992 to 2006. Of these, 134 patients evolved to acute leukaemia in a median time of 11 months. We analyzed the presenting features of patients who did evolve and of those who did not, reclassified as per WHO criteria, with the exclusion of RAEB-t and CMML patients. Between the two groups no differences were revealed as to age (67 years in evolved patients vs 68 in patients with no evolution, p= ns), male/female ratio (1.6 vs 1.58, p=ns), median WBC count (3.9x10^9/L in both groups), median Hb level (9.5 vs 10 gr/dl, p=ns), median platelet count (100 vs 128x10^9/L, p=ns). Differences were noted instead in peripheral and bone marrow blast cell percentage (4% vs 0% and 15% vs 7%, p=0.01), in the rate of RAEB patients (76% vs 64%, p=0.03) and in cytogenetic alterations (normal karyotype 15% vs 30%, p=0.05; monosomy 7, 5% vs 0%, p=0.03; trisomy 8, 3% vs 11%, p=0.02). Similar percentage of patients in both groups developed infections (42% vs 44%, p=ns); a difference was noted in hemorrhagic symptoms (23% vs 13%, p=0.04). Stratification of patients according to clinical prognostic scoring systems (Bournemouth and Spanish) showed a higher incidence of intermediate/high risk in patients who evolved versus those who did not (p=0.001); stratification according to WPSS, that evaluates also the transfusional requirement, showed a higher percentage of patients with intermediate/high risk among those who evolved to AML. In conclusion, trilinear dysplasia is not per se a negative prognostic factor as it adversely affects prognosis only when is associated at diagnosis to signs established as of disease progression.

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REVISED WHO 2008 CLASSIFICATION OF PATIENTS WITH REFRACTORY CYTOPENIA WITH UNILINEAGE DYSPLASIA (RCUD)

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Dysplasia >or=10% in one bone marrow lineage and one cytopenia constitute the low-risk myelodysplastic syndromes (MDS) category UCUD or Unilineage Cytopenia and Unilineage Dysplasia in the WHO 2008. We retrospectively reclassified 126 patients with these features at diagnosis: 79 as refractory anemia (RA), 23 as refractory neutropenia (RN), 24 as refractory thrombocytopenia (RT). We did not find differences as regards sex, age, Hb level, WBC count, and karyotype between the 3 groups. Low PMN count (0.8x10^9/L) was observed in the RN category and low platelet count in the RT category (51x10^9/L). We did observe different transfusion requirement in RT category (45.8%) compared to RA (62%) and RN group (69%) (p=0.05); less infections in RT category (20.8%) compared to RA (32%) and RN categories (43%) (p=0.03); more haemorrhagic symptoms in RT category (41.6%) and RN category (26%) compared to RA group (5%) (p=0.001). Application of different scoring systems revealed a lower incidence of patients at low risk in RT category compared to RA and RN, thus explaining the lower overall survival observed in the former group. In fact, RT category had an overall survival of 15.9 months compared to 48.2 months of RA and 35.9 months of RN category (p=0.001). Seven out of 79 (8%) RA patients evolved into AML, compared to 4/23 (17%) RN and 1/24 (4%) RT patients, with progression developing in a median time of 89, 33.8 and 12.8 months, respectively (p=0.03). In conclusion, in our study the revised WHO 2008 classification confirmed the importance of separating patients with unilineage dysplasia for prognostic assessment, while suggesting that RT category has a worse prognosis.

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WPSS VERSUS SIMPLIFIED MYELODYSPLASTIC SYNDROME RISK SCORE: WHICH IS THE BEST TOOL FOR PREDICTION OF SURVIVAL?

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WPSS was based on the prognostic role of transfusion requirement in MDS patients. We applied this system to 411/650 patients by excluding those without available karyotype, or FAB RAEB-t and CMML. Patients were stratified into five risk groups: very low (32 patients), low (76 patients), intermediate (86 patients), high (187 patients) and very high (30 patients). We found statistically significant correlations between risk category and haemorrhagic symptoms (p=0.034), platelet count (p=0.010), neutrophil count (p=0.0001), trilinear dysplasia (p=0.0001), infections at presentation (p=0.006). WPSS also showed an impact on prediction of leukemic evolution (p=0.001) and survival (p=0.0001). Overall survival ranged from 57 months for patients with very low risk to 23 months for patients with very high-risk score (p=0.001). We also tested the new score proposed by Kantarjian et al, which considered poor performance status, older age, thrombocytopenia (<30, 30-49, 50-199 x 109/l), anemia (< 12 gr/dl), increased bone marrow blasts (5-10%, 11-29%), leukocytosis, chromosome 7 or complex (>/=3) abnormalities, and prior transfusions, as prognostic factors. We applied the score in 569 patients including CMML and secondary MDS. We identified four risk groups: low risk (estimated median survival 44.9 months, 3-year survival rate 61%), intermediate 1 (estimated median survival 33 months, 3-year survival rate 34%), intermediate 2 (estimated median survival 20 months, 3-year survival rate 15%), high-risk (estimated median survival 9 months, 3-year survival rate 5%). We also found a significant correlation between this score and probability of evolution: 20.8% in low, 30% in intermediate 1, 50% in intermediate 2 and 59% in high risk (p=0.0001). In conclusion, our study confirmed the importance of including transfusion requirement in the prognostic disease assessment, and also confirmed that the new simplified score proposed by Kantarjian *et al.*, is applicable to all patients, not only to those with untreated primary MDS.

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WT1 AND CXCR4 EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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WT1 is highly expressed in most acute leukemias, and its level of expression is associated with the presence, persistence, or reappearance of leukemic hematopoiesis. Stromal cell-derived factor-1 (SDF-1) is a homeostatic chemokine that is constitutively secreted by marrow stromal cells. SDF-1 signals through CXCR4, which plays an important role in hematopoiesis, development and organization of the immune system. Prognostic impact of CXCR4 expression levels on the neoplastic cells has been demonstrated in breast cancer, renal cell cancer and AML. We investigated WT1 gene expression and its association with the expression of the chemokine receptor CXCR4 on bone marrow $CD34^+$ cells of MDS patients. BM samples from 46 MDS patients (according to WHO classification: 20 RA, 10 RAEB I, 5 RAEB II, 4 RARS, 4 deletion of 5q, 3 MDS unclass) were tested for WT1 expression at diagnosis and every 6 months. WT1 gene expression was evaluated by methods of real-time quantitative PCR (RQ-PCR). Surface CXCR4 expression were measured flow cytometrically. At diagnosis, 29 BM samples (12 RA, 10 RAEB I, 5 RAEB II, 1 RARS, 1 MDS unclass) expressed WT1 transcript amounts greater than the ranges level. The degree of WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. Moreover, a significant correlation was found between WT1 expression levels, blast cell percentage and CXCR4 over-expression on blast cells (as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5). The patients received only a supportive therapy if necessary. After 6 months, 11 patients (2 RA, 5 RAEB I, 4 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 and CXCR4 expression and a further elevation of WT1 expression level after 6 months. Our data show that in most MDS, including a large percentage of RA and almost the total number of RAEB I and II, WT1 is expressed above the range observed in normal controls in BM and that its expression is directly correlated with the type of MDS. A strong association is present between the level of WT1 expression and the blast percentage and the CXCR4 over-expression. Our results justify further investigation into the role of CXCR4 in MDS and suggest that WT1 and CXCR4 should be incorporated into the risk assessment of MDS patients.

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PLATELET COUNT IS AN INDEPENDENT PROGNOSTIC FACTOR IN MYELODYSPLASTIC SYNDROMES CONSIDERED AT LOW RISK BY FAB AND WHO CLASSIFICATIONS

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Bowles *et al.* in 2006 reported on the importance of platelet mass (PM, determined as mean platelet volume (MPV) x platelet count) as an independent prognostic information for survival in myelodysplastic syndromes: by evaluating 58 patients, median survival was 5 months for subjects with PM < 0.6 ml/L, >82 months for patients with PM > 1.2 ml/L and 30 months for patients with PM intermediate values. We reviewed 536 patients diagnosed as having MDS according to FAB and WHO classifications at our Institute in a period between July 1983 and December 2000. Data regarding MPV were recorded from 1995 and were available for 123 patients. In our series the platelet count independently predicted survival with both classifications, when considered in several ways: as per IPSS definition (< 100×10^9/L versus higher values), by using the FAB classification a median survival of 31 months was observed in patients with platelets >100×10^9/L versus a median survival of 16.2 months in patients with platelets less than 100×10^9/L (p=0.001). When applied the WHO classification, median survival was 34 months for patients with platelets >100×10^9/L versus 14 months for patients with platelets less than 100×10^9/L (p=0.0001). When dividing patients in three groups depending on whether platelet count was below, within or above the normal range (<150, 150-450, >450×10^9/L), we found a difference only for RA and RARS according to FAB classification and for RA, RCMD, RARS, RCMD-RS and isolated 5q- syndrome, but not for RAEB, according to WHO classification. As regards disease evolution, as per IPSS definition (<100×10^9/Lversus higher values) we did not find a significant prognostic correlation: it was 40% in patients with platelets < 100 x 109/l versus 25% in patients with platelets > 100×10^9/L (p=0.058). The sub-division of platelet count in three categories (<150, 150-450, >450×10^9/L) identified 36.7% of leukemic evolution in patients with platelet count < 150 vs 22.8% and 22% (p=0.01) in the second and third group, respectively. Considering MPV value in our series of 123 patients with available data, we identified 2 categories of subjects according to a dichotomous separation at 8.5 fL: we did not find a correlation with survival, differently from that reported by Bowles et al. In conclusion, platelet count is prognostically important and may represent an independent factor especially for MDS usually considered at low risk; in our series of MDS patients, as also suggested by Palmer et al and by Germing et al, the MPV value did not add significant information.

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FAMILIAR OCCURRENCE OF MYELODYSPLASTIC SYDROME WITH DEL(5Q)

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Myelodysplastic Syndromes (MDS) are a heterogeneous group of disorders which have very rarely been reported to occur in the same family. We herein describe 2 sisters affected by MDS with the same karyotypic abnormality consisting in a partial deletion of chromosome 5 long arm, del(5q). The 1st patient was diagnosed as having RA (WHO) with Int-1 IPSS score (marrow blasts 2%, anemia and neutropenia) in 7/1996 when aged 61 years; at first bone marrow (BM) examination karyotype was normal. From diagnosis to 3/2004 the patient received only treatment with folinic acid; cytogenetic analysis showed again a normal karyotype in 1/2004. From 4/2004 to 8/2004 the patient received high-dose rHuEPO (80,000 IU weekly) without clinical improvement; from 3/2005 she started to need transfusional support of about 3 red packed cell units monthly. In 6/2006 a new BM aspirate showed a stable morphological picture, but karyotype was 46, XX, del(5)(q13;q31) in 70% of metaphases. A progressive pancytopenia developed since 8/2006, with worsening general conditions and increase of transfusional requirement; the patient died in 12/2007 from broncopneumonia while in pancytopenia without signs of progression to acute myelogenous leukaemia (AML). The 2nd patient was diagnosed as having RAEB-II (WHO) with high-risk IPSS score (marrow blasts 18%, anaemia and neutropenia) in 1/2008 when aged 77.5 years; karyotype was 46 ,XX, del(5)(q13;q31) (7 cells), 46, XX, idem, -6, + mar (5 cells) and 46, XX (9 cells). She started to have transfusional requirement in 5/2008 and rapidly evolved into AML in 7/2008; due to a concomitant broncopneumonia with poor performance status, she received a conservative approach only, with both antibiotics and hydroxyurea, but died in 9/2008 from disease progression. Familiar anamnesis did not reveal any toxic exposure in both sisters; however, it is worth of note that their parents were cousins. To our best knowledge, this is the 1st report on a familiar occurrence of MDS with del(5q). The lack of a common anamnestic exposure to environmental mutagenic agents lead us to speculate on a common genetic background of instability predisposing to develop abnormalities of chromosome 5 at the same region involved in sporadic acquired MDS cases.

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REFRACTORY ANEMIA WITH ANTI-ERYTHROBLAST AUTOIMMUNITY: INCREASED EXPRESSION OF BAX AND EPO-R

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Anti-erythroblast autoimmunity has been demonstrated in bone marrow (BM) aspirates of roughly half patients with refractory anemia (RA) by mitogen-stimulated-direct antiglobulin test (MS-DAT), together with increased erythroblast counts and signs of hemolysis. This autoimmu-

nity, rather than being cytopathic, was found to induce a hyperplastic and diserythropoietic growth in vitro of BM progenitors. The aims were to study erythropoietin levels (EPO) and the expression of EPO receptor (EPO-R), and to investigate the level of the pro-apoptotic marker Bax and the anti-apoptotic marker Bcl2 in BM cultures from MS-DAT positive and negative patients. We investigated 51 patients with RA. MS-DAT was performed by stimulating BM with PMA and PHA; antibodies were detected in culture supernatants by competitive solid phase ELISA. The EPO levels and EPO-R expression were evaluated by ELISA. The apoptotic markers Bax (pro-apoptotic) and Bcl2 (anti-apoptotic) were evaluated by ELISA in total cell lysates. Twenty-nine out of 51 (57%) patients displayed positive MS-DAT in BM. BM-MS-DAT positive patients showed reduced EPO levels (13.8±3.8 versus 30.3±8.2 mUI/ml, mean+/-SE) and significantly increased EPO-R expression (45.6±9.5 versus 19.7±3.3 pg/mL, p=0.05), compared with MS-DAT negative ones. The level of the pro-apoptotic marker Bax was significantly higher in MS-DAT positive versus negative patients (66.9±14.1 versus 21.3+/-3.3 pg/mL, p=0.03), and consistently, Bcl2 level was lower, although not significantly (10.9±2.4 versus 15±3.2 pg/mL). Our results showed that RA patients with anti-erythroblast autoimmunity displayed reduced EPO levels, increased apoptosis and expression of EPO-R. These findings suggest that antibodies may have a "stimulating" effect on EPO-R inducing erythroblastosis and ultimately leading to an increased apoptosis.

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APPLICATION OF WPSS FOR THE PROGNOSTIC STRATIFICATION IN A SINGLE-CENTRE COHORT OF MDS PATIENTS TREATED WITH INTENSIVE CHEMOTHERAPY

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The WHO classification-based prognostic scoring system (WPSS) is widely accepted in patients with myelodysplastic syndromes (MDS). A recent retrospective study by GITMO confirmed it as a prognostic indicator in the subgroup of MDS patients undergoing allogeneic stem cell transplant, in particular in terms of post-transplant relapse-free survival (RFS; Alessandrino *et al*., Blood 2008). Given its time-dependency, this prognostic score could be similarly calculated at time of initiating other therapies. We retrospectively evaluated the influence of WPSS in a small cohort of MDS patients, not eligible to allogeneic stem cell transplant, treated with intensive chemotherapy at our institution. Fifteen patients (9 m; 6 f) were treated with intensive chemotherapy at our institution between 1999 and 2009. Mean age was 60.5 y (range 51-70). WHO subgroups were: RA (1 pt), RCMD (1 pt), RAEB I (6 pts), RAEB II (7 pts). No LMMC or RAEB-t according to FAB was included. Nine pts with int-2 or high risk MDS according to IPSS were treated at diagnosis, the others after progression. First line course was FLAG (Fludarabine 25 milligrams/squaremeter/day for 5 days, ARA-C 2 grams/squaremeter/day for 5 days, G-CSF) in 13 pts, Idarubicine plus ARA-C in 1 pt, Topotecan plus ARA-C in 1 pt. At least one high dose ARA-C based consolidation course was administered to 7 of 9 patients in CR after one /two cycles; the others were excluded due to poor clinical condition. High dose Busulphan intensification followed by autologous stem cell rescue was administered in 2 pts. WPSS was calculated at time of starting therapy. Complete remission (CR) was obtained in nine patients; partial remission (PR) in 3, while no response was observed in 3 (NR). All patients in PR received a second line therapy and two of them had a CR at that time, while the third had disease progression. Two NR patients underwent a salvage course, both with no response. Overall CR rate was 73.3%. No treatment-related mortality was observed. Mean RFS was 17.8 m, while mean OS was 40 m. According to age, RFS rate was 20.6 and 6.7 m for patients aged <65y and >65y, respectively. RFS was 25.5 and 13.8 m for good and intermediate-high risk cytogenetics, respectively. Only pts on intermediate, high and very high WPSS were treated, with a RFS of 14.1, 22.5, 10.5 m respectively. In our small series chemotherapy alone shows a good CR rate, but with short-term response. RFS was influenced by age and cytogenetics, but not by WPSS.

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IMPACT OF PROGNOSTIC SCORING SYSTEMS AND OTHER DISEASE PARAMETERS ON RESPONSE TO ERYTHROPOIETIN IN *DE NOVO* MDS

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IPSS score is a good predictor even for erythroid response to Erythropoietin (Epo) in MDS patients. A new prognostic score (WHO related prognostic scoring system, WPSS) and new prognostic factors as ferritin and LDH (*Sanz et al, Kantarjian et al; ASH 2008*) , emerged in recent studies, could contribute to response rates definition. We retrospectively reported overall response to Epo in a series of low and int-1 IPSS risk MDS; we correlated erythroid response to recently proposed prognostic factors. Forty-two low or int-1 risk MDS pts were treated with Epo at our institution between February 2001 and March 2009 (M=23; F=19). Median age was 73 y (range 30-83); 30 RA+RARS, 9 RCMD+RCMD-RS, 2 RAEB-1, 1 MDS-Unclassified according to WHO were reported. IPSS was low in 29 pts and int-1 in 13; WPSS was very low in 21 pts, low in 12, intermediate in 8, high in 1. Bone marrow blast percentage was <5 in 39 pts, 5-10 in 3 pts. Cytogenetics was good in 37 cases, intermediate in 4, poor in 1. At time of therapy initiation, 21 pts were transfusion dependent (mean transfusional demand = 2.1 erythroid units/m). Mean reticolocytes percentage on peripheral blood was 2.3 (range 0-7.1); mean LDH was 410.9 international units/liter (range 178-1660); mean serum erythropoietin was 133.3 milli-international units/microliter (range 3.8-841). Among transfusion-free patients, mean ferritin level was 406.5 nanograms/milliliter (range 21-1681). IWG 2006 response criteria defined erythroid response. Each pt received initially 30000 international units weekly of Epo β, or 40000 weekly of Epo Alfa. Statistical analysis was performed using a linear model with ordinal logit link function. Thirty pts (71.4%) achieved an erythroid response to Epo: 21 had a maximum gain in hemoglobin >1.5 grams/deciliter, 9 had it <1.5. Among previously transfused pts, 9/21 had a demand reduction of more than 50%, 2 had it inferior to 50%. Mean time-to-maximum response was 11.9 w (range 3-59). Only 4 mild adverse events possibly related to Epo were recorded. Mean therapy duration was 17.2 m (range 1-88). Results of univariate analysis are shown on Table 1. In our monocentric series overall response rate to Epo and mean therapy duration is comparable to previous studies; frequency of adverse events is acceptable. Impact of baseline EPO, transfusional demand, IPSS and WPSS on hematological response seems to be relevant. Apparently none of the considered variables influences transfusional demand reduction.

Table 1.

	END-POINT	
Variable	*Hematologic response*	*Transfusional response*
Baseline Epo	OR = 0.995; P = 0.045	OR = 0.998; P = 0.414
Transfusional demand	OR = 0.203; P = 0.011	OR = 0.169; P = 0.142
Baseline ferritin	OR = 0.999; P = 0.380	OR = 1.000; P = 0.995
IPSS (low risk vs. int-1)	OR = 0.321; P = 0.014	OR = 0.349; P = 0.089
Baseline LDH	OR = 1.001; P = 0.419	OR = 0.997; P = 0.282
BM blast percentage (<5 vs 5-10)	OR = 0.192; P = 0.057	OR = 0.160; P = 0.108
Reticolocytes	OR = 0.739; P = 0.089	OR = 0.824; P = 0.448
WPSS	OR = 0.522; P = 0.036	OR = 0.685; P = 0.305

P201

EVALUATION OF BONE MARROW EXPRESSION OF CD34 AND CD117 IN THE ASSESSMENT OF ERYTHROID RESPONSE TO ERITHROPOIETIN IN THE USUAL CLINICAL PRACTICE OF LOW- AND INT-1 IPSS MYELODYSPLASTIC SYNDROMES

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A possible role of flow cytometry as an additive predictive parameter for response to growth factors in low and int-1 risk myelodysplastic syndromes (MDS) has been pointed out in a recent prospective phase II study (*van der Vorst et al, ASH 2008*). Still, it is unclear which myeloid blast markers deserve particular attention. We evaluated how bone marrow blast expression of markers of immaturity (CD117) and of myeloid commitment (CD34) in patients with low- and int-1 MDS influences response to standard therapy with erythropoietin (Epo). Expression of CD34 and CD117 antigens in not-enriched BM samples taken at time of

Epo initiation was retrospectively investigated in 23 low- (17, 73.9%) and int-1 (6, 26.1%) MDS pts. According to WHO, 19 pts had RA+RARS (82.6%) and 4 had RCMD (17.4%). Erythropoietin was routinely started in anaemic patients with hemoglobin <10 grams/deciliter at the initial dose of 30.000 international units weekly Epo-β, or 40.000 weekly Epo-α. Fourteen pts (60.9%) were transfusion-dependent at time of Epo initiation. Immunophenotypic analysis was carried out by using a panel including monoclonal antibodies CD34 and CD117, conjugated with the fluorochromes FITC and PE, respectively. Acquisition of information on 1x10(e)5 stained cells corresponding to the whole BM cellularity was assessed on a dual-laser FACSCalibur flow cytometer using the CellQUEST software (Becton Dickinson, San José CA USA). IWG2006 criteria defined erythroid response; definition of transfusional response is reported on Table 1. Multiple group comparison was made using general linear model with ordinal logit link function. Sixteen pts(69.6%) had an erythroid response: 12 had a complete response (CR), the remaining a partial (PR). A transfusional demand reduction was observed in 7/14 pts (50%; CR in 6 pts, PR in 1 pt). Mean time to maximum response was 12.9 weeks (range 3-59). Mean duration of Epo therapy was 22.6 weeks (11.7 for NR; 26.2 for PR; 27.7 for CR); mean observational time was 30 weeks (22.9 for NR; 28 for PR; 34.8 for CR). Result of univariate analysis on CD34 and CD117 bone marrow expression is shown on Tab.1. Our retrospective study confirms previous data on erythroid and transfusional response to Epo in low- and int-1 MDS, but fails to demonstrate a prognostic impact of routinal immunophenotypic markers like CD34 and CD117 on those responses within usual clinical practice as defined by Italian authorities.

Table 1.

	ENDPOINT	
Variable	*Hematologic response*	*Transfusional response*
Baseline Epo	OR = 0.995; *p*=0.045	OR = 0.998; *p*=0.414
Transfusional demand	OR = 0.203; *p*=0.011	OR = 0.169; *p*=0.142
Baseline ferritin	OR = 0.999; *p*=0.380	OR = 1.000; *p*=0.995
IPSS (low risk vs. int-1)	OR = 0.321; *p*=0.014	OR = 0.349; *p*=0.089
Baseline LDH	OR = 1.001; *p*=0.419	OR = 0.997; *p*=0.282
BM blast percentage (<5 vs 5-10)	OR = 0.192; *p*=0.057	OR = 0.160; *p*=0.108
Reticolocytes	OR = 0.739; *p*=0.089	OR = 0.824; *p*=0.448
WPSS	OR = 0.522; *p*=0.036	OR = 0.685; *p*=0.305

P202

AZACITIDINE IN HIGH AND LOW RISK MYELODYSPLASTIC SYNDROMES. RETROSPECTIVE EVALUATION OF 4 DIFFERENT THERAPEUTIC REGIMENS

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Azacitidine (AZA) has proven effective in Myelodysplastic Syndromes (MDS), as it has been shown to induce: a) significant reduction of transfusion dependence; b) decreased risk of evolution into acute myeloid leukemia (AML); c) improvement of quality of life; d) significant increase of overall survival, as compared to conventional care regimens, in high risk patients (pts.) (*Silverman, 2002 and 2006; Fenaux, 2009*). The currently approved AZA regimen is 75 mg/sqm/die subcutaneously (SC) or intravenously (IV) for 7 days every 28 days. Recently 3 different AZA dosing regimens, which avoid week-end dosing, have shown to induce therapeutic responses consistent with the currently approved schedule (Lyons, 2009). Moreover, the combination of AZA with valproic acid (VPA) and all-trans-retinoic acid (ATRA), based on epigenetic biology, has proven safe and feasible in AML and high risk MDS (*Soriano, 2007; Voso, submitted*). From September 2004, in our Institution, 27 MDS pts. (20 males), with a median age of 69 (50-84) yrs, were treated with AZA, following 4 differerent treatment regimens. Group 1 (9 pts.), with IPSS risk high-or-int-2, received the currently approved regimen (AZA: 75 mg/sqm/die SC for 7 days/28 days). Group 2 (6 pts.), with high-or-int-2 risk MDS, received the combination of AZA (75 mg/sqm/die SC for 7 days/28 days) with VPA (600-1.500 mg/die orally) and ATRA (30 mg/sqm/die, orally, from cycle n. 5, in case of non-response). Group 3 (6 pts.), with high-or-int-2 risk MDS, received the alternative 5-2-5 AZA regimen: AZA 50 mg/sqm/die SC for 5 days, followed by 2 days no treatment, then 50 mg/sqm/die for 5 days. Group 4 (6 pts.), with low risk disease (IPSS low-or-int-1), refractory to erythropoietin (EPO) or with a poor EPO-response profile or with severe neutropenia or thrombocytopenia, were treated with the alternative AZA 5 regimen: AZA 75 mg/sqm/die SC for 5 days. 22 pts. (81%) completed at least 6 AZA cycles, and were considered evaluable for response. 13 pts. (59%) showed a favourable response, following IWG criteria (Cheson, 2000 and 2006): 1 Complete Remission (CR), 1 Partial Remission (PR) and 11 Hematologic Improvement (HI). 9 pts. did not respond, and 5 pts. are not evaluable (NE) (less than 6 cycles: 2 of them still under treatment). In Group 1: 5 responses (55%), (1 CR, 1 PR and 3 HI), 3 failures and 1 NE. Group 2 : 3 HI (50%), 3 failures. Group 3: 3 HI (50%), 1 failure, 2 NE. Group 4: 2 HI (33%), 2 failures, 2 NE.

P203

EFFICACY AND TOXICITY OF INTRAVENOUS 5-AZACITIDINE (5-AZA) AS SINGLE AGENT IN THE TREATMENT OF MYELODISPLASTIC SYNDROMES (MDS) AND UNFIT ELDERLY ACUTE MYELOID LEUKEMIA (AML): PRELIMINARY DATA OF SINGLE-CENTER EXPERIENCE

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Most of pts with MDS or AML are elderly or had significant co-morbidities at diagnosis resulting ineligible for standard chemotherapy. 5-Aza a ring analog of the pyrimidine nucleoside cytidine inducing re-expression of silenced genes in cancer cells may represent a good therapeutic option in this subset of pts. Recently 5-Aza given subcutaneous as single-agent has been shown to improve OS of pts with MDS when compared to BSC. In a phase III study comparing 5-Aza vs. conventional care regimens (CCR) or BSC in high-risk MDS, the OS resulted significantly better in pts treated with 5-Aza regardless of IPSS score or cytogenetic group. MDS or unfit AML patients were treated with 5-Aza,75 milligrams per square meter given as 1-hour IV infusion once daily on days 1-7 every 4 weeks for at least 6 courses. Up to date, 18 pts, median age 75 yrs (66-84 yrs) have been treated; median time from diagnosis to treatment was 3 mos (1-40). According to WHO classification 5 pts had AML,3 RCMD, 5 RAEB-1, 3 RAEB-2 and 2 CMMoL. IPSS score was Low-Int-1 in 3 pts and Int-2-High risk in 10. All but one had co-morbidities: recent cardiac infarction (n=2), hypertensive cardiomyopathy (n=7), chronic renal failure (n=3) and insulin-dependent diabetes (n=5). 13 pts (75%) were RBC transfusion-dependent and 22% both RBC and PLTs transfusion-dependent. In median pts received 6 cycles of 5-Aza (range:2-15). Sixteen pts are evaluable for response and toxicity. Two pts achieved CR (12%) and 6 (37%) an HI with an ORR of 49%. Four pts (25%) had major erythroid response with three of them becoming RBC transfusion independent (18%) while two pts had a minor erythroid response (12%). The median time to response was 4,5 mos. Two pts failed to achieve at least an HI, 4 had PD, 2 SD. Median duration of response was 4 mos (2-10). Seven pts died (4 PD, 1 fungal infection, 1 re-infarction and 1 acute pulmonary edema). After a median F-up of 8 mos (2-18), 9 pts (56%) are alive (LMA=2, AREB1-2=3, CMMoL=2 and RCMD=2), with a median survival time of +10 mos (4-18). No 5-Aza related deaths were recorded. Efficacy and toxicity profile of 5-Aza IV seems to be similar to that of 5-Aza SC. WHO grade 3-4 neutropenia and thrombocytopenia was observed in 38% and 33% of pts rescpectively. Non hematologic toxicity was mild and uncommon. These preliminary data show that 5-Aza IV is safe and effective as well as 5-Aza SC in the treatment of pts with MDS or unfit elderly AML improving also their compliance.

Anemias and Erythroyd Disorders

P204

GENETIC COUNSELLING DIFFICULTIES IN THALASSEMIA: NEWBORN PHENOTYPE UNCERTAINTY IN ASSOCIATION WITH A NOVEL β GLOBIN MUTATION

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Background. The risk determination for β thalassemia carriers of having affected children represents a significant genetic counseling question. Phenotypic predictions are difficult in the presence of rare or novel mutations. Prenatal diagnosis seeks to predict the child's clinical phenotype however this may be variable, depending on the parental mutations or the co-inheritance of other genetic determinants (i.e. α and γ genes defects). Aims. We report the case of a non-consanguineous pregnant couple who are both β globin mutation heterozygotes (novel in the mother and previously reported in the father). Methods. Routine hematology, separation of the Hb fractions with measurement of the Hb A2 and Hb F levels and globin chain synthesis; molecular analysis by A.R.M.S.-PCR and direct sequencing (Beckman Coulter CEQTM 8000 Genetic Analysis System). Results. The Italian father was a heterozygote for the Mediterranean IVS I-6 (T->C) mutation, detected by A.R.M.S.-PCR. The mother, an Australian woman, with paternal Indian ancestry, presented with microcytic hypochromic parameters without iron deficiency, a high Hb A2 level and Hb F consistent with β globin gene mutation heterozygosity carrier. The globin chain synthesis ratio was 1.55 also consistent with β thalassemia carrier status despite the absence of additional investigations (mRNA and cDNA tests) or family studies. β globin gene sequencing detected a 12 base deletion located at the start of the second intron of the β globin gene [IVS II-2 (-12 bp)]. The analysis of the most common defects in the α and in the gamma genes did not detect additional abnormalities. Conclusions. The novel 12 bp deletion is assessed as resulting in a severe β thalassemia phenotype when in combination with known pathogenic β globin mutations due to hematological parameters, mutation position within intron 2 (splicing site) and an α/non-α globin ratio of 1.55. As this mutation has not been reported previously and the mRNA has not characterized, we have classified it as a "probable" mutation. The combination of this mutation and the IVS I-6 mutation is likely to result in a full β thalassaemia phenotype.

P205

C-TERMINAL DELETION IN THE ALAS2 GENE AND X-LINKED DOMINANT PROTOPORPHYRIA

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Erythropoietic protoporphyria is an inherited disorder caused by partial mitochondrial deficiency of ferrochelatase (FECH), the terminal enzyme of heme biosynthesis. Most patients have autosomal-dominant EPP (dEPP), in which clinical expression normally requires coinheritance of a FECH mutation that abolishes or markedly reduces FECH activity trans to a hypomorphic FECH IVS3-48C allele. About 4% of families have autosomal-recessive EPP. In Italy, mutational analysis fails to detect FECH mutations in about 20% of EPP families, of which about 50% are homozygous for the wild-type FECH IVS3-48T allele, suggesting possible involvement of another locus. Recently, Whatley et al. described a previously unreported form of X-linked dominant protoporphyria (XLPP) associated to c.1706–1709 delAGTG and c.1699–1700delAT deletions in ALAS2 exon 11. In their paper the authors suggested that a modification of the C-terminal region of ALAS2 may be responsible for a protophorphyria phenotype by gain of function mechanism. The marked increased ALAS2 activity in these cases that could explain the over-production of protoporphyrin IX, despite of normal FECH activity, and the reduction of iron stores that are often observed in these patients. In view of this report we re-examined 7 Italian unrelated FECH-negative EPP families for a total of 19 subjects: in 4 families, 6 males and 3 females carried the deletion c.1706–1709 delAGTG. This supports Whatley observation and suggest that defects in ALAS2 could be an alternative genetic background for protoporphyria. In contrast to the authors, we found a remarkable heterogeneity of phenotypes between females that could result from X-chromosome inactivation. Out of three unrelated females with the ALAS2 deletion, one, mother of a proband, was asymptomatic while the other two showed increased level of protoporphyrin causing severe photosensitivity, despite normal FECH activity and homozygosis for the wild-type FECH IVS3-48T allele. Otherwise we found no evidences that x-inactivation could lead to a milder disease in symptomatic females. In fact, these latter showed a similar erythrocytes protoporphyrin concentration and liver involvement as symptomatic males. The molecular defect in 3 FECH-negative EPP families remain still unknown, indicating that new gene targets can potentially offer new opportunities for diagnosis and treatment of EPP.

P206

A NEW FRAMESHIFT MUTATION IN TPI GENE ASSOCIATED WITH SEVERE TRIOSE PHOSPATE ISOMERASE DEFICIENCY

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Triosephosphate isomerase (TPI) deficiency is a rare autosomal recessive disease characterized by non-spherocytic hemolytic anemia, severe and progressive neuromuscolar impairment and increased susceptibility to infections. The gene, localized at 12p13, encodes for 248 aminoacids and is expressed in all tissues. 15 different mutations have been so far reported. Among them Glu104Asp is the most frequent, having been described in more than 20 families. We describe a case of severe TPI deficiency due to compound heterozygosity for Glu104Asp mutation and a new TPI null mutant. The propositus was a 7 weeks old baby suffering with haemolytic anemia, failure to thrive and poor neuromuscolar development. He was born at term and had some respiratory distress at birth requiring CPAP for 24 hours; he was noticed to be jaundiced and investigations showed hyperbilirubinemia with hemolysis and no evidence of allo- or auto- immunity. At 4 weeks of age he was investigated for jaundice and failure to thrive; he was found to have hemolysis. He had reduced muscle tone, with abnormal posturing. He developed breathing difficulties, with evidence of a chest infection. He required intubation and ventilation for 15 days and frequent blood transfusions. He was extubated and discharged home but readmitted after 3 days with diarrhea and worsening respiratory failure; his condition gradually deteriorated and he required reintubation. Brain MRI scan showed no diagnostic abnormalities, and a muscle biopsy showed non-specific changes. When the diagnosis of TPI deficiency was made, he was extubated and died shortly afterwards at the age of 10 weeks from respiratory failure. At the time of the study the hemoglobin was 7.4 g/dL, and reticulocyte count $400x10^9$L. TPI activity was at the lower limit of normal (1332 IU/gHb, normal values 1317-2905) in spite of the presence of a large number of transfused cells, whereas both parents displayed low activity in the heterozygote range (1080 IU/gHb, and 821 IU/gHb). The sequence of the complete coding region of TPI gene in the patient showed the presence of the missense mutation GAG-GAC (nt315, Glu104Asp) and a new variant consisting in a G insertion at codon 10; this latter causes a frameshift and a premature stop codon at residue 71, resulting in a nonfunctional protein product. This alteration is the more upstream frameshift mutation so far reported in TPI gene and likely account for the very severe clinical pattern observed in the patient.

P207

LOW-DOSE RITUXIMAB IN IDIOPATHIC AUTOIMMUNE HEMOLYTIC ANEMIA

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Conventional therapy of warm autoimmune haemolytic anaemia (WAIHA) include administration of corticosteroids and immunosuppressive agents, or splenectomy, whereas no effective treatment exists for cold hemagglutinin disease (CHD). A substantial proportion of patients with WAIHA do not respond to or relapse after corticosteroid therapy and may experience clinically relevant side effects. Favourable responses to rituximab at standard doses (375 mg/m^2 weekly for 4-6 courses)

have been reported in both WAIHA and CHD, idiopathic or secondary, as well as in other autoimmune diseases, such as rheumatoid arthritis and primary immune thrombocytopenia. Recently, low dose (LD) rituximab (100 mg fixed dose weekly for 4 courses) has been proven effective in patients with autoimmune cytopenias, particularly immune thrombocytopenia. The aims of this study were to evaluate the safety, activity and the duration of the response of LD rituximab associated with standard oral prednisone (PDN) as first line therapy in newly diagnosed WAIHA and CHD, and as second line therapy in WAIHA relapsed after standard oral PDN. *Methods*. in this single-arm prospective pilot study, LD rituximab was administered at 100 mg fixed dose weekly on days +7, +14, +21, +28 along with standard oral PDN (1 mg/kg/die p.o. from day +1 to + 30, followed by quick tapering: 10 mg/week until 0.5/mg/kg/die, then 5 mg/week until stop). Complete and partial initial responses (iCR and iPR)) were defined as Hb >/= 12 g/dL and >/= 10 g/dL at month +2 from the beginning of therapy, respectively; sustained response (SR) was defined as Hb >/= 10 g/dL at month +6, in the absence of any treatment. Nine patients (5 female, 4 male; median age 49 yrs, range 28-65) were enrolled. A iCR and iPR were observed in 5 and 3 out of 9 patients, respectively; the median Hb level increased from 9.25 g/dL (range 6.1-12.2) at enrolment to 11.7 g/dL (range 9.6-14.6) at month +2. A SR at month +6 was observed in 5 out 5 evaluable patients. No side effects or serious adverse events were observed. These preliminary results seem to indicate that the addition of LD rituximab to standard corticosteroid therapy is a feasible and active treatment in AIHA. Data on SR are intriguing, particularly regarding the possible steroid sparing effect of LD rituximab, but need to be confirmed in larger survey and after longer follow up.

P208

ECULIZUMAB TREATMENT DURING PREGNANCY IN A PATIENT AFFECTED BY PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia in which a defect of glycophosphatidylinositol (GPI)-anchored proteins of the hematopoietic stem cell leads to increased sensitivity of the red cells to complement-mediated lysis, causing intravascular hemolysis and hemoglobinuria. Other clinical features of this disease are cytopenia and an increased frequency of thrombotic events. Venous thrombo-embolism (VTE) is the leading cause of morbidity and mortality, accounting for about 1/3 of all death. Pregnancy in PNH women significantly increase risk for the mother and baby: in particular related to VTE and anemia. The risk of maternal mortality is estimated in 10-20% of PNH pregnancy, principally due to VTE complications. As consequence, female patients in reproductive age are advised to avoid pregnancy. Eculizumab is a humanized monoclonal antibody anti terminal complement C5 protein, with the inhibition of complement-mediated cell lysis. Eculizumab is very effective in controlling intravascular hemolysis. Moreover, Eculizumab stabilizes Hb levels, reduces thrombotic events, decreases transfusion requirements and improves quality of life of PNH patients. Actually, very partial and few experiences have been reported about Eculizumab therapy during pregnancy. We report the case of a 35-year–old woman with PNH under treatment with Eculizumab (900 mg every two weeks) since 2005. Good haemoglobin levels and normalization of serum LDH were achieved with the therapy. The patient became pregnant in October 2008. We decided to continue Eculizumab during pregnancy in order to reduce the VTE risk. Moreover, we started a prophylactic anticoagulant therapy with LMWH and antiplatelets inhibitors after 12 weeks of pregnancy. At the moment, after 30 weeks of gestation, the patient's condition and tolerability of the treatment are good; Hb values remain around 9 g/dl and LDH results in normal range. Maternal-fetal conditions are strictly monitored, fetal growth is normal and side effects of therapy not appeared. Patient monitoring will continue in the post-partum period to obtain an intensive care of the high risk of thrombotic events. We can assert that Eculizumab therapy during pregnancy could effectively reduce VTE risk although more studies are necessary to confirm it.

P209

COMPARISON OF THE EOSIN-5-MALEIMIDE FLOW CYTOMETRIC METHOD WITH OSMOTIC FRAGILITY TESTS USED IN DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

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Hereditary Spherocytosis (HS) is a rare disease caused by defects of red cell membrane proteins (spectrin, ankyrin, band 3 and band 4.2) and associated with hemolytic anemia of variable degree. The diagnosis is based on clinical history, blood smear examination, and red cell osmotic fragility tests whose sensitivity have been reported to range from 48 to 95%. A flow cytometric method (EMA-binding test) based on the fluorescence of red blood cells after incubation with eosin-5-maleimide dye which binds specifically to band 3 has been recently proposed as a diagnostic test for HS (Bolton-Maggs, 2004). The aim of this study was to compare the sensitivity of the EMA-binding test with that of the osmotic fragility tests most commonly used for the diagnosis of HS, i.e. NaCl osmotic fragility on both fresh and incubated blood, standard (GLT) and acidified (AGLT) glycerol lysis tests, and pink test on 108 consecutive HS patients (60 spectrin, 1 spectrin+ankyrin, 1 spectrin+4.2, 1 ankyrin, 32 band3, 2 band 3+4.2, 11 with unclassified defect). 42 patients with other haemolytic anaemias (11 unknown, 6 autoimmune, 9 erythroenzymopaties, 3 hereditary elliptocytosis, 13 congenital dyseryhthropoietic anemia) were also evaluated. EMA-binding results were expressed as percentage of the fluorescence reduction compared to the mean fluorescence of five normal controls tested in parallel (*Girodon et al, 2008*). According to the ROC curve analysis, the optimum decrease in fluorescence to separate normal subjects form HS patients was 10%. The test's specificity, computed on 400 normal subjects, was 98%. The overall results are reported in the table. The sensitivity of the EMA-binding was 87 %. Of the 14 EMA binding-negative HS patients, 8 had spectrin deficiency, 3 band 3 deficiency, and 2 unclassified and 1 ankyrin HS. 11 of them were AGLT and pink-positive, 1 only positive for NaCl fresh, whereas 2 (spectrin deficiency) were negative to all tests. In conclusion, EMA-binding test is slightly less sensitive but more specific for HS than AGLT. The association of EMA and AGLT gives a sensitivity to 97%.

Table 1. EMA binding.

	Positive pts/total HS	Sensitivity %	Positive pts/ total other anemias
EMA binding	94/108	87	11/42 (CDA II)
GLT	60/108	55	-
AGLT	99/108	91	16/37
NaCl fresh	58/93	63	-
NaCl incubated	70/93	75	5/37
Pink test	91/105	86	10/37

P210

COMPLETE HEMATOLOGICAL RESPONSE AFTER LOW DOSE RITUXIMAB IN A PATIENT WITH REFRACTORY WARM-TYPE AUTOIMMUNE HAEMOLYTIC ANEMIA

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The use of rituximab administered with the same schedule used for treatment of B-cell non Hodgkin's lymphomas (375 mg/sqm weekly for 4 weeks) has been extended to the management of autoimmune cytopenias such as the idiopathic thrombocytopenic purpura (ITP), the cold agglutinin disease and the warm-type autoimmune haemolytic anaemia (AIHA). The optimal schedule has not been established yet and standard dose might be an overtreatment. Low dose rituximab (100 mg weekly for 4 weeks) has been recently tested in patients with ITP and in a case with AIHA. In that latter case, a partial response was reported but no further details on degree and duration of response has been described. We report here the effects of low dose rituximab (LD-R) in a patient with relapsed/refractory AIHA. The absence of an underlying lymphoproliferative disease and the purpose to reduce the immunosuppressive effects

of rituximab were the reasons of the use of LD-R. LD-R (100 mg weekly for 4 weeks) was administered in a 78-year old man with a long lasting and symptomatic IgG warm-type idiopathic AIHA who was ineligible to splenectomy and resistant to steroids, immunosuppressive agents and high-dose intravenous immunoglobulins. The haematological and laboratory data before rituximab therapy were the following: haemoglobin (Hb) 8.9 g/dL (supported with 4 packed red-cell transfusions in the previous 2 months), lactate dehydrogenase (LDH) 250 U/L, indirect bilirubin 3.8 mg/dL, haptoglobin 0 mg/dL, reticulocyte count 84‰. According to the criteria reported by D'Arena *et al.,* complete remission (CR) was defined as stable Hb level > 12 g/dL, transfusion independence and absence of clinical and laboratory signs of haemolysis for at least 4 weeks after rituximab treatment, irrespective of direct antiglobulin test positivity. CR was rapidly achieved at the 6th week and maintained until the 29th week. After 6 months of CR, AIHA relapsed (Hb 8.3 gr/dL, LDH 170 U/L, indirect bilirubin 2,27 mg/dl, haptoglobin 24 mg/dL and reticulocyte count 35‰) and a second course of LD-R (100 mg weekly for 4 weeks) was administered. A second CR was documented after 15 weeks and maintained until 24th week. After 6 months from the second course, a maintenance therapy with 100 mg of rituximab every 2 months was started and up to now (17 months from the first course of therapy and 11 months from the second course) the patient is still in CR. A deep B-cell depletion was documented during all the period of the first and second course of treatment but neither infections nor other toxicities were observed. Our report suggests that LD-R may induce a CR in refractory/relapsed warm AIHA as well as the standard dose. A new CR can be obtained in case of relapse. In our case, we observed a slower time of response than the one observed during the first course (6th vs 15th week). Considering the efficacy and the good safety profile, LD-R could be tested in a larger series of patients with warm AIHA.

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CHELATION THERAPY WITH DEFERASIROX: EFFECTS ON CARDIAC IRON OVERLOAD MEASURED BY T2* MRI

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We report the effects of deferasirox (DFX) on cardiac iron in five patients with transfusion-dependent β-thalassemia, (mean age: 22.6 years), undergoing to 4 years of chelation therapy with deferasirox (10-30 mg/kg/die) and evaluated by multislice multiecho T2* MRI. Systolic global function (left ventricular ejection fraction - LVEF) was also monitored by MRI, to further document the possible correlation between myocardial T2* values and clinical outcomes. Other conventional parameters of iron overload such as serum ferritin levels and LIC were monitored. Each patient received a baseline cardiac MRI evaluation for clinical reasons (mean time of exposure to DFX at baseline = 18 months - range 6-45; mean DFX dose received at baseline = 15.1 mg/kg/die) and was evaluated at regular interval (18 months). Liver iron was assessed (baseline LIC by SQUID in pediatric pts or biopsy in adult pts, then also MRI T2*). Serum ferritin levels and safety parameters were regularly measured during all DFX exposure. The overall MRI results showed a decrease of the cardiac iron levels in all five patients during treatment with DFX (mean basal T2* value: 18.4 ms±SD, range 13-24; mean final T2* value: 31 ms±SD, range 21-41). Similar results were recorded for cardiac function: LVEF progressively increased in 3 of our 5 pts (from 56% to 60%; from 57% to 60%; from 60% to 70%) and was maintained stable in the other 2 pts. Serum ferritin levels showed a trend in reduction after DFX dose adjustment (mean final dose set according to pt response = 23 mg/kg/die) and reached or maintained levels < 1000 ng/mL in four patients. Overall the treatment was well tolerated. No patient experienced rash, gastrointestinal disturbance and increases in serum creatinine, which were the most common adverse events associated to DFX in the registration trials. One patient discontinued the treatment for two months because of post-infective proteinuria. However, after the resolution of this event, DFX treatment was continued with a further improvement of T2* cardiac values. *Conclusions*. This report shows a decrease of the cardiac iron overload associated with improvement of LVEF and decrease of serum ferritin levels, in our five patients treated with deferasirox.

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PREVALENT HEMOLYTIC PRESENTATION IN A WOMAN HOMOZYGOTE FOR α2 MUTATION OF TERMINATION CODON TAA --> AAA (HB ICARIA)

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A woman, aged 27 years, born in Sicily, was admitted in our hospital for hemolytic anemia (Hb 8.7 grams/deciliter). She was anemic since childhood. Constant normocytosis, (MCV 84-90 fentoliters), hypochromia (MCH<27 grams/deciliter) and high RDW characterized the erythrocyte indices. The reticulocyte absolute count and the percentage were high. Moreover, hemolysis signs were present (high bilirubin and LDH, low aptoglobin and glycosilated hemoglobin). The spleen was enlarged (longitudinal diameter 18 centimeters) and an accessory spleen (2 centimeters) was present. Immunologic tests were negative, so a congenital anemia was suspected. At the observation of peripheral blood smear, evident anisocytosis, hypochromia and polychromasia of erythrocytes were present but no morphologic alterations suggestive for a membrane defect. Glucose-6-phosphate dehydrogenase and Pyruvate kinase were high. Iron balance indicates a moderate iron overload. The hemolysate High Performance Liquid Chromathography, performed with a dedicated instrument, showed an abnormal injection peak, HbF 0.7%, HbA and low HbA2 (1.5%). The sample was referred to a specialized laboratory for molecular analysis of globin genes. The α genes were examined for many molecular defects. The presence of Hb Icaria mutation was detected by Reverse Dot Blot hybridization using oligonucleotides specific for wild-type and mutated sequences (Foglietta *et al.* 2003). Hb Icaria is a non deletional form of α-thalassemia (Clegg *et al.*, 1974) caused by a single base substitution in the termination codon of the α2 globin gene TAA AAA. The protein is an unstable elongation with a lysine at position 142. This case has some particular aspects to be discussed. The clinical features and the red blood cell indices induce to suspect an hemolytic disorder more than a thalassemia. The MCV in the normal range could be confounding but the constant hypochromia, in this case, should indicate a globin chain defect. The high hemolytic rate, as it has been reported (Kanavakis *et al.*, 2000), is often present in HbH disease. It is the consequence of hemichrome binding to the erythrocyte membrane. This mechanism, leading to a perturbation of membrane permeability and of red blood cells hydratation, would be partially responsible of the observed MCV, high for a thalassemic syndrome. Moreover, reticulocytosis contributes to the MCV elevation.

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TICLOPIDINE-INDUCED APLASTIC ANEMIA IN A PATIENT WITH ERYTHROCYTOSIS AND CEREBROVASCULAR DISEASE

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A white male aged 67 is reported who had been diagnosed with erythrocytosis of unknown cause by his GP in 2000. Blood counts were controlled by phlebotomy alone. The patient was intolerant of non-steroidal anti-inflammatory drugs and received no anti-platelet treatment. He had no other general cardiovascular risk factors but being a former smoker. In 2009, following a transitory ischemic attack, he was started on ticlopidine (250 milligram twice a day). He was then referred to the hematology clinic; JAK2-V617F mutation resulted negative with normal erythropoietin level and normal estimated splenic volume. Forty days after the patient started taking Ticlopidine, he was hospitalized because of fever and leukopenia. Blood cell count showed 1080 white blood cells per cubic millimeter, hemoglobin levels of 16 grams per deciliter, hematocrit of 46%, 254000 platelets per cubic millimeter. Ticlopidine was stopped and empirical antibiotic and G-CSF treatment started. Bacterial and non-bacterial infections were never substantiated in the laboratory or by radiology. On day +11 after stopping Ticlopidine, white blood cells fell to 520 per cubic millimeter, hemoglobin to 12 gram per deciliter, and PLT to 71000 per cubic millimeter. Paroxysmal nocturnal hemoglobinuria was suspected and excluded by phenotypic study of lymphocyte subpopulations. Bone marrow examination showed a hypocellular (less than 5%) bone marrow with T lymphocytes and poly-

clonal plasm B cells and without blasts, suggestive of aplastic anemia. The patient received treatment with intravenous Methyprednisolone 1 milligram per kilogram per day since day +12 and also per os Cyclosporine 5 per kilogram per day since day +19. Steroid diabetes was a side effect. One platelet transfusion was required on day +18 (nadir platelet count of 10000 per cubic millimiter). Hemoglobin levels never fell below 10 gram per deciliter. On day +21 blood cell count started improving and the fever disappeared. G-CSF was continued until day +27 when blood cell count showed 4810 white blood cells per cubic millimeter, haemoglobin levels of 10.4 grams per deciliter, 24000 platelets per cubic millimetre. The patient was discharged and immunosuppressive treatment was continued at home until day +39, when it was stopped for a week due to perianal abscess; then it was continued with a lower dose. On day +70 a normal blood count was achieved.

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BEYOND ECULIZUMAB (EC): CAN WE IMPROVE THE HEMATOLOGICAL RESPONSE IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS? WHAT, TO WHOM AND WHY

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PNH is characterized by intravascular hemolysis due to the lack of the complement inhibitors CD55 and CD59 from RBC surface; EC has proven effective for the treatment of hemolysis in PNH. We assessed the hematological response (HR) in 22 PNH patients, with the aim of investigating strategies to optimize the benefit from EC. HR was defined as optimal (transfusion independence [TI], Hb>11) in 36% of patients, major (TI, Hb>8) in 41%, partial (>50% reduction of transfusional need [TN]) in 9% and minor (LDH reduction with unchanged TN) in 14%, these latter due to aplastic anemia. All patients showed a complete control of intravascular hemolysis, with the exception of 4 cases with persistently high LDH and hemosiderinuria: this was attributed to pharmacokinetic reasons (breakthrough). The reduction of the interval administration to 12 days or the increase of EC dose to 1050 mg led to LDH reduction and Hb increase in all of them. Among the 16 patients lacking an optimal HR, in 4 cases we demonstrated iron deficiency due to pretreatment iron loss; iron supplementation increased Hb in all cases. In 3 cases we demonstrated erythropoietin (Epo) level not adequate to anemia, in absence of renal failure; treatment by recombinant Epo led to improved HR in all cases. Three patients had impaired marrow function (not adequate ARC, neutropenia and thrombocytopenia); two of them, fulfilling the criteria of aplastic anemia (AA), were treated by immunosuppression (while on EC). One achieved a complete remission of AA (leading to normal Hb), while the other finally undergo a curative allogeneic transplant. In some patients, more than one mechanism potentially explaining their suboptimal HR were identified. Furthermore, all patients showed a proportion of PNH RBCs with C3 on their surface, suggesting an ongoing extravascular hemolysis. In some patients, *in vivo* RBC survival by 51Cr labeling showed markedly reduced RBC half-life with high spleen and liver counts; a paradigmatic case was treated by splenectomy achieving an optimal HR. EC is effective in controlling intravascular hemolysis in PNH, but the hematological benefit is heterogeneous; some patients may show persistent anemia, which may be due to different causes, even in the same patient. We demonstrate that a critical clinical assessment of PNH patients receiving EC is needed to identify such causes, possibly leading to specific additional treatments able to improve the hematological benefit from EC.

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CLINICAL BENEFIT FROM ECULIZUMAB IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS (PNH) WHO HAVE NOT RECEIVED PREVIOUS TRANSFUSIONS

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PNH is a rare and debilitating disease arising from a clonal expansion of PIG-A mutated HSCs, which lack from their surface all GPI-linked proteins. On RBCs, the absence of the complement inhibitors CD59 and CD55 leads to chronic intravascular hemolysis, which subsequently causes anemia, hemoglobinuria, fatigue and other disabling symptoms due to free Hb. The anti-C5 humanized monoclonal antibody Eculizumab (Ecu) has proven effective for the treatment of hemolysis in transfusion-dependent PNH patients. We report the efficacy and safety of Ecu in a cohort of 9 PNH patients who have not received any previous transfusion. Their median age was 39 years (range 16-59); all of them showed massive hemolysis, as demonstrated by increased LDH. The indication to the treatment was either a severe anemia (not transfused due to personal belief or recent diagnosis), or moderate anemia with frequent paroxysms and severe hemolysis-related symptoms; two patients with mild anemia were included because of their history of life-threatening thromboembolic events. All the patients received Ecu after anti-meningococcal vaccination, according to the standard schedule: 600 mg for 4 weeks (induction), followed by 900 mg every other week (maintenance); the median treatment duration was 16 months (range 1-39). Ecu induced a dramatic LDH reduction in all patients (median LDH from 1500 to 356 U/L, p=0,001), leading to a significant increase of Hb level (median Hb from 9 to 10,7 g/dL, p=0.0003; median increase 2 g/dL). There was a significant increase of PNH RBC population size from a median of 23,2% to 58,1%(p=0.003). No thromboembolic or severe adverse events were observed. All patients achieved the resolution of paroxysms and disease-related symptoms, resulting in a evident improvement of quality of life. These results were in agreement with those from the SHEPHERD study, showing that minimally transfused patients achieved clinical benefit similar to those requiring more transfusions. We conclude that eculizumab is safe and effective even in PNH patients not receiving transfusions; the blockade of intravascular hemolysis in these patients led to improvement of anemia, as well as to improvement of disease-related symptoms and quality of life. Even if the indication to the treatment should be assessed in individual patients, eculizumab results in a significant clinical benefit even in "mild" PNH patients, possibly impacting on disease-associated long-term morbidity and mortality.

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SURFACE EXPRESSION AND PLASMA LEVELS OF ADHESION MOLECULES ICAM-1 VCAM-1, E-SELECTIN AND PLASMA LEVELS OF PROTEIN S AND PROTEIN C IN PATIENTS WITH β-THALASSEMIA INTERMEDIA AND SICKLE CELL DISEASE. THE EFFECT HYDROXYUREA

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Background. The pathophysiological mechanism of vaso-occlusion in Sickle Cell disease is complex, involving adhesive processes between sickle red blood, leukocyte, and the activated endothelium. Reduced activity of the protein C and protein S may contribute to vaso-occlusion. We investigated the effect of Hydroxyurea (HU) on surface expression of the circulating endothelial cells (CEC) of ICAM-1 (CD54), VCAM-1 (CD106), E-SELECTIN (CD62E), on plasma levels of soluble sICAM-1, sVCAM-1, SE-SELECTIN, and on plasma levels of protein C and protein S of SCA and -Thalassemia Intermedia (TI) patients. Methods: We studied 10 patients with SCA disease, 15 patients with TI, and 12 healthy controls. Activated CEC were defined as CD45 negative, CD54, CD106, CD62E positive. Plasma was separated from blood of patients and controls, and sICAM-1, sVCAM-1, sE-SELECTIN levels were quantified using specific kit according to the manufacturer's instructions. *Results*. The percentage (%) of positive CEC what express the markers of endothelial-cell activation were significantly higher in SCA and TI patients not Taking HU comparated with healthy controls, so as plasma levels (ng/ml) of sICAM-1, sVCAM-1, sE-SELECTIN. SCA patients: CD54+ = 10,9 vs 1,4 respectively; CD106+ = 19,7 vs 2,1; CD62E+ = 21,2 *vs.* 3,7; sICAM-1=1263,12vs102,24;sVCAM=1978, 67vs300,81;sE-SELECTIN=935,04 vs 352,59; TI patients: CD54+(%) = 12,1 vs 1,4; CD106+=3,68 vs 2,1; CD62E$^+$ = 7,08 *vs.* 3,7; sICAM-1 (ng/ml) = 1911,16 vs 102,24 respectively; sVCAM=1067,61 *vs.* 300,81; sE-SELECTIN = 746,92 *vs.* 352,59. The expression of adhesion molecules on CEC and plasma levels of soluble sICAM-1, sVCAM-1, sE-SELECTIN were significantly reduced during HU therapy. SCA patients: CD54$^+$ (%) = 5,01; CD106$^+$(%) = 9,9; CD62E+(%)= 11,6; sICAM-1(ng/ml) = 511,81; sVCAM (ng/m)=870,41; sE-SELECTIN (ng/ml)=587,49. TI patients: CD54$^+$(%)=2,0; CD106$^+$(%)= 2,1; CD62E+(%) = 1,8; sICAM-1(ng/ml) = 1294,18; sVCAM (ng/ml) =1067,61; sE-SELECTIN(ng/ml) = 746,92. The plasma levels of protein C and protein S were increased in SCA and TI patients on HU terapy comparated to those not taking HU; however, this difference was not significant. (SCA patients: Protein C(%) = 106,76vs 77,59; Protein S(%) = 79,070 vs 65,96; TI patients: Protein C(%) = 83,46vs 64,65; Protein S (%)= 80,50vs 74,33. *Conclusion*. Our results confirm that the vascular endothelium is activated in patients with SCA and TI disease, as evidenced from the higher expression of adhesion molecules CD54, CD106, CD62E on the surface of CEC and from the higher levels of solubile sICAM-1, sVCAM-1, sE-SELECTIN, that reflect an increased capacity for the adhesion of sickle erythrocytes and leucocytes to the endothelium. HU therapy reduced the expression on the endothelial cell surface, so as plasma levels of adhesion molecules, reducing the activation state of the endothelium, besides increase plasma levels of anticoagulants protein C and protein S, reducing hypercoagulable state, which means smaller endothelial damages to level of the endothelium and then in a reduction of the events-vaso-occlusive.

Chronic Myeloproliferative Syndromes I

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POLYCYTHEMIA VERA AS A PREDISPOSING FACTOR FOR AORTIC STENOSIS: INCIDENCE AND CORRELATION WITH BLOOD CELLS COUNT AND JAK2 V617F MUTATIONAL STATUS

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Background. The association between Polycythemia Vera (PV) and thrombosis is multi-factorial involving the complex interaction between activated leukocytes, platelets and endothelium. Recent reports have postulated that PV patients may over express adhesive molecules on red cell surface, likely by JAK2V617F mutation (*Wautier M et al. Blood. 2007;110(3):894-901*). This process activates endothelium with production of vascular growth factors and other mechanisms leading to atherosclerosis. Aortic Stenosis (AS) is the commonest valvular heart disease in western countries; its pathogenesis is mainly related to a degenerative process sharing many characteristics with atherosclerosis. At the present is not known whether patients with PV are at high risk of developing AS. Objective of the study. We perform a prospective study for evaluating rate of AS and its correlation with blood cells count and mutational status in patients with PV. Materials and methods. Incidence rate of AS among PV patients have been compared with control patients matched for age, cardiovascular risk factors (hypertension, hyperlipidemia, diabetes, smoke and alcohol abuse) and coexisting cardiac diseases (i.e. heart failure). Diagnosis of PV has been posted accordingly to PVSG criteria. Diagnosis and severity of AS has been posted by echocardiography: stenosis with a valve area <1.0 cm^2 has been considered severe. *Results.* Over a period of 18 months we recruited 43 PV patients (28 males and 15 females) and 74 controls. No differences were found in regard of the above cited characteristics; mean age was 66.7 among PV patients and 68.2 among controls. The average duration of PV was 5.7 years with an average follow-up of 2.5 years. Most of the PV patients were on antiplatelet/anticoagulant therapy (27/43, 62.7%) and have been treated with cytoreductive therapy. Twelve (27.9%) had a thrombotic event before PV diagnosis; 4 (9.3%) developed thrombosis during the follow-up (median 1.3 years). A moderate/severe AS was found in 11 PV patients (25.6%) in comparison to 4 (5.4%) in control group (p= 0.004), thus giving a Relative Risk of 4.7 (CI 95%: 1.61-13.95). Among PV patients, the multivariate analysis did not show any correlation regarding JAK2V617F mutational status, duration of disease, previous thrombosis, cytoreductive therapy and other common cardiovascular factors. A significant trend was demonstrated in favor of patients with elevated haematocrit (>55%) (p= 0.001). *Conclusions.* Our study clearly shows that PV patients carry a fourfold risk of developing AS, mainly related to the presence of high haematocrit level. No clear association were found regarding white blood cell or platelets count, effect of cytoreductive therapy or previous thrombosis. Whether high incidence of AS may be related to expression of adhesive molecules on red cells or altered share stress is currently under investigation.

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ACTIVATING MUTATION VAL617PHE OF JANUS KINASE 2 GENE AND CXCR4 EXPRESSION IN PH1-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES (CMPD)

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Traditionally, Ph1-negative CMPD include Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Myelofibrosis with Myeloid Metaplasia (MMM). The Val617Phe point mutation of Janus Kinase 2 gene (JAK2V617F) is believed to participate in the pathogenesis of Ph1-negative CMPD and occurs in the majority of patients with PV and approximately half of those with either ET or MMM. Disregulation of CXCR4 and disruption of the CXCR4/SDF-1 axis may play a role in the pathogenesis and disease progression of CMPD. In our institution we are following 28 patients with PV, 18 patients with ET and 15 patients with

MMM. We used the allele specific polymerase chain technique for detection of Val617Phe mutation in all 61 patients with chronic myeloproliferative syndrome. Surface CXCR4 expression on circulating and bone marrow $CD34^+$ cells was measured flow cytometrically. We measured Val617Phe frequency as 82% (23/28) in PV, 50% (9/18) in ET, and 40% (6/15) in MMM. We found significantly elevated hemoglobin levels and platelet count together with very low serum level of erythropoietin in Val617Phe-positive polycythaemia vera and essential thrombocythaemia patient groups. However, white blood cell count and the frequencies of splenomegaly and other complications (thrombosis, bleeding, transformation to acute leukemia) were not significantly different between the mutation-positive and negative groups. The expression of CXCR4 on circulating $CD34^+$ cells was significantly reduced in patients with MMM as compared to normal controls and patients with PV and ET. By analysing immunophenotypic pattern of bone marrow $CD34^+$ cells we found in 10 out of 61 CMPD patients (i.e. 3 PV, 2 ET, 5 MMM) an over-expression of CXCR4 (as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5). This subset of patients showed significantly higher levels of bone marrow blast cells and serum lactate dehydrogenase (LDH). No statistical association was found between JAK2V617F mutational status and the CXCR4 expression. The non-invasive mutation analysis of the Janus Kinase 2 Val617Phe is suitable for routine laboratory application and helps the differential diagnosis of chronic myeloproliferative syndrome. However current informations on disease-specific prognostic relevance of JAK2V617F are inconclusive, while our results warrant further investigation into the role of CXCR4 in CMPD and suggest that CXCR4 should be incorporated into the risk assessment of CMPD patients.

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COEXISTING JAK2 V617F-POSITIVE ESSENTIAL THROMBOCYTHEMIA AND BCR-ABL-POSITIVE CHRONIC MYELOID LEUKEMIA IN A PATIENT AT INITIAL PRESENTATION

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Coexistence of BCR-ABL fusion transcript and JAK2V617F mutation has been previously reported in a few cases of Polycythemia Vera and Primary Myelofibrosis, mainly as metachronous phenomena; in rare cases retrospective analisys revealed their coexistence from the beginning of the disease. Only one case of concomitant JAK2 V617F mutation and BCR-ABL1 rearrangement at initial presentation has been described in a patient showing clinical features of both CML an PV. A 46-year old woman presented with marked thrombocytosis (1406000/microL PLTS) and leukocytosis (27900/microL WBC); haemoglobin levels were normal and differential leukocyte count showed: neutrophils 70%, eosinophils 1%, basophils 4%, lymphocytes 14%, monocytes 7%, metamyelocytes 1%, myelocytes 3%. She complained of erythromelalgia at both feet and intermittent left limbs paresthesia; no splenomegaly was observed. BCR-ABL fusion transcript b2a2 type and JAK2V617F mutation were detected. Conventional karyotype and FISH analysis confirmed the presence of t(9;22) in 100% of cells. Acetylsalycilic acid and imatinib mesilate (400 mg/die) therapy was promptly started, obtaining rapid regression of symptoms. After 2 months complete hematologic response was observed, with normal platelets values, and after 6 months CML complete cytogenetic remission was obtained. To evaluate if BCR-ABL rearrangement and JAK2V617F mutation occurred in the same clone, we performed clonogenic assays. To assess response to imatinib therapy of CML and its possible influence on the JAK2V617F clone the patient was monitored using real-time quantitative-PCR for both molecular abnormalities. None of the CFU-GM colonies showed the coexistence of both molecular abnormalities. During the follow-up, quantitative-PCR analysis showed a 3.18 logarithmic decreased of BCR/ABL copy number in bone marrow, reaching molecular remission at last follow-up, whereas the JAK2 V617F allele burden decreased progressively from 46% at diagnosis to 33% in the last sample. This the first case of ET with V617F mutation and concomitant Ph+ CML in a patient at initial presentation. Clonogenic assays suggest the coexistence of two distinct myeloproliferative disorders involving different clones. On the other hand, imatinib therapy induced thrombocytosis regression paralleled by a decrease of JAK2V617F allele burden, in contrast with previous observations showing no effect on or even expansion of the JAK2 V617F clone in patients treated with imatinib.

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IMPACT OF JAK2V617F MUTATION ON THROMBOTIC RISK AND OTHER CLINICAL FEATURES IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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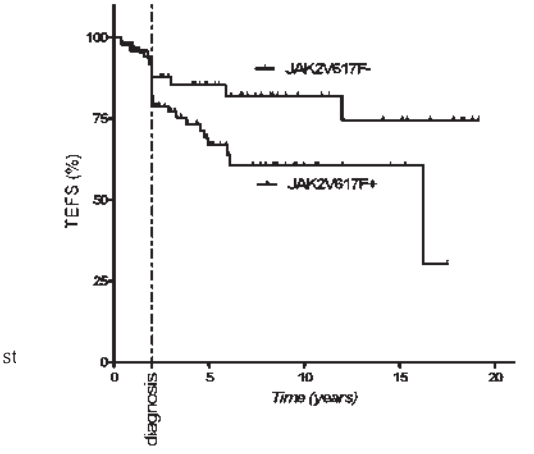
Background. Acquired JAK2V617F mutation in Essential thrombocythemia (ET) can be found in approximately 50–60% of patients (pt). We investigated the possible correlation between JAK2V617F mutational status and clinical features, particularly the impact on primary and recurrent thrombotic risk, still controversial in literature. *Methods.* Allele-specific PCR for JAK2V617F mutational status was performed on genomic DNA from bone marrow cells or peripheral blood granulocytes in 115 ET (49 males, 66 females) pt, referred to our division from 1987 to 2008. Clinical features were evaluated at diagnosis and during follow-up, comparing JAK2 positive and wild-type (WT) pt. Data were processed for statistical analysis with Graph Pad PRISM 5 DEMO Software performing non-parametric methods (χ^2 and Fisher's exact tests for categorical nominal variables and the Mann-Whitney rank test for ordinal variables). Thrombotic risk and thrombotic event-free-survival (TEFS) were estimated performing log-rank (Mantel-Cox) test applied to Kaplan-Meyer method. *Results.* 66 ET pt (57.4%) were positive for JAK2V617F mutation. We observed an increased thrombotic risk in JAK2 positive pt at diagnosis. In fact, considering JAK2 WT ET pt as reference group, the relative risk (RR) of primary thrombotic event was 2,143 (95% CI: 1.053-4.360, p=0.035) for JAK2 mutated pt, with a TEFS significantly lower than WT pt. Altogether arterial events were more frequent than venous events without statistical difference between two ET groups. Moreover a trend of higher risk of recurrent thrombosis (p=0,056) was showed for JAK2 positive pt. There was no significant difference about bleeding risk and evolution in myelofibrosis between JAK2 mutated or WT ET pt. Last, we confirmed that JAK2 positive pt were older (median age 59 vs 48 years, p=0.0016) and presented at diagnosis higher hemoglobin (Hb 14,3 vs 13,3 g/dL, p=0.0027), higher hematocrit (Ht 43% vs 39.8%, p<0.0001) and lower platelet count (PLT 725 vs 841×10^9/L, p=0.005) respect to WT group. These PV-like features have also maintained statistical significance during the course of disease *Conclusion*. JAK2V617F positive compared to WT pt display an increased risk of thrombosis at diagnosis and most likely during the follow-up, with a PV-like phenotype. This could suggest a more aggressive phenotype since the diagnosis in JAK2 mutated pt with consequently a potential different therapeutic approach that will be investigated in future studies.



Thrombotic event free survival (TEFS) in JAK2V617F positive and negative patients

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EFFICACY OF DASATINIB IN A PATIENT WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML), KIT-D816V POSITIVE, REFRACTORY TO STANDARD THERAPY: A CASE REPORT

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Recent characterization of Philadelphia–negative chronic MPD provided a clear rationale for investigating the efficacy of novel targeted therapies, in particular tyrosine kinase (TK) inhibitors. Dasatinib significantly inhibits either wild-type TKs as KIT and PDGFR alfa-β, or their mutants, including KIT-D816V gene, which is resistant to Imatinib. Long-term hematologic and molecular remission have been reported in Systemic Mastocytosis (SM) and acute myeloid leukaemia with mutant KIT-D816V after combined treatment with chemotherapy and Dasatinib. In this report we describe the efficacy of Dasatinib in one patient (pt) affected by CMML, KIT-D816V positive, refractory to standard cytoreductive therapy. A 78 years old male pt was diagnosed to CMML according to WHO criteria in September 2006. The onset presentation was mild anemia, normal leucocyte (WBC) and platelet count, high Erythropoietin level and mild spleen enlargement, 3 cm below costal margin (BCM). Initially no cytoreductive treatment but only transfusional support was performed. In October 2007 a progressive monocytosis, severe anemia and increasing splenomegaly (8 cm BCM) appeared. Therefore, different cytoreductive agents (Hydroxyurea, Etoposide and Busulfan) have been used to control myeloproliferation and constitutional symptoms without significant improvement. In march 2008, a disease restaging with bone marrow aspirate and biopsy, showed a picture still compatible with a chronic phase of CMML without SM features associated. Molecular analysis showed c-kit D816V mutation in the exon 17 without mutation of JAK2 and PDGFR genes. Considering this molecular result associated to rapid and progressive clinical worsening (spleen 15 cm BCM, severe constitutional symptoms, hyper-leucocytosis), experimental treatment with Dasatinib 70 mg twice daily started. A mild improvement of constitutional symptoms and a control of splenomegaly and WBC count, but not a complete remission of disease was obtained. Indeed analysis of c-kit D816V mutation, performed after 6 months of Dasatinib, was still positive. Dasatinib may be considered a valid approach in CMML carrying gain of function TK mutation. It can transiently improve constitutional symptoms and control the myeloproliferation as in our pt. Duration and powerful of its effect remain to be disclosed. Large clinical studies and molecular essays for evaluation of disease burden could be helpful in this setting

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ANGIOGENESIS IN ESSENTIAL THROMBOCYTHEMIA: FIBROBLAST GROWTH FACTOR-2 AND VASCULAR ENDOTHELIAL GROWTH FACTOR CROSS-TALK

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Essential thrombocythemia (ET) is a clonal disease characterized by thrombocytosis and platelet pro- and antiangiogenic releasates, such as platelet factor 4 (PF4) and fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF), respectively. We therefore evaluated platelets, PF4, FGF and VEGF in fifty ET patients (24 males and 26 females, mean age 58 years) who fulfilled PVSG and WHO. Their mean duration of disease was 7 years. Of 50 patients, 18 were on hydroxyurea whereas 32 were not receiving any cytoreduction. All patients were on antiplatelets. Platelets were measured by automated analyser. PF4, FGF and VEGF were assayed by ELISA. Considering that FGF and VEGF may be produced by platelets, we adjusted FGF and VEGF per platelet (FGFPLT/106 and VEGFPLT/106). All patients had thrombocytosis (684±299 $x10^9$/L) and high PF4 (113±39 IU/mL vs 6.9±2.2 IU/mL) (p<.0001), FGFPLT(0.10±0.09 pg /106 vs 0.034±0.002 pg/106) (p<.0001) and VEGFPLT (1.8±1.6 pg/106 vs 0.5±0.4 pg/106) (p<.0001). No correlation there was between PF4 and FGF and VEGF whereas a positive correlation was found between FGF and VEGF (p=0.044). These data suggest that angiogenesis is upregulated by FGF/VEGF crosstalk and that targeting these cytokines may be a potential therapeutic strategy in ET.

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A CASE OF CYCLIC THROMBOCYTOPENIA/THROMBOCYTOSIS.

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Cyclic fluctuations in platelet count are reported in few cases of polycythemia vera treated with hydroxyurea or in cyclic thrombocytopenia-thrombocytosis of autoimmune origin. We report on a 64-year-old woman in whom severe thrombocytopenia was regularly followed by thrombocytosis: each cycle was about 42 days long with platelet count ranging from 7 to 700-9,800x10^6/L. The amplitude of platelet fluctuation increased during follow-up. A progressive hyperleukocytosis, up to 256x10^6/L, was observed without qualitative abnormalities in peripheral blood smears. Leukocytes showed a similar periodicity in count fluctuation. No liver or spleen enlargement was present. Morphological examination of bone marrow revealed cyclic depletion and expansion of megakaryocytes and erythrocytes progenitor cells, whereas, hyperplasia of leukocyte progenitors was always present. Bone marrow immunophenotype was normal. No cytogenetic abnormalities were found. BCR/ABL translocation was not present. Bone marrow coltures were performed at platelet peak: the in vitro growth of CFU-GEMM, CFU-GM and BFU-E colonies was normal in the presence of specific growth factors, while no growth of BFU-E was observed in the absence of erythropoietin. *In vitro* bone marrow coltures from healthy volunteers were not inhibited nor stimulated by patient's serum collected in any time of platelet cycle. Serum level of thrombopoietin (TPO) was serially determined: TPO level increased as platelet count decreased below 100 x106/L and decreased rapidly as platelet count started to rise. Because of multiple episodes of organ infarctions (bowel, kidney, spleen, brain) in the previous two years, prophylactic short course treatment with low molecular weight heparin and antiplatelet drug was given during thrombocytosis phases: neither additional thrombotic events nor bleeding complications were observed. Bleeding episodes occurred during the thrombocytopenic phase were treated with platelet concentrates. The patient died for haemorrhagic shock 4 years after the onset of the disease. Patient's cryopreserved cells were tested for the somatic acquired JAK2 V617F mutation: the presence of the mutation allowed the disease classification as chronic myeloproliferative neoplasm. We can hypothesize that JAK2 V617F mutation had induced a hypersensitivity to cytokines in particular to TPO but the reciprocal relationship between platelet count and TPO level suggests the persistence of a feedback control.

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CORRELATION BETWEEN ASPIRIN (ASA) NON-RESPONSIVITY AND JAK2 MUTATIONAL STATUS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN): PRELIMINARY DATA

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ASA is widely used in general population to prevent cardiovascular complications. However, some individuals exhibit a reduced antiplatelet response to ASA and continue to suffer from thrombotic events. Polycythemia Vera (PV) and Essential Thrombocythemia (ET) are MPN which carry JAK2V617F mutation, a recent discovered biological marker which drives the excess of proliferation. At present, mutated patients seem to have a higher thrombotic risk compared to WT cases. Low dose aspirin (100 mg/die) is widely used to prevent thrombosis in MPN, even if a clear evidence of its utility is available only in PV patients. The aim of our study is to correlate thromboses of MPN, mutational status and aggregation pattern. We studied 75 MPN patients (29 PV,46 ET, 27 M, 48 F mean age 58.7±10 y) diagnosed in agreement with WHO 2008 criteria. All patients were under ASA 100 mg/day. Major thrombotic complications separate as arterial (stroke, myocardial infarction, peripheral artery disease) or venous (deep vein and splanchnic thrombosis) were registered. As controls we used 22 individuals (7 M,15 F, mean age 69,4±10 y), under ASA 100 mg/day for secondary prevention of cardiovascular events. The JAK2V617F mutation was searched with allele-specific PCR and aggregation study (Born's method) under arachidonic acid stimulus was performed. We defined *ASA non-responders* the cases with normal aggregation in spite of verified ASA intake. The Pearson's χ^2 test

(two by two table) was used to compare categorical variables. 22 (76%) PV and 32 (68.5%) ET patients carry JAK2V617F mutation. The relation between thrombosis and JAK2 characteristics is summarized in the Table. Thromboticic complications occurred in 20 patients; 17 (85%) were mutated and 3 (15%) WT (statistically not different). Four mutated MPN (30.7%) were ASA non-responders and had new thrombosis. In contrast, none of the controls nor WT MPN resulted ASA non-responders. In our study, all ASA non-responders patients with thrombosis are JAK2V617F even if we did not found statistical significance. We surmise that increasing the number of patients a significance should be found.

Table 1.

	No thrombosis		*Thrombosis*					
			TOTAL		*Arterial*		*Venous*	
	V617F	WT	V617F	WT	V617F	WT	V617F	WT
PV	13	5	9	2	8	2	1	0
ET	24	13	8	1	6	0	2	1
Tot	37	18	17	3	14	2	3	1

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LONG-TERM OUTCOMES AND CLINICAL RELEVANCE OF JAK2V617F MUTATION IN YOUNG POLYCYTHEMIA VERA PATIENTS AGED LESS THAN 50 YEARS

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Several studies have reported on the occurrence of JAK2V617F mutation in the majority of patients with polycythemia vera (PV). The clinical relevance on long-term outcomes of this molecular aberration in the subset of PV patients aged less than 50 years, is currently not known and under investigation. In a single institutional study, mutational screening for JAK2 was performed in DNA derived from peripheral mononuclear cells from 74 consecutive PV patients aged less than 50 years, diagnosed according to PVSG, and followed for a median time of 96 months. Homozygosis was defined by the presence of the V617F mutation in more than 50% JAK2 alleles. Univariate analysis performed with Mann-Withney test, included all clinical features at diagnosis. There were 42 males and 32 females, median age was 43 yrs (range 23-50). The mutation was detected in 51 of the 74 patients (68.9%), with 41.8% homozygosis. Comparison between JAK2 V617F wild-type, heterozygosis and homozygosis status, did not reveal significant association with gender, haemoglobin level and haematocrit at the time of diagnosis. As regards other clinical features, in patients with leukocytosis the JAK2V617F occurred in heterozygous and homozygous state in 26.3% and 52.7%, respectively. In patients with high platelet count the mutation was detected in the 91.9% of cases (37.8% heterozygous, 54.1% homozygous). The frequency of homozygosis in patients with spleen enlargement and aquagenic pruritus was 53.6% and 52.4% respectively. During the course of PV, thrombotic events occurred in nine patients (12.0%): all patients carried the JAK2V617F mutation (3 heterozygous, 6 homozygous). Three patients had progression to myelofibrosis (PMF): two of these were heterozygous for JAK2 mutation, and one was wild-type. No patient had evolution to acute myeloid leukemia (AML) and at the time of this analysis all patients were still alive. The results of the current analysis confirm the link between JAK2V617F and PV (approximately 95-99% of cases) also in the subset of younger patients, although this acquired mutation was detected in a smaller proportion of cases (68.9%) as compared to general PV population (90%). In our series of patients we observed a rate of thrombosis of 12%, which was relatively lower than reported in previous studies. As regards the disease evolution, we showed a higher rate of PMF (4% of cases) than that reported in previous analyses, in which this complication was not observed. In conclusion, present analysis showed that JAK2 mutational status in younger patients influenced disease phenotype and was predictive for clinical events.

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ESSENTIAL THROMBOCYTHAEMIA (ET) IN YOUNGER PATIENTS: CLINICAL AND BIOLOGICAL FEATURES

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Less than 20% of ET patients are below 40 years of age at diagnosis and there are very few reports addressing this subset of younger patients. We retrospectively evaluated a cohort of ET patients aged < 40 years and compared their clinical and biological features with those of older patients (>40 years). From 1/1980 to 6/2008, 218 patients (median age 50.7, M/F 81/137) were consecutively diagnosed at our Institute based on the PVSG criteria; of them, 61 patients (M/F 23/38) were aged < 40 years (group A) and 157 patients (M/F 58/99) were aged > 40 years (group B). There was no statistical difference as to smoke attitude and incidence of thrombosis in the family; incidence of previous thrombotic events was lower than expected in younger patients (2/61 vs 21/157, p=0.02) Haematological characteristics at onset (mean Hb, Ht, WBC and PLT counts) as well as gender distribution did not differ significantly between the 2 age subgroups. Bone marrow cellularity was greater in young patients (median 60%, IR 46-69) than in elderly (50%, IR 40-60) (p=0.001) as observed in normal subjects; a lymphoid interstitial infiltration occurred in 3/61 younger patients vs 26/157 elderly (p=0.025). On the contrary, reticulinic fibrosis was not significantly different in the 2 age groups. JAK-2 V617F mutation was present in 22/59 (37.2%) and 82/145 (56.5%) evaluable patients in the group A and group B, respectively, with a statistically significant difference (p=0.04). During follow-up, 33/61 younger patients (54.1%) started a PLT-lowering treatment (11 with IFN, 8 with anagrelide, 14 with Hydroxyurea or Pipobroman) compared to 115/157 patients (73.2%) aged > 40 years (p=0.007); median period from diagnosis to treatment was 2.9 months (IR 1.2-23.2) in the group A and 2.3 months (IR 0.3-13.1) in the group B (p=0.17). It is worth of note that there was no statistical difference as to thrombotic events during the course of disease (6/61 in group A vs 26/157 in group B, p=0.1) as well as to evolution in myelofibrotic phase. In conclusion, younger patients with ET seem to have a similar clinical presentation when compared with patients aged > 40 years; JAK-2 V617F mutation seems to occur in a lower rate in younger patients, but it does not seem to affect the incidence of thrombotic events.

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CORRELATIONS BETWEEN JAK-2 STATUS AND HYSTOLOGICAL FEATURES IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET)

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The V617F JAK-2 mutation has been recently described in about 50% of patients with ET: however, its correlation with the most common ET histological features has not yet been reported in details. We evaluated JAK-2 mutational status in 225 patients with ET, diagnosed at our Institution from 12/80 to 12/2008 according to PVSG criteria, and correlated this finding with the main ET histological characteristics at onset. There were 79 males (35.4%) and 146 females (64.6%) with a median age of 50.4 years (interquartile range 38.5-62.3); quantitative mutational analysis revealed a wild-type JAK-2 in 108 patients (48%) and a V617F JAK-2 mutation in 117 patients (52%), with a mutant allele burden < 50% (heterozygous status) in 98 (43.4%) and > 50% (homozygous status) in 19 (8.6%). As concern histological characteristics at onset, median percentage of marrow cellularity was 50% (interquartile range 40-60). A megakaryocytic hyperplasia was found in all but one patients (99.6%), with the presence of megakaryocyte clusters in 76/225 patients (33.8%); in addition, a granulocytic or a trilinear hyperplasia was detected in 83

patients (36.9%). Dysplastic features were observed in 212/225 patients (94.2%); in 166 patients only megakaryocytic line was involved, while in 46 patients dysplastic features were present also in granulopoietic and/or erythropoietic lines. A grade 0/0-I marrow fibrosis was reported in 151 patients (67.1%) while a grade I/I-II marrow fibrosis was present in the remaining 74 (32.9%) patients; a reactive interstitial or nodular lymphocytic infiltration was observed in 29/225 patients (12.9%). Correlating these histological features with JAK-2 mutational status, wild-type and heterozygous patients did not differ significantly for any histological characteristic evaluated. On the other hand, patients with > 50% V617F JAK-2 allele burden showed a statistically significant increase in median marrow cellularity (p=0.003 vs heterozygous patients; p=0.01 vs wild-type patients) and megakaryocyte clusters (p=0.031); in addition, homozygous patients showed a significant lower incidence of associated granulopoietic dysplastic changes (p=0.015). Interestingly, no difference was observed as to marrow fibrosis as well as to lymphocytic infiltration. In conclusion, these preliminary data seem to show that ET patients with wild-type and heterozygous V617F JAK-2 mutation constitute a unique disease, while homozygous patients seem to have a different histological pattern as also reported for clinical characteristics.

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SEQUENTIAL EVALUATION OF JAK-2 V617F ALLELE BURDEN IN HETEROZYGOUS PATIENTS WITH MYELOPROLIFERATIVE SYNDROMES (MPS)

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JAK-2 V617F mutation is an emerging tool in the diagnosis and management of Myeloproliferative Syndromes (MPS); however, prospective evolution of JAK-2 V617F allele burden and its correlation with clinical characteristics and treatment are still unclear. To address this issue, 86 patients with MPS and a 1st evidence of JAK-2 V617F mutation in a heterozygous state (allele burden < 50%) were prospectively studied for allele burden variations There were 36 males and 50 females, median age was 56.7 years, interquartile range (IR) 46.4-66.5 (M/F 57/69); according to MPS type, there were 45 Essential Thrombocythemia (ET), 35 Policythemia Vera (PV) and 6 Idiopatic Myelofibrosis (IM). Median time from diagnosis to 1st JAK-2 allele burden evaluation was 6.1 years (IR 2.3-14.1); median interval between 1st and 2nd JAK-2 allele burden evaluation was 12.0 months (IR 11.1-13.9). As to treatment, 33 patients (38.3%) had not received any treatment while 53 patients (61.7%) were under chemotherapy (46 with Hydroxyurea, 6 with Pipobroman and 1 with Melphalan) when both JAK-2 allele burden evaluations were performed. JAK-2 V617F allele burden median values at 1st and 2nd analysis in the whole population were 23.1% (IR 7.5-34.7) and 27.1% (IR 13.1 -38.4), respectively (p<0.001). In patients with ET, JAK-2 V617F allele burden median values at 1st and 2nd analysis were 17.9% (IR 4.4-32.6) and 28.5% (11.0-39.2), respectively (p=0.001), while in patients with PV they were 26.9% (IR 9.5-35.5) and 23.0% (14.5-38.9), respectively (p=0.035). In patients without therapy, there was only a trend to allele burden increase from 1st to 2nd evaluation (median values 29.3% and 32.9% respectively, p=0.07), while the increase was statistically significant in patients receiving chemotherapy (median values 17.9% and 22.4% respectively, p=0.002). Patients with a shorter disease duration (< 6 years) showed a V617F allele burden increase from the 1st median value of 22.3% (IR 6.1-33.7) to the 2nd median value of 24.2% (IR 11.8–36.3) (p=0.023); patients with a longer previous disease duration (>6 years) had an allele burden increase from the 1st median value of 24.1% (IR 9.2– 35.7) to the 2nd median value of 29.2% (IR 13.6–40.5) (p=0.003). In conclusion, JAK-2 V617F allele burden appears to increase significantly over time in patients with MPS; this increase seems to occur in any type of disease and at any time from diagnosis. In addition, chemotherapy does not seem to reduce the allele burden increase. However, a larger number of patients is needed to examine the role of the different types of chemotherapy.

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JAK2V617F MUTATION IN BUDD-CHIARI SYNDROME AND NON-CIRRHOTIC EXTRA-HEPATIC PORTAL VEIN OBSTRUCTION

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The Budd-Chiari Syndrome (BCS) and Non-Cirrhotic Extra-Hepatic Portal Vein Obstruction (EHPVO) are characterized by hepatic venous outflow obstruction, generally due to venous thrombosis. These rare diseases are usually caused by multiple concurrent factors, including acquired and inherited thrombophilias. Since the diagnosis of myeloproliferative neoplasms is often difficult in patients with BCS and EHPVO because of spleen enlargement, secondary pancitopenia and bleeding disorders, recent observations have included in their diagnostic work-up the analysis of the JAK2 mutation. The aim of this study was to evaluate the prevalence and the levels of the JAK2V617F mutation in the patient population affected by BCS and EHPVO followed at our Hepatology Division. The JAK2 mutation was evaluated by allele-specific real-time TaqMan polymerase chain reaction. We enrolled in this study 34 patients (17 males and 17 females; median age: 38±9.8 years,) affected by BCS (9 patients), EHPVO (22 patients), or both (3 patients). The JAK2V617F was detected in 20 patients (58.8%): 11 (55%) in the heterozygous status and 9 (45%) in the homozygous status. Among these, 3 had a diagnosis of Polycythemia Vera (2 heterozygous and 1 homozygous), 6 of Essential Thrombocythemia (3 heterozygous and 3 homozygous), 5 of Primary Myelofibrosis (1 heterozygous and 4 homozygous). In the remaining 5/6 patients the bone marrow biopsy revealed the presence of initial marrow fibrosis: grade 0-1 (4 patients affected by EHPVO, 3 of them with heterozygous deletion and 1 homozygous) and grade 1 (heterozygous patient affected by EHPVO). One refused the bone marrow biopsy. Our results suggest that although JAK2V617F plays an important role in patients with BCS and EHPVO patient, additional factors are implicated in the pathogenesis of these diseases prompting further studies on others related acquired genetic alterations.

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A SIMPLE SCORING SYSTEM BASED ON LEUKOCYTE ALKALINE PHOSPHATASE ACTIVITY AND PERIPHERAL GRANULOCYTE PRECURSOR PERCENTAGE PREDICTS JAK2 V617F MUTATION IN PATIENTS WITH PRIMARY MYELOFIBROSIS

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A recurrent mutation in the *JAK2* gene consisting of a valine-to-phenylalanine change at position 617 (JAK2 V617F) was reported in half and more of the patients with primary myelofibrosis (PMF). This mutation increases JAK2 kinase activity affecting phenotype and clinical outcome. Recently, some of us observed that in PMF JAK2 V617F mutation is significantly associated with higher white blood cell count and granulocyte activation and it independently predicts the evolution toward large splenomegaly, need of splenectomy and leukemic transformation. Since increased leukocyte alkaline phosphatase (LAP) expression is considered a granulocyte activation marker and variable LAP levels are found in PMF, we evaluated LAP activity by cytochemistry in peripheral blood smears from 266 PMF patients at diagnosis searching for possible correlations among LAP score, JAK2 V617F mutation status, as assessed by allele-specific PCR, and clinical-pathological features. JAK2 V617F mutation was identified in 162 patients (61%). In these patients LAP scores were significantly higher than in nonmutated cases (p<0.0001), without difference between homozygous and heterozygous mutations. Score values above the normal range were observed only in patients carrying the mutation. No correlation was found between LAP score and white blood cell or platelet count, hemoglobin concentration, splenomegaly, Lille prognostic score, while there was a significant inverse correlation between LAP score and peripheral granulocyte precursor or blast percentage (p=0.0003). On the other hand, a multivariate analysis showed a sig-

nificant association of JAK2 V617F mutation with lower peripheral granulocyte precursor percentage. A ROC curve analysis allowed us to identify a LAP score of 100 (AUC= 0.81, 95% CI 0.76-0.86) and a peripheral granulocyte precursor percentage of 10 (AUC= 0.80, 95% CI 0.64-0.79) as optimal cut-off to discriminate mutated patients with good sensitivity and specificity (range 84-98%). On the basis of these variables, we defined a simple scoring system to predict JAK2 V617F mutation (Table). All cases with a score of 3 carried the mutation, while 94% of patients with a score of 0 showed a wild-type gene. Then, we prospectively tested this score system in a new cohort of 105 PMF patients obtaining superimposable results. In conclusion, we suggest a very simple, low expensive and reproducible method based on old techniques to predict a novel mutation.

Score		
0	1	2
LAP score <100	--	LAP score ≥100
Peripheral granulocyte precursors ≥10%	Peripheral granulocyte precursors <10%	--

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A RETROSPECTIVE SURVEY ON ROUTINE CLINICAL MANAGEMENT OF POLYCYTHEMIA VERA PATIENTS OUTSIDE OF CLINICAL TRIALS: COMPLIANCE TO DIAGNOSTIC/THERAPEUTIC GUIDELINES AND PROGNOSTIC FACTORS

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Several guidelines have been published for polycythemia vera (PV) diagnosis and therapy. However, their impact on routine clinical practice is still undetermined. Moreover, the need of haematocrit (Ht) values < 0.45 for thrombosis prevention has been recently questioned. We retrospectively revised, according to 2001 or 2007 WHO criteria, 300 patients with a polycythemia vera diagnosis. The impact of median Ht, chemotherapy and known prognostic factors on overall and thrombosis-free survival was evaluated. Twenty-five percent of PV diagnosis, all established before the availability of JAK2 tests, did not satisfy WHO criteria; conversely 94% of recent diagnosis were confirmed by JAK2 V617F mutation. Further evaluations were made on the 226 patients with a confirmed PV diagnosis. Median age was 66 and 70% were in the high thrombotic risk group according to Finazzi and Barbui (Blood Rev 2005;19:243-252). Ninety-eight % of patients received anti-platelet and/or anticoagulant therapy. Cytoreductive treatments (hydroxyurea alone in 82% of cases) was given to 151 patients. Survival rate was 91% at the median follow-up of 5.84 years and projected to 89% at 13 years, with a 4% cumulative incidence of acute myeloid leukemia. Median Ht level was evaluated on 211 patients with at least 3 blood counts/year. Only 22% of patients maintained an Ht < 0.45: their overall and thrombosis-free survival did not significantly differ from those of patients with a 0.45-0.48 value. Conversely, an Ht > 0.48 was significantly associated, by both univariate and multivariate analysis, to shorter survival and higher risk of major thrombosis. *High thrombotic risk* according to Finazzi and Barbui significantly correlated to shorter overall and thrombosis-free survivals. Chemotherapy reduced thrombotic risk without affecting survival. Our study evidenced incomplete compliance to diagnostic and therapeutic guidelines outside of clinical trials. Wide use of anti-platelet and avoidance of alchilating drugs possibly improved survival. Some prognostic factors have been confirmed, whereas the optimal Ht to be pursued was not defined, although a value < 0.48 looks highly advisable.

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INCIDENCE OF LEUKEMIC TRANSFORMATION AND SECOND NEOPLASIA IN CMPDS PH -

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Introduction. The aim of this study was to analyse the incidence of blastic transformation during Ph- chronic myeloproliferative disorders (CMPD Ph-) and the occurrence of second tumors. *Materials and Methods* We analysed the clinical and hematological characteristics of 694 patients (pts) diagnosed as having CMPD Ph- between 1977 and 2008 and followed up for a median of 144 months (range: 12-376): 95 with chronic idiopathic myelofibrosis (CIMF) (56 M and 39 F, M/F ratio 1.44; median age 65 years, range: 27-85); 153 with polycythemia vera (PV) (79 M and 74 F, M/F ratio 1.06; median age 58 years, range: 18-86), and 446 with essential thrombocythemia (ET) (169 M and 277 F, M/F ratio 0.6; median age 61 years, range: 18-87). *Results*: The incidence of acute transformation was of 8/95 CIMF pts (8.4%) (4 M and 4 F, M/F ratio 1; median age 63 years, range: 58-78); 4/153 PV pts (2.6%) (2 M and 2 F, M/F ratio 1; median age 63 years, range: 47-65); and 17/446 ET pts (3.8%) (8 M and 9 F, M/F ratio 0.9; median age 62 years, range: 39-81). All of the patients with CIMF in blastic transformation died, two of the PV pts and one ET pt are still alive. The blastic transformation was of myeloid/lymphoid lineage in 8/0 CIMF, 4/0 PV, and 15/2 ET pts. Second tumors developed in 13 CIMF pts (14%) (5 M and 8 F, M/F ratio 0.6; median age 72 years, range: 42-84), one PV pt (0.6%) (1 F; median age 68 years), and 32 ET pts (7.2%) (15 M and 17 F, M/F ratio 0.8; median age 62 years, range: 43-81). Nine (69%) of the CIMF pts with a second tumour are still alive, as are the one PV patient (100%) and four of the ET pts (12.5%). The second tumor was of epithelial lineage in all 13 CIMF pts, the one PV pt, and 16/32 ET pts. *Conclusions*: Our second malignancies, hematological or other, data are in line with our previous results [Radaelli *et al*., Hematology 2008] and those of Passamonti et al. [Haematologica, 2008] and Abdulkarim *et al*. [Eur J Haematol, 2009].

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LEUKOCYTOSIS IS A RISK FACTOR FOR RECURRENT THROMBOSIS IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background. Leukocytosis is associated with an increased risk of first thrombosis in patients with polycythemia vera (PV) and essential thrombocythemia (ET). Whether it is a risk factor for recurrent thrombosis too is currently unknown. *Aims.* In order to investigate the impact of leukocytosis on the risk of recurrent thrombosis in patients with PV and ET, we carried out a multicenter retrospective cohort study. *Patients and Methods.* We recruited 253 patients with PV (n=133) or ET (n=120), with previous arterial (70%) or venous major thrombosis (27.6%) or both (2.4%), and not receiving cytoreduction at the time of thrombosis. The median leukocyte count at that time was $10.2x10^9$/L (range 3.1-24.9). The total observation time after thrombosis was 1,602 pt-years (medi-

an 5.5). The hazard ratio (HR) for recurrent thrombosis was adjusted for gender, diagnosis (PV or ET), age at the time of the initial thrombosis (>60 or <60 years), presence of one or more vascular risk factors, history of remote thromboses, type of first thrombosis (arterial or venous), haematological parameters at the time of the first thrombosis (hematocrit, white blood cell count, and platelet count > the respective upper quartile), and type of treatment following thrombosis. *Results.* Thrombosis recurred in 78 patients (30.7%); age >60 years at the time of the first thrombosis was an independent predictor of recurrence (HR 2.00, 95%CI 1.23-3.37) and recurrence was prevented either by antiplatelet treatment (HR 0.38, 95%CI 0.19-0.77) and cytoreduction (HR 0.45, 95%CI 0.28-0.73). The patients with a leukocyte count in the highest quartile >12.4x10^9/L at the time of their first thrombosis had an increased risk for arterial recurrence in respect to all the remaining patients (HR 2.16, 95%CI 1.12-4.18).The increased risk for arterial recurrence associated with leukocytosis was confirmed among patients <60 years (HR 3.35, 95%CI 1.22-9.19). Leukocytosis was not significantly associated with the risk for recurrence among the patients >60 years neither predisposed to venous recurrences. *Conclusions*. In the younger patients with PV or ET leukocytosis at the time of first thrombosis is associated with an increased risk of future arterial thrombotic events. It is suggested that leukocytosis could be an important tool of patient stratification not only among the low risk individuals without history of thrombosis but also among the young high risk individuals with a previous history of thrombosis.

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THE RISK OF RECURRENT THROMBOSIS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA IS INCREASED IN CARRIERS OF HOMOZYGOUS JAK2 V617F MUTATION

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Background. Some evidence suggests that the JAK2 V617F mutation is associated with an increased risk of first thrombosis in patients with essential thrombocythemia (ET). Whether is a risk factor for recurrent thrombosis too is currently unknown. *Aims*. In order to investigate the impact of the JAK2 V617F mutation on the risk of recurrent thrombosis in patients with ET, we carried out a multicenter retrospective cohort study. *Patients and Methods.* We recruited 143 patients with ET, with previous arterial (64.4%) or venous major thrombosis (34.8%) or both (0.8%). The total observation time after thrombosis was 922 pt-years (median 5.5). All patients were tested for the presence of the JAK2 V617F mutation in granulocytes by allele-specific polymerase chain reaction; 98 of them (68.5%) carried the mutation. Information about the patient's mutant allele burden was recorded in 47 mutated patients. Heterozygous (n=42) or homozygous (n=5) status was defined as a mutant allele burden < 50% or > 50%, respectively. The hazard ratio (HR) for recurrent thrombosis was adjusted for gender, age at the time of the initial thrombosis (>60 or <60 years), presence of one or more vascular risk factors, history of remote thromboses, type of first thrombosis (arterial or venous), presence of the JAK2 mutation, and type of treatment following thrombosis. *Results*. Thrombosis recurred in 43 patients (30%); the presence of the JAK2 mutation did not predict recurrence (multivariable HR 0.88, 95%CI 0.46-1.68). This finding was substantially unchanged after stratification of the patients according to the age at the time of the first thrombosis (<60 years or >60 years), the type of first thrombosis (arterial or venous), and administration of cytoreduction after the first thrombosis. Indeed, homozygotes for JAK2 V617F had an increased risk of recurrence in comparison with wild-type patients (HR 6.15, 95%CI 1.51-24.92); in respect to the heterozygous patients, the risk was increased but without reaching the statistical significance (HR 2.94, 95%CI 0.73-11.78). The heterozygous patients had a risk of recurrence quite similar to that of the wild-type patients (HR 0.99, 95%CI 0.45-2.14) *Conclusion*. In the patients with ET and carrying the JAK2 V617F mutation with a mutant allele burden > 50% the risk of recurrent thrombosis is increased, whereas a lower mutant allele burden is not associated with recurrent thrombosis.

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INCREASED RISK OF SPLANCHNIC OR CEREBRAL VENOUS THROMBOSIS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA CARRYING THE JAK2 V617F MUTATION

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Background. We previously reported in a cohort of patients with splanchnic venous thrombosis (SVT) or cerebral venous thrombosis (CVT) the presence of the JAK2 V617F mutation in 95% of those with overt myeloproliferative neoplasms (MPN), in 21% of those with SVT and without overt MPN, and in 5% of those with CVT and without overt MPN (De Stefano et al, J Thromb Haemost 2007, 5: 708). Moreover, the mutation is actually known to be associated with an increased risk of thrombosis in patients with essential thrombocythemia (ET). Aims: In order to investigate the impact of the JAK2 V617F mutation on the risk of thrombosis in unusual sites in patients with ET, we carried out a retrospective cohort study. *Patients and Methods.* We recruited 201 patients with ET (M/F 68/133, median age at diagnosis 55 years, range 20-92): 142 were asymptomatic and the remaining ones had suffered from arterial thrombosis (n=34,16.9%), venous thrombosis in common sites (n=12, 5.9%), SVT (n=10, 4.9%), and CVT (n=3, 1.5%). All the patients were tested for the presence of the JAK2 V617F mutation and for inherited thrombophilia. Results: The mutation was present in 122 patients (60.6%), namely in 74 asymptomatic ones, in 37 with arterial thrombosis or venous thrombosis in common sites, in eight with SVT, and in all the three patients with CVT. Therefore, the relative risk (RR) of thrombosis in unusual sites (SVT or CVT) associated with the mutation in ET patients was 1.62 (95%CI 1.22-2.14) in comparison with the patients without history of thrombosis, namely 1.53 (95%CI 1.08-2.17) for SVT, and 1.91 (95%CI 1.63-2.24) for CVT. All the patients with SVT or CVT were aged <60 years: among the patients within this age range, the RR associated with the mutation in respect to the asymptomatic individuals was 1.99 (95%CI 1.41-2.81) for overall thromboses in unusual sites, 1.88 (95%CI 1.26-2.81) for SVT, and 2.35 (95%CI 1.82-3.03) for CVT. Those results did not substantially change after exclusion of the patients with inherited thrombophilia: one with SVT, one with CVT, and three without thrombosis (*data not shown*). It is notheworthy that the presence of the mutation was not associated with any significant increase in the risk of deep venous thrombosis in common sites neither in the overall cohort (RR 1.27, 95%CI 0.71-2.30) neither in the patients <60 years (RR 1.56, 95%CI 0.84-2.91). *Conclusion*. The JAK2 V617F mutation is a risk factor for venous thrombosis in unusual sites (splanchnic or cerebral veins) in young patients with ET.

Non-Hodgkin's Lymphoma II

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RADIOIMMUNOTHERAPY IN FOLLICULAR NON-HODGKINS LYMPHOMA PATIENTS: RELEVANCE OF THE TIME OF TREATMENT

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Background. Radioimmunotherapy (RIT) with Zevalin is a novel treatment option for non-Hodgkin lymphoma (NHL). Eoncouraging results have been reported, even though due to the different settings of pts that have been treated, they are extremily heterogeneous. Most recent experiences have shown better results when the treatment was started in an early and more sensitive phase of the disease. *Aims*. The aim of our study is to verify the RIT efficacy, safety and toxicity in pts affected by follicular lymphoma (FL). Pts were stratified according to diagnosis time, disease status and to the quality and quantity of previous therapeuthic approaches received. *Methods*. Since December 2005 to date, 20 pts (M/F 10/10; mean age 57 years – range 45-73) with FL in different phases of the disease have been treated with RIT: one patient at diagnosis (I group); 7 pts in PR after first line therapy (II group); 5 relapsed pts after a line of treatment (III group); 4 refractory/relapsed pts after 2 or more lines of treatment (IV group); 3 refractory/relapsed pts after multiple lines of treatment including autologus stem cell transplantation (ASCT) (V group). All pts received the classic scheduled combination of Rituximab 250 mg/mq on days 1 and 8 followed by Zevalin (15 MBq/kg). All pts received Cotrimoxazole, Itraconazole and Acyclovir as anti-infective prophylaxis. *Results*. We observed 17 CR (85%) distributed as follows: 13 (100%) in the first 3 groups; 2 out of 4 (50%) and 2 out of 5 (66%) in the 4th and 5th group, respectively. In 8 pts molecular responses were also documented. Out of the 17 pts in CR, 6 relapsed between +5 and +20 months from RIT. RIT was well tolerated and a WHO grade 3/4 hematological toxicity was observed in 8 pts (40%). One patient underwent RIT after post ASCT relapse, showed a secondary myelodysplastic syndrome. *Conclusions*. Treatment with RIT has been well tolerated. Hematologic and molecular responses were satisfactory in terms of long lasting remission. Hematologic toxicity was acceptable. There were no documented infections and no hospitalization was necessary after RIT. Our experience has shown that RIT efficacy was inversely proportional to the number of previous therapeuthic approaches

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ROLE OF CEREBROSPINAL FLUID FLOW CYTOMETRY ANALYSIS IN NEWLY DIAGNOSED AGGRESSIVE NON-HODGKIN LYMPHOMAS AT HIGH RISK FOR LEPTOMENINGEAL DISEASE (LD): RESULT OF A MULTICENTRIC PROSPECTIVE ITALIAN STUDY

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Background. Flow cytometry (FCM) assessment of cerebrospinal fluid (CSF) has recently been known to increase the rate of positivity of occult LD in comparison to conventional cytologic examination (CC). However it's still unknown its prognostic value. *Design and Methods*. The aim of this study was to compare CC vs FCM in a large cohort of patients with newly diagnosed aggressive NHL at high risk for LD (diffuse large B-cell lymphoma (DLBCL) IPI 2-3 and elevated LDH with at least two extranodal sites or with bone marrow or testis or palate or eye-socket involvement; Burkitt lymphoma (BL); blastoid variant of mantle cell lymphoma (B-MCL); B-cell precursor lymphoblastic lymphoma (B-LL); HIV+ patients) All patients were required to have no evidence or signs of neurological disease. All patients received intrathecal standard prophylactic therapy. The incidence of positive test for occult LD with both FCM and CC was compared using the McNemar test for paired data.We also assessed the impact of detecting occult LD with FCM on progression-free survival (PFS). *Results*. From August 2004 to June 2008, 118 patients were enrolled by 10 centres. Clinical characteristics were: median age 55 years (IQR:43-63); DLBCL 88 patients (75%); BL 18 pts (15%); B-MCL 8 pts (7%); B-LL 4 pts (3%); 18 pts (15%) were HIV positive. FCM was able to detect a clonal population in 16 out of 118 patients (14%) whereas CC detected abnormal cells only among 7 pts (6%)(p= 0.0002, McNemar's 2). Therefore, 9 patients (8%) were discordant: FCM positive/CC negative.

Table 1. Patient characteristic.

PATIENT CHARACTERISTIC	FCM+/CC+	FCM+/CC- (DISCORDANT)
Histology		
DLCL-B	5	5
BL	2	3
B-MCL	0	0
B-LL	0	1
HIV+	2	1
Stage		
I-II	1	0
III-IV	6	9
Extranodal disease		
0-1	3	3
>1	4	6
BM	5	6
Testis	1	0
Palate/socket	0	1
IPI		
1-2	0	0
>3	7	9
LDH > normal	5	8

From date of diagnosis, overall median follow up of survivors was 11 months (IQR:5-18). We observed 22(19%) systemic progressions, 5(4%) CNS progressions and 19(16%) deaths (17 PD, 2 infections). PFS at 6 months was 94% (95%CI:86-97) in pts both negative in FCM and CC, 57% (95%CI:17-84) in pts both positive, 83% (95%CI:27-97) in pts discordant (p= 0.0029, log-rank test). *Conclusions*. FCM assessment of CSF has an higher sensitivity than CC but it's non yet clear what is the clinical relevance of a positive FCM. Our preliminary data suggest that patients both positive for FCM and CC have an higher risk of progression compared with those both negative, whereas discordant cases may have an intermediate prognosis. Data with longer follow up actually in evaluation will provide futher information regarding this issue.

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GEMCITABINE AS SINGLE AGENT IN PRETREATED T-CELL LYMPHOMA PATIENTS: LONG-TERM OUTCOME OF 38 PATIENTS

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Among the several second-line and experimental drugs, gemcitabine should be considered among the most suitable options for pretreated peripheral T cell lymphoma unspecified (PTCLU) and mycosis fungoides (MF) patients. Gemcitabine has been demonstrated to be an effective monotherapy with a 60-70% overall response rate in advanced, heavily pretreated patients. We report the long-term update of the outcome of 38 heavily pretreated T-cell lymphoma patients after salvage treatment with gemcitabine. Between May 1997 and September 2007, 38 patients with previously treated MF and PTCLU completed treatment with gemcitabine at our institute. Inclusion criteria included the following: histologic diagnosis of MF or PTCLU; relapsed/refractory disease after at least two conventional therapeutic approaches; age greater than 18 years; WHO performance status ≤ 2. Nineteen of 38 patients had a diagnosis of MF and 19 PTCLU. The median age of the patients was 54

years (range, 32-78 years); 28 patients were male and 10 were female. The median number of prior treatments was three (range, 2 to 8). Gemcitabine was given to all patients on days 1, 8, and 15 of a 28-day schedule at a dose of 1200 mg/m^2 per day for a total of three-six cycles. The overall response rate was 50% (19 of 38 patients); the CR and PR rates were 21% (8 of 38 patients) and 29% (11 of 38 patients), respectively. According to the histologic subtypes, patients with MF had a CR rate of 16% (three of 19 patients) compared with 26% (five of 19 patients) among the patients with PTCLU. Patients with MF had a PR rate of 31.5% (6 of 19 patients) compared with 26.5% (5 of 19 patients) among the patients with PTCLU. Among the CR patients, 6/8 are in CCR with a disease-free interval of 120, 43, 34, 22, 18 and 15 months respectively; two of them were MF and 5 PTCLU. This study confirms the high efficacy of gemcitabine in heavily pretreated T cell lymphoma (MF and PTCLU) patients with the possibility to have a subset of real long-term responders.

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THE HISTONE DEACETYLASE INHIBITOR ITF2357 (GIVINOSTAT) PROMOTES BURKITT'S LYMPHOMA CELL LINE DEATH MODULATING MICRO-RNA AND TISSUE TRANSGLUTAMINASE 2 EXPRESSION

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Background. Epstein-Barr virus infection and c-myc overexpression are the hallmarks of Burkitt's lymphoma (BL). Recent studies support the existence of a c-myc-microRNA (miRNA) interaction within the genesis and the maintenance of the lymphoma phenotype. Furthermore, myc oncoproteins have been found to inhibit the transcription of tumor suppressor genes, including tissue transglutaminase 2 (TG2), a multifunctional protein that promotes apoptosis and differentiation in normal tissues. This can occur by recruiting histone deacetylase (HDAC) 1 proteins to target genes. Therefore, we tested ITF2357 (Givinostat), a new hydroxamate inhibitor of HDAC, on BL cell lines with respect to its effects on cell viability and on c-myc mRNA, miRNAs and TG2 expression modulation. *Methods and Results*. ITF2357 induced late and early apoptosis respectively on Namalwa and Raji BL cell lines, exhibiting an IC50 of 200nM after 48h from drug administration. Accordingly, ITF2357 induced subG1 peak formation in Namalwa and G1 arrest in Raji cells. Notably, c-myc mRNA decreased only in Namalwa cells after treatment. ITF2357 treated Raji cells instead showed a gradual increase of c-myc mRNA, however paralleled by a reduction of c-myc protein. The profound miRNA modulations, particularly evident in treated Raji cells as assessed by array analyses and quantitative real-time PCR, could explain these apparently inconsistent observations. MiR-155 and miR-98, known for their oncogenic activity in myc-associated tumors, were significantly down regulated after treatment. Conversely, ITF2357 induced the expression of Let-7a, which has been shown to negatively affect c-myc at posttranscriptional level. Finally, immunohistochemical analysis revealed an increased cytoplasmatic expression of the tumor suppressor TG2 in ITF2357-treated Raji cells, compared to their untreated counterparts. *Conclusions*. ITF2357 demonstrated potent cytotoxic and growth inhibitory activities on BL cell lines. These effects might be related to the restoration of the expression of different c-myc targets, including oncogenic and tumor-suppressing miRNAs and the tumor suppressor TG2. Accordingly, the reversion of c-myc abnormal expression was achieved in both BL cell lines studied. The potential candidacy of ITF2357 as a therapeutic agent for BL awaits further evidences from ongoing analyses in animal models.

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EFFICACY OF 18F-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY (FDG-PET) AT THE END OF THERAPY IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) TO DEFINE COMPLETE REMISSION (CR)

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Background. The response evaluation is performed by computer tomography (CT) as a standard tool in DLBCL. The introduction the FDG-PET performed for post treatment response assessment of aggressive Non Hodgkin's Lymphoma (NHL) is advised by the Revised International Workshop Criteria (IWC). We explored the predictive value by FDG-PET scan performed at the end of therapy after 6 cycles of R-CHOP14 in patients (pts) with DLBCL. *Patients*. From 2002 at 2008 we treated according to R-CHOP14 schedule for 6 cycles 49 pts with DLBCL. The FDG-PET and CT was mandatory at baseline and at the end of therapy to include pts in the study. We evaluated the progression free survival (PFS) of pts starting from the time of diagnosis to relapse or progression of disease or last follow-up. *Results*. Median age was 57 years (34-73), 22 pts were female and 27 male, 25 pts presented stage I-II and 24 stage III-IV. The International Prognostic Index (IPI) was low in 51% of pts, low-intermediate in 27%, intermediate-high in 14% and high risk in 8%. Bulky was reported in 16 pts; fourty-two pts attained complete remission (CR) (86%) and 7 pts (14%) partial remission (PR). The FDG-PET and CT performed at the end of therapy were both negative in 34 pts (70%); both positive in 6 pts (12%) and in 9 pts (18%) the FDG-PET and CT scan performed post therapy were discordant (FDG-PET negative and CT positive). Thus the positive predictive value of a FDG-PET at the end of therapy was 67% and the negative predictive value was 84%. The sensitivity and specificity of FDG-PET at the end of therapy were 36% and 95% respectively. Sixteen pts showed bulky disease at the diagnosis; 7 out 16 of these pts showed disease progression or relapse. The FDG-PET performed at the end of therapy was positive in 2 pts with bulky disease moreover 5 out 14 bulky pts with FDG-PET negative relapsed. Therefore an high rate of false negative FDG-PET was observed in this group of pts. In the same group of bulky disease pts the CT was positive in 4 out 5 relapsed pts. The overall survival at 24 months was 92% with a median follow up of 20 months (range 9-83) and the PFS was 77% with a median follow up of 13 months (range 4-78). *Conclusions*. The FDG-PET shows an high specificity but a very low sensibility in DLBCL. Obviously larger study are needed but at the moment CT remain the most sensible instrument to define CR. A negative FDG-PET at the end of therapy is not necessary associated with a long PFS.

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SAFETY AND EFFICACY REDUCING GRANULOCYTE COLONY-STIMULATING FACTORS (G-CSF) IN R-CHOP14 SCHEMA

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Background. Rituximab plus CHOP14 is increasingly used in the treatment of diffuse large B cell lymphoma (DLBCL). Some recent trials (Mint and RICOVER 60) lead to the conclusions that R-CHOP14 could be considered the standard of care for young patients (pts) with good prognosis and for elderly pts with DLBCL. Dose dense therapy is feasible with GCS-F support which is recommended for10 days. *Aims and Methods*. Starting from 2002 we have included 60 pts with DLBCL and 5 with follicular lymphoma grade IIIb, median age was 61 years (range 34-79), 60% had an high-intermediate or high IPI. We prospectively decided to use 7 vials of G-CSF (from +5 to +11) in 1rst 10 pts and in absence of infections or delays the following pts were treated with 5 vials (from +7 to +11). Moreover if pts reached a number of leucocytes over 20.000/mm3 we reduced again the number of vials until 3 for cycles. CHOP was administered every 14 days, preceded on day 1 by rituximab and followed by 7 (in the 1st 10 pts), 5 or 3 days (3 vials in 40% of pts) of G-CSF (filgastrim). Haematological toxicity and feasibility was calculated over 381 cycles administered. *Results*. We have used 1526 GCS-F vials, 5 vials (range 2-7) for cycle and a median of 24 vials (range 10-35) for every pts. The programmed therapy was completed in 62 out 65 pts (96%); 3 pts swichted to a different scheduling (R-CHOP21). Twelve cycles (3%) have been delayed in 10 pts for severe adverse events. Neutropenia grade 3-4 developed in 2.3% of cycles, febrile episodes in 1.2% of cycles, thrombocytopenia grade 3 or 4 in 1.2% of cycles and hospedalization in 1% of pts. Of the 381 cycles considered, the median nadir of leucocyte was 3850×10^9/L (range 400-8400), the median nadir of haemoglobin was 11.3 gr/dl (range 5.8-15.7) and the median nadir of platelets was 135000 (range 43000-328000). The complete remission rate was 87% and after a median period of 29 months overall survival was 81%. *Conclusions*. In our experience, dose dense chemotherapy (R-CHOP14) supported by G-CSF has been well tolerated also elderly pts. We confirm that the reduction from 10 to 5 or 3 GCS-F vials has not determined an increase of neutropenia, febrile episodes,

delays in the treatment and hospedalizations confirming and high rate of response to therapy. Thus R-CHOP14 can be performed with a lower number of G-CSF vials with a clinical and biological advantage for the pts.

P242

ALLOTRANSPLANT IN RELAPSED REFRACTORY AGGRESSIVE T-CELL LYMPHOMAS: RETROSPECTIVE ANALYSIS OF 14 PATIENTS

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Background. Relapsed aggressive peripheral T-cell lymphomas show unsatisfactory long-term survival. In these patients, allogeneic HSCT (allo-HSCT) is associated with low relapse rate but high transplant-related mortality (TRM). *Patients and methods*. We retrospectively analyzed the outcome of 14 resistant/relapsed aggressive T-cell lymphomas (5 peripheral T cell lymphoma-unspecified, 2 mycosis fungoides, 7 T-cell lymphoblastic lymphoma) who underwent to allo-HSCT between June 1983 and June 2008: 7 patients were in partial response (PR), 6 in progressive disease (PD) and 1 in stable disease (SD) at the time of transplantation. Nine (64%) patients received myeloablative conditioning (MA) regimens, whereas 5 (36%) patients underwent to a reduced-intensity conditioning (RIC) regimen, having a HLA-identical sibling donor in 10 (71%) cases, while a HLA-identical unrelated donor in 4 (29%). *Results*. Overall response rate was 79%. Eight patients achieved a complete response (CR): 4 T-LBL, 3 PTCL-U, 1 MF; 3 patients were in PR. Six out of the 8 patients in CR had received MA regimen, while 2 a RIC one. Among the 11 responding patients (CR+PR) after transplantation, 6 showed a PR and 5 a PD before allo-HSCT. Two of the 8 patients in CR after transplantation relapsed, both dying of disease progression. Six patients are alive and in continuous CR (median follow-up of 24 months). Estimated 10-year overall survival (OS) and relapse-free survival (RFS) rates are 43% and 75%, respectively. The 1-year TRM was 14%. Six patients developed acute GVHD (43%) and only one showed limited chronic GVHD. *Conclusions*. We show that allo-HSCT can be safe in patients with relapsed aggressive peripheral T cell lymphoma with a satisfactory estimated 10-year OS and RFS rates.

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LIVER BIOPSY DURING SPLENECTOMY REVEALS THAT SPLENIC MARGINAL ZONE LYMPHOMA IS OFTEN AN HEPATO-SPLENIC DISEASE

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Primary hepatic lymphomas are rare while secondary liver involvement by systemic lymphoma is relatively common. Except for hepatosplenic T-cell lymphoma, the incidence and clinical relevance of secondary liver involvement by other lymphoma subtypes, that primarily arise in the spleen, have not been so far evaluated. In particular, splenic marginal zone lymphoma (SMZL) is usually characterized by isolated splenomegaly and bone marrow infiltration, but the incidence of liver involvement isn't known. We analyzed splenectomies performed in pts affected by lymphomas to evaluate the incidence and features of liver involvement by lymphoma in wedge liver biopsies. We studied 45 pts with lymphoma who underwent a laparotomic splenectomy from 2001 to 2008: 19 SMZL, 15 primary splenic DLBCL, 1 secondary splenic DLBCL, 8 classic HL, 1 lymphoplasmocytic lymphoma, 1 hepatosplenic T-cell lymphoma. A concurrent wedge biopsy of the liver was available for all pts except for 5 (4 SMZL, 1 DLBCL). Liver involvement was detected in 18 pts: 11/15 pts (73 per cent) with SMZL, 4/15 primary splenic DLBCL, 3/8 HL, 1 hepatosplenic T-cell lymphoma. In SMZL a portal pattern of liver involvement was detected in 10/11 pts; in 7 pts it was associated to sinusoidal pattern, in 2 pts to a nodular/lobular pattern; in a single case the lymphoma infiltrate was confined to hepatic sinusoids. Bile duct lympho-epithelial lesions were detected in 6 cases, cholestatic changes in 5, ductopenia in 2 and hepatitic changes in 4. No case showed progression to DLBCL in hepatic infiltrates. HCV serology was positive in 5/15 SMZL. Three HCV+ cases showed liver involvement by lymphoma and were HCV-RNA; in these cases pattern of liver infiltration was portal; 1 had a double pattern of infiltration, portal and sinusoidal. The remaining 8 pts with SMZL involving the liver were HCV-. Phenotype was $CD20^+$, $CD79a^+$, $CD5^-$, $CD10^-$, bcl-6-, cyclin D1- in 15/15, $bcl2^+$ in 14/15, CD23- in 14/15 SMZL. Among the 12 cases of primary splenic DLBCL, 7 were HCV^+; 6 cases showed a germinal center (GC) phenotype, whereas 6 had a non-GC phenotype. Liver involvement was detected in 2 HCV- pts, with a nodular to diffuse pattern. Our data demonstrate that SMZL is characterized by a high incidence of liver involvement with a frequent portal and sinusoidal pattern. Interestingly, detection of hepatic localization by SMZL seems to be irrespective to HCV status infection and appears as an intrinsic feature of this lymphoma subtype.

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ASSOCIATION OF THE -174G>C POLYMORPHISM AND PLASMA LEVELS OF IL-6 WITH PROGNOSIS IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Diffuse large B-cell lymphomas (DLBCLs) are a subtype of non-Hodgkin's lymphoma characterized by a striking heterogeneity in clinical and biological behaviour. Recent studies of gene expression profiling suggest a critical role of tumour microenvironment and host inflammatory response in DLBCLs pointing to new promising biomarkers to better define patients' outcome. High serum levels of IL-6, a critical pro-inflammatory cytokine, have been reported to be associated to a poor prognosis in DLBCLs. Expression of IL-6 may be modulated by single nucleotide polymorphism (SNP) in the promoter region, such as G>C SNP at position -174 (IL-6/-174G>C). We analyzed genotype-phenotype associations for IL-6 in patients with DLBCLs, and their potential impact on clinical characteristics and outcome. Our study included 118 patients with DLBCL and 100 healthy controls. All subjects were genotyped for IL-6/-174 using a mismatch-RFLP-PCR. Plasma was available for 98 patients enrolled at diagnosis and before the start of any therapy and 75 controls. IL-6 plasma levels were measured by standard ELISA assay, with a limit of sensitivity of 0.001 ng/ml. For statistical analysis, we dichotomized IL-6 levels using the detection limit as cut-point. *Results.* Plasma levels of IL-6 were below the detectable limit of our assay in 42/75 (56%) controls and 49/99 (49.5%) patients. Within our control group, we found a significant correlation between the minor allele -174C and elevated levels of IL-6 in the plasma (OR, 4.3; C.I. 95%, 1.65-11.60; p=0.003) suggesting a genotype-phenotype association. Plasma levels of IL-6 were significantly higher in patients than in controls (p= 0.003), while no differences were found in genotype distributions between controls and patients. Patients who were carriers of the IL-6/-174 C gene variant were at higher risk for DLBCL with an unfavourable prognosis (IPI-score > 3; OR, 2.4; C.I. 95%, 1.0-5.65; p=0.05). In addition, IL-6 plasma levels were associated with an IPI-score > 3 (OR, 4.64; C.I. 95%, 1.65-13.07; p=0.004). As a consequence, elevated levels of IL-6 and the IL-6/-174 C gene variant were significantly correlated to an inferior overall survival (both p=0.04). Our results indicate that genetic determination of IL-6 production is associated with characteristics of disease at diagnosis and shows a prognostic impact in DLBCL.

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ROLE OF 18F-FDG-PET IN FOLLICULAR LYMPHOMA: A SINGLE CENTRE EXPERIENCE

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Follicular lymphoma (FL) is one of the most frequently occurring lymphoma entities in Europe and North America and is the most common indolent lymphoma. 18F-FDG-PET is becoming a routine measure for staging and follow-up of patients with aggressive lymphoma. By contrast, there are more limited data on its use in indolent lymphomas and, in particular, the role of this methodology in FL is still under investigation. We employed 18F-FDG-PET/CT (PET) in patients with FL referred to our Institution during the last three-year period. Overall, study population included 21 patients (11 males, 10 females; median age 58 years, range 42-77) with FL WHO grade I-II-III (5, 8 and 6 patients, respectively), plus 2 patients with mixed diffuse large B-cell lymphoma/FL. A total of 28 PET scans (9 before therapy and 19 for response assessment after treatment) were evaluated and compared with conventional staging. In 7 patients PET evaluation was longitudinally performed before and after therapy. Treatments were heterogeneous and given according to local policy, age and clinical conditions of patients, and research protocols applied: they included FND with or without Rituximab, R-CHOP or R-CHOP-like regimens, R-CVP, R-FM. Two patients received Rituximab or Chlorambucil alone, respectively. At diagnosis, seven cases demonstrated FDG avidity (78%), with a mean maximum standardized uptake value (SUV) of 8.2 (range 6.1-10.4). In one of the PET negative cases, disease had been completely excised. PET scan showed less diffused involvement than computed tomography (CT) in 2 of 9 patients, without, however, changing in the stage. At re-staging, twelve out of 19 evaluated patients had both CT and PET negative: all these patients maintain complete remission after a median follow-up of 26 months (range 11 to 43). By contrast, three patients who had CT negative/PET positive findings at re-staging experienced relapse/progression 12 to 23 months after completing induction therapy. Interestingly, one of these patients had achieved molecular remission after therapy, as showed by negative PCR for t (14;18) transcript. Four patients were PET and CT positive after therapy. All had progressive disease within few months and underwent alternative approaches. Though still preliminary and requiring confirmation on larger numbers of patients, our data indicate that FL is frequently FDG-avid and that persisting positive PET after treatment can contribute to identify patients with poorer prognosis.

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EFFICACY AND SAFETY OF YTTRIUM-90 IBRITUMOMAB TIUXETAN IN OLDER PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMA

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Radioimmunotherapy (RIT) has emerged as an important treatment options for patients with non-Hodgkin lymphoma (NHL). (e)90Y-ibritumomab tiuxetan (Zevalin®) consist of ibritumomab, a murine monoclonal antibody to CD20, conjugated to the metal chelator tiuxetan for retention of the β emitter (e)90Y. Clinical trials with this agent have demonstrated significant activity in indolent NHL with mild toxicity. The median age of NHL patients included in these trials is mainly < 65 years. Our aim was to evaluate the effectiveness of Zevalin as treatment option for patient > 65 years old with indolent NHL. Between November 2005 to January 2009 thirteen patients, three male and ten female, median age 76 years (range 67-82), with indolent NHL (11 follicular and 2 small lymphocytic) were treated with Zevalin. Six patients had stage IV disease, four stage III and three stage II. All patients received an initial infusion of rituximab at a dose of 250 mg/m^2 on day 1 and a second infusion at same dose on day 8 followed by a weight-based dose of Zevalin (median dose 1006 MBq; range 668-1260). Seven patients perfomed Zevalin as consolidation after first line therapy with Rituximab plus chemotherapy (5 R-CHOP, 1 R-FN, 1 R-COMP): of these two were in complete remission (CR) and five in partial remission (PR). Five patients perfomed Zevalin in relapse (three in first end two in second relapse). After RIT the overall response rate was 92% (10 CR and 2 PR); in particular all patients in first line of treatment achieved CR. One patient had stable disease. At a median follow-up of 11 months (range 4-30), all patients are alive and maintained the response. One of two patients in PR achieved CR after following therapy. Treatment was well tolerated; grade 3-4 thrombocytopenia were seen in 8 patients while grade 3-4 neutropenia in 5 patients. Only one patient developed herpex-zoster infection. In conclusion, in our experience, Zevalin produces high response rate (up to 90%) and durable remission in patients with indolent NHL with mild toxicity. Notably, in first-line treatment, consolidation with RIT resulting in PR-to-CR conversion in all five patients in PR after the R-chemotherapy. The favourable safety profile of the regimen makes it an effective treatment for older patients who, for age and comorbidity, are not eligible for intensive treatment as high-dose therapy and stem cell transplantation

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OTHER TUMORS IN PRIMARY CUTANEOUS LYMPHOMAS

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Aims. Mycosis Fungoides/Sézary Syndrome (MF/SS) patients are usually treated with multiple therapeutic regimens, increasing the risk of other neoplasms. We aimed to assess the incidence of other neoplasms in our MF/SS series collected in an regional academic hospital in Central Italy which is a referral center. *Patients and Methods.* The clinical records of 217 MF/SS patients diagnosed from March 1994 and with at least 24 months of follow up, were reviewed in order to detect the time of occurrence and the incidence of other neoplasms. *Results*. We studied 217 patients, 207 MF/10 SS, 144 M/3 F, with a median age 56 yrs (14-84). Overall, 52 other neoplasms were diagnosed in 46 patients (21.2%), 40 solid/12 haematological, 19 occurring before, 7 simultaneously and 25 after MF/SS diagnosis. Solid tumors were located more frequently in skin (11), colon (5), breast (5) and lung (4). Plasma cell disorders (8), acute leukemias (3) and B-cell lymphoma (1) were the haematological neoplasms. Patients with multiple other neoplasms showed lung carcinomas associated with solid tumors (3) and plasma cell disorders (1). Skin cancers were all diagnosed before MF/SS except one. Occurrence of other neoplasms was not related to stage, but concurrent/subsequent neoplasms were observed more frequently in late stages (14%) than in early ones (4.7%; p=0.01). The interval of occurrence was longer for tumors preceding MF/SS (median 53 mo.s, range 8-284 mo.s) than for tumors following MF/SS (median 46 mo.s, range 6-123 mo.s). Administration of chemotherapy for MF/SS was not associated with an increased incidence of subsequent neoplasms. *Discussion*. Other tumors occur in at least 21% of MF/SS patients, suggesting the need for an accurate clinical work-up, particularly for skin tumors and other hematological malignancies. The mechanisms underlying this event is still unclear. Skin tumors were observed more frequently before the diagnosis of MF/SS and cannot be related to skin-directed therapy side effects. Both a genetic predisposition for oncogenic events in different cell types as well as a dysregulation of the immune anti-cancer response may explain the occurrence of multiple tumors in MF/SS patients, maybe playing different roles in different stages, as early and advanced cases differed more for the time of occurrence than for the incidence of second tumors. Multicentric studies may help in elucidating the pathogenesis of multiple neoplasms in MF/SS patients.

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THE INTENSIFICATION OF THE CONDITIONING WITH RADIO-CONJUGATED ANTIBODY, IMPROVES THE OUTCOME IN AUTO-TRANSPLANT PATIENTS WITH NON-HODGKIN LYMPHOMA?

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The Autologous transplantation is an effective therapy in the treatment of non-Hodgkin lymphoma (NHL). Over the last decade the advent of monoclonal antibodies (anti-CD20 conjugated or not) has triggered the possibility of changing the transplantation procedure, or conducting an in purging vivo or intensifying the conditioning with radio-conjugated. From May 2005, at our division, have been auto transplanted 24 patients with CD20 + NHL (16 have done a purging *in vivo* (BEAM-R) and 8 have intensified the conditioning with the addition of radio-conjugated at day -14 to transplantation (Z-BEAM). The aim of our study is to assess whether there are differences in terms of DFS, OS and EFS between these 2 groups of patients. The characteristics of patients in the 2 groups were: 11 M and 5 F median age of 58 years (range 17-65) in the BEAM-R and 5 M and 3 F median age of 51 years (range 40-69) in Z-BEAM. In the BEAM-R group 10 patients had high grade lymphomas, 4 mantle cells and 2 follicular, the status at transplant has been: CR: 8; PR: 6 and NR: 2. In Z-BEAM group, 4 patients had high grade lymphomas, 3 follicular, and 1 mantle cells, the status disease at the transplant were: CR in 3 patients; PR: 4 and NR: 1. The median of CD34 cells infused was 4 x 106/Kg in both groups. The Haematological recovery assessed with N>1000/mm^3 and PLT> 20,000/mm^3 were 11 and 12 days respectively in groups BEAM-R and Z-BEAM. The TRM assessed in the 2 groups has been 2 / 16 (12.5%) in the BEAM-R and 1 / 8 (12.5%) of Z-BEAM group, this high incidence is due to patients highly treated or in active disease. with a median follow-up of 30 and 14 months after auto-transplant, 8 / 14 (57%) and 4 / 7 (57%) of patients were in CR, respectively, for groups BEAM-R and Z-BEAM. The EFS median are 26 and 13 months respectively for the BEAM-R and Z-BEAM group. This difference is not statistically significant (p: 0.8, Cox F-test). In conclusion, even if the number of the patients is small, the difference in terms of DFS, OS and EFS doesn't seem significant among the two regimes of conditioning. Is necessary a randomized study to confirm these preliminary data.

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MACOP-B REGIMEN IN THE TREATMENT OF ADULT LANGERHANS CELL HISTIOCYTOSIS: EXPERIENCE ON 7 PATIENTS

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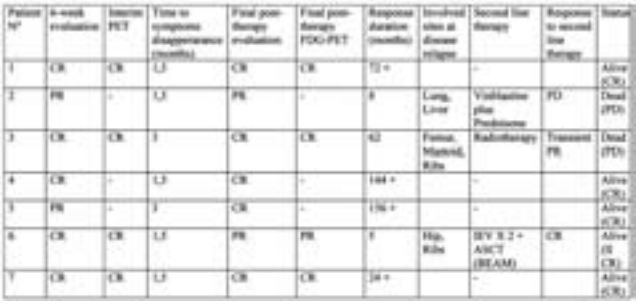
Background. Adult Langerhans cell histiocytosis (LCH) is a rare disease and prospective randomized trials are lacking. The combination of Vinblastine and Prednisone, given in a 6-months course, is the standard of care. However, the results particularly in case of high risk disease are disappointing. *Patients and Methods.* We report our monocentric experience in the treatment of 7 adult patients with multisystem (MS) LCH (n=3) or single system multifocal (SS-m) LCH (n=4) with the third generation short course intensive chemotherapy regimen MACOP-B. *Results*. The overall response rate was 100% (5 CR and 2 PR). After a median follow-up of 6.5 years, four patients are in first continuous CR and 3 patients relapsed after 5, 8, and 62 months respectively. Among the relapsed patients, two of them died for disease progression and the remaining patient underwent to high dose treatment obtaining a second CR. Four patients were evaluated with Positron Emission Tomography (PET) scan: considering the posttherapy PET evaluation, all three PET negative patients at the end of chemotherapy had a long lasting response with only one patient relapsing after 5 years. PET scan also detected additional bone lesions at diagnosis in 2 out of 4 patients, changing the treatment program in one of them. *Conclusions*. MACOP-B regimen seems to be very active in the treatment of adult MS or SS-m LCH, with long lasting responses in 5 out of 7 patients. In addition, PET scan merits further evaluation in the initial staging and in the evaluation of the response to chemotherapy in adult LCH.

Table 1. Rapid 6-week response, final response, FDG-PET evaluation and final outcome.



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INTENSIFIED THERAPY PROGRAM FOLLOWED BY HIGH-DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION AS FIRST-LINE TREATMENT FOR PERIPHERAL T-CELL LYMPHOMA: PRELIMINARY RESULTS OF A PROSPECTIVE STUDY

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Background. Patients (pts) with mature peripheral T-cell lymphoma (PTCL) are known to have a poor prognosis when receiving standard conventional chemotherapy. Therefore, many physicians consider high dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) a standard therapy for these pts. Aims: To evaluate the effectiveness and safety of an intensified first-line chemotherapy approach including a conventional MACOP-B regimen followed by alternated IVE/DHAP and HDT-ASCT as consolidation therapy in newly diagnosed pts with PTCL. *Patients and Methods.* Between September 2006 and December 2008, 10 adult pts with newly diagnosed PTCL, aged less than 65 years and eligible for intensive treatment, were treated with 12 courses of standard MACOP-B followed by one course of IVE (ifosfamide, etoposide, epirubicin), alternated with one course DHAP (dexamethasone, cytosine-arabinoside, platinum). Stem cells were harvested after IVE and pts who achieved a complete response (CR) were consolidated with a myeloablative HDT-ASCT using BEAM as conditioning chemotherapy regimen. Pts with primary cutaneous, immature lymphoblastic lymphoma or with anaplastic large cell kinase (ALK) positive or negative patients were not included. Histological subtypes were peripheral T-cell lymphoma unspecified (PTCLu) in 9 pts and enteropathy associated T-cell lymphoma (EATL) in the remaining patient. The median age at diagnosis was 46 years (35-64), 7/10 were males, 7/10 pts had advanced (III-IV) stage disease. B symptoms were present in 5/10 pts, 5/10 had high LDH levels, 1/10 a bulky mass and 3/10 presented an extranodal involvement. According to the age-adjusted IPI, 5 pts had an IPI score of 0-1 and 5 a score of 2-3. *Results*. To date, 6/10 pts have completed the full program, while 4 pts are still on therapy. After the MACOP-B regimen, 5/7 pts (71%), achieved a CR/Cru and 2/7 (29%) a partial response (PR). Six pts received the planned intensification therapy; of these, 5 (83%) witnessed a CR/Cru and underwent an ASCT, while 1 (17%) progressed during the IVE regimen. After a median follow-up of 12 months, 2 pts have relapsed at 17 and 13 months from the ASCT and have died of disease progression despite salvage therapy. The patient who progressed during intensification therapy is still alive with lymphoma. There were no treatment-related deaths and all pts experienced mild or moderate toxicities compatible with the planned procedure. The 2-year OS and PFS are 60% and 41%, respectively. Conclusions: These preliminary results indicate that an intensified first-line chemotherapy program with HDT-ASCT is effective and safe, and may lead to a potential cure of pts with PTCL. Further prospective studies on larger series of pts are warranted to better define the curative impact of this intensive therapeutic approach for PTCL.

P251

INTRALESIONAL RITUXIMAB IS A NEW SAFE AND ACTIVE TREATMENT IN CONJUNCTIVAL LYMPHOMAS

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Background. Rituximab is a chimeric monoclonal antibody directed against CD20 antigen. Systemic administration of rituximab is associated with variable response rates among different B-cell lymphoma entities. Intralesional administration of rituximab could be associated with an increased local disease control as reported in a few cases of cutaneous MALT lymphoma. Rituximab could exhibit a lower activity in ocular adnexal lymphoma (OAL) due to a poorer bioavailability or lack of adequate concentration of effectors in the tumor microenvironment. Thus, intralesional injection of rituximab could be a suitable strategy to overcome these limitations. Method: We report the use of intralesional injections of rituximab (four weekly injections followed by six monthly injections) in three patients with relapsed CD20$^+$ OAL. Every single dose included 1.5 mL of undiluted rituximab (10 mg/mL) plus 1 ml of epinephrine-free 2% xylocaina. *Results*: Two patients had conjunctival MALT lymphoma refractory to previous treatment with systemic rituximab; they achieved a complete remission and a partial remission after intraconjunctival rituximab. The third patient had a follicular lymphoma of the eyelid refractory to doxycycline treatment that did not regress after the first intralesional injections of rituximab. In this patient, the addition of autologous serum to rituximab improved therapeutic activity, and led to a fast complete response. *Conclusions*: These preliminary findings suggest that intralesional rituximab is a well tolerated and active strategy in marginal zone and follicular lymphomas of the ocular adnexae, even in patients primarily refractory to systemic rituximab. Supplementation with autologous serum can be introduced to increase local concentration of proteins C3 and C4 and improve rituximab cytotoxicity. Duration of response and potential late effects remain to be defined. This promising therapeutic approach deserves to be further investigated both in OAL and other extranodal lymphomas.

P252

SIX-MONTH CLARITHROMYCIN REGIMEN IS SAFE AND ACTIVE IN EXTRANODAL MARGINAL ZONE B-CELL LMPHOMAS (EMZL): A SINGLE-CENTER PHASE II TRIAL

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Background. Clarithromycin has immunomodulatory properties, partially mediated by the suppression of inflammatory cytokines. In combination with other immunomodulatory agents, this macrolide exhibits high tumour regression rates in multiple myeloma and Waldenstrom's macroglobulinemia, and a few case reports suggest some antineoplastic activity of clarithromycin in EMZL of the colon and the lung. *Method*. 13 HIV- patients (pts) with relapsed EMZL were enrolled in a phase II trial addressing monotherapy with oral clarithromycin (500 mg, twice daily, 6 months). Baseline evaluation included physical examination, blood exams, HIV, HBV and HCV serology, gastroscopy, orbit MRI, total body CT scan, and bone marrow biopsy. *Results*. Median age was 57 ys (range 36-80; 7 males). Clarithromycin was the first salvage line in 7 pts and the 3-6th line in the others. At registration, disease was local in 11 pts (bilateral in 2) and systemic in 2. Extranodal sites were orbit (n=6), conjunctiva (4), stomach (2), and breast (1). No pt had increased LDH serum levels, only one had B symptoms. Two pts had HCV+ serology. Five pts had previously eradicated gastric *H. pylori* infection (n=1) or orbit *C. psittaci* infection (n=4). All pts received clarithromycin as scheduled; two pts had episodic G1 stomatitis and nausea. Response was complete in two pts and partial in 3, lasting between 9+ and 31+ months, with an ORR of 38%; responses were observed in the 4 pts with conjunctival lymphoma and in one pt with orbit lymphoma. Five pts had SD and 3 pts experienced PD. Seven pts are progression free at a median follow-up of 17 months, with a 2-yr PFS of 53%. The pt with B symptoms experienced high-grade transformation; all pts but 2 (HCV+ cirrhosis and stroke) are alive. *Conclusions*. This six-month clarithromycin regimen is safe and active in EMZL, mostly in conjunctival forms. This exploratory trial provides a rationale for future investigations on the anti-lymphoma activity of clarithromycin.

P253

LACK OF PREDICTIVE VALUE OF EARLY EVALUATION OF 18-FDG-POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY (PET) ON THE OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS (PTS) TREATED WITH R–CHOP

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The predictive value of early PET in DLBCL patients is unclear. The clinical analysis of PET results by dichotomous interpretation as positive or negative is often difficult to apply. Moreover, published data are often based on retrospective studies. Our study was addressed to evaluate the predictive value of the early and final PET on PFS of DLBCL pts. From April 2004 to December 2008, 81 newly diagnosed DLBCL or follicular grade IIIb pts were included. Clinical characteristics were: 49 males and 32 females; median age 56 years (22-82); 18 pts stage I-II and 63 stage III-IV; IPI score 0-1 52 pts and score 2-3 28. All pts were treated according to the planned treatment, not modified by PET results: 55 with 6-8 R-CHOP and 26 with 4 R-CHOP followed by HDC and ASCT. All pts had PET scan performed at the diagnosis, after 2-4 courses of therapy (PET-2) and at the completion of treatment (PET-3): all PET results were defined as positive or negative with visual dichotomous consensus response criteria. Seventy pts completed the planned therapy and were evaluable. PET-2 was perfomed after 2 R-CHOP in 41 pts, after 3 in 15 and after 4 in 22. At the end of therapy 64 pts (91%) achieved a CR and 6 (9%) were non responders. Forty-nine pts (62%) were negative and 30 (38%) positive at the PET-2 and 64 pts(91%) were negative and 6 (9%) positive at the PET-3. The concordance between clinical evaluation of CR and PET-3 negativity was 97%: two CR were false PET-3 positive due to a second neoplasia.

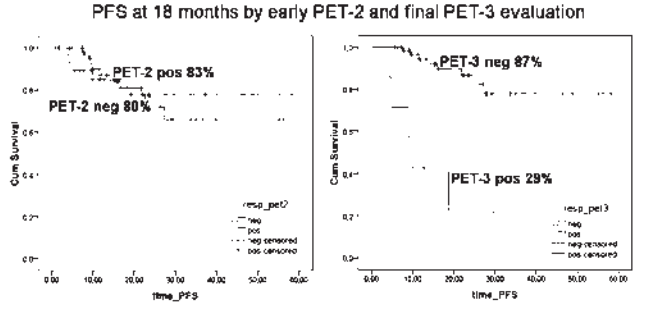

Figure 1.

Correlation between PET results and outcome was evaluated. No correlation between PET-2 results and CR rate was found: CR 95% in PET-2 negative pts vs 81% in PET-2 positive (p=ns). With a median FU of 18 months, PFS was 76%. PET-2 did not correlate with PFS (p=.88), conversely PET-3 strongly predicted PFS (p=.001). (Figure 1). Indeed, the lack of predictive value of PET-2 was observed both in the subgroup of pts treated with/without HDC+ASCT. 2y-PFS rates PET-2 negative vs PET-2 positive pts were: HDC+ASCT group 77% vs 91%; no HDC+ASCT group 81% vs 78%. Finally, PPV and NPV of PET-2 were 17% and 80% respectively; conversely PPV and NPV of PET-3 were 71% and 87% respectively. Our results indicate that in DLBCL pts treated with R-CHOP early PET does not correlate with the PFS. Conversely, final PET results correlate with it. Prospective larger studies will be needed to establish the real role of interim PET to predict the outcome in this subset of pts.

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MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS WITH THE DHAP REGIMEN WITH OR WITHOUT RITUXIMAB IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMAS

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Mobilized peripheral blood stem cells (PBSC) are widely employed in the management of patients with diffuse large B-cell lymphomas (DLBCL). The DHAP regimen was thus integrated into various treatment plans tailored for DLBCL patients as a second line therapy and salvage chemotherapy for PR patients, or as an intensification and mobilizing regimen in CR HiRisk. Treatment with rituximab is widely used for DLBCL. However, its effects on peripheral blood stem cell mobilization are not completely known. In our study we retrospectively evaluated 58 patients with DLBCL mobilized with the DHAP regimen, 36 patients receiving and 22 not receiving rituximab, in order to evaluate whether there were any differences in the number of $CD34^+$ cells ($x10^6$/kg) or engraftment kinetics. Patients mean age was 42 years (range 16-67). 44 patients (75.8%) had stage III-IV disease. 12 patients (20.7%) had bone marrow involvement; systemic B symptoms were present in 35 patients (60.3%). At the time of PBSC mobilization 35 patients (60.4%) were considered to be responsive (complete remission, partial remission or sensitive relapse) and 23 (39.6%) not responsive (refractory relapse or refractory to therapy). The median CD34+ cells collected was 8.2 $x10^6$/kg in patients receiving rituximab vs $10.3x10^6$/kg CD34+ cells (p=n.s.) in the non rituximab treated group. Failure to mobilize, defined as failure to reach a circulating $CD34^+$ cell count of 10/mcl, occurred in 5 patients (13.8%) in the rituximab group and 7 (19.4%) in the non rituximab group. All patients were transplanted using myeloablative chemotherapy conditioning regimen (BEAM); G-CSF was administered subcutaneously from day +3 at a dose of 5 microg/kg body weight/day. Comparison of the two groups showed no statistical significant difference between median days to absolute neutrophil $>0.5x10^9$/L and platelet $>20x10^9$/L counts after autologous stem cell transplantation, and no differences in incidence and severity of infections, days of fever or duration of antibiotic treatment between groups. In conclusion, the use of rituximab in association with DHAP does not affect the ability to collect an adequate number of PBSC for autologous stem cell transplantation in DLBCL. Further studies are warranted in larger populations to determine the impact of rituximab on collection, engraftment and survival.

P255

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) POLYMORPHISMS AND THEIR RELATIONSHIP WITH CLINICAL OUTCOME OF MANTLE CELL LYMPHOMA

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The vascular endothelial growth factor (VEGF) has a critical role in vasculogenesis and vascular permeability in several tumors. VEGF G+405C, C-2578A and C-460T SNPs are known to be related to VEGF production. We determined the allele and genotype frequencies of VEGF G+405C, C-460T, C+936T and C-2578A SNPs in MCL patients and compare it with the clinical outcome. We used quantitative real-time PCR method for the determination of the four VEGF SNPs. Blood samples were collected from 30 MCL patients and 58 healthy controls. There were a significant differences in the allele frequency of C-2578A (p=0,0001) and G+405C (p=0.0139). Linkage disequilibrium was noted among loci +2578 and +936. MCL patients were treated with R-Hyper-CVAD or R-CHOP regimens. The overall response rate for the entire series was 87%, with 4 cases of resistance (1 in the Hyper-CVAD and 3 in the R-CHOP subgroup). Complete remissions were achieved by the 60% of patients, without any significant difference between patients receiving R-Hyper-CVAD and R-CHOP (65% *vs.* 50%, p=0.4). The median time to progression (TTP) of the entire series was 33 months, with 47% of patients alive and free from disease progression at 36 months. No clinical characteristics assessed at diagnosis did significantly influence the achievement of clinical response or TTP. On the contrary, median TTP was 27 months in the subgroup treated with R-CHOP versus 43 months for those receiving R-Hyper-CVAD (p=0.043) (Figure 1). VEGF genotypes or allelic frequencies did not condition the response to treatments or survival. The TTP was significantly conditioned only by the quality of response, being shorter for patients who did not achieve the complete response. All cases were assayed at diagnosis for either IgH or BCL1/JH rearrangements on bone marrow samples: in 15 cases (50%) of cases, a molecular marker was found. After treatment, 9 of them (60%) achieved PCR-negativity. This result was not significantly conditioned by VEGF polymorphisms and did not significantly condition survivals. This study suggest that VEGF polymorphisms are not useful molecular tests in the clinical practice, at least in MCL patients treated with R-Hyper-CVAD and R-CHOP regimens.

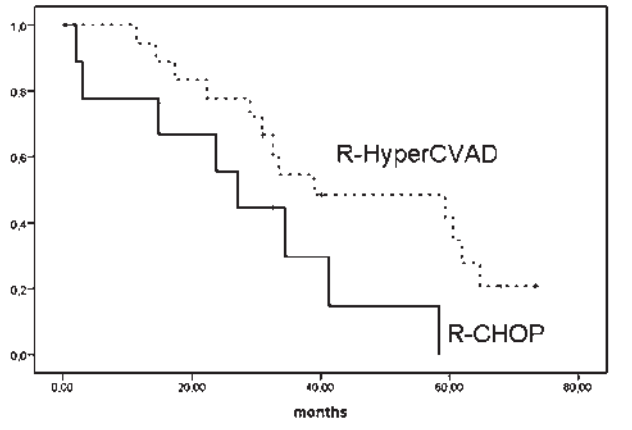

Figure 1.

P256

THROMBO-EMBOLIC EVENTS (TE) IN ADULT HODGKIN AND NON-HODGKIN LYMPHOMA PATIENTS (LP): A METANALYTIC APPROACH TO THE RISK EVALUATION

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Coagulation activation is common in haematological malignancies and can result in systemic and cerebral arterial or venous thrombosis. Cancer treatments, CVC, mechanical obstructions, erythropoietin may contribute to the onset. In LP the TE have been described with an incidence ranging between 3 and 13% reaching up to 60% in primary brain locations. In a retrospective analysis of our experience at Catholic University of Rome and Campobasso the incidence was 15% (87/564). Excluding the CNS lymphoma, at present, no subpopulation of LP at higher risk for thrombosis has been identified as a preferential candidate for antithrombotic prophylaxis. This study aimed at obtaining, using a meta-analytic approach, accurate estimates of the thrombotic risk in Hodgkin Disease (HD) and non-Hodgkin's lymphoma (NHL) patients. Eighteen articles dealing with thrombotic complications in LP were identified in electronic databases and references (29 independent cohorts, 18,018 patients and 1,149 events). Pooled incidence rates (IR) were calculated using a method based on the exact maximum likelihood binomial distribution. The global IR of thrombosis was 6.4% (95% CI:6.0-6.8). The global IRs of venous and arterial events were 5.3% (95%CI: 5.0-5.7) and 1.1% (95%CI: 0.9-1.2) respectively. The IR of thrombosis observed in subjects with NHL was 6.3% (95%CI: 5.9-6.7), significantly higher than that observed for HD patients (4.7%; 95%CI: 3.9-5.6). Within NHL, patients with aggressive histology had the most elevated risk of events (IR: 8.3%; 95%CI: 7.0-9.9) when compared with indolent NHL (IR:6.3%; 95%CI:4.5-8.9). The incidence of thrombosis in SNC LP

was extremely high (IR: 48.1%; 95%CI: 32.8-70.7). No study evaluated the effect of congenital thrombophilia; in 2 series antiphospholipid antibodies failed to show any significant role on the thrombotic risk. Our meta-analysis shows that the IR of thrombosis in LP is quite high especially in aggressive histology and advanced stage of the disease. These results may help better defining lymphoma populations at high thrombotic risk, candidate to antithrombotic prophylaxis, even though associated to increased hemorrhagic risk for the possible therapy related thrombocytopenia. On the other hand the overt TE have important implications including need for chronic anticoagulation with the associated risk of bleeding, possible delays in delivering chemotherapy, a high risk of recurrent thrombosis, along with a decreased quality of life.

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LIMITED STAGE LOW-GRADE LYMPHOMA PATIENTS RECEIVING RITUXIMAB COMBINED WITH LOCALIZED RADIOTHERAPY: THE LONG-TERM FOLLOW-UP SHOWS PROLONGED SURVIVAL WITH LOW INCIDENCE OF DISEASE RECURRENCE

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Background. Involved field radiotherapy (IF-RT) is the most commonly used treatment in localized low-grade lymphomas, allowing an excellent overall survival (OS), with failure-free survival (FFS) ranging between 40 to 55% at long-term. The addition of chemotherapy to IF-RT has been shown to markedly improve the outcome, with FFS rates approximately 20-25% higher than that achieved with IF-RT alone. However, due to the potential long-term toxicity of chemotherapy, IF-RT alone remains the standard option. The association of the anti-CD20 Rituximab with IF-RT may be an alternative approach, allowing the delivery of an effective systemic drug while sparing the toxicity of chemotherapy. The long-term outcome of a series of localized low-grade lymphomas treated front-line with Rituximab and IF-RT is here reported. *Patients and Methods.* Since March 1999, 24 consecutive low-grade lymphoma patients with stage I-II presentation received the Rituximab+IF-RT treatment program. Their median age was 61 yrs. (range 25-75), 14 were Female, the histological diagnosis was grade I-II Follicular Lymphoma (15 patients) or Marginal-zone Lymphoma (9 patients); they all had a favorable presentation (IPI 0-1), without high tumor burden, 13 patients had an extranodal involvement. Treatment included 4 weekly doses of Rituximab (375 mg/sqm) followed by IF-RT (median radiation dose: 36 Gy, range 25-40). Additional Rituximab was given as consolidation to one slowly-responding patient (2 doses) and to two more patients (4 doses). *Results*. Rituximab followed by IF-RT was well tolerated without severe complications, complete remission (CR) was achieved in all patients. At present, at a median follow-up of 4 yrs. (range: 0.5-9 yrs.), 20 patients are alive in continuous CR, while four patients displayed disease recurrence, at 24, 37, 51, and 97 mos. since diagnosis, respectively. Three of these patients are now alive in 2nd CR following rescue with chemoimmunotherapy; the fourth patients died for lung cancer. At present, the 5-yr. OS and FFS projections are 93% and 81%, respectively. Conclusion: The association of Rituximab and IF-RT is an effective front-line treatment for limited stage low-grade lymphoma. The projected 5-yr FFS is similar to that reported with the combination of chemotherapy and RT, and markedly higher compared to that observed with IF-RT alone. The good tolerability makes the combination of Rituximab and radiotherapy a strong alternative to the standard IF-RT approach.

Acute Leukemias II

P258

ONE OR TWO LEUKEMIA-SPECIFIC PROBES ARE EQUALLY EFFECTIVE FOR MINIMAL RESIDUAL DISEASE EVALUATION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Several trials in childhood ALL demonstrated that MRD correlates well with clinical outcome. In these studies two or more highly sensitive leukemia specific probes were required for MRD evaluation, to support the primary objective of treatment de-intensification in MRD negative patients. Fewer studies evaluated the clinical significance of MRD in adult ALL, mainly in a retrospective manner. We performed a prospective phase II trial in which MRD measurements obtained during consolidation were used to define patient risk class and allocate them to MRD-guided treatment including or not hematopoietic stem cell transplantation. This study demonstrated that MRD was the single most important predictive factor for relapse and clinical outcome. Aim of the present analysis is to review whether an MRD assessment using one or two sensitive leukemia-specific probes is equally effective for the detection of residual disease and prediction of patient outcome. MRD analysis was performed in 130 remission patients out of 142 who completed consolidation therapy. A single probe was available in 61.8% of the cases and two probes in 38.2%; probe sensitivity was 10-4 in 89.3% of the cases. Five year overall survival and disease-free survival were 80% and 77% compared to 69% and 68% in patients who were defined MRD negative using two and one sensitive probes, respectively (p=NS). Furthermore, in patients studied with 2 probes, only in few cases there were discordant results. In 11 cases both probes had the same sensitivity and MRD positivity was noted at the detection limit; this may be considered an expected statistical variation. In 4 more cases MRD positivity was close to the detection limit of the more sensitive probe. In only one case a clearly positive and negative result was obtained. These results suggest that during the first months of MRD-oriented treatment of adult ALL, the major technical concern is on probe sensitivity rather than number. Moreover, in adult ALL studies, where an important objective remains the early recognition of patients with high risk of relapse, low-sensitivity probes (10-3) are useful for detection of high levels of MRD. In conclusion, 1) MRD analysis performed with 1 sensitive, leukemia-specific probe is as effective and accurate as with two probes, increasing the number of patients potentially evaluable for a MRD-guided consolidation therapy, 2) low-sensitivity probes can be used to detect persistence of high levels of MRD and guide therapeutic decisions.

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USE OF FLUDARABINE IN INDUCTION THERAPY DOES NOT OVERCOME THE NEGATIVE EFFECT OF ABCG2 (BCRP) OVER-EXPRESSION ON DISEASE-FREE AND OVERALL SURVIVAL IN ADULT AML PATIENTS.

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Breast cancer resistance protein (BCRP), a multidrug resistance (MDR) protein of the ATP-binding cassette family, is associated with a worse

prognosis in AML patients. Inclusion of fludarabine in induction therapy for AML results in high rates of complete remission (CR) and has been shown to overcome the negative effect of P-glycoprotein (PGP), another MDR-related protein, over-expression. No specific data are available about fludarabine effect on the outcome of AML patients with BCRP over-expression. We measured the expression of BCRP in 138 cases of "*de novo*" adult AML, treated with a fludarabine-based induction therapy, to evaluate the effect of this regimen on CR rates and long-term survival according to BCRP status. Median age was 56 years (range: 16-84), 21 patients had an unfavourable cytogenetics and 47 (37%) displayed a mutation of FLT-3 gene (33 ITD and 14 TKD). All patients were treated with an induction regimen containing fludarabine, cytarabine and idarubicin, and at least one consolidation course with high-dose cytarabine and idarubicin. BCRP expression was measured at diagnosis with flow cytometric analysis. BCRP protein was over-expressed in 67/138 (48%) patients. A strong correlation was found between BCRP positivity and PGP (p<0.01) and MRP (p<0.001), and with immature (i.e. M0-M1) morphology. Ninety patients (65%) attained a CR after induction. Advanced age, unfavourable cytogenetics and FLT3-ITD negatively affected remission rate. As expected, neither BCRP or other MDR-related protein over-expression was associated with CR obtainment. Relapse occurred in 30/90 patients (33%), with a higher relapse rate in BCRP+ cases (20/45) than in BCRP- patients (10/45, p=0.04). Moreover, relapse occurred earlier in the BCRP+ group (12 vs 24 months, p=0.01). As for disease-free survival, also overall survival (OS) was affected by BCRP status (RR 2.2, p=0.005), with a particularly negative prognosis in patients with BCRP and PGP co-overexpression. BCRP did not influence achievement of CR in fludarabine-treated AML patients, but significantly affected relapse rate, CR duration and OS. Fludarabine it's able to overcome the negative effect of MDR over-expression on CR rates, but BCRP+ patients have an unfavourable long-term prognosis, and could benefit of an intensive post-remission therapy.

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EARLY REDUCTION OF WT1 TRANSCRIPTS ON PERIPHERAL BLOOD DURING INDUCTION CHEMOTHERAPY PREDICTS FOR OUTCOME OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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We investigated the prognostic significance of early peripheral blast clearance as assessed by WT1 copy reduction during the first days of "3+7" induction course in 57 adult patients aged less than 65 years with acute myeloid leukemia (AML). After first cycle, CR was achieved in 35 patients; of the 22 patients not achieving CR (NCR), 7 patients obtained partial remission while 15 patients were refractory. Quantification of WT1 transcripts by realtime quantitative PCR (ProfileQuant Kit from Ipsogen, Marseille, France) in peripheral blood was performed on day 1 (immediately before starting of therapy) and on day 5 of therapy and expressed as WT1 copy number every 10,000 ABL copies. WT1 ratio was defined as the ratio of WT1 copy number measured on day 1 and day 5. The number of day-1 WT1 copies was not predictive of CR. On the contrary, the median number of day-5 WT1 copies resulted significantly lower in patients obtaining a CR compared to those who did not (p=0.003). The median WT1 ratio was greater in patients attaining CR as compared to non responders (11.68 vs 2.14, respectively; p=0.0006). Furthermore, Disease Free Survival (DFS) and Overall Survival (OS) were significantly longer in patients displaying a WT1 ratio > 5.82 (i.e. the median value of whole cohort) than in patients with WT1 ratio ≤ 5.82 (p=0.024, and p<0.001, respectively) (Figure 1). In a multivariate analysis including age, white blood cells (WBC) count, karyotype and FLT3/NPM status, DFS was predicted by both WT1 ratio (p=0.01) and WBC count (p=0.041), while WT1 ratio was the only variable predicting for the likelihood to achieving CR and for longer OS (p=0.009 and p=0.007, respectively). Overall, these data support that early clearance of leukemic blasts, as measured by WT1 ratio, represents an important predictor of outcome. Specifically, the kinetic of WT1 copies reduction correctly predicts the bone marrow response to induction therapy in the majority of cases. On the other hand, there is a group of responder patients wrongly predicted as refractory on the basis of low WT1 ratios. It is noteworthy that these patients showed a short DFS, that is a proof of poor quality of CR. The fact that measurement of WT1 ratio can be easily reproducible and accomplished using PB samples anticipates the relevance of these findings for management of AML patients. In fact, an early outcome prediction might provide a tool to customizing therapeutic strategy since the first days of induction therapy.

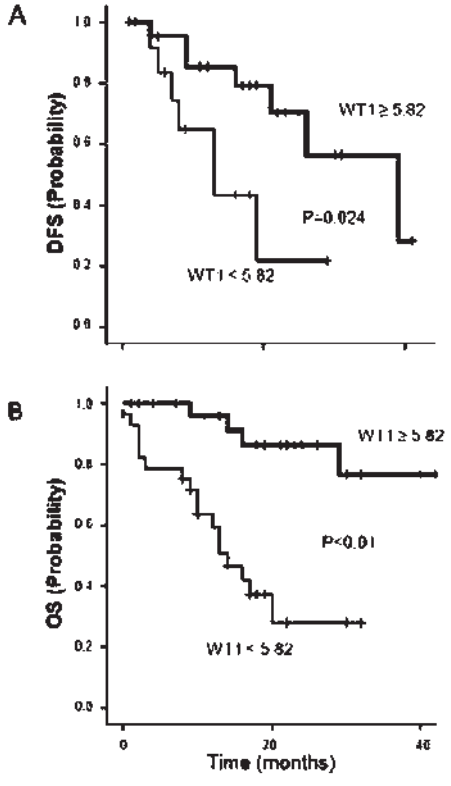

Figure 1

P261

EVALUATION OF EFFICACY OF CLOFARABINE AS TREATMENT FOR RELAPSED OR REFRACTORY ACUTE LEUKEMIA: DESCRIPTION OF 33 CASE REPORTS

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Background. Clofarabine is a modified new generation nucleoside analogue containing a fluorine which both confers reduced susceptibility and reduced toxicity. Clofarabine inhibits not only DNA polymerases and DNA synthesis but also it is able to inhibit ribonucleotide reductase. Previous trials have shown that Clofarabine is effective as first line therapy in older patients with acute myeloid leukemia not suitable for intensive chemotherapy. *Study Aim*. To evaluate efficacy and tolerability of Clofarabine in patients with a relapse or refractory acute myeloid (AML) or lymphocytic (ALL) leukemia in terms of clinical remission. *Patients and Methods*. A total of 33 patients (19 men and 14 women; mean age 48±16 years, range 19-75) with relapsed (25 patients: 19 with one and 6 with two or more relapses) or refractory (8 patients) acute leukemia were included in this descriptive report. Twenty-five patients had AML, 7 ALL and 1 patient suffered from a blast crisis in a chronic myeloid leukemia form. In six patients with AML, leukemia was secondary to a myelodisplastic condition. Clofarabine was administered at 20-52 milligrams/m^2 (mean dosage 27 milligrams/m^2) over 1 hour daily for 3-5 days every for 5 cycles, in association with other drugs. Cytosine arabinoside was the most frequently associated drug. Complete Remission (CR) was defined as a normalization of the marrow (with 5% or less blasts in a normocellular marrow) and peripheral counts. Partial remis-

sion (PR) was defined as for CR but with the persistence of 6% to 25% marrow blasts. *Results*. After Clofarabine therapy a CR was observed in 12 (36%) patients, a PR in 8 (25%), no response or not evaluable in 8 (25%) patients. In five (15%) patients it was too early to assess response. In the evaluable patients at follow up (n=26) 11 patients died (42%), whereas CR was observed in 11 patients (42%) and a PR in 3 (11%). One patient had a relapse. Death was more common in patients with no or partial response after Clofarabine treatment. Among the responding patients, 8 (24%) were transplanted (allogeneic or autologous). Conclusion: In this descriptive report, Clofarabine used in subjects with relapsed or refractory leukemia is able to obtain a clinical remission (complete and partial) in up to 60% of patients. Moreover a clofarabine-containing schedule could be considered as a feasible bridge to transplantation in such patients.

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BONE MARROW OCCURRENCE OF CYTOTOXIC AUTOLOGOUS BCR-ABL SPECIFIC T CELLS DURING IMATINIB TREATMENT IN PH+ ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS WITH PROLONGED DISEASE-FREE SURVIVAL.

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Recent reports have well evidenced the existence of a broad T cell immunity against chronic myeloid leukemia (CML), also during Imatinib mesylate (IM) treatment, supporting the notion that anti-tumor T lymphocytes may effectively participate in the control of Philadelphia chromosome positive (Ph+) leukemic proliferation. However, immunological surveys are still lacking in the setting of Ph+ acute lymphoblastic leukemia (Ph+ALL), with some patients unexpectedly showing prolonged disease free survival (DFS) under IM alone, as maintenance therapy (800 mg/die). In this study, we sought for BCR-ABL specific T cell responses in bone marrow (BM) and peripheral blood (PB) samples from 9 consecutive Ph+ALL patients under IM alone, to find out correlations of anti-leukemia immune dynamics with minimal residual disease (MRD) and clinical outcome. In our series, median DFS was 63 months and OS was 60% at 48 months. Indeed, 9/9 Ph+ALL patients showed robust BM anti-leukemia immune responses, varying from 20 to 350 SFCs/10(e)6 cells, as detected by IFNg-ELISPOT screening with pools of peptides (9-20mers long), deriving from the complete spanning of p190 BCR-ABL fusion region. Such T cell responses were rare in PB. Strikingly, p190-specific BM T cell responses were associated with lower MRD values, but waned when disease relapsed ($p<0.001$). Furthermore, cytokine production and memory T cell profiles were analyzed by multiparametric flow cytometry, showing the expansion of p190-specific cytotoxic BM T cells producing IFNg (median 1.3%) and TNFa (median 0.5%), mainly Effector Memory T cells, both $CD8^+$ and $CD4^+$. In addition, 51chromium-releasing cytotoxicity assays directly demonstrated the functional lytic activities of p190-specific BM-derived CTL clones against autologous/allogeneic Ph^+ blasts (or target cells pulsed with p190-derived peptides) in 6/9 patients (median 1600 LU10/10^6, range 0-3300). This study discloses, for the first time, that BM-homing, BCR-ABL specific autologous T cells are allowed to develop in Ph+ B-ALL patients, possibly synergising with high-dose IM treatment during long-term maintenance therapy, possibly representing a novel factor for disease control and prognostic evaluations. To improve patients' outcome under IM therapy, ex-vivo expansion and re-infusion of anti-Ph autologous T clones could worthily be explored, chancing the reduction of residual disease burden to prevent the development of IM resistance and clinical leukemia relapse.

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FLOW CYTOMETRIC ANALYSIS OF CD135 MEMBRANE EXPRESSION IN ACUTE MYELOID LEUKEMIA

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Background. It has widely been proved that FLT3 mutations in Acute Myeloid Leukemia (AML) are significantly associated with unfavourable prognosis. The aim of this study is to verify the role of FLT3 tyrosine kinase receptor (CD135)expressed by leukemic cells of patients with AML and to correlate it with FLT3 molecular expression and other biological and clinical parameters. *Patients and Methods.* The membrane expression of CD135 has been analysed by flow cytometry in 42 patients with AML (M/F 28/14: median age 64 years; range 27-84; FAB M1/M2: no. 20; FAB M4/M5 no. 22. The results have been correlated with the molecular expression of FLT3 mutation, with the bone marrow and the peripheral blood leukemic involvement, with FAB cytotype and with the immunophenotype including the surface expression of CD34, CD117 CD56 antigens. *Results.* Out of the 42 patients evaluated, only 14 cases showed that the membrane expression of CD135 resulted less than 20%. Both the bone marrow blastosis (67% vs 44%: p 0.01) and the peripheral leukocytosis (41 vs 12 x10^9/L: $p<0.01$) were more largely found in cases that showed CD135 expression. The results obtained with the flow cytometric analysis almost completely overlapped the results obtained by molecular analysis: genetic mutation was absent in the CD135 negative cases, whereas it was present in 86% (24/28) of CD135 positive cases. Overall, CD135 positive expression was found in 82% (18/22) of FAB M4/M5 AML and only in 50% (10/20) of FAB M1/M2 AML. Both the expression of CD34, CD117, CD56 and T and B lymphoid cell lines antigens resulted completely independent to the CD135 expression. *Conclusions*. According to our experience, the membrane antigenic expression of FLT3 receptor has represented: a) a high correlation marker with the AML aggressiveness evaluated both with the size of the marrow and peripheral leukemic mass; b) a marker significantly related to the cytotypes M4 and M5 AML of FAB classification; c) an indipendent marker from the expression of stem cell antigens or of B and T cell lines antigens; d) a marker closely related to the FLT3 genetic mutation. This last above mentioned characteristic describes the membrane antigenic expression of FLT3 receptor as a biologically and clinically valid parameter, easily replaceable to the molecular analysis in the AML prognostic evaluation.

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MITOXANTRONE, ETOPOSIDE, ARA-C AND LOW DOSE GEMTUZUMAB OZOGAMICIN (MY-MEC) AS SALVAGE THERAPY FOR RELAPSED-REFRACTORY AML PATIENTS.

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Background and aims. Gemtuzumab ozogamicin (GO) has shown promising activity in relapsed-refractory AML patients. We therefore explored the feasibility and the efficacy of a regimen combining mitoxantrone, etoposide and Ara-C (MEC) with low dose GO. *Methods*. We present the first analysis on 30 patients with CD33+ refractory-relapsed AML receiving mitoxantrone 12 mg/sqm, etoposide 100 mg/sqm, Ara-C 1 g/sqm (days 1-4 in patients < 65 years and 1-3 in patients ≥65) and GO 5 mg at day 5 or 4. *Patients*: The median age of patients was 64 (range 33-74); M / F ratio was 22 / 8; FAB subtypes were M0 in 4 patients, M1 in 8, M2 in 10, M4 in 3, M5 in 3, M6 in 2. Thirteen patients were refractory to first line induction therapy, 17 were in first relapse (median first CR length 6 months, range 3-19). Twenty-one patients had de-novo AML; in 9 patients AML was secondary to MDS (7) or myeloproliferative disorders.[2] Cytogenetic analysis revealed a poor prognosis alteration in 5 patients (complex karyotype) and an intermediate alteration in the other 25. *Results*. The neutrophil and platelet recovery required a median of 17 and 20 days. Therapy was well tolerated, with 2 deaths occurring during induction (7%). Thirteen infectious complications were observed (9 sepsis, 2 pulmonary aspergillosis, 2 broncopneumonia). No VOD were reported and mild and transient signs of liver toxicity were observed in 3 patients only. Fourteen pts (47%) achieved CR, 3 (10%)

showed a partial response, 11 (36%) did not respond; 2 (7%) patients died before response evaluation. Three patients underwent allogeneic stem cell transplant. Complete remission and survival lasted a median of 7 (range 3-26) and 8 months (range 1-27), respectively. Five out of 13 patients treated for refractory disease achieved CR (38%) whereas CRs have been 9 among 17 patients treated in first relapse (53%). Poor prognosis cytogenetics at diagnosis had a negative impact on CR rate (0% compared to 56% in patients with intermediate prognosis karyotype). CR rates have been 52% and 33%, in de-novo and secondary AML respectively. Eigth out of 14 patients in CR have relapsed. Overall 25 patients have died .

P265

SYNERGISTIC *IN VITRO* EFFECT OF SECOND MITOCHONDRIA-DERIVED ACTIVATOR OF CASPASES MIMETIC COMPOUNDS (SMAC) AND TUMOR NECROSIS FACTOR (TNF)-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) ON HUMAN ACUTE LEUKEMIA CELL LINES

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Dysregulation of apoptosis plays a central role in many human hematopoietic malignancies. Important apoptosis regulators are the Inhibitor of Apoptosis Proteins (IAP) among which XIAP (X-linked IAP) is the most potent. XIAP is characterized by 3 tandem BIR domains which selectively target caspases 3, 7 and 9 thus inhibiting the apoptotic process. This delicate equilibrium is altered in various tumors, including leukemia, where XIAP is often over-expressed with a consequent caspase-dependent resistance to enter apoptosis. The activity of XIAP is antagonized by Smac. It has been shown that small molecules mimicking Smac have the ability to induce apoptosis in tumor cells. It has also been demonstrated that Smac-mimetics sensitize a broad range of tumor cells to other death ligands and particularly to TRAIL, a recognized pro-apoptotic agent currently evaluated in clinical trials in solid tumors. Here we describe the cytotoxic effect of 56 newly synthesized monovalent and bivalent Smac-mimetics and their effects, alone or in combination with other drugs in human leukemic cell lines HL60, K562 and Jurkat as well as on normal $CD34^+$ hematopoietic progenitor cells. The ability of Smac-mimetics to bind to XIAP was detected with a fluorescent polarization-based binding assay and their effect on cell growth, alone or in combination, was evaluated by a colorimetric assay for the quantification of cell proliferation and viability based on the cleavage of the WST-8 tetrazolium salt by mitochondrial dehydrogenases. The more promising compounds showed IC_{50} ranging from 0.3 to 1 microM on the HL60 cell line. No significative cytotoxic effect was observed in normal controls. The Jurkat and K562 cell lines were less sensitive to Smac mimetic compounds with IC_{50} ranging from 11.8 microM to more than 50 microM. In combined treatment a synergistic effect was observed on the more resistant K562 cell line with cytarabine and etoposide 1 microM (R Kern Index = 1.5 and 1.3, respectively, with Smac5 20 μM), particularly with TRAIL 50 ng/mL (R=6.2, with Smac12 10 μM). As TRAIL is currently evaluated in clinical trials for its reported strong pro-apoptotic activity and many hematological malignancies are resistant to TRAIL alone, thus limiting its therapeutic effectiveness, its strong synergistic effect with our Smac mimetic compounds could be an important observation in the development of innovative and potent pro-apoptotic drug combination for leukemia treatment.

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NPM MUTATION MAY PREDICT A FAVORABLE OUTCOME IN AML ELDERLY PATIENTS WITH NORMAL KARYOTYPE

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NPM mutations represent the most common genetic lesion in *de novo* adult AML. They are generally associated with favorable outcome, in patients with a normal karyotype and without FLT3 ITD mutations. The prognostic value of NPM mutation in elderly is still unknown. Therefore we evaluated NPM mutation performing immunostaining on paraffine embedded biopsies of 50 AML patients aged over 60 years. Patients received an induction chemotherapy followed, in case of CR, by consolidation with chemotherapy alone (n:11), transplant (n: 15) or Mylotarg infusions (n: 8). Fifteen out of 50 patients (30%) had cytoplasmatic positive (NPMc+) AML, 3 of them had a mainly cytoplasmic NPM dislocation. Six of the 15 patients had normal karyotype, 3 had a complex karyotype. Karyotype was not evaluable in 6 pts. FLT3 mutational status was not available. We did not find any patient (gender and age) or disease characteristics (CD34 status, karyotype, FAB classification, secondary AML and WBC count) related to NPM positive status. NPM incidence was also equally distributed in the three different arms of consolidation. All 6 patients with normal karyotype and NPM positive status achieved a CR after induction, while a 67% CR rate was observed in the other patients (p: 0.08). A nearly significant (p=0.06) better OS was observed in NPM positive and normal karyotype patients (66.7%) compared with all other patients (17%). DFS was also much better in the NPM + patients with normal cytogenetic (66.7%) compared with other patients (24%) (p=0.18). The different outcome between the two elderly population showed only a trend of significance probably because of the low number of patients with normal karyotype and NPM^+. In conclusion NPM status seems to have a positive prognostic impact also in elderly patients with normal Karyotype. Larger studies and FLT3 mutational analysis are required in order to clarify the real impact of NPM on prognosis and treatment decision.

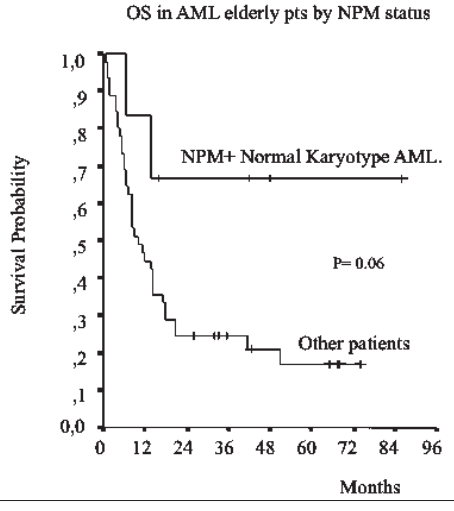

Figure.

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HIGH DOSE CYTARABINE, CLOFARABINE AND GEMTUZUMAB OZOGAMICIN (CLAC-MYL) IN RELAPSED OR REFRACTORY AML PATIENTS

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Background. Clofarabine has been shown to be effective in AML

patients, both as single agent and mainly in association with high dose cytarabine. *Aims*. On the basis of these reports, we conducted a preliminary study combining clofarabine, high dose cytarabine and gemtuzumab ozogamicin (Mylotarg) in AML patients who relapsed or failed to respond to at least two induction therapies. *Methods*. We treated 13 patients affected by relapsed/refractory AML with a regimen including clofarabine at 22.5 mg/m^2 daily on days 1-5, followed after three hours by cytarabine at 1 gr/m^2 daily on days 1-5, with the addition of gemtuzumab ozogamicin 6 mg/m^2 on day 6 (CLAC-Myl). Four patients received a further consolidation cycle with clofarabine at 22,5 mg/m^2 and cytarabine at 1 gr/m^2 day 1-4. *Results*. Among the thirteen patients, five were in first relapse, five in second relapse, three with resistant disease. The mean age was 53.2 years (range 33-68 years), the white blood count at the accrual was 31.500 m^3 (range 2140-153.000). 7/13 patients achieved a complete remission, 5/13 had resistant disease, 1/13 died of complications during the aplastic phase (multiorgan failure in a woman 62 years old, at the third relapse after allogeneic bone marrow tranplantation, diagnosed with liver GVHD before starting the treatment). The most frequent non haematologic adverse events were vomiting (4/13), diarrhea (6/13), transient liver toxicity (2/13 grade 3-4), infections microbiologically documented (7/13), febrile neutropenia (4/13). Comparing with other salvage strategies, in this small cohort of patients we did not observe a significant delay in bone marrow recovery (median time to ANC recovery 25 days), except in a patient (female, 34 years old) that experienced an unexpected, irreversible aplasia after the consolidation course, complicated by HHV6 reactivation. This patient is still under evaluation after receiving UCB transplantation. Among the seven responding patients, four underwent allogeneic bone marrow transplantation, one relapsed after 6 months, and two not eligible for transplant procedures are still in complete remission with a follow up of 9 months. *Conclusions*. Our very preliminary results suggest that the CLAC-Myl regimen is effective in this particularly poor prognosis category of patients, with safety data consistent with previously reported salvage therapies. Further studies are warranted.

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PROTEINASE 3 (PR3) GENE IS HIGHLY EXPRESSED IN CBF LEUKEMIAS AND IT CODES FOR A PROTEIN WITH ABNORMAL NUCLEAR LOCALIZATION WHICH INDUCES CHEMOSENSITIVITY

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Proteinase 3 (PR3) gene codes for a serine protease involved in the control of proliferation of myeloid leukemia cells. When abnormally expressed it confers factor-independent growth to hematopoietic cells. The aim of this study was to investigate the role of PR3 gene in leukemic haematopoiesis. We analyzed the expression levels of PR3 by RQ-PCR in 113 BM samples collected from AML patients at diagnosis. 19 patients were characterized by t(8;21) and 16 by inv(16). PR3 expression was also analyzed in 15 BM and 40 PB samples from healthy volunteers. PR3 protein was analyzed by western blot (WB) and immunofluorescence. The transcription factor C/EBPa, which negatively regulates PR3 expression was studied in parallel and its DNA binding was investigated by EMSA. Gain and loss of function experiments were performed by transfecting COS and 293T cell lines with a plasmid containing the full length PR3 sequence and HL60, Me-1, and Kasumi cell lines with specific shRNA. We found that PR3 is significantly overexpressed in AML samples. The median value of 2-DD Ct is 740, (range 15-5043). Interestingly, patients affected by CBF leukemias showed higher PR3 values compared to patients with normal karyotypes (NK) (p<0.0002 for t(8;21), p<0.001 for inv16) and lower C/EPBa levels. C/EBPa DNA binding activity in absent in CBF AML cells but not in NK AML. WB demonstrated the correlation between the mRNA and protein amount. Immunofluorescence demonstrated the de-localization of the protein within the nucleous in CBF AML but it is completely cytoplasmatic in leukemic cells with normal karyotype and in MDS. Transfection experiments with PR3 plasmid demonstrated that PR3 overexpression results into a significantly increased proliferation and reduced apoptosis. By contrast transfection with shRNA triggers apoptosis and cell growth inhibition. In addition, WB demonstrated that nuclear PR3 is able to cleavage the p65subunit of NF-kB into a p56 isoform which lacks any transcriptional activity as confirmed by EMSA. In conclusion, PR3 gene expression and protein are significantly increased in AML, particularly in CBF leukemias in which the protein is completely delocalized within the nucleous. Ectopic expression of PR3 induces increased proliferation and apoptosis arrest. The abnormal nuclear localization of PR3 in CBF leukemias results into the loss of function of NF-kB thus representing one mechanism of chemo sensitivity in this group of patients.

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THE ROLE OF HIGH-DOSE DAUNORUBICIN BASED INDUCTION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: LONG-TERM RESULTS OF THE GIMEMA ALL0496 TRIAL

Annino L.,[1] Camera A.,[2] Vignetti M.,[3] Fazi P.,[3] Paoloni F.,[3] Morra E.,[4] Fioritoni G.,[5] Di Raimondo F.,[6] Cantore N.,[7] Ferrara F.,[8] Vitolo U.,[9] Specchia G.,[10] Fabbiano F.,[11] Pizzolo G.,[12] Todeschini G.,[12] Vitale A.,[13] Mancini M.,[13] Elia L.,[13] Foà R.,[13] Amadori S.,[14] Rotoli B.[2] Mandelli F[13] on the behalf of the GIMEMA Acute Leukemia Working Party

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From 1996 to 2000, 498 adult ALL patients entered a multicenter trial aimed to investigate the effects of early intensive cytoreduction by high dose daunorubicin (HDD) (270 mg/m2 total dose) in induction, followed by high dose AraC and etoposide as consolidation and three-year maintenance, on CR rate and survival. The trial included centralized extensive biological studies. L3-ALL were excluded, and BRC/ABL rearranged patients were shifted to the European EIGLE protocol after induction. CNS prophylaxis included intrathecal therapy and cranial irradiation. Eighty percent of patients had B-ALL and 20% T-ALL. BCR/ABL was detected in 25% of patients, ALL/AF4 in 5%. The median follow-up of the trial is now 5.26 years (min. 0.5 – max 10.0). CR was achieved in 80% of patients; 12% were resistant to induction and 8% died during induction. CR rate was 80% in B-ALL and 73% in T-ALL. Among BCR/ABL rearranged cases, 65% attained CR, with 21% primary refractory and 14% early deaths. Of the 24 ALL1/AF4 patients, 21 (87%) achieved CR. Factors affecting CR rate by multivariate analysis were age (p=0.0019), BCR/ABL (p=0.0029) and MDR (p=0.0183) status, CD34 (p=0.0554) and T-phenotype (p=0.0004). Consolidation was given to 281 patients, and 215 started maintenance treatment; 218 patients relapsed (84% hematological) within a median time of 26 months. Thirty BCR/ABL-ve and 38 BCR/ABL+ve patients underwent stem cell transplant. At the last follow up, 177 patients are alive, OS and DFS being influenced by age (p<0.0001, p=0.0088) BCR/ABL status (p=0.0001, p=0.0001) and WBC at diagnosis (p<0.0001, p=0.0099). Unlike the previous Gimema ALL0183 and 0288 studies, in this trial T phenotype resulted an independent prognostic factor for CR achievement, but not for DFS. These results are not different from those achieved in other large trials for adult ALL, suggesting that early intensive cytoreduction is insufficient to improve disease outcome. However, a better stratification of patients according to biological characteristics followed by differentiated therapeutic approaches may identify subsets of ALL patients benefiting from intensive induction with HDD.

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CXCR4 AS A PREDICTOR OF RESPONSE IN ACUTE MYELOID LEUKEMIA

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The expression of CXCR4 (CD184) has been associated with poor prognosis in Acute Myeloid Leukemia (AML) and it has been suggested that the CXCL12(SDF-1a)/CXCR4 interaction contributes to the resistance of leukemia cells to chemotherapy-induced apoptosis. Inhibition of CXCR4 was found to enhance chemotherapy-induced apoptosis in a

subset of leukemic myeloblasts that carry Flt3 mutations and to overcome chemoresistance associated with stromal activity.NPM variants with a cytoplasmic localization represent the most common mutation detected in myeloid malignancies and are associated with a favourable clinical outcome.A recent study provides biological evidence for a novel role for NPM as a negative regulator of CXCR4 signalling induced by CXCL12:suppression of NPM expression enhanced chemotactic responses to CXCL12,and conversely,over-expression of a cytosolic NPM mutant reduced chemotaxis induced by CXCL12. We investigated whether CD184 expression is a negative predictor factor for response to chemotherapy and if there is clinical evidence that NPM mutations could overcome chemoresistance to induction therapy in this subset of patients.The expression of CD184 was analyzed by flow cytometric methods in a group of 81 cases of adult AML at onset of disease,diagnosed since January 2006.The diagnosis was performed according to FAB/WHO criteria;all patients received intensive chemotherapy according to institutional protocols.There were 42 males and 39 females and median age was 56 years (range 15-75). AML cells were considered positive if CD184 was expressed by more than 20% of blasts.RESULTS CD184 was positive in 56 (69%) and negative in 25 cases (31%).There was no significant difference between the two groups in terms of sex,age,Hb level,WBC and Plt counts,percentage of blasts and occurrence of NPM mutation. CR rate was 44% in CD184$^+$ and 70% in CD184-(p=0.03);among CD184$^+$ cases, CR rate was significantly higher in NPMc+ cases,(p=0.002).Our results show that CD184 expression is associated with a lower rate of CR after induction therapy and this association is stronger in NPM unmutated cases, suggesting that CD184 expression is a negative predictive factor for response to chemotherapy. Further data are needed to verify if the biological role of NPM mutation as a negative regulator of CXCR4 signalling induced by CXCL12 could have a clinical impact contributing to overcome the resistance of leukemic cells to chemotherapy.

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A NEW SUBSET OF ADULT B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENT WITH PREDNISONE-INDUCED MONOCYTIC DIFFERENTIATION?

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Background. Despite treatment results have improved in the past decade, prognosis of ALL in adult age is still dismal. The identification of genetic alterations through gene expression profile (GEP), and deep molecular cytogenetic analysis by SNPs Array, is leading to a more defined characterization of this heterogeneous disease, in order to obtain an optimized risk stratification and develop targeted therapies in different subgroups of ALL. Ph positive, hypodiploidy, and the involvement of MLL gene on chromosome 11, are associated to an unfavourable outcome. Recently, in pediatric ALL, two newly recognized subsets have been identified: 1) the "BCR-ABL like", a group of cases characterized by very high incidence of relapse, genetic alteration of IKZF1, of VPREB-1 and a gene expression profile similar to BCR-ABL1 ALL (Mullighan *et al.*; N Engl J Med 2009); 2) the "ALL with prednisone induced monocytic differentiation", a novel subtype of childhood B cell precursor recently described (Mejstrikova *et al.*; personal communication at BFM meeting Bergamo, 2009). However, it is unknown if these two subtypes of ALL are present also in the adult subset. *Methods and result.* A 43 years woman was hospitalized due to fever and severe asthenia. Peripheral blood count revealed a moderate leucopenia and a severe anemia. A bone marrow biopsy and the immunophenotype signature led to the diagnosis of pre-B ALL. The conventional cytogenetic analysis showed the trisomy of chromosome 8 in 14 out of 20 metaphases. Molecularly, the search for E2A-PBX, BCR-ABL and TEL-AML1 fusion transcripts was negative. After informed consent was obtained, the patient received a prednisone pre-treatment for 8 days, according to our institutional experimental clinical trial based on AIEOP ALL 2000 Protocol. A progressive increasing of white blood cells count was observed, reaching a severe hyperleucocytosis (WBC 140000/mm^3) at the end of steroid therapy. A morphological and immunophenotype analysis of peripheral WBC revealed a wide population constituted by CD14 positive mature monocyte cells. Detailed GEP and SNPs Array analysis were performed both before and after steroid treatment. Experiments for cloning the genomic alterations are ongoing and results will be presented. *Conclusion*. This report supports the presence of a new subset of adult patients with "ALL with prednisone induced monocytic differentiation", previously described only in the pediatric context.

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CONCOMITANT OVEREXPRESSION OF BCRP AND FLT3-ITD MUTATION ARE ASSOCIATED WITH POOR PROGNOSIS IN ACUTE MYELOID LEUKEMIA PATIENTS

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Overexpression of various multidrug resistance (MDR) proteins in acute myeloid leukemia (AML) cells is associated with clinical resistance to chemotherapy and, consequently, treatment failure. Among MDR proteins, breast cancer resistance protein (*BCRP*, or *ABCG2*) is associated with a worse prognosis in AML patients. Another well-known prognostic factors is *FLT3* mutational status, as patients with internal tandem duplication (ITD) mutation display a worse outcome. We analyzed 118 cases of adult AML patients, homogeneously treated with a fludarabine-based induction therapy, to test the role of BCRP and FLT3 on disease outcome and the correlations between these two factors. BCRP was overexpressed in 56/118 (47%) patients, and FLT3-ITD mutation was found in 33/118 (28%) cases. A significant correlation was found between BCRP positivity and FLT3 mutation, with 23 ITD in 56 BCRP+ cases (41%) compared to 10 ITD in 62 BRCP- patients (16%) (p=0.004). After induction therapy, 78 patients (66%) attained a complete remission (CR). CR rate was negatively affected by FLT3-ITD (p=0.02) but not by BCRP expression (p=0.13). Conversely, BCRP status had a strong impact on disease-free survival (DFS), with 18 relapses in 41 BCRP$^+$ cases (44%) and 8 relapses in 37 BCRP- patients (22%) (p=0.03). FLT3 mutation did not impacted on relapse rate (p=0.14) when considered alone, but its impact on DFS was additive to BCRP overexpression, as BCRP$^+$/FLT3$^+$ patients had a highest relapse rate (10/19, 53%), compared to BCRP$^+$/FLT3$^-$ (8/22, 36%) and BCRP- cases, independently of FLT3 status (8/37, 22%) (p=0.04) [Figure 1].

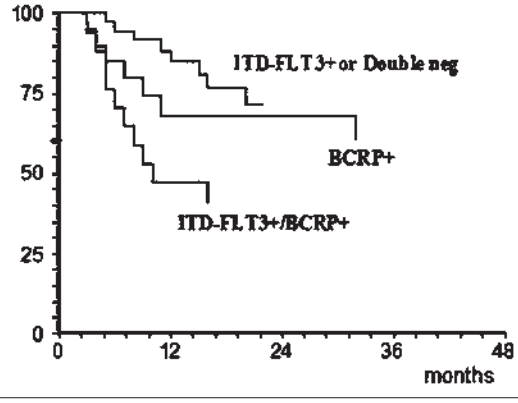

Figure 1.

Overall survival (OS) was affected by BCRP status (p=0.04), but not by FLT3 mutation (p=0.6). FLT3-ITD positivity did not significantly impact on OS in BCRP+ patients, but this can be due to the high transplant rate in our population (70/118 patients were transplanted, 60%). BCRP and FLT3-ITD have a different negative impact on AML prognosis, as the first affect DFS and the latter CR obtainment, but the two factors are often co-expressed and BCRP$^+$/FLT3$^+$ patients display a significantly higher relapse rate and shorter DFS, with FLT3 mutation having an additive effect in BCRP$^+$ cases. In our cohort, co-expression of high BCRP levels and FLT-ITD mutation identifies a subgroup of AML patients with worse prognosis, that can beneficiate of an aggressive, post-consolidation therapy.

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RITUXIMAB IMPROVE LONG-TERM DISEASE-FREE SURVIVAL(DFS) IN OLDER PATIENTS WITH ACUTE LIMPHOBLASTIC LEUKAEMIA (ALL)

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The cure rate of ALL in adults remains unsatisfactory. The remarkable progress in childhood ALL has not been replicated in adult ALL and approximately two thirds of patients younger than 60 years, and more than 90% of those over 60 years, are expected to succumb to their disease. Over 80% of adults can achieve a complete remission; however, the majority of such patients relapse. Nevertheless, significant developments have occurred over the past decade one of those is targeted therapy with monoclonal antibodies. We examined two patients, 73-years old man and 68-year old woman, affected by high risk precursor B-lineage ALL for abnormalities of cariotype and CD20 positive blasts. In medical history, the 1st patient presented epilepsy and Parkinson disease on medical treatment, the 2nd reported breast cancer treated with surgery and radiotherapy The patients were treated with induction therapy according to 0183 GIMEMA protocol modified with rituximab admistered at dose of 375 mg/mq on day 0 of therapy for 4 doses monthly. After therapy both patients obtained hematological remission, althought the female subject developed pulmonary embolism and the male patient had pseudomonas aeruginosa pneumonia. Consolidation therapy consisted in L-VAMP regimen adapted with reduction dose of 30% for previous complications. Now both patients are on standard maintenance therapy at reduced dose of 50% for low performance status and advanced age. Today patients are in complete hematologic and cytogenetic remission with median follow up of 24 months. Their median hospitalization time was 70 days .Previous reports suggest that CD20 expression in *de novo* adult precursor B-lineage ALL appears to be associated with a poor prognosis. Incorporation of Rituximab into frontline chemotherapy regimens might ameliorate clinical outcome of these patients and change these investigations. In our experience it is feasible and safe and it could have improved long-term disease-free survival by the control of malignant clone which escape to conventional chemotherapy Nevertheless these data need further confirmation in a larger patient cohort.

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EXTRAMEDULLARY INFILTRATES IN ADULT ACUTE MYELOID LEUKEMIA AT ONSET: BIOLOGICAL FEATURES AND OUTCOME

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*Background.*Acute myeloid leukaemia (AML) may be associated with extramedullary infiltrates (EMI) of malignant precursor cells at diagnosis. To explore the frequency, biological features and outcome of adult AML patients with EMI at diagnosis,we evaluated 261 consecutive untreated patients with *de novo* adult AML (median age 47 years, range 15-60). *Methods.* The diagnosis was performed according to FAB/WHO criteria (excluding APL);all patients received intensive chemotherapy according to institutional protocols.Four categories of EMI were found:skin,gingival infiltration,central nervous system involvement and soft tissue infiltration.Of 261 patients, 31(11.8%) had EMI at diagnosis:no differences in terms of sex, age,LDH,Hb,Plt,WBC,percentage of blasts were found between patients with EMI and without EMI.Results.Comparing these 261 patients with a historical group of 258 elderly (> 60 years), EMI was shown to be more frequent in younger patients (p<0.009). EMI had a higher incidence in the M4/M5 subtype (58%vs30.4%,p<0.002).There was no significant correlation with cytogenetic risk subgroups.Antigens more significantly expressed in patients with than without EMI were CD7, CD14 (p=0.001,p=0.02) and the CD56/CD14 combination with or without CD4 (p=0.005, p=0.001).In our cases CD56 alone cannot account for most instances of tissue infiltration.CD34, CD117 and CD13 were more significantly expressed in patients without EMI (p=0.02, p=0.001, p=0.008).Only CD14 was positive in more M4/M5 cases with EMI than without (p=0.03).Analysis of antigen expression by the histologically evaluated sites of EMI showed the same phenotypic profile.There was no difference in terms of CR rate (61.5% vs 55%; p>0.05) between cases with and without EMI,although DFS was shorter in EMI cases (p=0.001).However,overall survival (OS) did not differ significantly between the two groups. Conclusions.Our data suggest that AML with EMI has specific clinical and biological characteristics:age under 60 years,M4/M5 subtype,expression of CD7, CD14,CD56/CD14 with or without CD4.The presence of EMI adversely affects DFS but not the CR rate,suggesting a high cellular resistance of blast cells to chemotherapy in AML with EMI. Analysis in a larger series of adult AML is needed to evaluate interactions among other prognostic factors (FLT3 mutations, NPM1, CXCR4, etc.) in this AML subgroup, and to allocate these patients to a specific prognostic group and a more intensive treatment arm.

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A SIMPLE MOLECULAR PROFILE AT DIAGNOSIS MAY PREDICT THE PROBABILITY OF ACHIEVING COMPLETE REMISSION IN UNTREATED NON-M3 AML PATIENTS RECEIVING INDUCTION CHEMOTHERAPY: A PRELIMINARY ANALYSIS ON 85 PATIENTS

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Background. FLT3 mutations and expression of NPM, WT1, BAALC and other genes may exert a relevant prognostic role in AML patients. *Methods*. A molecular profile (FLT3 mutations and expression of WT1, NPM-A, NPM-B and BAALC) was performed in 85 untreated non M3 AML patients receiving induction chemotherapy with the aim of predicting CR rate and long term outcome. FLT3 mutations were analyzed by standard PCR method. WT1, NPM-A, NPM-B and BAALC were analyzed by quantitative Real-Time-PCR normalized on ABL expression. *Patients*. The median age was 60 years (17-80), FAB subtypes were M0 in 15 patients, M1 in 25, M2 in 26, M4 in 12, M5 in 6 and M6 in 1. Sixty-one patients (72%) had denovo AML, 24 (28%) had a secondary disease. The karyotype was favourable (FK) in 2 patients (2%), intermediate (IK) in 64 (75%, including 59 patients with normal karyotype, NK), unfavourable (UK) in 17 (21%). In 2 patients (2%) karyotype could not be evaluated. FLT3 (85 patients): 12 (14%) had ITD and 6 (7%) had exon 17 mutations. WT1 (83 patients): in 10 (12%) expression was < 100, in 10 (12%) between 100 and 500, in 17 (20%) between 500 and 1000, in 46 (56%) was > 1000. NPMA (81 patients): in 56 (69%) expression was 1000 (66% with NK, 4% with FK, 25% with UK); in 9 (11%) ranged between 1000 and 10 000 (78% with NK, 22% with UK); in 16 (20%) as > 10000 (94% with NK, 6% with UK). NPM B (81 patients): in 66 (81%) expression was ≤ 1000 (67 % with NK, 3% with FK, 30% with UK); in 8 (10%) between 1000 and 10 000 (88% with NK, 12% with UK); in 7 (9%) was > 10000 (100% with NK). BAALC (84 patients): in 34 (41%) expression was < 1000, in 28 (33%) between 1000 and 10 000, in 22 (26%) was > 10 000. *Results*. 11/12 patients with FLT3 ITD achieved CR (91%) and 7 have relapsed (58%). 4/6 patients with FLT3 mutations in exon 17 reached CR (67%) and 1 (25%) relapsed. Level of WT1 expression did not correlate with CR rate. 31/56 (55%) with NPM A ≤ 1000 achieved CR (15 relapsed); 5/9 (55%) with NPM A between 1000 and 10 000 reached CR (2 relapsed); 13/16 (81%) with NPM A > 10 000 reached CR (4 relapsed, 2 of these with FLT3ITD). 34/66 (51%) with NPM B ≤ 1000 achieved CR (15 relapsed); 8/8 (100%) with NPM A between 1000 and 10 000 reached CR (5 relapsed); 7/7 (100%) with NPM B > 10 000 reached CR (2 relapsed, 1 of these with FLT3ITD). 29/34 (85%) with BAALC < 1000 achieved CR (28 had NK; 11/29 relapsed); 11/28 (39%) with BAALC between 1000 and 10 000 achieved CR (11 relapsed); 11/22 with BAALC > 10 000 (50%) reached CR (5 relapsed). The highest CR rates have been reported in patients with BAALC < 1000, NPM A >10 000 (13/14, 93%, with 4 relapses); BAALC <1000, NPM A >10 000 , without FLT3 mutations (11/11, 100 %, with 1 relapse); BAALC <1000, NPM B > 10 000 (6/6, 100 %, with 1 relapse). *Conclusions*. A simple molecular analysis at diagnosis based on BAALC and NPM expression may predict the probability of achieving CR. A longer observation is needed to evaluate if this will be associated with improved outcome also.

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INTENSIVE TREATMENT OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA OR MYELODYSPLASTIC SYNDROME: A SINGLE CENTER EXPERIENCE

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Background. acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) peak incidence is in the seventh decade of life. The outcome of elderly patients (pts) with AML or advanced MDS is dismal because of the unfavourable characteristics of the disease and the frequent co-morbidities. Intensive chemotherapeutic programs, with or without haematopoietic stem cells transplantation (HSCT), are not usually offered to pts older than 60-65 as most of them are considered too frail to tolerate the side effects of such treatments; alternatively, they receive palliative and supportive care, or experimental targeted molecules. We here review data from elderly pts with AML or MDS, treated at our Center with an intensive approach. *Aim*. retrospective evaluation of feasibility and efficacy of different intensive treatments administered to AML/MDS elderly pts, at our Center. *Methods*. period March 1999-Jenuary 2009, 52 pts, median age 69 (range 65-78), PS 0-2, renal, hepatic, cardiac and pulmonary function were within normal limits. Diagnosis (WHO): RAEB1 2, RAEB2 9, MDS/MPD 1, AML MD 23, AML 17. Cytogenetic risk (47 pts): high 7, intermediate 38, low 2. All pts received at least one cytarabine-containing induction cycle. Pts in CR were addressed to post-remission treatments according to their age, clinical condition, prognosis and donor availability. *Results*. CR rate was 63.4% (33 pts) after 1 or 2 cycles without significative difference comparing subgroups of pts according to diagnosis and cytogenetics. Induction mortality was 13.4% (7 pts). Post-remission treatment: 15 pts received ≥ 2 cycles of standard or high-dose chemotherapy, 18 pts (54.5%) received an HSCT. HSCT (20 pts, 2 with residual disease after induction): 15 autologous (AUTO), 1 allogeneic from a sibling donor (SIB), 2 from an haploidentical familiar donor (HAPLO). Relapses: 19 pts (57.5%), 18 received salvage treatments, 10 (55.5%) obtained a second CR. At last up-date 18 pts were alive (34.6%), 13 in CR, with a median follow-up of 696 days (range 86-2677). Median survival of all pts from start of treatment was 408 days (range 20-2657). *Conclusions*. different intensive approaches proved feasible and effective in our elderly pts with AML or poor prognosis MDS. A significative rate of long term survivors free from disease was documented. Our results suggest that transplantation can be a therapeutic option for selected elderly pts. In our opinion, palliative and supportive care should be offered only to pts with serious comorbidities; alternatively, these pts could be enrolled in experimental trials with new targeted molecules.

Multiple Myeloma II

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SERUM CYTOKINES EXPRESSION IN YOUNG PATIENTS WITH MGUS AND IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMID COMBINATION REGIMENS

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Multiple Myeloma (MM) is an hematologic malignancy characterized by a network of antiapoptotic, pro-inflammatory and proangiogenetic cytokines produced by bone marrow stromal cells and plasma cells. Have been shown in vitro that Bortezomid (proteosome inhibitor) induce plasma cells apoptosis overcoming and inhibiting the plasma cells binding to bone marrow stromal cells and related cytokines setting. The aim of this study is to analyse the expression of cytokines serum level in patients with nonoclonal gammopathy of undetermined significance (MGUS) at oncet and the modification of cytokines serum level in patients with MM during treatment with combination regimens incorporating bortezomid .We analized 15 patients with MGUS and age < 50 years old and 30 patients with relapsed/refractory MM trated with 1 prior line of chemotherapy. Serum samples were collected monthly to investigate cytokine expression (IL2, IL2r, TNFα, TNF β, sTFR, IL6, IL6r) by ELISA .Of 30 MM patients (stadio I-II-III) 18(group A) were <65 years old (treated 13 with CVD and 5 with PAD regimen) and 12 were >65 years old (group B) treated with BMP regimen . CVD treatment consisted of i.v. Bortezomid (1.3 mg/m2 on days 1,4,8,11)plus dexa (20 mg)in the same days and i.v.cyclophosphamide 150 mg/m^2 on days 1,8,15 for 4-6 cycles each 21 days,PAD regimen consisted of i.v. Bortezomid (1.3 mg/m2 on days 1,4,8,11)plus dexa (20 mg)in the same days and i.v.adriamycin 9 mg/m2 on days 1-4 for 4-6 cycles each 21 days.and BMP consisted of i.v. Bortezomid (1.3 mg/m^2 on days 1,4,8,11, 22,25,29, 32)plus oral melphalan 6 mg/m^2 and oral prednisone 60 mg/m2 on days 1-5, for 4 cycles each 40 days. The overall response rate was in group A 10/18 nearly complete response+partial response (55, 7%), 5/18 stable disease (27,7%), 3/18 progressive disease (16,7%) and in group B 7/12 nearly complete response+partial response(58,3%), 2/12 stable disease (16,7%), 3/12 progressive disease (25%). In all patients with MM and in 3/15(20%) patients with MGUS that evolved in MM IL2 was ele- vated. In group A and B the patients that reached nearly complete response or partial response IL2 decreased during the monthly serum monitoring, while in patients with stable disease or progressive disease IL6r changed from normal range to incresed level. It is necessary confirmation in larger population to warrant the results of our study that serum level of IL2 is correlated with short duration of smoldering condition of MGUS and fast evolution in MM, besides that high IL6r during chemotherapy can identify a subset of MM patients with a high risk of combination regimens failure.

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THE MAINTENANCE ROLE WITH VERY LOW DOSE THALIDOMIDE AFTER AUTO-SCT IN MULTIPLE MYELOMA: SEVEN YEARS OF EXPERIENCE AND OBSERVATION

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New drugs and high dose therapy with auto-transplantation (auto-SCT) has improved prognosis of multiple myeloma (MM). New drugs are promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is been investigated as first line therapy, the limit of this drug is the toxicity dependent dose and this determines a poor compliance. It could be useful in the control of minimal residual disease. We used low dose of thal as maintenance after autologous transplantation in patient with MM from January 2002 and here we bring our experience after seven years of observation. From January 2002 to April 2009 17 patients (8 males and 9 females) with MM have been treated in our institution. Median age was 59 years (range 48-72). 10 were IgG, 3 IgA, 3 light chains and 1 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 4/17 performed double auto-SCT. Three months after SCT these patients has begun the maintenance with thal 50 mg/die,

to start thal maintenance 6 patients were in CR, 7 in PR and 4 in resistant disease and the median somministration of thal has been of 12 months (range 3-36 months). Median follow up from the beginning of maintenance therapy was 44 months (range 15-84) with 11/17 (65%) patients in CR or stable disease, with progression free survival (PFS) and overall survival (OS) projected at 84 months of 55% from to start thal. In our experience we have observed a neurological toxicity (grade I-III) in the 65% of the patients but only 4 have had to suspend the treatment; a haematological toxicity of grade I in the 55% of the patients that have not behaved interruption of the treatment and finally in any case we have documented thrombotic episodes. Finally we have compared this group of patients with another group (18 patients) with the same clinical characteristics that we have observed in the same period but that have not effected maintenance with thal. In this last group 15/18 patients (83%) relapsed with median follow-up of 40 months (range 16-66) and median PFS and OS of 16 and 43 months respectively. The difference between the 2 groups is statistically significant for PFS (p: 0,003), and OS (p: 0, 04). The median overall survival observed after progression, in the two groups, has been of 13 months, this difference is not statistically different (p:0,8). In conclusion in 7 years of observation our experience has shown that maintenance with low doses of thal, after auto-transplantation, it not only has a good compliance but it improves the PFS and OS and it doesn't worsens the survival from the relapse.

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CORRELATION BETWEEN IN VITRO AND *IN VIVO* ANTI-ANGIOGENIC EFFECTS AND CLINICAL RESPONSE IN MYELOMA PATIENTS TREATED WITH LENALIDOMIDE

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Lenalidomide is approved for the treatment of relapsed/refractory Multiple Myeloma (MM). Experimental studies are ongoing for its use as frontline therapy in MM. We examined the clinical response to lenalidomide in a cohort of patients affected by MM and studied the angiogenic phenotype of MM patients-derived endothelial cells (MMECs) to define anti-angiogenic properties of this drug and its correlation with clinical response. Nine patients with symptomatic MM, according to the IMWG have been studied. Four were treated as first-line therapy according RV-MM-PI-209 GIMEMA study and five were treated for a progressive disease. The median age was 62 years; two IgAk, four IgGk and three IgG . International Staging System score was 2 in all pts. Relapsed pts had previously received a Bortezomib-conteining regimen. The median treatment-free interval in relapsed patients was 4,2 months (range 1-15 months).

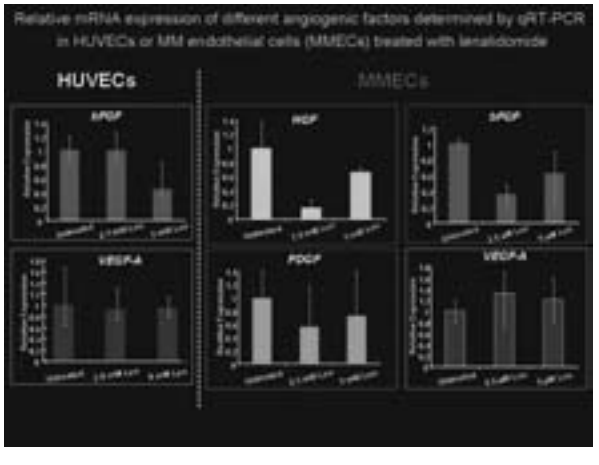

Figure.

The clinical response to treatment was evaluated, according to the IMWG criteria, in all patients after the fourth course of lenalidomide (orally 25 mg/day for 21 consecutive days every 28 days) plus low-dose Desamethasone. Before starting lenalidomide, endothelial cells from bone marrow aspirates were collected and isolated. The effects of lenalidomide on vessel morphogenesis has been evaluated in vitro after exposure to different doses of the drug and *in vivo* in the chorioallantoic membrane (CAM) assay. We also investigated whether lenalidomide affects the expression of genes involved in the angiogenic network, i.e. bFGF, HGF and PDGF. After four courses of therapy, two patients achieved a very good partial response and seven a partial response. Lenalidomide inhibited capillary formation on Matrigel as well as angiogenesis in the CAM in all analysed cases. The inhibition was more intense in the two patients who achieve VGPR and in one PR patient. A higher down-regulation of bFGF, HGF and PDGF expression in the MMECs has been demonstrated in the same cases. These results show that lenalidomide exerts an anti-angiogenic activity in vitro and *in vivo* thought down-regulation of pro-angiogenic genes. These preliminary data may be indicative of a direct correlation between antiangiogenic activity and clinical response in MM patients. Further investigation are ongoing for expand the number of patients and evaluate the correlation after 6 months and at the end of therapy.

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COMBINED TREATMENT WITH THE MEK INHIBITOR PD0325901 AND ARSENIC TRIOXIDE HAS POTENT ANTITUMOR ACTIVITY IN NOD-SCID MICE BEARING ADVANCED TUMORS OF HUMAN MULTIPLE MYELOMA

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Despite recent advances in therapy, Multiple Myeloma (MM) remains incurable because of the high resistance to apoptosis and both intrinsic and acquired drug resistance. Therefore, new therapeutic strategies are needed to improve patient outcome. We recently demonstrated that blockade of the MEK/ERK signaling module, using the small-molecule inhibitors PD184352 or PD0325901 (hence called PD, Pfizer, Ann Arbor, the second compound is the present clinical candidate), strikingly enhances arsenic trioxide (ATO)-induced cytotoxicity in MM cells through a multiple modulation of apoptotic regulatory proteins, including p53 family proteins, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors, several Bcl-2 family proteins and caspases, that depend on the functionality of the p53 pathway. Furthermore, we also demonstrated that PD plus ATO treatment induces early tumor (volume approximately 200 mm^3) regression, prolongs survival and is well tolerated *in vivo* in a human plasmacytoma xenograft model. The aim of this study was to investigate whether the combined treatment with PD and ATO is effective in animals with more advanced tumors; thus, we used a murine model in which MM RPMI 8226 cells were injected subcutaneously into NOD-SCID mice and when the tumors reached approximately 1000-1200 mm^3, mice were randomized (n=4/group) to receive vehicle or PD0325901 at 10 mg/kg administered by oral gavage or ATO (3.75 or 5.0 mg/kg) injected intraperitoneally or PD/ATO on a 5-days-a-week schedule for 3 consecutive weeks. Treatment of RPMI 8226 MM-advanced tumor-bearing mice with PD0325901 (10 mg/kg) significantly reduced MM-tumor growth as compared to control ($p<.01$, Tukey-Kramer test), ATO (3.75 or 5.0 mg/kg) had minimal effect on the growth of tumors, which increased as in control mice. Importantly, when PD (10 mg/kg) was combined with ATO (3.75 mg/Kg), there was a significant reduction in tumor size and growth rate relative to untreated or PD treated mice ($p<0.001$ for PD/ATO versus control, and $p<0.01$ for PD/ATO versus PD, Tukey-Kramer test). The combination of PD and ATO (3.75 mg/Kg) significantly prolonged survival compared with treatment with either drug alone and was well tolerated *in vivo* because no differences in body weight and general appearance was noted in mice during the treatment. We next investigated the *in vivo* effects of the drug combination on proliferation and apoptosis; whole tumor-cell tissues and tumor lysates from mice treated for five days (n=2/group) were subjected to immunohistochemical staining and immunoblotting to assess *in vivo* phosphorylation of ERK, the proliferative antigen, Ki-67, and cleaved caspase-3. Tumor tissues from

PD0325901 (10 mg/kg) treatments resulted in profound p-ERK inhibition compared with tumor tissues from vehicle control or ATO-treated animals. In agreement with these data, a significant decrease in the number of Ki-67 positive plasma cells was noted in tumor sections from PD-treated mice relative to tumors from mice receiving either vehicle control or ATO (3.75 mg/Kg) treatment alone, thereby confirming the tumors growth retardation observed in PD-treated mice. Either PD (10 mg/kg) or ATO (3.75 mg/Kg) alone did not increase caspase activation compared with tumors from control cohorts. However, the combination PD/ATO dramatically activated caspase-3 in advanced tumors. Notably, consistent with our previous in vitro study demonstrating the involvement of the Bim pathway in MM PD/ATO-induced apoptosis, immunoblotting of MM tumors from PD plus ATO-treated mice showed an elevated ratio of proapoptotic Bim to antiapoptotic Mcl-1 when compared with treatment with either drug alone. Collectively, our previous and present findings suggest that combining PD with ATO induces both cytostatic and cytotoxic responses *in vivo*, resulting in regression of early or advanced tumors, prolongs survival *in vivo*, and is well tolerated *in vivo*. In conclusion, our preclinical *in vivo* studies provide the framework for testing PD0325901 and ATO combination therapy in clinical trials aimed to improve patient outcome in MM.

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BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE DECREASE BONE RESORPTION WHILE SPARING BONE FORMATION AS COMPARED TO THALIDOMIDE-DEXAMETHASONE IN NEWLY DIAGNOSED MULTIPLE MYELOMA IRRESPECTIVE OF RESPONSE TO THERAPY

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Bone disease occurs in approximately 80% of multiple myeloma (MM) patients at diagnosis and it is due to an altered balance between bone resorption and bone formation caused by the interaction of the neoplastic clone with bone marrow microenvironment. Eradication of the myeloma clone could contribute to decrease bone resorption; bone formation, however, remains often impaired due to the use of high-dose steroids. It has been recently demonstrated, both in vitro and in animal models, that Bortezomib can stimulate osteoblastogenesis. In order to verify this finding also *in vivo*, we evaluated markers of bone resorption (crosslaps) and bone formation (osteocalcin-OC and bone alkaline phosphatase - BAP) in a series of patients who were enrolled in the "Bologna 2005" clinical trial at our Center. By study design, patients were randomized to receive three 21-days courses of induction therapy with either VTD (Bortezomib, 1.3 mg/m^2 on d 1, 4, 8, and 11, plus Dexamethasone, 40 mg on each day of and after Bortezomib administration plus Thalidomide 200 mg/d from d 1 to 63) or TD (Thalidomide as in VTD and Dexamethasone 40 mg/d on d 1-4 and 9-12 of every 21-d cycle), prior to stem cell collection and double autologous stem cell transplantation. As of July 2008, 32 patients (23 male and 9 female, median age = 57 yrs) entered the sub-study; of these, 16 patients were randomized in each treatment arm. At diagnosis, both groups of patients showed a marked increase in serum crosslaps (7978 1436pmol/L in the VTD arm and 8679 1924pmol/L in the TD arm) while both OC and BAP were reduced as compared to normal values. After completion of the induction therapy, serum crosslaps were significantly decreased in both treatment groups (2582 476pmol/L in VTD arm, p=0.000; 2649 557pmol/L in the TD arm, p=0.005). In the TD group a significant further reduction in bone formation markers was also observed (44% reduction in serum OC and 35% in BAP, p=0.02 and 0.03 as compared to pre-treatment values); on the contrary, in the VTD arm both OC and BAP were not significantly decreased as compared to baseline (0% and 20% for OC and BAP, respectively). These results were confirmed also comparing markers of bone remodeling in patients obtaining either ≥VGPR or less in both treatment arms, thus suggesting that the effect of bortezomib on bone turnover is not only mediated by the inhibitory effect on the neoplastic clone but also by a direct action on osteoblastogenesis.

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TWENTY-FIVE MG LENALIDOMIDE EVERY OTHER DAY IS FEASIBLE AND EFFECTIVE IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND IMPAIRED CREATININE CLEARANCE OR EXCESSIVE MYELOTOXICITY

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The serum half life of a single oral dose of 25 mg lenalidomide in patients with multiple myeloma increases from a mean of 3 hours (with normal renal function) up to 9 hours if moderate/severe renal impairment is present (creatinine clearance < 50 or < 30 mL/min, respectively). In the latter case - or in patients showing significant myelotoxicity – a reduction of the daily dose is recommended. However, no theoretical assumption encumbers the possibility that delayed full standard doses could equally be effective and tolerated in patients requiring reduced doses, similarly to what is generally done when using antibiotics with prevalent renal elimination. Six patients (2 males and 4 females, mean age, 64 yrs, r.: 49-85) affected by advanced, resistant and progressive multiple myeloma (mean number of previous treatment lines: 5 r.: 3-6) were treated with monthly 21-day courses of 25 mg lenalidomide e.o.d. and dexamethasone (low-dex.), due to renal insufficiency (2 pts.; calculated creatinine clearance 36.7 and 21.4 ml/min, respectively) or excessive marrow toxicity verified during previous 25mg/d lenalidomide courses (2 pts with melphalan added). The two patients with impaired renal function received 6 and 1 courses respectively: one patient responded completely (disappearance of urinary light chain and normal hemogram after the first course), whereas it is too early for the evaluations of the second patient. A partial minimal response was achieved in two of the 4 patients with myelotoxicity (after 1 course in both patients, as treatment was then interrupted for complications following pathological bone fractures). The disease remained stable in the third patient and progressed in the fourth (3 and 4 courses plus melphalan, respectively). The patients with kidney impairment did not experience significant myelotoxicity, which instead occurred in 3 of the other 4 patients, who required red cell transfusions and G-CSF support. No SAE occurred in any of the patients. These preliminary observations need to be validated by controlled studies enrolling larger number of patients.

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ASSOCIATION OF THALIDOMIDE AND STEROIDS FOR THE TREATMENT OF PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A MONOCENTRIC EXPERIENCE

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Thalidomide, an immunomodulating drug with antiangiogenic activity, is an efficacious therapeutic option for unfit patients with multiple myeloma. Its efficacy may be increased by the addiction of steroids, even in association with other drugs such melphalan or cyclophosphamide. In this study we assessed the efficacy and toxicity of thalidomide in combination with steroids in a series of patients with relapsed or refractory multiple myeloma pretreated with at least one previous therapy (range 1-4), including high dose dexamethasone, alkylating agents, anthracyclines, IFN- and autologous graft. Thalidomide 50-150 mg/die was administered orally in a total of 15 patients (median age 74.8 years, range 67-84) with relapsed or refractory multiple myeloma observed between May 2004 and June 2008. Oral dexamethasone or prednisone was added to the treatment. All patients continued therapy until relapse or progression and were prospectively followed-up including accurate monitoring of side effects. Response to thalidomide was assessed according to the European Group for Blood and Marrow Transplantation criteria. The median follow-up time was 26.8 months (range 7-52). Response rate to thalidomide was 80% (12/15 patients) with a median duration of response of 28 months (range 7-52): 2 patients showed a very good partial remission, 10 partial response, 1 stable disease and 2 progression of disease. During follow-up, 5 patients died (2 due to progression, 2 due to other neoplasm, 1 due to hearth failure), 10 patients are still alive (1 VGPR and 8 PR in continuous therapy, 1 PD off therapy). No response was observed in 3/15 patients (20%). Despite the

following side effects, mild to moderate bradycardia (20%, 1 needed PMK positioning), peripheral sensitive polyneuropathy (20%) and constipation (6%), no patient discontinued therapy. This study shows that thalidomide in combination with steroids is an effective salvage therapy with a high response rate and manageable side effects for patients with relapsed and refractory multiple myeloma.

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RECONSTRUCTION OF TRANSCRIPTIONAL REGULATORY NETWORKS IN MULTIPLE MYELOMA

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Microarray experiments can be used to detect patterns in gene expression that stem from regulatory interactions. However, traditional techniques used for detecting co-regulated genes on high-throughput gene arrays are rarely capable of distinguishing between direct and indirect interactions. In this study we applied ARACNe algorithm to decipher transcriptional regulatory relationships linking genes involved in multiple myeloma (MM). ARACNe utilizes information and data transmission concepts to identify statistically significant co-regulations among genes from microarray expression profiles. It allows reconstructing gene-gene relationships which most likely represent either direct regulatory interactions or interactions mediated by post-transcriptional modifiers. We applied ARACNe to a proprietary dataset including 5 normal donors, 11 MGUS, 133 MM, and 9 PCL for a total of 158 samples, and to a publicly available dataset accounting for 162 samples (i.e., 15 normal donors, 22 MGUS, 125 MM). Both datasets were profiled on U133A Affymetrix expression microarrays. ARACNe analyses derived a network of inferred regulatory interactions between genes, easily and interactively browsed using Cytoscape. For each gene, a list of correlated genes (neighbours) was obtained; these genes can be either targets or regulators of the gene of interest, and can be further tested for enrichment in functional categories or studied as common putative targets/regulators of relevant genes. The algorithm was applied to identify novel candidate genes which may play a role in the MM pathogenesis, such as genes with a great number of connections. Specifically, the analysis of the structure of inferred regulatory networks derived from both analyses revealed similar topological characteristics, and identified 27 common genes which show up to more than 100 interactions. Concerning the sub networks related to genes critically involved in MM (*CCND1, CCND2, CCND3, MAF, MAFB, MYC, FGFR3, WHSC1*), the analysis confirmed putative regulatory interactions: a total of 14 targets of these genes were conserved in both networks, notably including a relation between CCND1 and CCND2. In addition, genes implicated in hematological malignancies such as MLL or CD163, were identified. The adoption of such reverse engineering approach can thus suggest novel regulatory relationships between specific genes or can identify regulatory sub-networks of genes with a likely biological meaning in MM pathogenesis.

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RESOLUTION OF PURE RED CELL APLASIA AFTER REDUCED INTENCITY ALLOGENIC STEM CELL TRANSPLANTATION WITH MAJORE ABO MISMATCH DURING THERAPY WITH LENALIDOMIDE

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We report a case of pure red cell aplasia (PRCA) following a reduced intensity allogeneic stem cell transplantation with major ABO mismatch, resistant to standard treatment and resolved during Lenalidomide therapy. A 57 years old man was diagnosed of Multiple Myeloma (IgD/Lamda , stage IIIA) in August 2003. After treatment with 4 cycles of VAD regimen and autologous stem cell transplantation, he received a reduced intensity allogeneic transplant from his HLA identical sister with major ABO incompatibility (donor B+ / recipient 0+). Post transplant period was uneventful but 40 days after transplantation patient progressively developed a pure red cell aplasia with high immune anti-donor isohemagglutinin titers (immune anti B, 1:512). Erythropoietin level was 203 milliunits/ml. Analysis of chimerism revealed allogeneic engraftment. In vitro bone marrow cultures showed CFU-E absence. Since transplant patient remain transfusion dependent receiving 4 units/months of 0 + Red Blood Cells Units (RBC-U) and chelation. Subsequently he was treated with several immunosuppressive treatment: cyclosporine and steroids, plasma exchange (4 courses), 4 cycles of Rituximab plus Cyclophosphamide, and finally with escalation doses of donor lymphocyte infusions (DLI) in association with 150 micrograms/weekly of erythropoietin (EPO). No clinical or laboratoristic improvement was observed. Extramedullary Myeloma relapse occurred in February 2007; he was treated with Bortezomib, Liposomial Doxorubicin and Dexamethasone for 8 cycles. Complete remission was obtained. PRCA was unchanged. A second extramedullary relapse was occurred in December 2007. He was submitted to a local radiotherapy and Lenalidomide (25 milligrams/day for 3 weeks) associated with low dose of Dexamethasone (Rd). During the forth Rd cycle (in September 2008), together with Myeloma response, a stable increase of the hemoglobin value was observed (about 5 grams in two month). The anti-donor titers isohemagglutinin became undetectable, the direct ABO blood group converted to B+. Analysis of chimerism revealed a permanent allogeneic engraftment. Erythropoietin level was 35.8 milliunits/mL. This patient has been remaining transfusion–dependent 47 months after transplant, (total units of 0+ RBCU 184 since transplant), without conversion to the donor ABO blood group; only after the Lenalidomide treatment he was able to recover a normal haemoglobin value. This case indicates a possible immunomodulatory effect of Lenalidomide in the immunomediated PRCA.

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EXTREME SERUM FREE LIGHT CHAINS RATIO IS AN INDEPENDENT PROGNOSTIC FACTOR FOR PROGRESSION IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Recently Mayo Clinic group found extreme free light chains ratio (eFLCr): <0.03 or >32) and ISS to be independent prognostic factors for survival in newly diagnosed MM. Despite we use factors found to be prognostic in newly diagnosed MM, those predicting outcome of patients with relapsed/refractory MM are not known so far and it will be helpful to dispose of them to tailored therapy. In an attenpt to address this issue we explored the impact of eFLCr on survival parameters in 87 patients with relapsed/refractory MM treated within controlled trials with regimens including thalidomide (8%), Bortezomib (40%), Thalidomide-Bortezomib (36%) and Lenalidomide (16%). The median age of patients was 65 years (range 31-82), β2-microglobulin 3.6 mg/L (range 0.2-21,5), albumin 3.6 g/dL (range 2.4-4.5) and creatinine 1.0 mg/dl (0.6-8.0). ISS stage 2-3, kappa and lambda monoclonality, bone marrow plasma cell infiltration > 50% and first remission duration < 12 months were observed in 69%, 64%, 36%, 45% and 44%, respectively. Moreover, 32% of patients had received > 2 lines of prior therapy. Considering the whole group of patients, 62% of them obtained at least PR, 40% at least VGPR and 21% CR whereas 7% and 7% progressed and died early, respectively. Median TTP, PFS and OS were 16 and 15 months, respectively while 5-years OS was 42%. Median FLC value was 307 mg/L (range 1.6-10000) while FLCr ranged from 0 to 14200. Among the 87 patients included in this study, 42 (48%) presented eFLCr. These latter patients had a significantly lower CR rate (18% vs 41%; p= 0.041) and a significantly shorter TTP and PFS (median 10 vs 22 months; p= 0.019) and OS (median 17 months vs NR; p= 0.030). eFLCr was not associated with none of the abovementioned variables particularly ISS stage, β2-microglobulin, first remission duration and prior lines of therapy. Univariate Cox regression analysis of the above parameters selected eFLCr (HR=1.97; p=0.020) and ISS stage 2-3 (HR=1.74; p=0.078) as the covari-

ates associated with shorter TPP. Stepwise Cox regression analysis selected eFLCr alone (HR= 1.91; 95%CI=1.2-3.4; p=0.030) as factor significantly affecting TTP. In conclusion, as well as patients with newly diagnosed MM, those with relapsed/refractory MM can be usefully stratified according to eFLCr.If these results should be confirmed by large prospective studies, the FLCr should be included in the work up of patients with advanced disease and helpful in tailoring their treatment.

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INCREASED SERUM BILIRUBIN LEVEL (SBL) WITHOUT JAUNDICE IN PATIENTS WITH MULTIPLE MYELOMA

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Anecdotal cases have been reported on the artifactual increase of the SBL due to the interference of monoclonal immunoglobulins in automated analyzers. We report on 3 pts with MM showing hyperbilirubinemia without jaundice due to paraprotein interference. Pt no. 1 was a 51-year male, IgG/lambda, CS IIA, treated with VAD regimen followed by double autologous peripheral blood stem cell transplantation. He relapsed after 4 years and was treated with PAD regimen without showing any response. At that time, he had protein serum level 14,3 g/dL, monoclonal gammopathy 10 g/dL and total SBL 19,6 mg/dL without jaundice. Lenalidomide and oral dexamethasone (dexa) was promptly started with a slow but constant reduction of paraprotein levels and normalization of BSL just before the end of the first cycle. At the last follow-up (March, 2008), the pt had total bilirubin level of 0,35 mg/dL and monoclonal gammopathy 4,9 g/dL. Pt no. 2 was a 67-year female, IgG/lambda, CS IIIA. She received melphalan and methylprednisolone as first-line therapy. The disease relapsed after 14 months and the pt was refractory to several lines of chemotherapy (cyclophosphamide and dexa, bortezomib and dexa, thalidomide and dexa, VAD regimen). At that point, protein serum level was 13 g/dL, monoclonal gammopathy 8,3 g/dL and total SBL 23 mg/dL without evidence of jaundice. Pt was scheduled to receive bortezomib, melphalan, and methylprednisolone (VMP regimen) and, because of refractoriness of the disease, the pt started treatment with lenalidomide and dexa, with initial reduction of both paraprotein and total SBL. The pt died because of disease progression with cutaneous involvement in August 2008. Pt no. 3 was a 79 year man diagnosed in January 2009, IgG/lambda, CS IA. At that time he had protein serum level 10,4 g/dL, monoclonal gammopathy 3,7 g/dL and total SBL 11,9 mg/dl without jaundice. Because of the early stage of the disease the patient underwent to therapy with zoledronic acid given monthly. At the last follow-up (April, 2009), the pt had total SBL of 14,5 mg/dl and stable paraprotein. An artifactual increase of total bilirubin concentration was reported as an uncommon feature. To avoid misdiagnosis in the setting of pts with monoclonal immunoglobulins, hyperbilirubinemia needs to be carefully evaluated. Moreover, incorrect diagnoses due to technical interferences in laboratory determination of SBL may involve inappropriate treatments and longer hospital stays.

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A NEW INDUCTION COMBINATION THERAPY (THALDODEX) FOR MULTIPLE MYELOMA. PRELIMINARY RESULTS OF A PHASE II STUDY

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Treatment for multiple myeloma is dramatically changed over the past 5 years. Before the advent of the new drugs, antracycline containing regimens were considered the gold standard in induction. We investigate the efficacy and safety of an antracycline based treatment combining liposomal doxorubicin, Myocet, to steroids, Dexamethasone, and a novel agent, Thalidomide (ThalDoDex). From June 2007 to November 2008, ThalDoDex combination was delivered to 17 previously untreated multiple myeloma patients. Median age was 59 years (range 44-71). Treatment schedule was as follows: Thalidomide 100 mg/day for 14 days then 200 mg/day until the end of induction; Dexamethasone 40 mg from day 1 to day 4; Liposomal Antracycline (MYOCET) 50 mg/sqm day 1 for 4 cycles at 4 weekly intervals. LMWH 100UI/kg/day was added to all patients as DTV prophylaxis. The response criteria were defined according to Bladè et al. The population presented as follows: 4 patients were stage IIA, 11 patients stage IIIA and 2 IIIB; 8, 5 and 4 patients were ISS I, II, III respectively. Monoclonal immunoglobulin subtypes were as follows: IgG in 9 patients, IgA in 5 patients and light chains in 3 patients. The overall response rate (CR, nCR, VGPR, PR) was 86%: CR and nCR 30%. One patient was not evaluable for response because of a sudden death after 2nd cycle. Toxicities of grade III/IV according to CTCAE v3.0 was bradycardia in 1 patient, pneumonia in 2 patients and neutropenia in 4 patients (only in 2 of them was necessary to delay the next cycle and reduce the antracycline dose of 50%). Our experience suggest that ThalDoDex is effective and safe in newly diagnosed multiple myeloma patients. These preliminary results are comparable to those reported with other new regimens. The liposomal doxorubicin in combination to Thalidomide and steroids in induction may permit the use of alkylating agents, proteosome inhibitors and other IMIDs in successive treatments.

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PERCUTANEUS KYPHOPLASTIC AND VERTEBROPLASTY FOR A PAINFUL VERTEBRAL BODY FRACTURE IN HAEMATOLOGICAL PATIENTS

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Background. Bone disease is frequently associated with haematological malignancies for primary localization of disease (Multiple Myeloma and rarely Lymphoma) or as a secondary effect of intensive corticosteroid therapy. Vertebroplasty (PV) and percutaneous kyphoplasty (PK) are indicated in the treatment of pain due to nerve root compression after vertebral body collapse. *Method.* PV and PK consist in the percutaneos injection of PMMA (polimetilmetacrylate) into fractured vertebral body under fluoroscopy vision. A needle was introduced through a small dermatotomy and advanced to the posterior aspect of each pedicle along its superolateral cortex. Using a hand-mounted drill, the bilateral channel were created to reach the posterior one third of vertebral body. Trough the channel a high-pressure balloon was introduced and inflated to reduce the vertebral body back to its original height; subsequently we filled the cavity with the PMMA. *Patients.* Between 2003 and 2008 thirty three patients underwent to PK or PV; they were affected by vertebral body fractures or collapses secondary to MM, NHL localization (group A:25 patients) or to high dose corticosteroid treatments (group B:8 patients); patients had pain refractory to opiate analgesia and in treatment with biphosphonates and nobody showed spinal cord compression. All patients were valuated for grade of pain and disability before and after intervention according the VA-score (VAS:Visual analogue pain score, range 0-5) and the Barthel Index (score of the ability to do activities of daily living). In group A the median age was 62 years (range 44-83); 4/25 patients had multiple fractures ;the median value of pre-treatment VAS and BI was 20 (range 19-25) and 9 (4-11) respectively. In group B the median age was 65 years (range 60-73); 1/8 patient had two vertebral body fractures; the median value of pre-treatment VAS and

BI was 17 (range 12-21) and 10 (7- 14) respectively. Results:PK e PV was performed in 6 and 27 patients respectively. In 5 patients PV was performed on multiple vertebrae in the same treatment. After six weeks in the group A the median of VA-score and BI was 5 (range 2-11) and 17(range 14-20) respectively; 5 patients maintained low dose of analgesia. In the group B the median of VA-score and BI was 3 (range 0-8) and 18(range 17-20) respectively; one patient maintained analgesia. *Conclusion.* PK and PV were effective in relieving pain and improving life quality of the patients.

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EVOLUTION OF OSTEONECROSIS OF THE JAW (ONJ) IN PATIENTS WITH MULTIPLE MYELOMA (MM) AND WALDENSTRÖM'S MACROGLOBULINEMIA: A RETROSPECTIVE MULTICENTRIC STUDY

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Introduction. Bisphosphonates (Bsf) are a recognized and effective class of drugs used intravenously to treat cancer-related conditions, such as multiple myeloma (MM) and others solid tumours for the prevention of pathologic fractures, and in oral form to prevent osteoporosis and osteopenia. Some other activities are described as immunomodulating effects. Evolution of osteonecrosis of the jaw (ONJ) during bsf treatment is a rare complication with the risk increasing the longer the patient uses the drug. Pamidronate and Zolendronic acid can induce ONJ in 0,8-12% of patients as described in different reports. In this study we want describe the evolution and outcome of the ONJ in a multicentric study. We observed 55 pts with Multiple Myeloma (MM) who developed ONJ; immunoglobulin isotype was: 25 pts IgG- ; 6 pts IgG- , 12 pts IgA- ; 3 pts IgA- , 3 pts MM , 1 pt MM and 5 pts with Waldenström's Macroglobulinemia (WM) IgM- . Median age was 72 years (range 56-95), male 16/ female 39. All patients were treated with Bsf: Pamidronate 1 pts (1,8 %), Zolendronic acid 36 pts (65,5 %), Pamidronate/zolendronic acid 18 pts (32,7%). The average dose of Pamidronate was 2.022 mg (range 90-6.750 mg) and of zolendronic acid was 84 mg (range 4-256 mg). Anatomic localisation of the ONJ was: mandible 29 pts (52.7%); maxilla 22 pts (40%); mandible/maxilla 4 cases (7,3 %). The most common trigger for ONJ was dento-alveolar surgery, including extractions (43 cases-78.4%), dental implant placement (3 cases-5.4%), periodontal disease (5 cases-9%), and in 3 patients with dental prothesis (5.4%); 1 patient (1.8%) developed ONJ spontaneously. All patients stopped bsf therapy after ONJ diagnosis. All patients were treated with conservative treatment such as antibiotic therapy. In 18 patients (32.7%) antibiotic therapy was the only treatment used. 6 patients (10.9%) received antibiotic associated with surgical debridement of necrotic bone. 16 patients (29%) were treated with antibiotic therapy in combination with hyperbaric oxygen therapy/ozonotherapy and curettage; 12 patients (21.8%) required sequestrectomy in association with antibiotic and oxygen/hyperbaric therapy. 3 patients (5.4%) refused any treatment. Resolution was observed in 19 cases (34.5%); 24 patients (43,6%) improved as pain and as control infection of the soft and hard tissue. The osteonecrosis was not changed in 9 patients (16.3%); 3 patients (5.4%) did not respond to treatment. Our retrospective study demonstrate that, in established ONJ, clinical improvement can be obtained in a high percentage (78%), with a complete resolution of bone necrosis in one third of patients. Surgical treatment, associated with antibiotic therapy, is the most effective treatment to eradicate the necrotic bone. The effectiveness of hyperbaric oxygen therapy is not nowadays well determined, but in our experience it demonstrated its utility. Because the most common trigger for ONJ was dental extractions, prior to treatment with bsf, all patients should have a thorough oral examination and should be completed all invasive dental procedures, achieving optimal periodontal health. With increased recognition and follow up of the ONJ, it is likely that our knowledge will improve the risk of developing ONJ and obtaining in more patients a complete remission.

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PERCUTANEOUS VERTEBROPLASTY IN MULTIPLE MYELOMA: LONG TERM OUTCOME IN A PATIENTS SERIES

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Introduction. Percutaneous vertebroplasty (PVP) is a minimally invasive radiological method for the treatment of back pain related to osteoporotic vertebral compression fractures, vertebral metastates and myeloma. The vertebral column is the most common site for secondary bone metastases and lesions arising from hematological malignancies such as multiple myeloma (MM). Patients with MM are at high risk for vertebral compression fracture, and the use of vertebroplasty is expanding in this patients population. *Aim*. We reviewed long term clinical outcome of 534 PVP performed on a MM patients population to evaluate the safety and effectiveness of this interventional radiology procedure. Material and methods: Between January 2002 and September 2005, 264 symptomatic MM patients (534 vertebral collapse) were treated by PVP. All patients were in systemic active treatment for the hematological disease and reported severe back pain. Patients were first evaluated by objective examination, pain visual analogue scale (VAS) and with X-ray and MR diagnostic investigations. The procedure was performed on fluoroscopy biplanes high resolution guide. The follow-up was conducted with objective review (associate with a VAS reassessment) at 30 days and with MR and CT at 3,6 and 12 months after procedure. *Results*. technical success was 94% with a mean VAS reduction from 8.2 mm to 2.4 mm. Documented post and peri-procedural complications were: 1 radiculopatia, 38 discal leak and 40 venous leak of percutaneous polymethylmethacrylate (PMMA), with 1 pulmonary embolism. During a mean follow up of 27.8 months (range: 6-48), previously treated vertebral levels were stable both morphologically and structurally. *Conclusions*. percutaneous vertebroplasty is a safe and effective procedure in the treatment of painful vertebral collapse, refractory to medical conservative-therapy, inducing long lasting pain relief, enhanced mobility, and reduced use of narcotic medication, for all stages of multiple myeloma patients.

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IS THERE A LINK BETWEEN MONOCLONAL COMPONENTS AND SOLID NEOPLASMS? AND IF SO, CAN PET-CT HELP DETECTING IT?

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Introduction. A review of the literature shows discordant data on the association between monoclonal components and solid neoplasms. *Patients and methods* - 30 patients (M: 17, F: 13, age range: 65-78yrs) with MGUS diagnosed by standard criteria have been included in this perspective study. 15 patients showed a serum IgG (8 ; 7 ;), 3 an IgM (2 ; 1), and 12 an IgA (5 ; 7 ;) monoclonal component. Total body PET-CT was performed in all patients, in addition to a standard radiographic scan of all bones. *Result*. An associated solid tumour was found in 8/30 cases (26,6%; 5M, 3F). An IgA monoclonal component has been detected in 5/8 (62,5%; 3M, 2F) patients. In this subgroup the associated neoplasms were: colorectal adenocarcinoma (3), non-small cell lung cancer (1), and clear cell renal carcinoma (1). Colorectal adenocarcinoma and infiltrating ductal breast carcinoma were respectively diagnosed in two patients presenting IgG monoclonal component. At last an IgM paraprotein was detected in a colorectal adenocarcinoma patient. *Conclusions*. Total body PET-CT allowed us to diagnose different neoplasms, in early stage, in patients with MGUS. We showed how, in our limited population, PET-CT is comparable in sensibility and specificity to complete bone standard radiography for the study of bone disease. Moreover, this diagnostic technique allowed the early detection of solid tumors. Interestingly, in 5/8 cases (62,5%) the associated neoplasia was localized at the bowel, and in this subgroup the monoclonal component was Ig A in 3/5 cases. This study, even if conducted on a limited population, suggests that PET-CT could be usefully considered in the diagnostic algorithm of MGUS in order to discover eventually associated solid neoplasms

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T-REG CELLS AND EXPRESSION OF CD200 ON LYMPHOCYTES OF PATIENTS WITH MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY UNDETERMINED SIGNIFICANCE (MGUS)

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Background. The monoclonal gammopathies include a group of clonal plasma cell disorders clinically heterogeneous. Among these, MGUS has an indolent/benign clinical course in most cases, while multiple myeloma (MM) is a malignant diseases associated with dysfunctional T-cell responses. The biologic basis of this dysfunction is not defined. Because T-cells regulatory $CD4^+$ $CD25^+Foxp3^+$ and CD200, a membrane glycoprotein belonging to the immunoglobulin superfamily, play an important role in regulating immune system, we evaluated in patients with multiple myeloma and MGUS: 1) the expression of CD200 on B and T- lymphocytes, 2) T-reg cells $CD4^+$ $CD25^+Foxp3^+$, by flow cytometry. *Methods*. We studied 21 patients with MM at diagnosis, 17 patients with MGUS, and 26 healthy controls (HC). Peripheral blood were collected in EDTA. For the evaluation of CD200, $1x10^6$ cells were stained with 10microlitre of the following monoclonal antibodies: CD3, CD19, CD200, CD45, for 20 minutes at room temperature. After lysing red cells with ammonium chloride, cells were analyzed by flow cytometry. The expression of CD200 was evaluated on gated side light scatter (SSC)/$CD19^+$ B and SSC/$CD3^+$ T cells. For the evaluation of T-reg, $1x10^6$ cells were stained with 10 µL of Moabs CD4 and CD25 for 20 minutes at room temperature. Subsequently, intracellular FoxP3 staining was performed according to the manufacturer's instructions. Using a sequential gating strategy, T-reg cells were identified as $CD4^+$/$CD25^+$/$FoxP3^+$ T cells and expressed as a percentage of the $CD4^+$T-cell population. *Results*. we observed a significant decrease in expression of CD200 on T and B-lymphocytes of patients with MM (2.8±1.22%, and 52.2±15.1%) compared to MGUS (4.56±1.94%, and 63±17%) (p=0.004 and p=0,005 respectively) and HC (5.95±2.96% and 65,67±11.64%) (p<0.0001, p=0.003). No statistical difference was observed in expression of CD200 on T and B- lymphocytes of patients with MGUS compared to controls. In addition, we have observed in patients with MM a significant decrease in T-reg cells $CD4^+$ $CD25^+Foxp3^+$ (5,5±1.9%) compared to MGUS (7.1±1.7%) and HC (8.03±1.6%) (p=0,01 and p=0.002 respectively), while this difference was not observed in patients with MGUS vs HC. *Conclusion*. These results suggest that both T-reg cells and expression of CD200 on lymphocytes could contribute to immune dysfunction in patients with MM, and this abnormality could drive the evolution of MGUS in MM.

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INTRAVENOUS CONTEMPORARY ADMINISTRATION OF BORTEZOMIB, MELPHALAN, AND DEXAMETHASONE IN RELAPSED/REFRACTORY MYELOMA

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We evaluated the combination of intravenous and contemporary administration of bortezomib, melphalan, and dexamethasone. At the beginning of the study,22 patients have received monthly courses of intravenously Bortezomib at the dose of 1.3 milligrams/mq and Melphalan at the dose of 5 milligrams/mq on day 1,4,8,11. Dexamethasone was given at the dose of 40 milligrams on days 1-2,4-5,8-9,11-12 and the route was intravenous on the same days of Bortezomib and Melphalan, while by mouth the other days. After an interim evaluation indicated an excessive toxicity, the protocol was amended and the remaining 28 patients received the same drugs, at the same doses but on days 1,8,15,22 for Bortezomib and Melphalan and 1-2,8-9,15-16,22-23 for Dexamethasone. Each cycle was repeated every 35 days for a total of 6 planned courses. Sixteen refractory and 34 relapsed patients were included in the study. Median number of the previous lines of treatment was 2 with a range from 1 to 6. Four patients were relapsed within 12 months from autologous stem cell transplantation. Two patients showed a progression of disease after the first cycle and 47 are evaluable for response. At the end of treatment 27% have achieved a CR,21% VGPR, and 17% each PR,SD,and PD.Only in 10 cases the maximal response was achieved in 2 first cycles. Six cases after an initial response at 2nd cycle (>PR) experienced a progression of disease during further treatment. On the whole, 57% completed the therapeutic program (6 cycles) while 20% changed schedule due to toxicity or concomitant infections. Toxicity of the first schedule was much higher than the second schedule and was statistically significant for haematological toxicity, platelet transfusion, G-CSF administration, and non haematological toxicity such as diarrhoea, constipation and anorexia. Total median EFS was 15 months and median TTP was 19 months. Although the first schedule induced a longer EFS and TTP, the difference was not statistically significant. After a median follow up of 17 months, median overall survival (OS) has not yet reached and there is no difference between the two schedules. In conclusion, the contemporary intravenous administration of Bortezomib, Melphalan and Prednisone is a very active combination in relapsed/refractory patients with prolonged EFS, TTP, and OS. However it needs intensive supportive care due to an high incidence of hematological toxicities and poor compliance in particular with the biweekly schedule.

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DISULFIRAM, AN OLD DRUG WITH NEW POTENTIAL THERAPEUTIC USES FOR HUMAN HAEMATOLOGICAL MALIGNANCIES

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Proteasome is a novel interesting target in cancer drug therapy and the proteasome inhibitor Bortezomib has been successfully used for its antitumor activity in multiple myeloma and other lymphoid malignancies. The commonly used thiocarbamate alcohol-abuse deterrent disulfiram (DSF) is an aldehyde dehydrogenase (ALDH) inhibitor with documented proteasome-inhibiting activity. Recent reports indicate that DSF and other dithiocarbamates may have a significant potential in the treatment of human cancer without significant side effects. It was previously shown that this compound is able to block the P-glycoprotein extrusion pump, to inhibit the transcription factor nuclear factor-kappaB (NF-kB), to sensitize tumors to chemotherapy, to reduce angiogenesis, and to inhibit tumor growth in mice. In addition a recent work has described several compounds with proteasome-inhibiting activity, among which in the hit was DSF. Here we show that MM (22 samples), AML (12 samples) and ALL (11 samples) primary cells from newly diagnosed and relapsed/resistant patients were significantly sensitive to DSF at doses < 5 microM (dose achievable *in vivo*). Median IC50 was DSF 0,5 microM. MM, AML and ALL primary cells were ALDH weakly positive and we did not find any difference in the sensitivity to DFS on the basis of amount of ALDH expression or status of disease (diagnosis versus relapse). Conversely, DSF, at the dose of 0,5 microM had only a weak effect on normal CD34 and peripheral blood mononuclear cells (<30% of cell death). We next exposed the cells to the combination of low dose DCF (0,5 microM) plus CuSO4 (a commonly used herbal medicinal product that makes complex with DCF and reduces the superoxide dismutase, an enzyme involved in the mitochondrial depolarization) and we found that this combination was able to induce about 80-90% of cell death after 48 hours of treatment. The apoptotic effect of the combination (DSF plus CuSO4 0,5 microM) was comparable to that exert by therapy with Bortezomib plus Dexamethasone for MM samples, Cytarabine, Etoposide, Daunorubicine alone or in combination for AML samples or Vincristine, Dexamethasone and Daunorubicine alone or in combination for ALL samples. All these drugs were used in vitro at doses compatible with the levels reached *in vivo* during cancer treatment. In addition we show that DSF plus CuSO4 induce loss of mithocondrial

membrane potential thus suggesting the involvement of mitochondrial apoptotic pathway. These results may suggest a novel strategy for treating multiple myeloma and acute leukemias by employing an old drug, with known and mild side-effect, toward a new therapeutic use, probably in combination with other agents.

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INFLUENCE OF ZOLEDRONIC ACID ON SIERIC LEVELS OF OSTEOCALCIN AND PARATHORMONE, IN PATIENTS AFFECTED BY MULTIPLE MYELOMA, TREATED WITH CYCLOPHOSPHAMIDE, BORTEZOMIB AND DEXAMETHASONE (CY-BOR)

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In previous studies we evaluated Parathormone (PTH) and Osteocalcin levels in patients with MM and treated with Bortezomib and Dexamethasone. Osteocalcin levels significantly decreased (77%, 83% and 73% of baseline levels on days 4, 8 and 11), while PTH levels increased (121% and 138% on days 8 and 11). This trend is observed to be the same at every course of therapy. These variations appear to be due to Dexamethasone administration: indeed they are not observed after BOR alone while they are more evident after Dexa alone. Zoledronic Acid (ZA) enhances osteoblasts activity and increases the levels of PTH in response to hypocalcemia. In this study we evaluated the effects of ZA administered with Cy-BOR chemotherapy on Osteocalcin and PTH in patients affected by MM. Cy-BOR schedule is: Bor + Dexa on days 1,4,8,11 and Cyclo (300 mg/mq) on days 1,8,15. ZA was administered at day1 every other course. At present 11 courses with ZA and 9 without ZA have been completed. We observed that without ZA, Osteocalcin decreases almost in the same way on days 4, 8 and 11 (92%, 75% and 84% respectively). PTH levels do not change significantly: 111%, 97% and 98% on days 4,8 and 11. It appears, however, that the most interesting data arise from the association ZA- Cy-BOR: 1. Osteocalcin levels, after a modest reduction on day 4 (91%) remain the same as the baseline levels on days 8,11 and 15 (112%, 110%, 116%) 2. PTH levels increase much more significantly than after BOR and DEXA (290%, 260%, 366%, 351% on days 4,8,11,15) and remain high at the beginning of the following course (257%). According to these preliminary findings it appears that ZA hinders inhibition on osteoblasts exerted by dexa + BOR, while amplifies the increase of PTH. We plan to analyze a wider population of patients and to perform a statistical analysis of these data in the next months. If they will be confirmed it will be interesting to define: 1. if variations of osteocalcin and PTh affect the clinical course of patients with MM. 2. if it is possible and useful to inhibit the increase of PTH with the concomitant administration of calcium and vitamine D.

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A RETROSPECTIVE ANALYSIS OF THE TREATMENT OF DE NOVO MULTIPLE MYELOMA PATIENTS WITH HIGH-DOSE CHEMOTHERAPY FOLLOWED BY EITHER ONE OR TWO SUCCESSIVE AUTOLOGOUS HAEMATOPOIETIC PROGENITOR CELLS TRANSPLANTATIONS

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In the past, the failure of conventional chemotherapy to improve the outlook in Multiple Myeloma has led to the treatment of this disease with high-dose chemotherapy plus autologous haematopoietic progenitor cell transplantation (AHPCT). We conducted a retrospective analysis of the treatment of multiple myeloma with high-dose melphalan (HDM) followed by either one or two successive autologous stem-cell transplantations. In our bone marrow unit, in the last 12 years, 152 patients with Durie-Salmon stage II-III MM, were intended to undergo two sequential cycles of HDM with AHPCT after response to primary chemotherapy. Treatment program based on 3 cycles VAD followed by Cyclophosphamide 3-4 gr/mq for HPC collection. The preparative regimen was HDM 200 mg/mq for the first and second transplant with a three-four months interval. 63 patients underwent a single HDM and 89 a tandem HDM. After a median follow up of 34 months (range 1-136) from the time of the first transplant , the median overall survival (OS) and progression-free survival was 51 and 38 months, respectively. The probabilities of PFS and OS eight years after the first transplant were 32 % and 45 %, respectively. On an intent-to-treat basis, the OS and PFS was significantly higher in patients that underwant a tandem transplant (p= 0.0014 and p=0.0001, respectively) and with a better response (compete remission/near complete remission) after the first transplant (p=0.002, p=0.02, respectively). The Cox analysis confirmed a significant association among the number of transplants (p=0.004 OS, p=0.000 PFS), disease status post-transplant (p=0.000 OS; p=0.032 PFS) and clinical results. In this retrospective analysis we found that two successive AHPCT, each preceded by HDM, improved overall survival among patients with myeloma more than did a single transplantation and that that CR/nCR achievement is crucial for long-lasting response and prolonged survival.

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EXTRAMEDULLARY RELAPSE OF MULTIPLE MYELOMA (MM) AFTER NOVEL THERAPEUTIC AGENTS

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Background. Multiple Myeloma is mostly confined to medullary sites and its predominant clinical manifestations are generally related to BM infiltration and destruction of bone. However extramedullary involvement is seen in some patients. During the course of disease evolution, MM cells depend on BM microenviroment for their growth and survival. Newer therapies, such as thalidomide and bortezomib, capitalize on recent advances in our understanding of biology of MM, including molecular mechanisms by which MM cell-host BM interactions regulate tumor-cell growth, survival and drug resistance in the BM microenviroment. *Aims.* To describe different patterns of relapse in MM patients treated with thalidomide and/or bortezomib in a retrospective analysis. *Methods*. At various moments of the clinical course 37 MM patients (M/F 19/18, median age 65, range 47-90) received regimens including Thalidomide or Bortezomib or both: T-group: Thali-Dex or MPT regimen: 14/37(37%); B-group: Bortezomib plus Dexamethasone or Bortezomib-Caelyx-Dex regimen: 20/37(54%); BT-group: Thalidomide and Bortezomib plus Dex or VMPT regimen: 3/37(8%). *Results*. In the T-group 5/14(35%) relapsed: Two out of them (40%) had both BM and extramedullary involvement. Today these patients are died. In 11/20 of B-group (55%) MM patients treated with Bortezomib relapsed; 3 (27%) with only extramedullary but not BM and bone litic lesion involvement. Today 2 of them are alive on salvage therapy. The 3 BT-group patients are alive and in complete remission (follow-up 12, 19 and 24 mo). *Conclusions.* Five patients relapsed with extramedullary localizations; in 3 patients of the B-group they were only extramedullary, inducing us to speculate that in such cases plasmacellular clone could escape from drug control not microenvironment- related. Probably this control hold over in BM and bone compartment where bortezomib continue to inhibit binding of myeloma cells to BM stromal cells and BM triggered angiogenesis. For these reasons in the follow-up of patients treated with new agents, we suggest to evaluate the possibility of extra-medullary disease even if BM biopsy and skeleton radiograms are negative.

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IN VIVO PURGING FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS WITH MULTIPLE MYELOMA (MM): PRELIMINARY RESULTS OF A PILOT STUDY

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Thalidomide (Thal), bortezomib (BOR), and lenalidomide are highly active in the treatment of MM. The optimal schedule and association of these drugs is still under investigation. BOR is not thrombogenic, does not affect PBSC collection. It has already been used with Melphalan (MEL) before ASCT. A synergistic effect of BOR and Cyclophosphamide (CY) has been demonstrated. We started a pilot study in high risk MM patients, fit for ASCT, combining BOR, CY and dexamethasone (DEX) as induction and mobilizing therapy (CY-BOR), followed by supplemented BOR-ASCT, to determine: 1-percentage of complete remission (CR); 2-clearance of Minimal Residual Disease (MRD) in PBSC harvest and in bone marrow by flow cytometry and PCR. Schedule is: four 3-weeks cycles of BOR 1.3 mg/squared-meter (days 1,4,8,11), DEX 40 mg/day i.v.(days 1,4,8,11) and CY i.v. 300 mg/squared-meter (days 1,8,15) followed by PBSC mobilization with BOR standard dose (days 1,4,8,11), DEX 40 mg/day (days 1,4,8,11) and CY 3000 mg/squared-meter (day 8) in patients achieving at least PR. GCS-F is administered from day 9. If an adequate PBSC amount is mobilized, patients undergo ASCT with high dose MEL (day -1) and BOR (1.3 mg/squared-meter on days -6, -3, +1, +4). 18 pts (median age: 69 years) have been enrolled: with a median follow up of 200 days (range 17-450) all of them are alive. 13 are evaluable for response before PBSC harvest: 12 achieved at least VGPR after 4 courses of CY-BOR, 1 did not respond. 9 patients have been mobilized: in 7 we collected a sufficient PBSC amount (median 11.1x10(e)6; range 3-11.2x10(e)6 CD34+ cells/kilogram) and performed ASCT. Conditioning was well tolerated and followed by quick engraftment. With a minimum follow-up of 41 days after ASCT, all patients are alive; the 5 evaluable ones are in VGPR (2), CR (2) and nCR (1). We did not observe neither thromboembolic events nor grade 3-4 neurotoxicity. Flow cytometry analysis on 3 PBSC harvests shows complete clearance of plasmacells; in the others MRD study is still ongoing. Complete data will be further presented. This preliminary experience shows that this schedule is well tolerated and very effective also in elderly patients, allowing to collect a clonal plasmacells free harvest. Whether this will translate in an "*in vivo*" MRD clearance after ASCT, it should be confirmed by a longer follow up and by a PCR monitoring with patients specific probes (which is still ongoing).

P300

WHOLE-BODY LOW-DOSE-CT IN PATIENTS WITH MULTIPLE MYELOMA

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Osteolytic lesions are very frequent in myeloma; patients with bone disease are considered to be affected by symptomatic myeloma and are candidate to treatment. Plain radiograph is still suggested as the routine test: computed tomography (CT) without contrast is more sensitive than conventional radiography but is not generally used routinely for screening patients with myeloma because of the high levels of radiation exposure. We have performed whole body CT using a low-dose technique. Patients received an effective dose of 3.3 mSv (compared to 2.5 mSv for plain radiograph of skull, vertebral column, pelvis, ribs and long bones). Sixty-six patients with myeloma received a low-dose whole-body CT at the diagnosis. The following areas were found to be involved: cervical column 14/66; thoracic column 29/66; lumbar column 22/66; sacral column 9/66; skull 20/66; humerus 7/66; femur 13/66; ribs 17/66; scapula 9/66; pelvis 25/66; clavicle 6/66; sternum 4/66. A bone lesion was the only criteria to classify myeloma as symptomatic in 7/52 patients. In 9 patients a lung or pleural lesions was detected; in 7 cases it was considered infectious, in two cases myeloma involvement. One case of asymptomatic sinusal infection and one case of kidney neoplasia were detected. Among 74 column involvement, a solid mass extending to extraosseous tissues was present in eight patients. A restaging CT has shown a complete or partial regression of the mass after induction therapy in 6/8 cases. In some cases, when plain radiograph was also performed for different reasons, CT was able to see bone lesions otherwise undetectable. Notably one case of cervical column involvement undetectable to plain radiograph was seen. 273 CT have been performed during follow up of patients, either studied with plain radiograph or with CT at diagnosis. In six patients a lung infection was detected: in two cases a picture typical for fungal infections was seen. Conclusions:, whole body low-dose CT is a reliable diagnostic tool in myeloma patients. When compared with plain radiograph, it is more sensitive especially for some bone segments, it allows a better definition of neoplastic masses and assessment of response; it can give additional informations about extraosseous tissues.

P301

SOLITARY PLASMACYTOMA: A SMALL CLINICAL SERIES

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Plasmacytomas are clonal proliferations of plasma cells, which manifest as localized osseous (P-bone) or extraosseous (P-extramedullary) growth patterns without evidence of systemic disease. Solitary plasmacytoma is a rare clinical entity, described in the literature as case-reports or small clinical series. We identified 10 patients [8 male/2 female; mean age 54 years (range, 37-76)], recruited from 5 hematology centers, and diagnosed as having solitary plasmacytoma (9 P-bone and 1 P-extramedullary) between 2000 and 2009. The diagnoses were performed on the basis of the morphological and immunohistochemical findings of the bioptic specimens. Bone marrow histopathology and immunophenotype, serum and urine protein electrophoresis and immunofixation, complete blood count, serum creatinine, calcium, LDH, β-2 microglobulin, C reactive protein, albumin levels were analyzed; imaging studies including standard bone X-ray examination of the skeleton, magnetic resonance, computed tomography (CT) and/or positron emission tomography/CT scans were performed to clarify the extent of bone or soft tissue disease. In all patients, the results of a comprehensive work-up, including cellular expression of CD138 and light chain restriction, fulfilled the criteria of solitary plasmacytoma. In particular, P-extramedullary demonstrated in a patient of ours (Pt 5) was localized at the nasopharynx whereas P-bone, detected in the remaining 9 patients, was localized at the skull (Pt 7), spine (Pt 1 and Pt 4), ribs (Pt 2), pelvis (Pt 3, Pt 6, Pt 8, Pt 9), or femur (Pt 10).

Table 1.

Pt no	*Age/Sex*	*Diagnosis*	*Plasmocytoma site*	*Treatment*	*Response to therapy*
1	40/M	Aug 2000	Spine	XRT, VAD, PBSCT	CR
2	39/M	Nov 2000	Ribs	XRT, VAD, PBSCT	CR
3	62/F	Nov 2007	Pelvis	XRT, VD, PBSCT	PR
4	76/M	Aug 2007	Spine	XRT, VTD	CR
5	55/M	May 2008	Nasopharynx	surgery only	CR
6	56/M	Sep 2008	Pelvis	XRT, Len/dex	on therapy
7	41/M	Nov 2008	Skull	XRT, VTD	on therapy
8	62/M	Dec 2008	Pelvis	VTD	on therapy
9	67/F	Mar 2009	Pelvis		
10	37/M	Apr 2009	Femur		

Two patients (Pt 1 and Pt 2) were treated with radiotherapy followed by a conventional combination of chemotherapy and dexamethasone (VAD: vincristine, adriamycin, dexamethasone) and autologous stem cell transplantation (PBSCT), another (Pt 3) was treated with radiotherapy followed by a combination of proteosome inhibitor and corticosteroids (VD: velcade, dexamethasone) and PBSCT; the 3 of them achieved CR, persisting at the time of writing. At present, 3 patients are on treatment that consists of new therapeutic approaches, such as

Len/dex (lenalidomide, dexamethasone) in 1 of them (Pt 6), and VTD (velcade, thalidomide and dexamethasone) in the other 2 (Pt 7, Pt 8), as shown in the Table. Consistently with the literature data, among our patients the incidence of P-bone was higher than P-extramedullary as well as the rate for plasmocytoma was higher among males than females; further, in our cases the majority of P-bone arose in the axial skeleton, while P-extramedullary was localized at the nasopharynx that, together with larynx and upper respiratory tract, is the site of predilection for such plasmacytomas. Over the past decades, solitary plasmacytomas generally showed a good response to the existing therapies. However, new insights into the biology of these rare plasma cell neoplasms may allow a therapeutic choice (adjuvant systemic therapy or local therapy alone) based on risk assessment for progression to multiple myeloma.

P302

THALIDOMIDE MAINTENANCE THERAPY AFTER AUTO-SCT IN PATIENTS WITH MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP RESULTS

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Randomized trials have addressed the question of thalidomide maintenance therapy after ASCT in patients with multiple myeloma. All studies showed a significant benefit in PFS and the majority of these in OS, too. The thalidomide dose used was variable from 50 to 400 mg. The range of incidence of grade 3-4 peripheral neuropathy was 27-4% related to doses and duration of treatment. We conducted a retrospective analysis of patients with MM who received ASCT in our institution and maintenance therapy with low-dose thalidomide. From January 2001 to December 2006, 30 patients, 18 male and 12 female, have been treated. Median age was 60.5 years (range 38-68 years). 25 were IgG , 3 IgA, 2 light chains. All patients received induction chemotherapy with DAV regimen (Dexamethasone, Adryamicin, Vincristine) 2-4 cycles followed by single or double ASCT. Conditioning treatment was Mel 100 for 15 patients and Mel 200 for other 15. Three months after transplantation thalidomide was administered as maintenance with a dose of 50 mg per day, until the observation of relapse or toxicity. The response after transplantation was: 10 (33%) of patients achieved CR, 18 (60%) PR and 2 (3%) SD. The median duration of maintenance therapy was 30 months (range 6- 60 months) The median time to progression was 33 months (range 10-60). 53% of the patients was alive after 4 years.Median follow-up from ASCT was 39 months (range 28-69). We observed a neurological toxicity (grade 1-2) in 13% . A grade 1 hematological toxicity in 26% of patients. One patient had superficial venous thrombosis of leg and the treatment was suspended. In our experience, thalidomide has been well tolerated for a long time, median duration of maintenance therapy being 30 months and the low dose of drug has been able to improve both PFS and OS.

Chronic Myeloid Leukemia II

P303

ABCB1 AND CYP3A5 GENETIC VARIANTS CORRELATE WITH RESPONSE TO IMATINIB IN NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA PATIENTS

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Imatinib mesylate (IM) is the first-choice treatment in chronic myeloid leukemia (CML). Some patients (pts), however, experience suboptimal response or resistance, which highlights the need to find biological predictors of outcome in order to guide therapy optimization. Since blood and tissue concentrations of drugs may be influenced by interindividual variations (single nucleotide polymorphisms, SNPs) in genes encoding drug metabolizing enzymes and drug transporters, we reasoned that SNPs influencing the extent to which IM is actually delivered to target cells may account for differences in response. To test this hypothesis, we have performed a pilot genotyping study for a panel of 16 SNPs known to affect the activity of the Cytochrome p450 family isoforms CYP3A4 and CYP3A5 and of MDR1 (ABCB1), BCRP (ABCG2) and hOCT1 transporters in DNA samples from 82 pts (minimum follow-up, 12 months; all ethnicities represented) enrolled in an international randomized phase III trial of 400mg vs 800mg IM in newly diagnosed previously untreated CML in chronic phase (Tyrosine Kinase Inhibitor Optimization and Selectivity Study, TOPS). MDR1 SNP rs1045642 was statistically significantly associated with cytogenetic response (CgR) in the first 12 months: 72% of the homozygous wild type (CC), 85% of the heterozygous (CT) and 100% of the homozygous mutant (TT) pts had optimal (i.e., major CgR at 6 months, complete CgR at 12 months) CgR (p=0.044). Associations between different MDR1 haplotypes and optimal CgR in the first 12 months had p-values between 0.064 and 0.18. If the same level of association were seen in a larger sample size the results could reach statistical significance. In all cases, the greater the level of mutant SNPs present, the higher the fraction having optimal CgR. In addition, CYP3A5 SNP rs776746 was statistically significantly associated with major molecular response (MMR) by 12 months: 36% of the homozygous wild type (AA), 62% of the heterozygous (AG) and 100% of the homozygous mutant (GG) pts achieved MMR (p=0.05) Neither BCRP nor hOCT1 SNPs were found to correlate with response at any level. MDR1 and CYP3A5 genotyping of 105 additional pts enrolled in the same trial is now ongoing to confirm these preliminary observations and to further explore the association of MDR1 haplotypes with CgR.

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P304

OCCURRENCE OF CLONAL CYTOGENETIC ABNORMALITIES IN CHRONIC MYELOID LEUKEMIA TREATED WITH FIRST AND SECOND GENERATION TKIS

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Introduction. Occurrence of Clonal Cytogenetic Abnormalities (CCA) arising in Ph- cells has been widely reported. Aim of this study was to retrospectively evaluate frequency of CCA in newly diagnosed CP-CML being treated with front-line standard-dose Imatinib and in all-phase CML pretreated pts requiring switch to a second-generation Tyrosine Kinase Inhibitor (TKI). *Patients and Methods.* CCA were assessed in 2 groups of pts: group 1 comprised 60 CP-CML pts on front-line standard-dose Imatinib; group 2 included 17 pre-treated all-phase CML pts who

required treatment with a second-generation TKI after failing Imatinib. Cytogenetic analysis was performed - using standard G-banding techniques - at diagnosis and during treatment as recommended by the European Leukemia Net expert panel (*Baccarani et al, Blood 2006*). At least 20 metaphases were analysed; chrom. aberrations were considered as clonal when present in 10% or more metaphases. Results: In group 1, CCA frequency was 2/60 pts (3.3%); chromosomal anomalies were tris-8 and t(X;13). In group 2, 5/17 pts (29.4%) had one or more CCA developing at different times during follow-up; the most frequent chromosomal anomaly was trisomy 8 (4/17 pts). Of notice, in our study population, trisomy 8 was found both as CCA and as adjunctive cytogenetic abnormality in the Ph+clone. {as example, during therapy: 46,XX,t(9;22)[14]/47,XX,t(9;22),+8[5]/47,XX,+8[1] 47,XX,+8[7]/47,XX,+9 [11]/46,XX [2]. *Discussion*. In our experience, CCA frequency was higher among pretreated pts requiring second-generation TKIs. It may be hypothesized that a subgroup of CML pts develop they myeloproliferative disorder on the background of a genomic instability which predisposes to and expresses itself as additional cumulative chromosomal abnormalities during the course of their disease.

P305

IMATINIB IS A REMARKABLY EFFECTIVE THERAPY FOR NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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We have reviewed our experience with Imatinib in five teams in Italy and we present here the results of a population-based study of CML patients. One hundred and seventy-five previously untreated patients entered in this analysis, one hundred-sixty were evaluated. The median age was 51 years (range, 21-84), 95 were male and 65 females. The patients have been diagnosed with Ph-positive CML in chronic phase and must not have received treatment for CML, except for hydroxyurea. Sixty-three patients (39,4%) had low risk, 71 patients (44,4%) intermediate and 26 patients (16,3%) high risk according to Sokal. Patients were recruited from June 2000 and were assigned to receive Imatinib at a dose of 400 mg orally per day. The median time from diagnosis to start Imatinib was 21 days (range, 4-35). Patients receiving Imatinib who did not have a complete hematologic response within 3 months or whose bone marrow contained more than 65% Ph-positive cells at 12 months could have a stepwise increase in the dose of Imatinib to 600 mg orally. The primary end point was event-free survival, which was referred to the time to progression or progression-free survival. Events were defined by the first occurrence of any of the following: death from any cause during treatment, progression to the accelerated phase or blast crisis of CML, or loss of a complete hematologic or major cytogenetic response, discontinuation of Imatinib (patients failure to achieve a MCyR but did not lose their CHR) and patients who cannot tolerate the drug. Secondary end points were the rate of complete hematologic response (defined as a leukocyte count $<10x10^9$ per liter, a platelet count of $<400x10^9$ per liter, no blasts or promyelocytes, no extramedullary involvement, and no signs of the accelerated phase or blast crisis of CML); a cytogenetic response in marrow cells, categorized as complete (no Ph-positive metaphases), partial (1 to 35% Ph-positive metaphases), or major (complete plus partial responses) on the basis of G-banding in at least 20 cells in metaphase per sample; progression to the accelerated phase or blast crisis; overall survival; safety; and tolerability. Signs of a molecular response were sought every 3 months after a complete cytogenetic response was obtained with the use of real-time quantitative polymerase chain reaction to measure the ratio of BCR-ABL transcripts to ABL transcripts. Results were expressed as "log reductions". At the date of this analysis (September 2008), 19 patients (11.9%) had permanently Imatinib discontinuation after a median time of 13 months (range, 0.3-38). Reasons for discontinuation included non responder (n=7), progressive disease (n=4), toxicity (n=3) and unknown (n=5). After Imatinib discontinuation, 4 patients underwent allogeneic stem cell transplatation and 15 patients received dasatanib, nilotinib, hydroxyurea or interferon-α. Among the 141 patients who continued receiving Imatinib, the last reported daily dose was 400 mg in 135 patients (94%) and 600 mg in 6 patients. The most commonly reported adverse events were edema (including peripheral and periorbital edema), muscle cramps, diarrhea, nausea, musculoskeletal pain, rash and other skin problems, abdominal pain, fatigue, joint pain, and headache. Grade 3 or 4 adverse events were extremely rare and consisted of neutropenia, thrombocytopenia, anemia, elevated liver enzymes, and other drug-related adverse events. In no patients congestive heart failure was reported. The estimate cumulative rates of complete cytogenetic remission (CcyR) was 153/160 (95%) at 60 months. The median time to achieve CCyR was 6 months (range, 3 to 10 months). There were differences in the rates of cytogenetic response, according to a scoring system devised by Sokal and colleagues; in patients who were deemed to be at low risk on the Sokal scoring system, the rate of CCyR was 100%; the rate among patients at intermediate risk was 98%; and for those at high risk, the rate was 80%. All 153 patients, who achieved CcyR, were measured BCR-ABL transcripts in the blood samples. At a median of 709 days (range, 151-2145 days), levels of BCR-ABL transcripts had decreased by at least 3 log in 104 patients (66.7%). Thirty-six of them (23%) achieved CMR at a median of 817 days (range, 698-1020 days). Estimated EFS at 60 months was 89%. These results confirm that Imatinib is highly effective in patients with early CP-CML. Differently from the HH experience, we was able to demonstrate a high number of MMR (66,7%) with 36 patients achieving CMR. This disparity may be difficult to be explained. Perhaps, it may be due to the higher number of low-intermediate Sokal in our patients and compliance of the administration of the drug. Our patients were strictly followed by our teams and the patients were alerted to avoid to reduce the dose of the drug. Our data confirm that achieving MMR/CMR correlated with an excellent EFS. We agree with HH that approximately 25-30% of patients still need better therapy.

P306

AURORA KINASE INHIBITORS REVOKE THE PROLIFERATIVE ADVANTAGE AND "NEGLIGENT" G2/M CHECKPOINT OF CHRONIC MYELOID LEUKEMIA STEM CELLS AND EARLY PROGENITORS

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The pool of leukemic stem cells and early progenitors recapitulates two critical traits of chronic myeloid leukemia (CML): genomic instability and drug resistance. Indeed, genomic instability is the major source of genetic aberrations that drive the disease progression, including BCR-ABL point mutations that cause imatinib (IM) resistance. The constitutive activation of Aurora kinases (AK) A and B associated with BCR-ABL expression leads to the abrogation of mitotic spindle checkpoint and may be therefore considered as a major source of genomic instability. The advantage of AK inhibitors in the treatment of either IM-responsive or -resistant CML is ascribed to their ability of binding and inhibiting p210 BCR-ABL protein in wild type (wt) or mutated conformations. Their impact on specific targets, the AK, and on the IM-resistant pool of leukemic stem cells and early progenitors is not known. We found that the phosphorylation of p210 BCR-ABL protein at Tyr245 (within the SH2-linker domain proceeding from Tyr412 activating phosphorylation in the activation loop) was abrogated by AK inhibitors MK-0457 (VX-680 from Merck Pharmaceuticals) or AS-703569 (from Merck Serono) in Ba/F3 and 32D cell lines expressing the wt p210 protein and strongly reduced in the same cell lines expressing the most deleterious mutation: T315I. Furthermore, both AK inhibitors induced the de-phosphorylation of histone H3 at Ser10 (a crucial step for the onset of mitosis in early G2) followed by multipolar chromosome segregation, microtubule misalignment and aberrant cytokinesis. Those events drive the so called "mitotic catastrophe", a cell death process alternative to apoptosis. The effects of AKB inhibition on cell progression throughout mitosis arise from post-transcriptional modifications of the chromosomal passenger complex (CPC) components: Survivin and inner centromere protein (INCENP). Moreover, the drug-induced inhibition of AKA induced the transcription of its downstream target, the growth-arrest/DNA damage gene 45 (GADD45), thereby promoting cell accumulation in the sub-G1 phase of cell cycle that indicates cell death. All above mentioned events in response to MK-0475 occurred in a pool of early hematopoietic pro-

genitors (CD34+) purified from bone marrow samples of CML patients at clinical diagnosis and responsive to IM in vitro. Further experiments are currently in progress to elucidate their role in AK inhibitor cytotoxic effects against leukemic stem cells and early progenitors driven towards IM-resistance by BCR-ABL- dependent and -independent mechanisms. In conclusion, our results suggest that AK inhibitors target many signals involved in the survival advantage and genomic instability of leukemic cells and may be therefore considered in the treatment of CML in advanced stages.

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CONTRIBUTION OF ABL KINASE DOMAIN MUTATIONS TO FAILURES AND SUBOPTIMAL RESPONSES TO IMATINIB IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS

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Bcr-Abl kinase domain (KD) mutations are regarded as the most frequent mechanism of resistance to the tyrosine kinase inhibitor (TKI) imatinib (IM) in patients (pts) with chronic myeloid leukemia (CML). Nearly all studies, however, have focused mainly on pts with advanced disease, where resistance is most often observed. Nowadays, the great majority of pts on IM are early chronic phase (ECP) pts receiving first-line IM. If, on one hand, the IRIS study demonstrated that response rates are high and relapse is infrequent in ECP, on the other hand we still know very little on the contribution of KD mutations to resistance in this subset of pts. We screened for Abl KD mutations one hundred and twenty-five ECP pts on IM who were referred to our laboratory because their response was defined either as *failure* (n=81 pts) or as 'suboptimal' (n=44 pts) according to ELN recommendations. Twenty mutations were detected in 20/81 (25%) pts who failed IM. Mutations were found in 1/2 pts who showed no hematologic response (HR) at 3 months, 2/15 (13%) pts who showed less than partial cytogenetic response (PCgR) at 12 months, 5/29 (17%) pts who showed less than complete cytogenetic response (CCgR) at 18 months, 7/24 (26%) pts who lost CCgR, 5/11 (50%) pts who lost HR. Mutations were M244V (n=2), G250E (n=1), Y253H (n=4), E255K (n=1), T277A (n=1), E279K (n=1), F311I (n=1), T315I (n=1), M351T (n=3), E355D (n=1), F359V (n=1), H396R (n=3). In 8 pts who progressed to accelerated or blastic phase shortly after, five had mutations: Y253H (n=2 pts), E255K (n=1 pt) and T315I (n=2 pts). Six mutations were detected in 6/44 (14%) pts who had a suboptimal response to IM. In particular, a mutation was observed in 1/14 (7%) pts who showed less than PCgR at 6 months and in 5/30 (17%) pts who showed less than CCgR at 12 months. Mutations were M244V, E255K, F317L, M351T, F359V, F486S. A higher incidence of mutations was observed in high Sokal risk pts. We conclude that in ECP pts who receive IM as front-line treatment, Abl KD mutations are not the major mechanism of resistance, probably because mutations tend to accumulate during the natural course of the disease as a result of a progressively increasing genetic instability. Our data highlight the need to find out which is the actual predominant mechanism(s) of resistance acting in the setting of ECP - which now gathers the majority of CML pts on IM. Supported by European LeukemiaNet, AIL, PRIN and Fondazione del Monte di Bologna e Ravenna.

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DASATINIB INDUCES NOTABLE CYTOGENETIC AND MOLECULAR RESPONSES IN IMATINIB-RESISTANT OR -INTOLERANT CML-CP

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Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia (Ph) chromosome, which results from a reciprocal translocation between the long arms of the chromosomes 9 and 22 t(9;22)(q34;q11). The BCR-ABL gene encodes for a 210-kD protein with deregulated tyrosine kinase (TK) activity, which is crucial for malignant transformation in CML. Imatinib is the first choice drug in chronic phase CML (CP-CML), but approximately more than 20% of patients (pts) will need alternative therapy, due to drug resistance or intolerance. Among 101 pts diagnosed as CP-CML and admitted at the Department of Hematology, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena from 1999 to march 2009, 15 pts (10 males and 5 females, M/F ratio 2; median age 47 years, range: 31-70) have switched from Imatinib to Dasatinb because of loss of response in 12 pts (80%, 7 cytogenetic and 5 molecular), severe hepatic toxicity in two and intolerance in one. At diagnosis, the CML clinical risk scores were as follows: Sokal/ Hasford high risk 4 pts (26%), intermediate risk 5 pts (34%), low risk 6 pts (40%). All patients were treated with Dasatinib 100 mg once daily. All of the pts underwent conventional cytogenetic and quantitative real time polymerase chain reaction (Q-RT-PCR) analyses using ABI PRISM 7900 before and during TKIs treatment. A major molecular response (a *3-log reduction* according to literature) was achieved by 11/12 (92%) pts, except for one patient who obtained a minor molecular response; in 8 pts the molecular response was better than that previously obtained with Imatinib. The major molecular response was obtained in a median period of 9 months. The seven pts who had lost cytogenetic response during Imatinib treatment, obtained a complete cytogenetic response in 3 months. Finally, 2 of the 3 pts who switched to Dasatinib due to intolerance/toxicity improved their molecular response by >2 log. Our data indicate that Dasatinib induces notable responses, both cytogenetic and molecular, in Imatinib-resistant/intolerant CP-CML. It therefore represents a good therapeutic option: no side effects were observed and dose change was never necessary with durable responses. Further studies are needed to confirm these data in a larger series of CP-CML pts with longer follow up.

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COEXISTANCE OF A JAK2 MUTATED CLONE MAY CAUSE HEMATOLOGIC RESISTANCE TO TIROSYNE-KINASE INHIBITORS (TKI) IN CHRONIC MYELOID LEUKEMIA (CML)

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Most systematic investigations on myeloproliferative neoplasms and CML showed that JAK2 mutation and BCR-ABL were exclusive and their coexistence has been described in rare patients (Pt). We report 3 such cases. Pt 1 was diagnosed of CML, following the discovery of thrombocytosis. She took imatinib (IM) with no platelet control and hydroxyurea (HU) was added. She did not tolerate IFN so started nilotinib for leucocytosis, thrombocytosis and 100% Ph-positivity. In six months she was in complete cytogenetic response (CCyR) but the improvement of the counts was transient. We thus researched the Jak-2 mutation that was positive. Interestingly the Jak2-V617F was present since nilotinib start, but its expression achieved the maximum with CCyR. Pt 2 was diagnosed of primary myelofibrosis with leukocytosis, thrombocytopenia, splenomegaly and trilinear dysplasia plus collagen

fibrosis in the bone marrow (BM). Ph-chromosome was not detected. After 2 years with no treatment, BM showed 15% myeloblasts with hyperplastic myeloid series and 100% Ph-positivity. The ptstarted IFN and then IM for hematologic resistance, although a partial cytogenetic response. After an initial improvement, a new increase of leucocytes and platelets followed, with normal karyotype. HU was added, but hematological control remained unsatisfactory and IM was stopped. The pt stayed on CCyR with insufficient hematologic response for more than one year, until he developed a Ph-negative myeloblast crisis with homozygosis for JAK2-V617F. We found that before IM the pt had a 50% JAK2 mutated phenotype and after 3 months of therapy, more than 70%. Pt 3 was diagnosed of essential thrombocytopenia with a normal karyotype. A good control of the thrombocytosis was obtained with HU, which continued for 10 years and then the pt refused therapy for the next 3 years of stable disease, until he developed bone pain, leukocytosis and enlarged spleen. Conventional cytogenetics was normal but FISH disclosed a 47% Ph-positivity. The pt started IM with a rapid decrease of leucocytes; however, the platelets raised and HU was added. After 3 months the BM showed 7.4% Ph-positive cells. JAK 2 was found mutated from imatinib start. This report suggests the presence of two different clones, one Ph-positive and the other JAK2 mutated and the prevalence of the latter when the former was inhibited by a TKI. The presence of a JAK2 positive clone may be a rare cause of hematologic resistence to TKI.

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A DECREASED LEVEL OF SHP1 PROVIDES AN ADDITIVE SURVIVAL ADVANTAGE TO THE PH+ CELLS OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS AND MAY ACCOUNT FOR RESISTANCE TO IMATINIB TREATMENT

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Imatinib is the current first-line therapy for all newly diagnosed CML. However, 15-20% of patients do not achieve durable complete cytogenetic responses and a small proportion of patients develop resistance to this drug during the treatment. While mutation of Bcr/Abl kinase domain as a cause of resistance has been described in around 50% of resistant patients, other mechanisms accounting resistance in the remaining are poorly known. Here we show that the protein-tyrosine phosphatase SHP1, a protein with a tumour suppressor activity, may play an important role in the resistance to Ima treatment. In the first part of our study, we identify the SHP1, both by gene profiling and proteomic bidimensional electrophoresis, as one of the most differentially expressed proteins between the parental KCL22s (Ima-sensitive) and the Ima-resistant KCL22r cell lines (in this cell line resistance is independent from BCR/ABL mutations). Consistently with epigenetic regulation of the SHP-1 expression, we found that aberrant methylation of its promoter is fundamental for the down-regulation found in KCL22r (0.2±0.1 SHP1/ABL copy numbers) compared to KCL22s (1.8±0.3). Then, taking Immunoprecipitation assay, we found that in these cells one of the main interactors of SHP1 is SHP2, another SH2 domain containing phosphatase protein that, differently from SHP1, acts as a positive regulator of Ras/MAPK pathway. We hypothesized that SHP1, through dephosphorylation of 542- and 580-tyrosine residues of SHP2, might modulate its downstream activity and therefore constitute an important mechanism for Ima-sensitivity. Indeed, we found that KCL22r cell line, that has low SHP1 levels, showed complete phosphorylation of both SHP2 tyrosine residues, while these residues are not phosphorylated into the KCL22s line. Consistently with this hypothesis, knocking-down of SHP2 phosphatase in KCL22r by a specific shRNA results in 60% inhibition of KCL22rSHP2- proliferation and, under these conditions, the KCL22rSHP2- cells showed a significant reduction of STAT3 (60%) and ERK1/2 (70%) phosphorylation. The role of SHP1 as Ima sensitivity determinant were further corroborated by the expression data in 60 CML patients classified, according to the ENL definitions, as optimal (35), suboptimal (17) and failure (8) Ima responder after 18 months of treatment. The levels of SHP1 mRNA were significantly lower in failure patients (3.2±1.04) respect to those of suboptimal and optimal responders (3.8±1.54 and 5.8±1.72, respectively. $p<0.004$). Thus, our data suggests that an aberrant balance between the Shp1 and 2 levels plays a role in the IMA-resistance through activation of Ras/MAPK pathway and that low levels of SHP1 may be associated to IMA-resistance in CML patients.

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THE EXPRESSION OF SHP1 AND SIT: A NOVEL POWERFUL PREDICTOR OF MAJOR MOLECULAR RESPONSE (MMR) ACHIEVEMENT IN CHRONIC MYELOID LEUKEMIA GLEEVEC-TREATED PATIENTS ENROLLED INTO THE TOPS CLINICAL TRIAL

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Imatinib (Ima) has dramatically improved the outcome of patients affected by chronic myeloid leukemia (CML) and became the standard care for patients with newly diagnosed CML in chronic phase (CP). In spite of treatment progresses and novel biological findings, Sokal index is still a dominant prognostic determinant of newly diagnosed CML patients also in the era of targeted therapies. In this study we investigated the predictive role of the levels of expression of SHP-constitutive non receptor protein tyrosine phosphatase SHP1 and transmembrane adapter protein SIT, in leukemia cells obtained from 48 newly diagnosed CML patients enrolled into the TOPS (Tyrosine kinase inhibitor Optimization and Selectivity) trial. TOPS is a prospective, open-label, randomized (2:1) Phase III trial that compared Ima 800mg/d to 400mg/d in CP-CML. The findings end point of the trial is the rate of major molecular response (MMR) indicated by several reports as a parameter that predict a benefit for progression free survival (PFS). Results indicate that the mRNA levels of both SHP1 and SIT assayed by QPCR in peripheral blood of newly diagnosed patients and expressed as ratio to ABL, are significantly different between those patients who do and do not achieved MMR by 12 months (7.4±3.8 vs 6.0±3.2, $p=0.017$ for SHP1/ABL and 0.19±0.15 vs 0.10±0.12, $p=0.017$ for SIT/ABL). Complete cytogenetic response, CCyR, was a secondary end point of the TOPS study, overall, 65% have achieved CCyR by 6 months, and 85% by 12 months, and although not statistically different, results indicate that both SHP1 and SIT levels tended to be higher in patients who obtained CCyR, and the our further study with a larger sample size will show if the differences might become significant. .SHP1/ABL and SIT/ABL are weakly correlated each other in the patients, therefore each independently acts as predictor of MMR at 12 months and logistic regression indicated that the combination increases their prognostic value for predicting MMR in the first 12 months and using logistic regression, Sokal score does not add any discriminating power to either of those markers (either alone or in combination). In conclusion, our results indicate, that expression levels of SHP1 and SIT are useful predictors of MMR in newly diagnosed CP-CML patients. These data confirm our prior findings from "in vitro" studies, which investigated the role of SHP1 and SIT in mechanisms which regulate Imatinib sensitivity.

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CONCEPTION OF HEALTHY CHILDRENS UNDER IMATINIB TREATMENT IN TWO MAN AFFECTED BY CHRONIC MYELOID LEUKEMIA

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Imatinib is presently the gold standard therapy of chronic myeloid leukemia (CML), with complete cytogenetic responses achieved in 70-

90% of patients receiving the drug as first-line treatment. Imatinib selectively inhibits BCR/ABL and other tyrosine kinases, such as c-kit and PDGF-R. Overall, Imatinib is well tolerated; however, due to the limited information available, the potential consequences of this drug on a developing foetus are still a matter of debate. *in vivo* animal studies have proven that Imatinib induces teratogenesis and dose-related effects on spermatogenesis. We present here our experience on two male patients (46 and 39 y.o.) who voluntarily conceived under Imatinib therapy, without consulting or informing the doctor. The two patients had received a standard dose of 400 mg/day, respective for 23 and 16 months prior to conception. Both had shown a good drug tolerance and had reached complete cytogenetic remission (CCR) at the time of conception. Sperm count performed in one patient shoved no evidence of damaged cells. An uneventful vaginal delivery occurred with the partner of one CML patient, the other patient's partner underwent a caesarean section for podalic position. Birth weights and lengths of the babies (a male and a female) were in the normal centiles and development was reported normal. Limited information exists regarding management of CML with Imatinib during pregnancy. Studies in male rats demonstrated that Imatinib decreases testicular weight and sperm motility while high doses, of more than 100 mg/kg produce total fetal loss and testosterone level reduction. As well, inhibition of c-kit and PDGF-R in testicular tissue has been suggested in patients under high doses of the drug. Whereas it seems that even brief exposure to Imatinib may increase the risk of spontaneous abortion, miscarriages and other birth abnormalities in women, the limited data available do not support the hypothesis that similar effects on pregnancies are produced when CML male patients are exposed. However, lacking more detailed and well definite evidence, present recommendation is that all CML patients should practice adequate methods of contraception while under Imatinib therapy. Specific studies on male fertility and relationship between drug dosage and testosterone production are warranted, and Imatinib interruption is recommended in all male patients before any conception attempt starts. Further studies will be necessary to validate our experience.

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IDENTIFICATION OF NEW GENES SUSTAINING BCR-ABL ONCOGENIC SIGNALLING AND CML PROGRESSION THROUGH A GENETIC TOOL BASED ON A HUMAN BCR-ABL TRANSGENIC DROSOPHILA MELANOGASTER (DM)

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Despite the role of Bcr-Abl in the pathogenesis of Chronic Myeloid Leukemia (CML) is well established, the biological bases of CML progression remain largely unknown. We aim at identifying new genes and pathways leading to CML progression through a genetic screening using an hBcr-Abl transgenic Dm. A wide modifier screening was performed by crossing our set-up hBcr-Abl Dm with 300 fly stocks carrying well characterized chromosome deletions within the whole genome. The resulting progeny was screened using eye phenotype as first read-out system. In order to exclude false positive such as genes involved in eye development, each deletion giving phenotype changes in the eye was analyzed either by expressing it into lymph gland, which represents the fly haematopoietic system. Furthermore, loss of function (LOF) mutants of each gene included in identified regions, were tested and data were finally validated by analyzing samples from CML patients. The analysis of phenotypes in progenies obtained from screening crosses, showed a first group of flies (38%) displaying a more aggressive phenotype since they lack genes encoding for Bcr-Abl negative regulators and a second group (32%) showing a mild phenotype due to the absence of genes involved in the oncogenic signalling. By now we have identified up to 35 new genes responsible for phenotype changes, including Fax, Dab, Pros, Dock and Rab5. LOF mutations of these genes induced a worsening, while their gain of function (GOF) rescued the severity of the phenotype, suggesting that they can represent enhancers of Bcr-Abl signalling. Further validation of findings in fly comes from the analysis of genes expression by Real Time PCR in 35 CML patients at diagnosis and during progression. They resulted highly down-regulated ($p<0,001$) in CML samples with respect to 20 BM from healthy donors and their expression reached normal value during remission. Moreover, transfection of CML cell lines with GOF constructs reduced proliferation and induced apoptosis. By contrast, deletions of ENA, HSP90 and genes involved in Wnt signalling, rescued Bcr-Abl phenotype while GOF mutants gave a worse phenotype, suggesting their involvement in CML progression. In particular LOF of ENA, the CRKL hortologous, induced phenotype changes, suggesting that ENA could play a role in Bcr-Abl signalling. In conclusion, through the Dm model, we have identified new genes, which seem to be crucial for human Bcr-Abl oncogenic signalling and CML progression.

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PHILADELPHIA-NEGATIVE CHRONIC MYELOID LEUKEMIA: CYTOGENETIC-MOLECULAR CHARACTERIZATION AND RESPONSE TO IMATINIB TREATMENT.

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Background. About 95% of Chronic Myeloid Leukemia (CML) patients (pts) show the reciprocal translocation t(9;22)(q34;q11). A minority of pts does not show Ph chromosome by conventional cytogenetic (CC) and is classified as Ph-negative (Ph-) CML. Dual-color dual fusion BCR/ABL FISH allows to detect the localization of the rearrangement in metaphase cells. In Ph- CML cases BCR/ABL rearrangement is usually observed on 22q11 region, meanwhile a smaller subset shows BCR/ABL fusion gene located on 9q34. These pts are usually followed with FISH and RQ-PCR. AIMS. To evaluate if different mechanisms leading to BCR/ABL rearrangement can have a role on Imatinib (IM) therapy, we have characterized 6 cases of Ph- CML. *Results*. At diagnosis pts were characterized and, then, monitored during IM therapy by CC, FISH and RQ-PCR. Bone marrow karyotype was normal in all observed metaphases in each pt. The transcript was b3a2 in 5 pts and b2a2 in 1 pt. Dual-color dual fusion FISH-metaphases showed 4 pts with localization of BCR-ABL rearrangement on derivative chromosome 9, 1 pt on derivative chromosome 22 and 1 pt on both derivative chromosomes 9 and 22. The successive analysis were performed to monitor the imatinib response. In one pt, with rearrangement on der(9q), during disease progression, metaphase-FISH revealed the appearance of a secondary clone with two fusion BCR/ABL signals on both chromosomes 9, indicating a rearrangement amplification as a consequence of an eventual duplication of derivative chromosome 9 and loss of the normal one. During IM therapy, FISH revealed a decrease of malignant cells in 4 pts with only one signal fusion; they reached Complete Cytogenetic Response (CCgR) after a period between 6 and 30 months and then the Major Molecular Response (MMolR). The pt with two fusion signal didn't achieved MMolR within 3 years of therapy. Finally, in the pt with the secondary amplification of BCR/ABL rearrangement persisted a high rate of positive cells by FISH, even if he was in Complete Haematologic Remission (CHR). *Conclusions*. Interphase-FISH is an efficient tool to characterize and to monitor therapy of Ph- CML pts. Four Ph^- CML pts reached CCgR and MMolR showing a prognosis similar to Ph^+ CML pts. Only one pt, with secondary change, failed IM therapy, confirming BCR/ABL amplification as "warning" feature, detectable by FISH.

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IDENTIFICATION OF DISABLED AS A GENE INVOLVED IN CHRONIC MYELOID LEUKEMIA PROGRESSION

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Despite the role of Bcr-Abl in the pathogenesis of Chronic Myeloid Leukemia (CML) is well established, the mechanisms leading to CML

progression remain unknown. The Drosophila's Disabled is a non receptor tyrosine kinase, identified as adaptor protein acting downstream of many receptor tyrosine kinases (RTK) signalling pathway. One of the human homologs of Disabled, Dab1 is a large common fragile site gene, involved in neural migration. The other homolog Dab2 is an adaptor protein implicated in growth factor signalling, endocytosis, cell adhesion, hematopoietic cells differentiation and cell signalling of various RTK. The expression of both genes is often decreased in many human cancer types suggesting their possible role in oncogenesis. To date, their involvement in haematological malignancies remains unknown. The aim of the study was to clarify the involvement of Dab1/2 in CML and to investigate their role as negative interactors of Bcr-Abl. Dab1/2 mRNA was analyzed by Real Time PCR in 64 samples from CML patients (44 BM and 20 PB) at diagnosis, in 20 patients in blast crisis and in 38 healthy donors (18 PB and 20 BM). In18 patients, genes expression was analyzed during remission as well. Protein expression was investigated by Western Blot and Immunofluorescence. In addition K562 was transfected with Dab plasmids to clarify Dab effects on cell growth and apoptosis. Finally, we used our already set up model of Dm transgenic for human Bcr-Abl to study the interaction between Dab and Bcr-Abl. We found that Dab1/2 expression levels were significantly decreased in CML patients either in BM or PB (p<0.002 and ^{P}<0.0004) as compared to healthy donors. Moreover in blast crisis we found lower transcripts levels and they returned at normal value during remission. Western Blot and immunoflourecence confirmed the absence of Dab protein in CML samples while it reappeared during remission. Moreover, transfection of gain of function constructs in K562 significantly reduced proliferation and induced apoptosis. Finally, the progeny obtained by crossing hBcr-Abl flies with flies carrying loss of function mutations of Dab showed a worsening, while Dab gain of function rescued Bcr-Abl phenotype, thus supporting the idea that Dab can represent a negative regulator of hBcr-Abl. Taken together these data suggest that Dab1/2 represent negative interactors of hBcr-Abl and are inactivated in CML cells, suggesting that their deregulation could be a crucial event in leukemogenesis.

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ACCURACY AND PRECISION OF RESULTS AFTER IMPLEMENTATION OF INTERNATIONAL SCALE TO EVALUATE MINIMAL RESIDUAL DISEASE IN CHRONIC MYELOID LEUKEMIA PATIENTS COORDINATED ON BEHALF OF GIMEMA-CML WP

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The best approach for achieving comparable BCR/ABL values is the use of an international reporting scale (IS), in which these values are anchored to major molecular remission (MMR) as established in the IRIS trial. Here we present a study to test the feasibility to implement and maintain the IS in a network of 20 Italian labs. Three of the labs had previously been aligned their results to the IS within an international initiative that included the IRIS participating labs, and therefore had the role of reference labs in the network. After a training period aimed to uniformly adopt the EAC method for MRD analysis, all network labs, in the first phase, analysed standard RNAs containing 4 levels of BCR/ABL using cell line dilutions. The log-transformed data of 9 replicates from each Standard RNA were plotted and the bias to the reference labs were calculated by the Bland&Altman algorithm for method comparison. The antilogs of method mean bias provide each lab-specific CF. The conversion to the IS greatly improve the accuracy of the results: indeed, the mean bias of participant labs passed from -0.22508 to 0.00013. A quality control test, based on the analysis of four patient samples at different levels of MRD, confirmed the high level of results alignment: the CV of data distribution were 22%, 12%, 11% and 30% for the four levels of MRD tested (BCR/ABLIS of 12.5, 1.52, 0.15 and 0.02). In a second step, we checked over time stability of lab CFs and compared the possibility to calculate CF using standard RNAs against the use of 15 patient derived RNAs at different levels of MRD. CFs were highly stable over time in all labs (n=12) that did not change instrument or reagents (CF differences = 0.07). We also tested lab performances for the consistent identification of MMR after IS conversion. To this aim, we divided the lab-result in 2 categories on the basis of accuracy and precision: 1) bias mean difference to reference lab within 1,2 fold and a range of results less than +/-5 fold (n=14); 2) average difference within 1,2 fold but a range of greater than 5 fold (n=4). Consistently with the high level of accuracy achieved within the network, no lab showed bias mean difference greater than 1.2. Results showed a 88% MMR concordance in the 14 laboratories of the first group, a 82% MMR concordance in the remaining four. This indicates that alignment to the IS may be achieved and maintained over time in a large number of labs and it ensures accurate measurements of MMR.

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ROLE OF HEME OXYGENASE 1 IN CML CELLS RESISTANT TO IMATINIB

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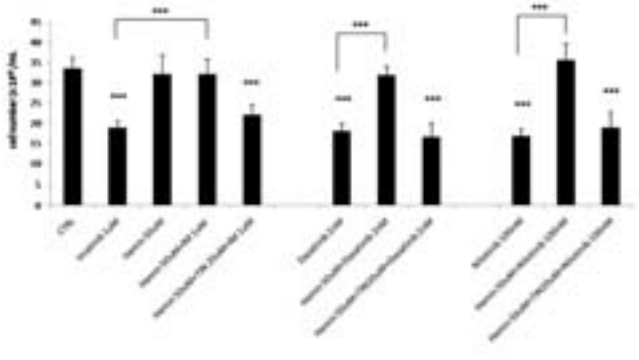
Background. Chronic myeloid leukemia (CML) is a stem cell disease in which BCR/ABL (tyrosine kinase (TK) activity) promotes the survival of leukemic cells. Imatinib mesylate is now the first-choice treatment for all newly diagnosed CML patients, but the initial striking efficacy of this drug has been overshadowed by the development of clinical resistance. The emergence of resistance to imatinib has prompted researchers to focus on strategies aimed at preventing or overcoming this phenomenon. Heme oxygenase-1 (HO-1) is an inducible rate-limiting enzyme which catalyzes group heme into carbon monoxide, iron and bilirubin. In the recent years, HO-1 expression has been reported as an important protective endogenous mechanism against physical, chemical and biological stress. The cytoprotective role of HO-1 has already been demonstrated for several solid tumors and acute leukemias. In addition, it has been recently showed that HO-1 is constitutively expressed in primary CML cells and that the BCR/ABL oncoprotein promotes expression of HO-1 in leukemic cells.



Therefore, HO-1 is considered to play an important role as a survival molecule in CML cells, and an overexpression of HO-1 was found to inhibit apoptosis induced by imatinib. *Methods.* In our laboratory, K562 cells were incubated for 24 hrs with imatinib 1 uM alone, or with an

inductor of HO-1 (Hemin 50 uM) or the combination of both. The same experiments were conducted with Dasatinib 2 nM and Nilotinib 100 nM. Cell viability was measured by trypan blue. Gene expression of HO-1 was assessed by Real time PCR (LightCycler, Roche). The results are expressed as mean±S.E.M. and the statistical analysis was performed using student's t test. A value of $p<0.05$ was considered as significant. Results.We found that HO-1 gene expression was increased about 3 fold after hemin treatment. The addition of hemin was able to overcome the inhibitory effect of IM (1 uM) on K562 cells ($p<0.002$) and the effect of IM was restored by adding an inhibitor of HO-1 (TIN) to the combination ($p<0.002$). Almost identical results were obtained with dasatinib and nilotinib ($p<0.002$). Conclusions. In conclusion, we confirm that HO-1 may represent a mechanism of resistance to IM and we showed that the same mechanism may apply to the other TKI (Dasatinib and Nilotinib). It remain to evaluate the role that HO-1 may have in the clinical setting.

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BRIT1 REGULATES G2/M CHECKPOINT IN CHRONIC MYELOID LEUKEMIA

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BRIT1 (BRCT-repeat inhibitor of hTERT expression), also known as microcephalin (MCPH1), acts as a regulator of both the intra-S and G2/M checkpoints. Regarding regulation of mitotic entry, BRIT1 co-ordinates the regulation of Cdc25A and Cdk1-cyclin B1 activity both in ATR-Chk1 and ATR-independent signalling. In our work we analyzed BRIT1 mRNA expression in K562 and in peripheral blood cells of chronic myeloid leukemia (CML) patients at diagnosis. Results were obtained using Taq-Man Gene Expression (Applied Biosystem) by Real-time PCR and were normalized using glucosio 6 phosphate dehydrogenase (G6PDH) as internal reference gene. The mean value of $2\Delta^{Ct}$ calculated on 18 patients was of 0,48 fold vs 20 healthy donors (control). K562 showed BRIT1 mRNA levels 0,7 fold lower than control. Because BRIT1 has a role in regulating mitotic entry, we assessed the capacity of K562 and CML cells to control G2/M checkpoint by cytokinesis block proliferation index (CBPI) assay. In particular, we used cells of three CML patients, one with BRIT1 mRNA levels as controls (p1) and the other two with a value of 2^- Ct respectively of 0.5 (p2) and 0,1 (p3) vs control (p2). Following DNA damage, CBPI assay distinguishes cells that have not divided (because of proper induction of the G2/M checkpoint) from those that have undergone one (binucleated) or more nuclear divisions (multinucleated) and are thus more likely to develop chromosomal abnormalities. K562 and CML cells were pre-incubated for 2 h with 0,2 mM hydroxyurea (HU) or irradiated with 2,5 J/m^2 UV. Then, cells was pelleted, washed and incubated for 72 h with 5 µg/ml cytochalasin B, an inhibitor of cytokinesis. Cells were processed using standard light microscopy after Giemsa staining. Treated and untreated conditions were compared about percentage of mononucleate cells respect to healthy donors cells. The percentage of mononucleate cells (PMNCs) increased approximately of 15±4% in healthy controls and of 13±8% in p1 after treatment with HU or UV, indicating G2/M checkpoint arrest. On the contrary K562, p2 and p3 showed this increase after UV only (PMNCs value in K562 = 46±2.7%; in p2= 44±3,5%; in p3= 25±5%) but not after HU treatment. In conclusion, our study reported a defective G2/M arrest in CML cells with low BRIT1 mRNA levels. The identification of new biomarkers of disease progression may be important for therapeutic management.

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IMATINIB AND DASATINIB INCREASE CYTOTOXICITY OF MELPHALAN AND DECREASES DELAY OF G2-M TRANSITION IN HUMAN K562 CHRONIC MYELOID LEUKEMIA CELLS

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BCR/ABL-positive cells activate DNA damage-dependent cell cycle checkpoints and repair damage more rapidly than non-transformed cells; this reason contributes to the higher resistance to anticancer agents. Treatment with tyrosine kinase inhibitors (TKIs) such as imatinib (IM) and dasatinib (DA), may reverse drug-resistance in BCR/ABL-positive cells. K562 (BCR/ABL positive cells) and HL60 (BCR/ABL negative cells) were incubated for 24h with IM 1 µM (pre-IM) or DA 5 nM (pre-DA) and then incubated for 1h (25th hour) with 20 µM of melphalan (ME), a DNA-damaging anticancer agent. Therefore, cells were pelleted, washed and resuspended in drug-free medium. Cellular viability and cell cycle were evaluated at 24, 48 and 72 hs from beginning of drug-free condition. 24 h pretreatment with TKIs (IM and DA) had no effect on HL60 cells, while reduced K562 cells of about 14,4±7.2% ($p<0.001$). Treatment with ME alone was able to kill HL-60, but this effect was not increased by pretreatment with TKIs. On the contrary, on K562 cells, ME alone reduced cell proliferation much less than on HL-60 while the TKI pretreatment sensitized cells to this DNA-damaging agent, thus resulting in a higher cytotoxicity. In Table 1 results are presented as % of variation of number of cells in respect to control at time 0. In addition, after treatment with ME, K562 cells in G2/M phase increased of 54±6 % and 43±10 %, in respect to CTRL (23±3 %) after 24 and 72 hs respectively ($p<0.001$). TKI pre-treatment reduced percentage of cells in G2/M phase at 24 h (pre-IM = 25±6 %; pre-DA = 18±5 %, $p<0.001$) and at 72 h (pre-IM = 10±5 %; pre-DA = 2±1 %, $p<0.001$). Pretreatment with IM or DA did not affect percentage of HL60 in G2/M phase. In conclusion, K562 cells pre-treated with IM or DA showed an increased sensitivity to ME and this was accompanied by a decreasing of time for DNA repair at the G2/M checkpoint. The same pre-treatment did not have effects on BCR/ABL negative cells (HL60). Our data suggest that inhibition of BCR-ABL signaling makes CML cells more sensitive to DNA- damaging drugs such as melphalan and this effect could be useful against resistant cancer cells or even CML stem cells.

Table 1.

	K562			HL60		
	24 hs	48 hs	72hs	24 hs	48 hs	72hs
Untreated	+ 50 ± 12	+ 111 ± 18	+ 144 ± 20	+ 69 ± 18	+138 ± 15	+ 169 ± 36
ME lone	+ 5 ± 10	+ 48 ± 15	+ 83 ± 16	+ 10 ± 8	- 39 ± 13	-68 ± 7
IM alone	+ 30 ± 14	+ 57 ± 18	+ 98 ± 16	+ 69 ± 16	+ 121 ± 19	+ 167 ± 18
DA alone	+ 50 ± 9	+ 104 ± 23	+ 138 ± 25	+ 69 ± 12	+ 121 ± 18	+ 160 ± 23
Pre-IM	- 47 ± 9 (*)	- 76 ± 7 (*)	- 69 ± 7 (*)	- 06 ± 7	- 25 ± 10	- 53 ± 9
Pre-DA	- 32 ± 10 (**)	- 40 ± 8 (*)	- 57 ± 8 (*)	+ 0,5 ± 8	-23 ± 11	- 53 ± 6

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RESISTANCE PROFILE OF THE BCR/ABL INHIBITOR BOSUTINIB USING A HIGH THROUGHPUT RANDOM MUTAGENESIS ASSAY

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The BCR/ABL fusion gene causes Chronic Myeloid Leukaemia (CML). Several selective BCR/ABL tyrosine kinase inhibitors have been successfully developed for the treatment of CML. Despite the dramatic results achieved, a limited number of patients experience resistance to these drugs, often through the occurrence of point mutations in the catalytic domain of BCR/ABL. Bosutinib (B) is a novel Src/Abl inhibitor presently in clinical development. Some mutations causing resitance to B such as V299L and T315I are known, but the number of mutants screened is limited. To

overcome this limitation we set up a High Throughput Mutation Profiling (HTMP) assay, based on the high-throughput clonal sequencing capabilities of the Next-Generation Sequencing instruments. We used the HTMP to investigate the resistance profile of BCR/ABL+ cells surviving a 7 day exposure to B (400 nM, Wyeth pharmaceuticals): the Cap, SH3, SH2 and kinase domain (KD) were analyzed. The average coverage for each nucleotide was 1531x, with a total of 3185422 bases sequenced. Globally, a total of 293 individual mutations were identified (transition to transversion ratio of 6.1/1). The majority of these mutations were clustered in a region comprised between the SH3 and the KD (177, 60.4%), with 21 mutations localized in the SH3 domain, 3 in the SH3-SH2 connector, 32 in the SH2 and 121 in the KD. The most frequently encountered mutations were: T315I (22.5%), V299L (2.9%), and L248R (0.91%) for a total of 344, 44 and 14 individual clones, respectively. To validate the results of the HTMP screening, 30 BCR/ABL mutants (*K51R, E60G, V67L, A136T, R170G, L184P, N231K, M244V, L248R, L248V, G250E, Q252H, E255V, K262R, D276G, K294E, V299L, T315A, T315I, F317L, F317V, M343T, S348P, M351T, F359I, F359V, H396P, H396R, G398R, E409G*) were generated using site-directed mutagenesis and their resistance profile to Bosutinib, Imatinib, Dasatinib and Nilotinib was analyzed using tritiated thymidine proliferation assays: as predicted by the HTMP analysis, T315I, V299L and L248R scored the highest resistance level, with a relative IC_{50} of 45.42, 26.10 and 18.36, respectively. The proliferation assays showed also that, similarly to mutant T315I, L248R is able to drive a complete resistance to all the 4 inhibitors tested (relative IC_{50}: 18.36, 22.18, 12.27, 29.95 for Bosutinib, Imatinib, Dasatinib and Nilotinib, respectively), while V299L led to an almost complete resistance to Bosutinib and Dasatinib (relative IC50: 26.10 and 8.65) and to a weak resistance to Imatinib and Nilotinib (relative IC50: 1.54 and 1.34, respectively).

P321

DASATINIB: OPTIMAL BRIDGE TO STEM CELL TRANSPLANT IN CHRONIC MYELOID LEUKEMIA BLAST CRISIS (CML-BC)

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Patients presenting CML-BC have a survival of 3-6 months and scarce response to imatinib. Dasatinib (BMS-354825) is an oral, multi-targeted kinase inhibitor, currently being used in pts with Imatinib-resistant advanced CML or relapsed/refractory Ph^+ ALL.Most of these pts will be evaluated for SCT, even though for them this curative therapy showed higher incidence of GVHD, VOD and TRM. We report here seven pts affected from CML-LB who received Dasatinib prior to alloSCT. Donors were matched siblings (2), matched unrelated (2) or blood cord unit (3). 5 were male and 2 female with a median age of 33,4 (18-55) years.First line therapies included Chemotherapy (VCR) plus high dose imatinib.All pts after 2-5 months from diagnosis received Dasatinib 70mg BID. T315I mutation occurred in 4 patients, Y253 and E255K in 2 patients, and a non codified mutation in 1 patient. Dasatinib induced complete hematological response (CHR) in 4 pts, and complete (n=3) and partial cytogenetic response (PCyR) (n=1) prior to SCT. 3 patients did not achieved a CHR presenting 25% marrow blasts and 65% respectively prior to SCT. All pts were conditioned with myeloablative protocol. GVHD prophylaxis consisted of CSA and MTX(n=4) or micofenolate association until +30(n=3). Pts received a mobilized peripheral blood stem cell graft with 3.52-11.04x10^6 CD34+ cells/kg (n=4) and cord blood unit with 0,18x10^6 $CD34^+$ cells/kg (n=3).Dasatinib was stopped 6 days before transplant procedure. 6/7 pts successfully engrafted reaching ANC>0.5x10^9/L on day +19(11-37) and PLT >20x10^9/L on day +21(11-50). Dasatinib was introduced again in 2 patients 30 days after SCT.One of them stopped therapy because of haematological toxicity after 2 weeks. 6/7 patients presented chimerism 97-100% on day+90. Transplant related toxicities were grade I/II.No pts developed hyperbilirubinemia or VOD. Hyperacute extensive GVHD(Gr III)was observed in only 1 pts at +9. 4 patients are alive, all of them in complete molecular response with a median follow-up of 14 (8-24)months, 1 died of aGVHD,1 for engraftment failure,1 for CMV pulmonary infection. We may suggest that in pts undergoing SCT following Dasatinib there is no evidence of adverse effect on SCT outcome, organ toxicities.Larger studies and longer follow-up are obviously indicated to confirm our preliminary results.2 of 3 T315I positive patients are alive in CHR.Dasatinib represents an efficient bridge to transplant to improve the outcome of this subset of patients.

Allogeneic Transplantation II

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RISKS OF ACUTE GVHD AND RELAPSE ARE ASSOCIATED WITH THE KINETICS OF CD3+ T CELLS ENGRAFTMENT AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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Objectives. This study investigated the kinetics of whole peripheral blood (PB) and $CD3^+$ T-cells chimerism in patients receiving reduced-intensity conditioning (RIC) allogeneic stem cell transplantation (SCT). The T-cell chimerism has been correlated with risk of grade II-IV acute graft-versus host disease (GvHD) and relapse. *Design and methods*. Thirty-three patients with a median age of 55 years (range 17-65) affected by lymphoma (20), multiple myeloma (8), acute myeloid leukaemia (4) or idiopathic myelofibrosis (1) received RIC allogeneic SCT at Hematology Division of Udine between January 2007 and January 2009. Source of stem cells was PB in all patients out 3, and donors were matched unrelated for 24 patients. Conditioning regimens were: thiotepa plus cyclophosphamide (23), 2 Gy total body irradiation (TBI) plus fludarabine (3), melphalan plus fludarabine (6), total lymphoid irradiation (1). In 22 cases anti-thymocyte globulin was used as part of GvHD prophylaxis. Hematopoietic chimerism has been serially assessed at 30, 60, 90 and 180 days after SCT in whole PB and sorted $CD3^+$ T-cells. The analysis have been performed by polymerase chain reaction (PCR) based amplification of short tandem repeats (STR) sequences using the AmpflSTR identifler kit (Applied Biosystems). Full donor chimerism (FDC) was defined as the presence of at least 95% donor cells. *Results.* Thirteen patients developed acute GvHD at median time of 21 days after RIC-SCT and 10 patients relapsed at a median time of 4 months after SCT. The percentage of patients achieving FDC was lower in $CD3^+$ T-cells in comparison with whole PB at day 30 and 60. Patients with grade II-IV acute GvHD had more frequently FDC in $CD3^+$ at day 30 than patients with grade 0-I acute GvHD (92%vs 64%, p=0.07), while there were no significant differences at 60 and 90 days. Patients who subsequently relapsed had a significant lower incidence of $CD3^+$ FDC at day 30 (55% vs 90%, p=0.03), at day 60 (50% vs 90%, p=0.014) and at day 90 (66% vs 100%, p<0.01) in comparison with patients with sustained remission. *Conclusions.* We conclude that mixed chimerism at days 30, 60 and 90 in $CD3^+$ T cells was associated with an increased risk of relapse. The development of acute GvHD seems to be correlated with FD T-cell chimerism at 30 day. The analysis of chimerism in $CD3^+$ cells is more sensitive than in whole PB and can supply useful indications for the management of immunotherapy after RIC-SCT.

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ALLOGENEIC TRANSPLANT IS SAFE IN HEMATOLOGICAL PATIENTS WITH PREVIOUS INVASIVE FUNGAL INFECTION RECEIVING SECONDARY ANTIFUNGAL PROPHYLAXIS

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Introduction. Patients (pts) with a previous diagnosis of Invasive Fungal Infection (IFI) who undergo allogeneic hematopoietic stem cell transplantation (Tx), have an increased risk of IFI due to immunosuppression secondary to conditioning, acute and chronic Graft versus host disease (GVHD) with their treatment. The aim of this study is to evaluate the efficacy of our secondary antifungal prophylaxis strategy to prevent Non Relapse Mortality (NRM) fungal related. *Materials*. all allogeneic transplants are performed in an highly protective environment, usually laminar air flow rooms, with highly sterile nursing procedures. Pts with a previous possible, probable, or proven IFI receive appropriate secondary prophylaxis during aplasia (usually Liposomal Amphotericine B) up to 3 months post-Tx. We reviewed the 188 records of all consecutive allogeneic Tx performed from January 1997 to May 2008 to select patients transplanted under secondary antifungal prophylaxis for previous IFI . (GVHD) prophylaxis was standard Cyclosporine plus short term methotrexate. No T-depletion or ATG were used. Observation for IFI

incidence has been extended to one year after Tx to include period at risk for GVHD. *Results*.23 transplanted pts had a previous diagnosis of IFI. Median age of this cohort was 48 years (range 20-62); stem cells source was: unrelated donor in 5 Tx, related in 18 Tx; conditioning intensity was reduced in 8 Tx, and full dose in 15 Tx. Pre-transplant IFI were classified as possible: 12 , probable: 5 or proven: 7 (1 non-Albicans Candidemia, 1 pulmonary Mucormycosis , 5 pulmonary Aspergillosis) . The pt with proven invasive non-Albicans Candidemia had coexisting possible lung fungal infection . Secondary antifungal prophylaxis was Liposomal Amphotericine B in 19/23 pts (two of whom after a pretransplant surgical resection of lesion), Voriconazole in 2 pts, Caspofungin and Itraconazole in 1 pt each. In the first year after Tx 7/23 pts needed steroid treatment for acute GVHD and 6/23 pts developed chronic GVHD, requiring steroids in 4. During the observation time 8/23 pts died: 5 for NRM (sepsis 2, GVHD 1, MOF 1, ARDS with possible IFI 1) and 3 for recurrence of haematological disease . Overall only one case of possible IFI (2 months after Tx) was recorded. *Conclusions*. Secondary antifungal prophylaxis makes allogeneic transplantation possible in pts with previous IFI and appears to keep additional NRM risk for fungal infection very acceptable.

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TREATMENT OF REFRACTORY CHRONIC GVHD WITH RITUXIMAB: A SINGLE CENTRE EXPERIENCE

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Introduction. Refractory chronic graft-versus-host disease (cGVHD) is an intractable complication of allogeneic stem cell transplantation, associated with considerable morbidity and mortality. Intensificatio of immunosuppressive therapy has failed to show any beneficial effect on survival. A therapeutic approach such as the anti-CD20 chimeric monoclonal antibody - Rituximab has been shown to induce significant clinical response in a proportion of patients with refractory cGVHD. We retrospectively analysed 18 patients treated with Rituximab for a refractory cGVHD. Methods. A total of 18 patients (11 males and 7 females; median age 56 years (34-61) received Rituximab for refractory cGVHD. Median duration of cGVHD before Rituximab was 10 months (2-76), the median number of failed treatment lines prior Rituximab was 3 (1 to ≥5). Rituximab was given intravenously at the conventional dose (375 mg/mq weekly) in 14 patients and at a reduction dose (100 mg/mq weekly) in 4 patients. The median number of administration was 6 (range 4-8). *Results*. the median follow-up after Rituximab was 12 months (2-112) Overall response rate was 50%: skin 10/16 (63%), mouth 10/18 (55%), eyes 6/16 (38%), liver 2/8 (25%), lung 0/6 (0%) and myastenia gravis 1/1. During the study period 8/18 (44%) patients died: causes of death were cGVHD progression (n=4) and infection (n=4). The actuarial 2 year survival is currently 77.5%. *Conclusions*. Our results confirm that Rituximab is effective in rather 50% of patients with refractory cGVHD and may have beneficial impact on survival.

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RAPAMYCIN HAS DIFFERENTIAL EFFECTS ON DISTINCT ANTIGEN PRESENTING CELLS (APC): INHIBITION OF THE SURVIVAL AND FUNCTION OF DENDRITIC CELLS BUT NOT MONOCYTE/MACROPHAGES.

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Dendritic cells (DC) are the most powerful APC and are essential to induce immune responses such as graft versus host disease (GVHD). Monocytes have been shown to differentiate to DC as well as macrophages in vitro and are considered the major source of APC *in vivo*. Rapamycin is a new immunosuppressive agent which is known to alter the function and survival of DC. In this study we tested the effects of rapamycin on the differentiation of monocytes to distinct APC subtypes. Purified monocytes from healthy donors were cultured in vitro with GM-CSF (50 µg/mL) only, GM-CSF and IL-4 (800U/mL) or GM-CSF and IFNa (1000U/mL) to induce their differentiation to macrophages and immature and mature DC, respectively, as previously described. Rapamycin was added at the start of culture at 10 ng/mL. After 6 days cells were harvested and checked for their (1) recovery, (2) apoptosis, (3) expression of costimulatory and DC-differentiation molecules, (4) uptake of soluble antigens and (5) production of TNFa and IL-12. Rapamycin decreased the recovery of APC cultured in the presence of IL-4 (by 72%+13, n=3) while monocytes cultured in GM-CSF with or without IFNa were not affected. Treatment with rapamycin was associated with increased apoptosis both in IL-4 and IFNa -containing cultures. Rapamycin decreased the expression of the costimulatory molecules CD86 and CD80 on the surface of cells cultured with IL-4 and IFNa but not with GM-CSF only. Interestingly, rapamycin increased also the expression of the CD1a molecule in cells cultured with IL-4, and even induced its novel expression in cells cultured with GM-CSF with or without IFNa . This did not correlate with an effect on antigen uptake, as rapamycin did not appear to alter uptake of either albumin or dextran by APC in any culture condition. Moreover, rapamycin blocked the secretion of IL-12 and TNFa by monocytes cultured with IL-4 (by 99+1% for both cytokines, n=3) and IFNa (97+1% for both cytokines, n=2) but not with GM-CSF only. Kinetic studies have shown that the effect of rapamycin can be observed only after at least 4 days of culture with either IL-4 or IFNa. These results suggest that rapamycin acts preferentially on DC-like APC rather than monocytes/macrophages. Further studies are ongoing to evaluate the molecular mechanisms of the differential effect of rapamycin on distinct APC subpopulations.

P326

ASSESSMENT OF LIVER DAMAGE WITH TRANSIENT HEPATIC ELASTOGRAPHY (FIBROSCAN) IN PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST DISEASE

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Introduction. Liver is a commonly target-organ of chronic GVHD (cGVHD), being often refractory to immunosuppressive therapy. Hepatic cGVHD develops generally as an indolent cholestatic disease or as an autoimmune hepatitis. Both primary biliary cirrhosis and cGVHD show some clinical and histological analogies. Liver biopsy specimens show lobular and/or portal inflammation, degeneration of the small bile ducts, portal fibrosis and are helpful to confirm the diagnosis of hepatic cGVHD. However, liver biopsy is not always feasible, because it may generate clinical complications, such as bleeding and infections. FibroScan is a new, fast and non-invasive technique to measure liver stiffness. FibroScan has been validated to detect significant positive relationship between liver stiffness values and histological fibrosis in chronic liver diseases such as chronic hepatitis B and C, cirrhosis and, primary biliary cirrhosis. Being fibrosis a hallmark of cGVHD, we prospectively evaluated the usefulness of FibroScan as non-invasive tool to make diagnosis of liver cGVHD. Patients and methods. Liver stiffness measurements were performed on 10 healthy subjects and on 20 patients undergoing allogeneic SCT (16 with related and 4 with unrelated donor) before transplantation, then every 3 months until 1 year after SCT and at the diagnosis of cGVHD. Median age was 42 years (range, 22-62). 72% received a reduced intensity conditioning and stem cells source was mainly peripheral blood (83%). One patient was HCV RNA-positive. No patients had any sign of liver disease at transplantation. In transient elastography (TE), only procedures with at least ten successful acquisitions and a success rate of at least 60% were considered reliable. The median value of successful measurements was considered representative of the liver stiffness in a given patient, only if the interquartile range (IQR) of all validated measurements was less than 30% of the median value. Results were expressed in kilopascals (kPa). Wilcoxon test was used for statistical analysis of the data. Results. Nine patients (45%) developed cGVHD at a median time of 5 months (range, 4-6). It was extensive in 6 patients (67%) with a median onset time of 6 months (range, 4-10). Liver was involved in 7 patients (78%). Liver stiffness values did not differ significantly in healthy subjects, patients before allo-SCT and patients after allo-SCT without cGVHD. Patients with hepatic cGVHD had higher stiffness values than patients without cGVHD (7.7±4,6 vs 4.6±1.9 kPa, p=0.03). A trend to higher liver stiffness in patients with cGVHD was observed at 3rd month as well (6.4±2.5 vs 4.6±1.5 kPa, p=0.05). *Discussion*. Our prospective study suggests that FibroScan could be a reliable non-invasive technique for the diagnosis of hepatic cGVHD, which could replace liver biopsy in the future. TE could be an effective procedure to

assess disease progression or response to therapy. However, it was not possible to demonstrate significantly different stiffness values in the setting of cGVHD between patients with and without liver involvement. The small number of patients evaluated or a clinically silent involvement of liver in all cases of cGVHD may account for this observation.

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HEPATITIS C AND B VIRUS INFECTION AND LIVER DYSFUNCTION AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION: RESULTS FROM A RETROSPECTIVE STUDY

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Acute and chronic liver dysfunction is common after allogeneic bone marrow transplantation (BMT). Although toxicity, graft versus-host disease (GVHD), and viral infections are the major causes. We conducted a retrospective study to establish the role of the infection with both the hepatitis C virus (HCV) and hepatitis B virus (HBV) in liver dysfunction after BMT. Between March1993 and January 2009, a total of 186 pairs donor/recipient were included in the study. HCV and HBV serologic markers, including HBsAg, HBeAg, HBsAb, HBeAb and HBcAb were tested for patients (pts) and donors. The cumulative incidence positive for at least 1 of markers was 4.8% (9) in pts and 3.2% (6) in donor group, respectively. The pts were positive: 1 HbsAg, HbcAb and Hbe Ab ,1 Hbs Ag with low level of HBVDNA,1 Hbc Ab, Hbe Ab,1 Hbe Ab e Hbs Ab , 1 HbcAb e Hbe Ab ,1 HBVDNA and AbHCV, 1 antiHCV e HCV RNA, 1 anti HCV, 1 HCV RNA positive. All positive pts for HBV markers or with donor positive, were treated with Lamivudine as prophylaxis. Two donors were HbcAb, HbEAb, HbsAb positive, 1 Hbe Ab, Hbsab positive, 3 ANTI HCV positive. The cumulative incidence for hepatic complication in all 186 pts was 23.6% (44 pts). In this group 24 pts (54.5%) had Acute Graft Vs Host Disease (aGVHD): 14 pts grade III IV,10 pts grade I-II. The incidence for chronic GVHD (cGVHD) was 13.2% (7pts), only 1 patient was suffering of extensively cGVHD. The other hepatic diseases were: 1 veno-occlusive disease (VOD), 6 Multiorgan Failure (MOF),1 Budd-Chiari syndrome, 2 acute hepatitis toxicity with high level of bilirubin and 3 chronic hepatitis. In the subset of positive pts or with positive donor, only 2 pts suffering of aGVHD and 2 patients with chronic hepatitis pre transplant maintained chronic state. No reactivation post transplant was observed in group of pts previously positive, although 1 reactivation with HCV chronic hepatitis was observed in 1 pt, negative for all markers pre-transplant, with donor positive for antibody HCV, at 3 years to transplant, 1 pt pre transplant negative with also donor negative, at 3 years to transplant, suffering of hepatitis type B with replication of HBVDNA. In conclusion HBV infection and HCV infection do not seem increase the rate of aGVHD, cGVHD, VOD. HBV and HCV infection do not prohibite BMT, but hepatitis virus infection can cause liver dysfunction and active prophylaxis of hepatitis virus infection remains necessary in accordance with guidelines.

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EARLY HELPER T-LYMPHOCYTE RECOVERY CORRELATES TO CLINICAL OUTCOME IN PATIENTS WHO UNDERWENT TO ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Aim. To assess the impact of early CD4$^+$ cell recovery after allogeneic bone marrow (BM) or peripheral blood stem cell (PBSC) transplant on acute graft versus host disease (aGVHD), overall survival (OS), transplant-related mortality (TRM). A multivariate analysis for OS as end point was performed to study factors associated to OS. *Materials and Methods* early CD4$^+$ cell count (median time 20 days after transplant, r. 12-34) was studied in 99 patients (pts), 51 female and 48 male with a median age of 46 years (r.11-67). The stem cell source was BM on 23 pts and PBSC on 76 pts. The donors were 83 matched sibling donors and 16 alternative donors (family mismatched or unrelated donors). Conditioning regimens were myeloablative in 48 pts or at reduced intensity in 51 pts. AGVHD grade II-IV was present on 44 pts. Median follow-up of pts was 46 months (r.12-86). *Results.* Univariate analysis showed a significant correlation between early CD4+ cell recovery and OS (R=0.389, p=0.000), TRM (R=-0.220 p=0.029). No correlation was between CD4+ cell count and aGVHD. Roc curve of CD4$^+$ cell count (area=72%, p=0.000) indicated that the cut-off was 115/microl. At 2 years follow-up, pts achieving this CD4$^+$ cell count had significantly lower cumulative TRM compared to pts who did not have this count (14.5%±5% vs 39.4%±8%, p=0.0026). At 5 years follow-up, OS was 77.5%±6.5% and 37.8%±7.5% (p=0.000) in pts with CD4$^+$ cell count more or less than 115/microl, respectively. We analyzed the predictive role of other factors for OS. With Kaplan-Meier method other than early CD4+ cell count (p=0.000), we found a significant correlation among donor type (p=0.000), aGVHD (p=0.000), AB0 identity (p=0.000), recipient sex (p=0.016), stem cell source (p=0.017) and OS. No correlation was found among conditioning, donor sex, disease type and status, recipient age and OS. The Cox analysis showed a significant association among early CD4$^+$ cell count (p=0.012), aGVHD (p=0.025), donor type (p=0.028) and OS. No correlation was found among AB0 identity, conditioning regimen, cell source and OS. *Conclusions*. the main predictive factor for clinical outcome of pts who underwent to allogeneic haematopoietic stem cell transplant is represented by early helper T cell count. Patients with low early CD4$^+$ count need to be followed more carefully to avoid transplant complications. In the future, manipulating the graft can improve early immune recovery and overall survival of patients after transplant.

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IMMUNE MEDIATED CYTOPENIA AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION

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A few cases of cytopenia after allogeneic stem cell transplantation (alloSCT) have an autoimmune etiology. Data of 175 consecutive patients allografted in our centre between 2001 and 2009 were reviewed. Immune cytopenia was defined as having clinical evidence of haemolytic anaemia or thrombocytopenia with positive autoantibodies. Other causes of cytopenia were excluded. Autoimmune cytopenia onset was correlated by multivariate logistic regression with donor, conditioning, A-B-0 incompatibility, disease, stem cell source and GVHD. Patients had lymphoma (98.56%), multiple myeloma (MM, 40.23%) or other malignancies (37.21%). All were allografted with reduced intensity conditioning from HLA matched (92.53%), mismatched siblings (13.7%), matched unrelated (39.22%) or haploidentical (31.18%) donors using mainly peripheral blood stem cells (96%). Median age at transplant was 49 (15-68). Conditioning included T-cell depletion with alemtuzumab in 73 cases (42%), antithymocyte globulin (ATG) in 29 (17%). Sixty-four patients (37%) had A-B-0 incompatibility. Twenty-three (13%) had mixed chimerism at day +30. Acute GVHD (aGVHD) occurred in 83 patients (47%), chronic GVHD (cGVHD) in 62 (35%). Eight cases (5%) of autoimmune cytopenia (7 haemolytic anemias and one thrombocytopenia) occurred at a median of 5.5 months after transplant. Four had a HLA identical sibling, 3 a matched unrelated donor, one a HLA mismatched related donor and one a haploidentical donor. T–cell depletion included alemtuzumab (4) or ATG (1). Four patients had grade 1 and one grade 2 aGVHD. Three had limited, 3 extensive cGVHD. None of the patients responded to treatment with steroids. Salvage treatment included high dose immunoglobulins, rituximab, vincristine and cyclosporine. One patient responded to second-line, 4 to third-line and 2 out of 3 to fourth-line therapy. More in detail, one patient responded to immunoglobulins and steroids, 1 to cyclosporine, 2 to rituximab and 3 to vincristine. Five patients are alive without recurrence of autoimmune cytopenia, 3 patients died of progression (1) or of extensive GVHD and infections (2). The multivariate analysis showed that aGVHD was the only covariate significantly correlated (p=0.03) with autoimmune cytopenia. In conclusion autoimmune cytopenia is a challenging com-

plication of alloSCT and aGVHD can be a significant risk factor in determining its occurrence. Most patients eventually respond to multiple lines of therapy.

P330

IDENTIFICATION OF MONOCLONAL B-CELL LYMPHOCYTOSIS AMONG SIBLING TRANSPLANT DONORS FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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In patients affected by chronic lymphocytic leukemia (CLL), a family history of CLL is recorded in 6% of cases (Mauro et al, 2006). Moreover, monoclonal B-cell lymphocytosis (MBL), identified in about 3-5% of adults with normal blood count, is present in 13% of first-degree apparently unaffected relatives of CLL patients (Rawstron et al, 2002). Since an increasing number of young CLL patients with adverse prognostic features are treated with allogeneic stem cell transplant (SCT), it might be reasonable to recommend that potential HLA-matched sibling donors are tested for the presence of MBL (Montserrat *et al.,* 2006). From January 2005, at our Institution we have routinely searched for a MBL in HLA-identical siblings of CLL patients candidate to an allogeneic SCT in the context of the donor eligibility screening. Thirteen HLA-matched siblings of 13 CLL patients have been so far evaluated (9 males, 4 females, median age 52 years, range 34-70). In 3 cases, the family history revealed the presence of a relative affected by a CLL, a chronic myeloid leukemia (CML) and a non-Hodgkin lymphoma, respectively. All donors showed a normal clinical examination, no significant comorbidities and peripheral blood (PB) counts within the normal range, with a lymphocyte count of $1.99x10^9$/l (range 1.06-3.06). A clonal B-cell population was searched by flow cytometry and polymerase chain reaction (PCR) on PB samples. Out of 13 HLA-matched sibling donors, 2 turned out to have a clonal B-cell population in the PB both by flow cytometry and PCR, giving an overall incidence of 15.4%. Both were males, of 40 and 70 years respectively. Family history of the first case was positive for CML. The B-cell clone accounted for $74x10^9$ and $77x10^9$ cells/l, respectively; both showed a lambda light chain restriction and a VH4 family usage by PCR. There was no concordance in the VH family usage between donor and patient pairs. The 2 HLA-matched siblings with evidence of a MLB were considered ineligible for a stem cell collection. Our decision was essentially based on the risk of transplanting potentially clonogenic B-cells. In conclusion, we recommend that the search for a MBL by an extended immunophenotypic analysis and PCR confirmation is added to the eligibility screening of HLA-matched siblings of CLL patients candidate to an allogeneic SCT, due to the potential high incidence of positive cases (15.4%). The eligibility evaluation of potential donors with MBL should be discussed.

P331

REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION IN PRIMARY CUTANEOUS T-CELL LYMPHOMAS

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Primary cutaneous T-cell lymphomas (CTCL) are an heterogeneous group of non Hodgkin's lymphomas, the most common forms are mycosis fungoides and Sezary syndrome. Patients with CTCL in advanced stage have a poor prognosis with conventional therapy; allogeneic hematopoietic stem cell transplantation (HSCT) provides a potentially curative approach for such patients, suggesting a role for graft-versus-tumor effect. Patients heavily pretreated, with advanced age and/or comorbidities are considered ineligible for a myeloablative allogeneic transplant, so the use of a reduced intensity conditioning (RIC), reducing transplant-related toxicities, made allogeneic transplant feasible in these unfit patients. We are reporting 6 patients (2 F; 4 M) with a median age of 52 years (range, 50-64 yrs) affected by CTCL who underwent a RIC transplant from HLA identical sibling. Diagnosis were: panniculitis-like T-cell lymphoma (n=1) large T-cell lymphoma (n=1), NK T-cell lymphoma (n=1), Sezary syndrome (n=3). At the time of transplant 4 patients had a relapsed/refractory disease, 1 patient was in partial remission and 1 in complete remission. All patients but 1 were heavily pretreated, having received at least 4 lines of prior therapies and 2 had undergone a previous autologous transplant. The RIC regimen consisted of fludarabine (80 mg/m^2), thiotepa (10 mg/kg) and cyclophosphamide (60 mg/kg) Cyclosporine and methotrexate were used as GVHD prophylaxis. Sources of stem cells were bone marrow and peripheral blood in 3 patients, respectively. All patients engrafted, a complete chimerism was obtained in 5 patients at day 30, in 1 patient at day 90 after transplant. All patients achieved a complete remission. They did not experience any early toxicities or infection complications other than CMV reactivations. A grade II acute GVHD was observed in 3 patients; 2 developed chronic GVHD: 1 limited and 1 extended with generalized scleroderma. One patient with Sezary syndrome relapsed at 9 months from transplant, a disease remission was achieved after donor lymphocyte infusions and she is still in remission at 4 years from transplant. One patient with NK T-cell lymphoma relapsed at 8 months from transplant. To date, with a median follow-up of 23 months (range 9-53) from RIC transplant, all pts are alive, 5 without evidence of lymphoma. Our results showed a low transplant-related toxicity and suggest a potential curative effect of allogeneic RIC transplant also in advanced CTCL.

P332

ALLOGRAFTING WITH NONMYELOABLATIVE CONDITIONING FOLLOWING CYTOREDUCTIVE AUTOGRAFTS FOR THE TREATMENT OF PATIENTS WITH MULTIPLE MYELOMA: AN UP-DATE IN THE ERA OF THE NEW BIOLOGIC AGENTS

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The full potential of a graft-versus myeloma effect after allogeneic hematopoietic cell transplantation (HCT) for patients with multiple myeloma (MM) has not been realized because of excessive early transplantation-related mortality with conventional HCT. Autologous HCT have been characterized by almost universal disease recurrences. In the last years, some trials combined autologous HCT with subsequent nonmyeloablative allogeneic HCT to maintain the benefits of both approaches with acceptable toxicity. In the era of the new agents (bortezomib and lenalidomide) for the treatment of MM patients, we evaluated an up-date of the combination of high-dose therapy and autologous HCT followed by a reduced intensity conditioning and allotransplantation (RICT) in this disease (*Martino et al. AJH 81:973–978, 2006*). 26 subjects with stage III MM (median age 52 years, range 40–65) received high dose melphalan (200 mg/m^2) followed by Autologous HCT (AHCT) previously collected after cyclophosphamide (4 g/m^2) and granulocyte colony-stimulating factor (G-CSF). After 3-4 months from AHCT, the patients underwent RICT, consisting of fludarabine 90 mg/m^2 + cyclophosphamide 900 mg/m^2 on days. Graft-versus-host disease (GVHD) occurred in 16 patients; 5 patients developed CMV antigenemia and were treated pre-emptively with ganciclovir. 1 patients died for transplant related mortality (GVHD). Response was simultaneously measured by both electrophoresis (EP) and immunofixation (IF); when IF was negative, patients were classified in complete remission (CR) and when it remained positive, near CR (nCR). After a median follow up of 54 months post RICT, Progression-free survival (PFS) and Overall Survival (OS) at 6 years was 36% and 76 %, respectively. Overall, the CR + nCR rate after dose-reduced allograft was enhanced from 19 to 77 %. No correlation between GVHD and OS and PFS was found. In conclusion, this up-date confirm that an up-front tandem strategy with RICT following autografting is feasible and induces high CR/nCR rate in MM. It is open the debates around the results gotten in terms of OS and EFS in the era of new drugs.

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RECOVERY OF SPECIFIC CMV CD8+ T LYMPHOCYTES AND T REG AFTER ALLOGENEIC PERIPHERAL STEM CELL TRANSPLANTATION: SINGLE CENTRE EXPERIENCE

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Background. Recovery of cytomegalovirus (CMV)-specific T-cells after allogeneic stem cell (alloSCT) is critical for protection against CMV disease;in humans the CMV-specific CD8+ must be regenerated after SCT in order to obtain protection against CMV infection and disease.Moreover CD4+CD25+Foxp3+ regulatory T cells(Tregs) are a major regulator of adaptive immunity. *Patients and Methods.* We used fluorochrome-conjugated tetrameric complexes of HLA-A101, HLA-A201, HLA-B702, HLA-B801, HLA B3501 to monitor recovery of CMV-specific CD8+ (according to the patient's HLA) in 60 patients after alloSCT.Patients were transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor(n=53) and an HLA identical unrelated donor(n=7). Median age was 36 years (range 18-61); diagnoses were acute myeloid leukaemia (n=48), acute lymphoblastic leukemia (n=8), chronic myeloid leukemia (n=3), myelofibrosis (n=1). *Results.* Median CMV-specificCD8+T lymphocytes were significantly higher in patients without than with CMV infection/disease at 1 (2 vs 0 cells/ mmc, p=0.05), 2 (5 vs 1 cells/mmc, p=0.05), 3 (12 vs 2 cells/mmc, p=0.03) and 6 months (22 vs 3 cells/mmc, p=0.03), respectively. Tetramer analysis showed that 31/60 (51%) patients reconstituted CMV-specific CD8+ at 2 months after transplantation; CMV infection/disease was observed in 2/33 (6%) patients with CMV-specific $CD8^+$ recovery and without GvHD, in 4/27(14%) patients with recovery of CMV-specific CD8+ T-cells with GvHD and in 23/27(85%) of patient without recovery of CMV-specific $CD8^+$ T-cells.In our experience no CMV infection/disease was observed in cases with recovery of CMV-specific CD8+ T-recovery cells >5/mmc. Moreover, we observed a good correlation between the recovery of CMV-specific $CD8^+$ lymphocytes and of $CD4^+/CD25^{high}$ Tregs at 2 (p=0.05, r=0.8) and 3 (p=0.06, r=0.7) months after alloSCT. However, median of Tregs values were significantly higher in patients without than with CMV infection/disease at 2 (15 vs 3/ mmc, p<0.03) and 3 months (22 vs 6/mmc, p=0.05). Acute GvHD grade II-IV was observed in 27/60 patients (45%); at univariate analysis, Tregs were significantly higher in patients without than with aGvHD (17 vs 4/mmc, p=0.05). *Conclusions.* In conclusion we suggest a good correlation between the recovery of CMV-specific $CD8^+$ T-cells and T regs which probably mediate the protective effects toward the aGvHD and the reconstitution of functional immunity. This support further consideration of T regs immunotherapy for clinical alloSCT.

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THE POTENTIAL APPLICATION OF THE ALLOGENEIC TRANSPLANT BY THE POLICY OF WIDESPREAD DONOR SEARCH: AN INTENTION-TO-TREAT ANALYSIS FROM THE ROME TRANSPLANT NETWORK.

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The policy of the Rome Transplant Network (RTN), a metropolitan network of transplant Centers, for patients candidates to an allogeneic hematopoietic stem cell transplant (HSCT) and lacking an HLA identical sibling is the contemporary search for one the HSC alternative sources such as Matched Unrelated Donor (MUD),Cord Blood Unit (CBU) or Haploidentical Related Donor (HRD). The main aim of the RTN policy is the identification of a suitable donor in order to perform transplant in adequate timing. The selection criteria for MUD consist of a 8/8 HLA loci matching tested at low resolution for class I HLA and at high resolution (HR) for class II; one difference in C Ag is considered acceptable in case of both I and II class HR identity. CBU's selection criteria are instead based on cell doses (TNC>2.5x10^7/kg and $CD34^+$>1x 10^5/kg) and on a HLA-compatibility >to 4/6 HLA Ag. From April 2006, the haplodentical option was also simultaneously considered, so all closer family members have been tested for the HLA. Here, we report the results of the intention to treat (ITT) analysis on the potential therapeutic impact of our transplant policy. Data were obtained from RTN database. From April 2006 to date, 196 pts have been candidated to receive an allogeneic HSCT for hematological disease. Sixty-six out of 196 (34%) underwent HSCT from HLA identical sibling, while a search process for an alternative donor was activated for 130 pts. Of 130 pts, 9 (7%) lost the eligibility to transplant early during the search process and 19 (15%) died of early disease progression in most cases: a suitable MUD or CBU had been identified for 13 of 19 within 3 months from the start of the search and only 6 pts (5%) died without an alternative donor had been found. To date, 73/102 evaluable pts (72%) lacking an HLA identical sibling have been transplanted (n=66: 23 MUD; 24 CBU; 19 HRD) or are willing to proceed towards the transplant (n=7: 3 MUD;2 CBU; 2 HRD). In summary, for all 196 candidates to an allogeneic transplant the eligibility was confirmed for 187 (95%), a suitable donor could be identified for 181 (92%) of all pts or 97% of the eligible ones and an allogeneic transplant could be performed for 168 (86%) of all candidates or 93% of those eligible. From this ITT analysis, we can conclude that, by adopting the RTN policy of widespread donor search and multiple transplant options, the allogeneic transplant can be offered as potential therapeutic procedure to a large majority of pts.

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IMPLEMENTING SYSTEMATIC SEARCH FOR ALTERNATIVE DONOR IN PATIENTS IN NEED OF ALLOGENEIC TRANSPLANTATION: AN INTENTION-TO-TREAT ANALYSIS OF 303 PATIENTS AT HSR BMT UNIT

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Background. Allogeneic transplantation of hematopoietic stem cells (allo-SCT) from an HLA-matched related (MRD) or unrelated donor (URD) is a curative option for patients (pts) with high-risk hematological disease (HRHD). In the absence of a MRD, pts have been offered investigational transplant strategies involving alternative donors such as umbilical cord blood (UCB) or family haploidentical SCT (haplo-SCT). In our Institution, since 2004 we adopted the policy to offer a family haplo-SCT to adult pts lacking an MRD or URD in order to adequately treat HRHD in the ideal appropriate time according to clinical indications to allo-SCT agreed in ongoing protocols for primary disease. *Methods*. Here we report the retrospective intention to treat (ITT) analysis of alternative donor transplantation in pts lacking a suitable MRD at our Institution. Data were obtained from local database. *Results.* Between January 2004 and March 2009, 303 pts (100% of the following ITT analysis) received indication to allo-SCT due to HRHD. Seventy-four pts (24%) received a transplant from a MRD. One hundred fifty-six pts (52%) activated an URD search in the IBMDR registry; 68 pts (22% of total pts, 44% of URD searching) received a URD transplant; 32 pts (11% of total pts, 20% of URD searching) received an haplo-SCT due to lacking of a suitable URD in the appropriate timing according to disease status, or absence of suitable criteria to engage an URD donor. Seven pts (2% of total pts, 4% of URD searching) received a UCB transplant because lacking a suitable haplo donor. Overall, 105 pts received a haplo-SCT (35%): 32 after IBMDR research, 73 up-front, due to urgency in performing transplant due to dismal disease. Sixteen pts died before receiving a transplant (5%), 16 (5%) are at present searching for a suitable donor, 18 (6%) have found a URD and a transplant is in evaluation. At the end, 254/303 pts with an indication to allo-SCT received a transplant, in a suitable timing, encounting for a global 83% ITT result. The median time from diagnosis to transplant was 156days in the MRD setting, 189days in the URD setting and 239days in the HAPLO setting. *Conclusions*. In our Institution, in ITT analysis, 83% of overall pts candidate received an allo-SCT: 59% from an alternative donor, 24% from a MRD. This rate arise to 90% for pts with AML, thanks to the highly committed policy performed in the alternative-SCT setting.

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A NEW APPROACH FOR THE DIAGNOSIS OF CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD) SCLERODERMA–LIKE: PRELIMINARY DATA AND CLINICAL IMPLICATIONS

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Background. cGVHD is the most important and disabling complication after allogeneic haemopoietic stem cell transplant (hsct); in recent years with the increased use of peripheral blood as stem cell source, there was a higher incidence of cgvhd involving skin and lungs (scleroderma-like). The only investigation that can pose diagnosis of cgvhd is still the biopsy of organ or tissue affected. Aim of the study: to diagnose early cgvhd scleroderma-like using a non-invasive and sensible test, the gene expression profile (gep), to treat patients in early and reversible phase of this disease. *Materials and Methods* we analyzed expression changes of 47 genes associated with alloreactivity in 6 patients affected by haematological malignancies submitted to allogeneic peripheral hsct from hla-sibling (n=5) or mud donors (n=1) and in a patient with classic scleroderma disease. We have used a Taqman® Low Density Array based on comparative ddct method to perform relative quantification of cDNA and proteomic kinasi-array. In all patients serial samples of peripheral blood mononuclear cells (pbmc) were collected between 2-4 years after transplant when a clinical feature compatible with cgvhd scleroderma-like was present. *Results*. In all samples of patients with cGVHD, confirmed by skin or lung biopsy, we found an up-regulation of the genes implicated in immune regulation(BCL2A1, CASP6, CCL7, CXCL1, CD52, CDCL9), Th1 response(EGR1, EGR2, IFNGR2, IL2, IRF1, IRF8, IL1A), TNF response (FAS, IKBKB, NFKB2), Th2 response (IL10, IL4, IL6), Th17) and Treg action (IL17A, IL7, FOS, FOXP3, ICOS). We found also an up-regulation of molecules with co-inflammatory (ICOS, SELP, SERPIN4, CD83, CD52) and vascular action (VEGF A, IL-8, adesine) and up-regulation of genes involved in collagen production and erk1/2 activation. The same results were observed in patient affected by classic scleroderma. *Conclusions*. our results demonstrate that our method can be an useful diagnostic tool of cgvhd scleroderma-like. It is necessary to increase the number of patients studied to sustain the importance of gep above all in patients who are developing a sub-clinical cgvhd. early diagnosis and proper treatment approach may allow, in this light, starting soon immunosuppressive therapy more effective, so as to preserve the quality of life in long survivor patients after allogeneic hsct.

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ALLOGENEIC TRANSPLANTATION FROM ALTERNATIVE DONORS IS FEASIBLE IN ELDERLY PATIENTS WITH POOR PROGNOSIS ACUTE MYELOID LEUKEMIA OR MYELODYSPLASTIC SYNDROME: THE SAN RAFFAELE INSTITUTE EXPERIENCE

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Background. Allogeneic (allo) stem cell transplantation (SCT) is the only potentially curative strategy for poor prognosis acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). Patients (pts) older than 60 are rarely offered an alloSCT because of the procedure-related toxicities and the difficulty to find a matched relative, fit to donate; SCT from alternative donors is usually controidicated in these pts because of the high rate of transplant related mortality (TRM). Since 2002, we have transplanted 25 pts, age ≥60, with advanced or high risk AML/MDS, from a matched unrelated (URD) or haploidenical related (HAPLO) donor. Data on feasibility and outcome are here reported. Aim:to retrospectively evaluate data on alloSCT from alternative donors in elderly AML and MDS pts, at our Institute. Methods:period 10/2002 to 10/2008, 25 pts, median age 63.7 (60-72.1). Diagnosis (WHO): AML 6, AMLMD 7, RAEB-1 2, RAEB-2 4, MDS/MPD 2, bifenotipic AL 1, t-related AML/MDS 3. Median number of chemo cycles before SCT: 2 (0-7). Donors:URD 5, HAPLO 20. Disease status at SCT: CR1 9, CR2 2, upfront 2, refractory 6, relapsed 6. Conditioning regimens contained fludarabine in 24 cases, treosulfan in 22, thiotepa in 2, cyclophosphamide, busulfan, melphalan in 1 case, ATG in all cases. GvHD prophylaxis: T-cell depletion in 11 cases, CSA/MTX in 11, rapamycin/MMF in 3. All pts received SCT from peripheral blood. *Results.* TRM before day+30: 2 (8%); 23 pts (92%) engrafted and were in CR at day+30, included 13 (93%) with active disease before SCT. Relapses were 5 (21.7%). Overall TRM was 44% (11 pts), 9 for infections, 2 for other causes; 4 pts died for disease progression, all deaths (15) occurring within the first year after SCT. aGvHD grade>II: 4 cases; extensive cGvHD: 3 cases. At last follow-up (FU) 10 pts (40%) are alive in CR with a median FU of 770 (56-1375) days, median OS from SCT of all 25 pts was 227 (3-1375) days. *Conclusions*. AlloSCT from alternative donors is feasible in elderly pts with AML or MDS. Long term survival free from disease has been obtained in several pts; notably, about one third of those transplanted with active disease are alive in CR at last follow up. Reduced-toxicity conditioning regimens showed limited early mortality; better prevention and management of infections could permit to reduce the overall TRM rate. We conclude that alloSCT from an alternative donor is an effective therapeutic option for pts with poor prognosis AML/MDS and advanced age.

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PERTURBED B-CELL HOMEOSTASIS DURING CHRONIC GRAFT-VERSUS-HOST DISEASE DOES NOT ASSOCIATE WITH LONG-TERM PERSISTENCE OF HOST B CELLS IN HUMANS

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Background. Chronic graft-versus-host disease (cGvHD) is a common complication of allogeneic hemopoietic cell transplantation (allo-HCT). The pathogenesis of cGvHD is poorly understood. In cGvHD, the homeostasis of B lymphocytes is perturbed, as demonstrated by the production of autoantibodies. B-cell depletion with monoclonal antibodies (mAb) interferes with autoantibody production and ameliorates signs and symptoms of cGvHD. In mouse models, cGvHD and autoantibodies associate with the long-term persistence of host B cells after allo-HCT (Sylvain Perruche *et al.*, Transplantation 2006). It has been postulated that host B cells may present alloantigens to donor T cells and, in turn, receive help for autoantibody production. This could be crucial to the pathogenesis of cGvHD. *Aim*. To investigate whether the long-term persistence of host B lymphocytes is associated with cGvHD and autoantibodies in humans. *Design and methods*. We recruited 13 consecutive patients with active cGvHD (4 mild, 5 moderate, 4 severe according to NIH classification) with a median time of onset from HLA-identical sibling (9 patients) and HLA-matched unrelated (4) allo-HCT of 6 months (range 3-36). As controls, we chose 10 patients that underwent HLA-identical sibling (2), HLA-matched unrelated (5) or haploidentical (3) allo-HCT and never experienced cGvHD. In the two groups, we studied: i) circulating autoantibodies, including anti-nuclear (ANA), anti-DNA, anti-extractable nuclear antigen, anti-β2 glycoprotein, anti-neutrophil cytoplasm, anti-thyroid, anti-mytocondria antibodies, rheumatoid factor, ii) absolute numbers of T ($CD3^+$, $CD4^+$, $CD8^+$), conventional B ($CD19^+$), B1 ($CD5^+CD19^+$) and NK cells ($CD16^+/CD56^+$) in the graft and in the peripheral blood, iii) microchimerism by short-tandem repeats (STR) on B, T and myeloid cells purified by immunomagnetic cell sorting (sensitivity 0,01%). Results. In our series of patients with cGvHD, we confirmed that B-cell homeostasis is perturbed. Patients with cGvHD had high-titer circulating ANA (>1:160) more frequently than controls (54% versus 10%, P<0,05). All other autoantibodies were negative. Peripheral T-cell counts were lower in patients with cGvHD than in controls (for $CD8^+$ cells p<0,05). This was not due to a difference in the absolute numbers of T lymphocytes within the graft between the two groups. Peripheral counts of conventional B and B1 cells in patients with cGvHD were similar to controls. Autoantibodies and cGvHD were not associated with the persistence of host B lymphocytes, since the analysis of STR on purified B cells revealed that they were all of donor origin. T and myeloid cells were also of donor origin. Of interest, in univariate analysis, *in vivo* B-cell depletion with mAb for the prophylaxis against Epstein-Barr virus-related lymphoproliferative disease showed a trend towards a lower risk of cGvHD (p=0.06). Conclusions. This study demonstrates that perturbed B-cell homeostasis during cGvHD does not associate with long-term persistence of host B cells in humans and is therefore donor-autonomous. Moreover, it suggests that the early depletion of donor B lymphocytes for cGvHD prophylaxis deserves further investigation.

Quality of Life and Support Therapy II

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HOMECARE HEMATOLOGICAL ASSISTANCE AS A MODEL OF CONTINUITY OF CARE: FROM ORGANIZATION TO COSTS

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At Ospedale Niguarda Ca'Granda (Milano) the structuring phase of Homecare Hematological Assistance (HHA) has been concluded in 2008. Regione Lombardia expressed a favorable opinion about both the humanization of the pathway of care and the implementation of a different level of care (de-hospitalization); it has been decided to expand this experience to all patients residents in Milano by further financing a 24 months project: the final objective has been identified in the definition of a new accounting system of this new level of care. So, a new methodology has been set up starting from the different clinical categories in order to quantify the resources needed for an appropriate care. By analyzing the 2007 Diagnosis Related Group forms of all patients resident in Milano, a patient pool of around 200 people in Milano has been estimated. Patients have been divided into three different categories according to their clinical and assistance needs: chronic patients (with or without physiotherapy or assistance for normal life activities), near-to-end patients (near-to-end and ending stage) and acute ones (protected discharge or chemotherapy). For each category different levels of care have been identified according to the types of care and providers, frequency, timing and complexity of assistance. Needs have been evaluated based on the previous experience of HHA and care provided to fragile patients from the Local Health District Milano. A proper dimensioning of the professional resources which are needed to provide the expected level of care is mandatory to evaluate the economical sustainability; this is particularly true for the homecare assistance where the primary cost driver are the human resources. From our analysis it is possible to define different profiles of care the cost of which is directly proportional to the assistance complexity of the patient type. The intensity of care coefficient (no. days of home-assistance / total no. of days of care) varies from 0,12 for chronic patients, to 0,77 for near-to-end patients up to 1 for acute ones. This project represents the bases to define a benchmark for HHA together with a tariff system based on the consumption of resources instead of a traditional clinical diagnosis.

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BIOGRAPHIES AND COMPARISON IN EXPERIENCES OF HEMATOLOGIC HOME CARE SERVICE

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Introduction. The essential characteristic of every therapeutic relationship should be personal contact and with domiciliary care this is taken to the extreme. Going into a patient's home requires respect, careful observation and close attention to detail. The health worker takes part in an unedited, live performance. Narrating facts alternates with life which expresses itself in real time before words can break through and form reality. In opening the door to his home, the patient reveals his inner world and his relational system, without metaphors and beyond his control, more than in a traditional setting. The story of his life begins. *Method*. In the holistic perspective that characterizes our work, listening empathetically to the patient and his family focuses on the most intimate needs, far beyond mere treatment of the illness. Since the patient is much more than just his illness, looking on each person as an individual means placing the person as a whole at the centre of the treatment, considering both his psychological and spiritual needs. Often the patient gives a destructured impression of himself and the relationship with the psychologist becomes an opportunity to rebuild his dignity. This can be done through biographical narration that goes over the significant moments in his life, redefining subjectivity whenever the illness has concretely represented its existence. *Discussion.* Our observations aim to underline the importance of biographical narration as a meaningful element in the treatment and care of the patient. This is a fundamental step because having one's feelings listened to and mirrored helps to tap into inner resources for facing the illness or the end of one's life. Although often hidden behind anger and depression, perhaps what the patient actually wants is to heal a feeling of faith in himself and in others. Moreover, the biographies gathered have given us the chance to reflect: there is a common thread that goes through some patients' lives, experiences of abandonment linked to early separation from parental figures (bereavement and institutionalization) and physical and psychological abuse both in childhood and in adulthood. In these situations the illness proves to be a reparatory phenomenon/representation for old wounds that have not been healed.

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NEUTROPENIC ENTEROCOLITIS: USEFULNESS OF ULTRASOUND SONOGRAPHY. SINGLE-CENTER EXPERIENCE

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Neutropenic enterocolitis (NEC) is a life threatening complication of patients treated with chemotherapy ranging from 2.6-33% . It is a necrotizing inflammatory disease of the ileo-cecal region but the terminal ileum, the small bowel and colon can also be involved by the disease. Perforation occurs in 5-10% of cases. Early diagnosis is crucial to start conservative medical management which appears the optimal strategy for most cases. Mortality rate is high up to 21-48% and should be always suspected in neutropenic patients with abdominal pain, fever and diarrhoea. Ultrasound was used to evaluate bowel-wall thickening (BWT). The degree of BWT correlated with the outcome and 60% of patients with BWT >10 mm died from this complication compared with 4.2% of those with BWT <10 mm. We evaluated retrospectively NEC cases occurred in the last two years and 32 fit patients were identified. We separated two sets of patients (ptsA and ptsB). In ptsA US was performed later in the course of the disease. in ptsB US was immediately performed as just one symptom presented (diarrhea and/or abdominal pain with or without fever) in neutropenic patients. Disease diagnosis was HD 10, ALL 4, AML5 MM 3 and NHL 10. Treatment were chemotherapy,[10] allogenic transplant with bu/cy (1) and cy/tbi (1), autologous transplant with bu/cy (1), BEAM (16) and , MEL 200 (3). All 32 patients had neutropenia grade 4 at time of diagnosis. Abdominal pain was present in 31/32 patients. Diarrhoea was present in 30/32 pts. Positive cultures were found in 12.5% (stool) and 25% (blood) of patients. US signs of NEC were: thickening or dilation of small and/or large intestine. US was informative in 8/14 patients in ptsA and in 18/18 patients in ptsB. When applied earlier (ptsB) US detected signs of NEC in 7 patients with abdominal pain and diarrhoea without fever with complete response to treatment in 6. Two patients within 12 hours from diagnosis underwent successful colon surgery during neutropenia guided by US features of potential wall rupture. Overall 3/32 patients died (all with positive blood colture). In two surgery was necessary and all the others were treated with conservative medical management. In conclusion early intestinal US in neutropenic patients performed in suspicion of NEC helped to detect early signs consistent with NEC and to start immediate treatment of this life threatening complication even before development of fever.

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MOTOR DISABILITY IN HEMATOLOGY: AN EPIDEMIOLOGICAL STUDY

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Background. Motor disability (MD), defined as reduction on ability to attend basic activities of daily living (ADL) was rarely explored and reported in hematological patients (pts). Hematological diseases and their treatment are able to cause a large variety of disabling conditions. *Methods*. On November 2007, we developed a study aiming to assess MD. Barthel index (BI), as basic ADL ability scale, was assessed weekly in

hospitalized pts. According to severity, MD was classified as mild (BI=67-99%), moderate (BI=33-66%), severe (BI=0-32%). The level of 10 disability-related items (muscle, bone, joint, central nervous system, peripheral nervous system, psychological aspects, lung, heart, movement-related pain, eyes) was defined to investigate the level of impairment. MD etiology was defined weekly. *Results*. 109 pts were examined. Median age was 60 (20-76). Diagnoses were: acute leukemia in 37, lymphoma/chronic lymphocytic leukemia in 35, multiple myeloma in 19, myelodisplastic/myeloproliferative disease and others in 18. Because of the possible modifications of disease phase or treatment over time, patients were evaluated weekly and assessed according to their disease phase. Of the total 354 weeks of evaluation, 110, 53, 42, 65, 49 and 35 were the number of weeks evaluated for complete remission (CR), partial remission (PR), relapse, progression, new diagnosis and undefined diagnosis, respectively. Similarly, 273, 22, 24 and 35 were the weeks for active, palliative treatment, no treatment and unknown treatment respectively. Disability was present in 48% (52/109) of pts; mild in 17%, moderate in 14% and severe in 17% of pts, respectively. MD was present in 34% (122/354) of the weeks; mild in 14%, moderate in 10% and severe in 10%, respectively. Statistically significant ($p<0.05$) lower risk of MD was noted in active vs other treatment (41% vs 27%) and in CR/PR vs other disease phases (30% vs 48%). Muscle impairment (18%), movement-related pain (10%) and bone lesion (10%) were the major mechanisms of MD. Etiology was: hematological disease in 32%, disease-related complications in 7%, therapy related in 46% and other causes in 15%. *Discussion*. MD is a frequent feature in hematological hospitalized pts. Extended data analysis is required to understand disability development and risk factors. A targeted rehabilitative approach to prevent and treat disability may lead to positive effects on both pts quality of life and caregiver work-load.

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NOCARDIA SPP INFECTIONS AMONG HEMATOLOGICAL PATIENTS: CLINICAL CHARACTERISTICS, RISK FACTORS AND OUTCOME OF FIVE CASES AT A SINGLE INSTITUTION

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Introduction. *Nocardia spp.* infections are rare but often life-threatening, especially for immunocompromised host. Most of the cases of nocardiosis reported among haematological patients were observed in those undergoing bone marrow transplantation. Lymphocytopenia and steroid therapy are considered underlying predisposing factors. Aims. We report five consecutive cases of nocardiosis in non transplant patients observed at our Institution, with the aim of better clarify clinical characteristics and impact on outcome. *Patients, Methods and Results*. During a period of 7 years (2002-2009), five cases of nocardiosis were observed at our Institution. Median age was 71 (range 67-82) and M/F ratio 4/1. Haematological underlying disease was: peripheral T-cell lymphoma + myelofibrosis (1), clonal T-lymphocytosis + hypereosinophilic syndrome (1), Castleman disease (1), B Diffuse Large Cell Non Hodgkin Lymphoma (1), Idyopathic Thrombocytopenic Purpura (1). Significant comorbidities were present in two patients (chronic obstructive pulmonary disease and chronic renal failure+diabetes mellitus respectively). Median time of clinical presentation from haematological diagnosis was 11 months (range 3-18). All the patients were refractory to therapy; moreover they were all on chronic steroid treatment (>3 weeks). None of them was neutropenic, whereas lymphopenia was present in all patients (median 0.89 x 10^9/L, range 0.51-1.38). Lung involvement by Nocardia was present in all patients; it was associated to disseminated lymphoadenopathy and brain abscess in two patients respectively. Species typing was available only in one case (Nocardia asteroides). Nocardia spp were isolated from blood in two cases, from sputum, bronchoalveolar and lymphonode drainage in one case each. In two cases nocardiosis was associated to a diagnosis of probable aspergillosis. Treatment of nocardiosis consisted in cotrimoxazole i.v. in 2 patients, imipenem/cilastatin in 2 cases and imipenem/cilastatin + cotrimoxazole in 1 case. All the patients died; in 4 cases nocardiosis was considered the cause of death.. Median survival was 35 days (range 2-35). *Conclusions*. Nocardiosis is a rare but often fatal infection among haematological patients. It has to be suspected also in non-malignant diseases, when severe immunodeficiency or chronic immunosuppressive therapy are associated. Refractory underlying disease, prolonged exposure to steroid treatment and lymphocytopenia seem to be predisposing factors.

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INTRAVENOUS CHEMOTHERAPY AT HOME IN HEMATOLOGY PATIENTS: A REPORT FROM THE A.I.L. HEMATOLOGY HOME CARE SERVICE IN MODENA

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Thanks to the availability of novel agents and the advances in supportive care, the use of antineoplastic treatments has recently increased in many blood malignancies, even in elderly patients and in palliative settings. In our department the fundraising organization A.I.L. sustains a home care (HC) program open to fragile hematology patients who need specialist and palliative care outside the standard in-hospital assistance. Eligibility criteria include poor performance status, appropriate home logistics, caregiver trainability, reasonable distance from hospital, patient's consent for domiciliary medications. In order to ensure a full continuity of care, intravenous chemotherapies are routinely provided at home. 36 patients out of 450 (8%) followed in the period 1999-2009 required intravenous chemotherapy, whose primary goal was to slow cancer progression and palliate symptoms related to advanced disease. Median age was 73 years (range 37-90). Gender distribution was balanced (male=16, female=20). Diagnosis were non-Hodgkin lymphoma (14), multiple myeloma (12), chronic lymphoid leukemia (4), acute myeloid leukemia (4), Hodgkin's disease (2). Single-agent treatments (cyclophosphamide, idarubicine, vincristine, etoposide, cytarabine, carmustine, mecloretamine, gemcitabine, alemtuzumab, bortezomib) regarded 25 patients for a total of 64 cycles and 150 home accesses. Polichemotherapy regimens (CVP, VBCMP, MOPP, carmustine/vinblastine) were administered in 14 patients for a total of 35 cycles and 36 domiciliary accesses. No relevant infusion side effect nor acute toxicity were reported. A treatment plan is produced on a intranet-based software. Antineoplastic drugs are prepared in the hospital pharmacy (located in the hematology day unit) and are collected by the HC hematologist in secure transport boxes. In the meanwhile the specialist nurse provides a venous access and administers ancillary therapy. An emergency kit containing antidotes to extravasation is always ready to use. At the end, infusion material is taken by the nurse for hospital waste disposal. In our model intravenous chemotherapy at home was feasible, sustainable and safe. Patient and family satisfaction was high, with special regard to the avoidance of discomfort due to driving distances, long waiting times and frequent hospital accesses. A greater effort should be made in promoting new legislations on domiciliary cancer chemotherapy and in sharing local experiences among international home care institutions.

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FREQUENCY OF PAIN AND EMOTIONAL DISTRESS IN LEUKEMIA AND OTHER MALIGNANCIES: A STUDY BY VAS, HADS AND ESAS

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Visual analog scale (VAS), Edmonton Symptoms Assessment System (ESAS) and Hospital Anxiety and Depression Scale (HADS) were used to evaluate the frequency of physical pain and emotional distress in 53 patients with acute leukemia, since diagnosis during the various clinical phases, as well as in 28 with haematologic malignancies other than acute leukemias, and in 76 with solid cancers, as controls. The diagnosis of depression and/or anxiety was made with a score of 8 of 21 or more in the HADS questionnaire. In the ESAS analysis, depression and anxiety were considered present with a score of 2 of 10 or more. According to the HADS score, during the induction phase, depression was reported in 21%, 36% and 33% of the acute leukemia patients, at diagnosis (time

0), at +15 and at discharge, respectively, while anxiety in 30%, 40%, 36% of the cases, at the same time intervals, respectively. Depression was reported in 32% while anxiety in 38% of all questionnaires, respectively, collected from patients at all time intervals during all clinical phases of leukemia. According to the VAS score, during the induction phase, mild pain was reported in 39.5%, 27%, and 16.6%, while moderate to sever pain in 11.6%, 20.4% and 5.5% of the cases, at diagnosis, at +15 and at discharge, respectively. According to the ESAS score during the induction phase, depression was reported in 45%, 38.6% and 27.7% of the patients, at diagnosis, at +15 and at discharge, while anxiety in 46.5%, 40.4%, 41.6% of the cases, at the same time intervals, respectively. ESAS showed a sensitivity of 57% and 74%, and a specificity of 67% and 67% for depression and anxiety, respectively, when all questionnaires are considered. In patients with other haematologic malignancies, HADS score was positive for depression in 21.4% while for anxiety in 25%, while ESAS score was positive for depression in 50% while for anxiety in 61% of the cases. In solid cancer patients, at diagnosis, mild to moderate to severe pain was reported in 38% of the cases. HADS score was positive for depression in 25% while for anxiety in 19.7% while ESAS score was positive for depression in 28.9% while for anxiety in 40.7% of the cases, with a sensitivity of 63% and 86.6% and a specificity of 82% and 70% for depression and anxiety, respectively. The data first show that not only solid cancer but also leukaemia patients may have physical pain and suffer from emotional distress since diagnosis, as revealed by HADS and ESAS. *Funding. This study was supported by A.I.L. Modena, ONLUS*

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PEGFILGRASTIM AS HEMATOPOIETIC STEM CELL MOBILIZING AGENT IN PATIENTS WITH MALIGNANT LYMPHOMAS. A SINGLE CENTRE EXPERIENCE

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Collection of peripheral blood hematopoietic stem cells (PBSC) is currently performed after mobilization with granulocyte colony-stimulating factors (G-CSF) alone or in association to chemotherapy (CHT). The pegylated form of G-CSF, pegfilgrastim, is currently used as growth factor for prophylaxis of febrile neutropenia. We investigated the use of pegfilgrastim in addition to CHT as a mobilizing regimen in patients (pts) with malignant lymphomas. From January 2007 to December 2008 40 pts were enrolled in this prospective study. Histology was: 16 Hodgkin's lymphoma, 16 DLBCL, 5 MCL, 2 PTCLu, 1 blastic/NK lymphoma. High-dose therapy was considered in 20 pts for refractoriness, in 13 pts for relapse, and in the last 7 high-risk pts according to internal guidelines. Median previous treatments was 1 (range 1-3). Salvage CHT was ESHAP, a 3-day DHAP-modified regimen consisting of cytarabine, etoposide, cisplatin; rituximab was added to ESHAP in 20 pts with B-cell lymphoma. Single-dose 6 mg pegfilgrastim was administered 24 hours after the end of ESHAP. Peripheral blood CD34+ cell count was evaluated when WBC were > $1x10^9$/L; pts started daily leukapheresis if peripheral blood $CD34^+$ were > 7/microL until a minimum target of $2x10^6$/Kg (actual body weight) $CD34^+$ cells were collected. A Cobe-Spectra cell separator was used to perform the procedures. Thirty-eight pts (95%) mobilized $CD34^+$ cells in peripheral blood, and performed leukapheresis successfully; in 36 pts a single apheresis was sufficient to reach the target. Median time from pegfilgrastim administration and collection was 9 days (range 7-11); median number of circulating $CD34^+$ cells at first collection was 71/microL (range 7-617). Total number of $CD34^+$ cells collected was $9,5x10^6$/Kg (range 2,4-47,6), and in 30 pts the number of cells harvested was higher than $5x10^6$/Kg. Two pts did not reach a sufficient peripheral blood $CD34^+$ cell count, a 69 years-old man who previously received high-dose Zevalin with autologous stem cell rescue and a 28 years-old woman who received chemotherapy for a Hodgkin's disease. Our data show that in pts with malignant lymphomas receiving CHT a single-dose of pegfilgrastim is a valid alternative to daily G-CSF as mobilizing agent, allowing optimal PBSC collection nearly all pts.

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PROSPECTIVE CYTOMEGALOVIRUS MONITORING IN ACUTE MYELOID LEUKEMIA PATIENTS DURING FIRST LINE CHEMOTHERAPY

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Very little is known about the incidence and clinical impact of cytomegalovirus (CMV) infection in acute myeloid leukemia (AML) patients at diagnosis and during chemotherapy. Aims of our study were to assess prospectively the incidence of active CMV infection in AML patients and to describe treatment outcome of CMV infection. Pp65 antigenemia monitoring was performed weekly at diagnosis, post-induction and post-consolidation chemotherapy, and whenever CMV reactivation was suspected. Patients with a positive antigenemia received anti-CMV pre-emptive treatment. Sixty-nine consecutive AML patients at diagnosis were evaluated. CMV serology at diagnosis was available in 56/59 patients achieving complete remission: 52 patients (93%) were IgG positive. The overall incidence of positive pp65-antigenemia in 59 evaluable patients after chemotherapy was 35%: 9 patients after induction and 12 post-consolidation. Sixteen of the 21 positive patients received anti-CMV treatment: 15 as pre-emptive therapy and 1 for CMV pneumonitis. The remaining 5 patients didn't receive anti-CMV treatment and didn't develop CMV disease. The incidence of CMV infection in patients receiving high dose or standard dose Ara-C was 46% (13/28) and 25% (8/31), respectively, this difference was not statistically significant (p=0.09). The 60-months overall survival is 60% vs 51%, for CMV pp65-antigenemia positive and negative patients (p=0.8). A high incidence (35%) of active CMV infection was recorded in adult AML undergoing first line treatment, while CMV disease was observed in 1 of the 59 (1%) patients monitored. Future randomized, placebo control studies are needed to assess the relevance of anti-CMV pre-emptive therapy in AML patients receiving chemotherapy.

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INFECTIOUS COMPLICATIONS DURING INDUCTION AND POST-REMISSIONAL TERATMENT OF ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Patients with Acute Myeloid Leukemia (AML) undergoing chemotherapy are at high risk for infections. The records of 81 consecutive patients (median age 49 years) with AML, treated with homogeneous induction and post-remission therapy, were restrospectively analysed. A total of 291 neutropenic episodes were studied. All patients were treated in an air-conditioned facility without EPA protection. Antiinfectious prophylaxis with levoxacin and itraconazole was given to all patients. Empiric antibiotic and antifungal therapy was started according to published guidelines. During the first induction course a neutropenic fever was observed in 69 patients (85.2%). Twenty-seven patients (33.4%) had fever without neither isolates nor clinical or instrumental sign of infection. They were classified as Fever of Unknown Origin (FUO). Ten patients (12.4%) had fever with clinical or instrumental signs of infection and were classified as Clinically Documented Infections (CDI). In 23 patients (28.4%) isolates of infectious agents were obtained and they were defined Microbiologically Documented Infections (MDI). "Possible" invasive fungal infection (IFI) was observed in 2 patients (2.4%), "probable" IFI in 3 patients (3.7%) and *proven* IFI was documented in 4 patients (4.9%). During the post-remissional courses the incidence of fever was significantly reduced compared to induction (85.2% vs. 48.7% , p=.0000). The number of days with fever was greater in induction in confront to post-remission courses (median 9.6 vs 2.3, p=.0000). In the post-remissional courses, the incidence of FUO, CDI, MDI, IFI were 19.1%, 2.5%, 29.2% and 0.5% respectively. We observed a difference between isolates obtained from blood cultures during induction or during the following post-remissional aplastic phases : the incidence of Gram+ and Gram- isolates was 70.8% and 29.2% respectively during induction versus 30.4% and 69.6% respectively during the

post-remissional courses (p=.0019). The response to empiric antibiotic therapy, defined as resolution of fever during neutropenia, was significantly lower in induction than in the post-remissional phase (52.2% vs 80.4%, p=.00018). Six patients (7.4%) died during induction because of infectious complications, while no additional infection related death was observed during post-remissional courses. The analysis of infections during repetitive courses of intensive chemotherapy in AML may guide more rational use of prophylaxis and empiric treatment.

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PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) IN HEMATOLOGY: A SINGLE CENTER EXPERIENCE

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Background. Reliable long-term vascular access is essential for the chemotherapy of patients with acute myeloid leukemia, refractory or relapsed lymphoma and for stem cell transplantion. Peripherally inserted central venous catheters (PICCs) are now widely used for intermediate and long-term access and they are increasingly supplanting conventional central venous catheters (CVCs). PICCs are non-tunnelled, central catheters inserted through a peripheral vein of the arm. PICCs can be used for prolonged, continuous or intermittent infusion therapies and the insertion doesn't need the anesthesiologist and operating theatre. They can be set up in hospital room and it is safer than conventional CVCs in thrombocytopenic patients. *Methods and results*. On June 2006 we formed a PICC-Team in our hematology. We retrospectively reviewed all haematological patients who received a PICC insertion between June 06 and March 09. We reviewed duration of catheterization, bloodstream infections PICC-related, safety of PICC and costs of management of PICCs as to conventional CVCs. One hundred sixty three PICCs were inserted in 63 patients with AML, 53 patients with NHL,10 patients with HL,15 patients with ALL, 22 patients with MM. Mean duration of catheterization was 180 days (total, 29.340 PICC-days). Bloodstream infections PICC-related (0,3/1000 PICCs-days) were less than bloodstream CVC-related (1-2/1000 CVCs-days). A questionnaire about quality of life and safety was submitted to each patient with PICC. The answers were compared with the same of patients with conventional CVC. PICC resulted more comfortable and safer, and it was better accepted than the conventional CVC. The prize of PICCs was calculated and the costs of total management of PICCs as to conventional CVCs and the mean duration of catheterization of PICCs as to CVCs was estimated. Even if PICC is more expensive than conventional CVC, the mean duration of catheterization with PICC is longer and the total management of PICCs is less expensive than the conventional CVCs (see table). *Conclusions*. Cumulative data analysis of PICCs confirmed that the management is economically sustainable and represents an improvement to the CVC, as far as the clinical indicators, financial costs and humanities are concerned.

Total cost (materials + time work)		Mean time of catheterization	Daily costs	
PICC	€ 176,79	180 days	PICC	€ 0,98
CVC	€ 105,81	30 days	CVC	€ 3,53
Difference	- € 70,98		Difference	+ € 2,54
Variation CVC vs PIC	- 40,1%		Variation CVC vs PICC	+ 259,1%

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HEPATITIS B VIRUS (HBV) REACTIVATION IN ANTI HBCAG POSITIVE PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES: A RETROSPECTIVE STUDY OF HEPATOLOGIST AND HAEMATOLOGIST (EP.EMA) COOPERATIVE GROUP

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Aims. To analyze the risk of HBV-related liver disease in isolated anti-HBcAg positive patients (pts) affected by hematological malignancies undergoing chemotherapy and hematological stem cell transplantations (HSCT). *Methods*. A retrospective study conducted between January 2006 and December 2007 in 4 Haematological Divisions in Rome. In each patient the determination of HBV markers and HBV–DNA were evaluate to recognize the occurrence of HBV reactivation in occult carriers ($HBsAg^-/HBcAb^+$). *Results*. Thirty-three pts were identified with occult HBV infection: 27 NHL 3 AML and 3 Myeloma. Median age was 67 years (range 25-88). The treatment schedule was different according to the diagnosis and the specific centre protocol: particularly 18 pts received Rituximab (375 mg/m^2) and 5 underwent HSCT (3 AutoSCT 2 Allo) Twenty-three pts were evaluable for the determination of HBV reactivation, while 10 were excluded because of early death (9 disease progression and 1 heart failure). Among the remaining 23 pts, 11 (48%) were $HBsAb^+/HBcAb^+$ and 12 (52%) were HBsAb-/$HBcAb^+$. HBV reactivation with acute hepatitis was diagnosed in 5 pts (22%): 2 Allo-SCT, 2 Auto-SCT and 1NHL treated with R-CHOP schedule. In these patients HBV-DNA was assessed: median serum value was 1,350,000 UI/ml (range 113,000-8,460,000). The median time between end of chemotherapy and HBV reactivation was 9 months (range 8-12). Antiviral treatment was started with lamivudine and adefovir in 4 pts, and entecavir alone in 1 case. At last follow up in March 2009 (median time of follow up 22 months, range 6-29) all pts were in clinical remission of hepatitis; HBV-DNA was not detectable in 4/5 treated pts. One pt shows a new seroconversion with loss of HBsAg and appearance of anti-HBsAg with protective title (>10 mUI/L). *Conclusions*. Our retrospective study indicates that pts with occult HBV-infection are at risk of reactivation; this problem is not limited to patients with lymphoma, but also affects patients with other haematological malignancies undergoing highly immunosuppressive therapy and/or SCT. All patients with HBV related disease showed a good response to antiviral therapy and prolonged treatment allowed the achievement of new seroconversion in 1/5 pts. A long term prospective study is actually ongoing in order to confirm the necessity of monitoring and to determine the role of antiviral prophylaxis in this group of patients.

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GENE EXPRESSION CHANGES OF UNFRACTIONATED PERIPHERAL BLOOD STEM CELLS (PBSC) OF DONORS DURING *IN VIVO* MOBILIZATION

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Introduction. Peripheral blood stem cells (PBSC) mobilisation requires the administration of haematopoietic cytokines ie rh granulocyte colony-stimulating factor (G-CSF). As more healthy donors are exposed to pharmacologic doses of rhG-CSF, it has become essential to study its effects to safeguard donor safety. Accumulating evidence now suggests that rhG-CSF effects in healthy subjects may be more complex and heterogeneous that originally thought. Therefore, changes in cell systems induced by mobilisation and homing are not yet.clear. To gain insight into the effects of G-CSF, the gene expression profile (GEP) of healthy donors was evaluated. In order to assess the duration of this effect, sequential studies on gene expression profile were also performed. *Meth-*

ods. Peripheral blood samples were harvested before and after of G-CSF somministration: at baseline, at + 5 days and at + 30 days post G-CSF. We used a TaqMan® Low Density Array based on comparative dd CT method to perform relative quantification of mRNA. Expression of each gene was normalized to the reference gene 18S mRNA, 1 unit was assumed as the normal reference value. About the project of macroarray card, we selected 47 candidate genes involved in immune network and inflammation pathogenesis. *Results.* After stimulation with G-CSF, we observed increased expression of genes which specifically acts on endothelial cells: VEGFA, eNOs, MMP9. Transcriptome of Th1, Th2, TH17 response was characterized by no significant modulation. Similar feature is showed by a cluster of genes coding for chemokines/cyclines, inflammation mediators. Only IL-10 was significantly up-regulated. Apoptosis regulator genes (*CASP1, CASP6, BCL2A1*) were inalterated, only transcription factors (EGR-1,2) were serially increased until +30 days. All others genes returned to baseline values at +30 days. It's interesting that onco-genes JUN and FOS did not change. *Conclusion*. Based on the totality of information currently available, we believe that the administration of rhG-CSF to healthy donors for the purpose of PBPC donation continues to have a favourable risk-benefit profile. The increase of endothelial pattern might be due to pore formation (increased endothelial fenestration). *In vivo*, release of VEGF by progenitor cells may result in a paracrine loop supporting proliferation of both endothelium and progenitors and may facilitate transendothelial migration during cytokine-induced progenitor cell mobilization.

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PRE-EMPTIVE TREATMENT WITH CIDOFOVIR FOR CYTOMEGALOVIRUS ANTIGENEMIA IN AUTOLOGOUS BONE MARROW RECIPIENTS AND CLL PATIENTS ON THERAPY WITH ALEMTUZUMAB

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Cytomegalovirus (CMV) is an important cause of morbidity and mortality in patients who have undergone severe immunosuppressive therapy. Ganciclovir continues to be the first choice for pre-emptive therapy, but it needs multiple intravenous daily administration for three weeks and may cause myelosuppression. Cidofovir is a non myelotoxic nucleotide analogue effective against CMV; its favourable pharmacokinetic profile allows a once-a-week dosing. We reviewed a database on 110 consecutive Autologous Stem Cell Transplant (ASCT) and that of 15 Chronic Lymphocytic Leukemia (CLL) patients treated with alemtuzumab. All patients were virologically monitored by quantification of pp65 antigenemia in peripheral blood. Cytomegalovirus infections were identified respectively, in 13 of 110 (12%) ASCT group and in 10 of 15 (66%) CLL group. Nine out 23 CMV reactivation showed manifestation of the infection. All patients were treated on outpatient basis. Patients with a positive pp65 assay were treated with cidofovir 5 mg/kg once-a-week for two weeks followed by one or two doses every two weeks. Twenty-three patients (13 autologus, 10 alemtuzumab) had 23 episodes of CMV-pp65 detection treated with cidofovir. The first positive antigenemia occurred after a median of 36 days from starting treatment (range 5-20) and the median antigenemia level at first appearance was 2 (range 1- 89). The treatment produced regression of symptoms in all cases and clearance of the virus in 21 (11 post-transplant 84%; 10 post alemtuzumab 100%), stained by CMV antigenemia. Median duration of therapy was 21 days (range 14-30 days) and the time to the first undetectable antigenemia was seven days (range7-28). We did not observe any further CMV reactivation, also in six of the ten patients who restarted treatment with alemtuzumab after the end of pre-emptive therapy. We did not observe any of the side effects potentially related to cidofovir administration: notably, none of the patients experienced renal toxicity, proteinuria, nausea or vomiting, ophthalmological or neurological toxicity. In our experience, pre-emptive therapy of CMV infection with cidofovir is safe and effective. In our opinion it could be considered an interesting alternative to Ganciclovir for pre emptive therapy, particularly advantageous for treatment of CLL and ASCT ambulatory patients at low risk of developing CMV disease.

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HOME CARE PROJECT IN NEUTROPENIC HEMATOLOGIC PATIENTS IN MONZA AREA: TWO YEAR ACTVITY

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Haematological patients receive chemotherapy that induces variable grade of neutropenia. The closeness to other patients during hospitalization or in a Day Hospital may be a major source of infections in these patients. Our home care (HC) project is directed to neutropenic patients with the aim to reduce the time of hospitalization, to improve quality of life and to allow a better use of our resources. The equipe is composed of a *physician leader*, five medical doctors , a nurse and a psychologist. To be eligible for this home care project called *protected discharge*, patients have to live in the Monza area, to be in a stable, noncritical clinical condition; they must have venous catheter and a care-giver suitable at home. Since performing transfusions is possible even in non-hospital environments in Italy, we decided to perform also blood and platelets transfusions at home besides first antibiotic line too, when necessary. The equipe meet every week and work on *individual care plan* (PAI:) from discharge to recovery of neutrophils (neutrophils plus than 1000/m^3). There is a constant contact with general practitioner thanks to a network. We give the patient a personal computer that is on line with hospital so that every health worker included general practitioner can be aware of clinical and laboratory situation in real time. Patients who receive multiple cycles of chemotherapy are included in the project during every neutropenic episode. Up to now, 22 patients have been included in the project. Two were affected by acute promyelocytic leukemia, 10 by acute myeloid leukaemia in NILG AML06 protocol, 3 by acute lymphoblastic leukaemia, 5 by non Hodgkin lymphoma, 2 by multiple myeloma. The median duration of the whole HC period for each patient is 13 days. Overall activity consisted of 130 blood drawings , 18 antibiotic, i.v. therapy (first line), 12 red blood cell and 30 platelet transfusions, 94 medical visits and 137 nurse visits. Just one patient asked for psicologist. Four patients were hospitalized and HC programs discontinued because of the onset of fever resistant to antibiotics in 3 patients and a heart problem in one patient. These preliminary results are encouraging and we keep on working on this program with a good compliance of patients and their family.

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FEBRILE NEUTROPENIA IN ACUTE MYELOID LEUKEMIA. EPIDEMIOLOGY OF A SINGLE INSTITUTION IN THE YEARS 2005-2008

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We have retrospectively analyzed 197 patients (pts) whose median age was 60.8 (range 20-96) affected by acute myeloid leukemia hospitalized in our institution between 2005 and 2008. During cytopenia all pts received antibiotic and antifungal prophylaxis therapy. A subset of 117 pts (59%) developed febrile neutropenia for a total of 187 events. In pts that received high doses of chemotherapy, the majority of febrile episodes occurred during induction phase (45%), consolidation (15%), and maintenance (5%). The highest rate of events was documented in relapsed – resistant pts (29%). Furthermore, in some pts (6%) the febrile event was observed at the hospitalization before starting chemotherapy. Forty-five febrile episodes (24%) were associated with gram-positive bacterial infections (30 patients, 66 %), gram-negative ones (13 patients, 29%) and polymicrobical organisms (5%). In 56 cases (30%) severe lung compromise was clinically documented, while 75 episodes (40%) were classifyed as fever unknown origin (FUO). Only 6% of febrile episodes was caused by fungal sepsis(5 *candida spp.* and 1 *fusarium*). All patients were treated with broad spectrum antibiotic therapy, furthermore in 85 febrile episodes (45,4%) an antifungal therapy was added: In particular, antifungal treatment was empirically initiated in 35 patients (11,71%), whereas it was started in cases of far and possible fungal infection (26 pts, 13,93%), in cases of far and probable fungal infection (13 pts, 6,95%) and in cases of far certain one (11 pts, 5,88%). Death related to infections was observed in 6 patients. Microbiologically documented infections were: bacteria in 1 pt, candidemia in 2 pts, geotricum capitatum

in1pt. Probable aspergillosis was hypotized in 2 pts. *Conclusion*. In this retrospective investigation we observed high mortality restricted to patients with resistant or relapse disease.

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EPIDEMIOLOGY OF FUNGAL INFECTIONS IN ADULTS WITH ACUTE MYELOID LEUKEMIA: A SINGLE INSTITUTION ANALYSIS

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Invasive fungal infection (IFI) remains a major cause of morbidity and mortality among patients with acute myeloid leukemia (AML). IFIs not only have increased in incidence in the last years but their aetiology has changed. The aims of this study are to assess the recent incidence and the factors contributing to the risk of IFI in 197 adults admitted to our department from 2005 to 2008. We retrospectively analyzed 187 consecutive febrile episodes occurred in 117 out of 197 AML patients. Among the 187 febrile episodes, 21 (11.2%), 14 (7.5%) and 10 (5.3%) cases were classified according to EORTC 2008 criteria as possible, probable and proven IFI, respectively. A total of 33 (17.3%) of episodes were treated with empirical antifungal therapy because fever persistence. Overall, antifungal therapy was used in 78 (42%) febrile episodes. The incidence rate of probable plus proven IFI was 12.8%. We observed a significant higher percentage (21%) in patient underwent intensive chemotherapy, while none of pts treated with supportive therapy or low dose chemotherapy had probable or proven IFIs. Antifungal therapy was more frequently used during induction therapy. In this phase, 28 percent of febrile episodes were classified as IFI: 12%, 9% and 7% as possible, probable and proven, respectively. Empirical therapy was done in 16 (18.8%) cases. A total of 14 IFIs were identified, including 8 invasive aspergillosis, 3 invasive candidiasis, 2 geotricum capitatum and 1 fusarium. During consolidation treatment, we observed 27 febrile episodes, which were classified 7%, 7% and 3% as possible, probable and proven IFI, respectively. Empirical treatment was done in 3 cases. In refractory AML or in patient with relapse, we observed 11% probable and 3% proven IFI. In conclusion, the incidence rate of IFI in our study is similar to that previously reported. The high incidence of IFIs, their continuing high mortality and the emergence of rare fungi, despite advances in antifungal therapy, require the development of adjunctive strategies.

Chronic Lymphocytic Leukemia II

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HEPATITIS B VIRUS (HBV) REACTIVATION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS WITH PRIOR RESOLVED HEPATITIS B EPISODE

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HBV reactivation is a well recognized complication during immunosuppressive treatment. In most cases reactivation occurs in pts showing HB surface antigen (HBsAg) but it is often observed also in cases with resolved infection who are HbsAg negative (-ve) but antibodies anti HB surface Ag (HbsAb) positive (+ve) and anti HBcore+ve (antiHBc). The aim of this study was to assess the rate of HBV reactivation in CLL pts HbsAg-ve and anti-Hbc+ve +/- HBsAb+ve. Hepatitis, defined as an increase in ALT level, was attributed to HBV in case of HBsAg serological reversion with an increase in HBV-DNA. In a group of 327 CLL pts, 75 (22,9%) were found to be HbsAg-ve and anti-Hbc+ve. Twenty-nine had never received CLL treatment while 46 pts had been treated with chemotherapy (CHT) or monoclonal antibodies. Seven treated pts received Lamivudine prophylaxis. HBV reactivation was observed in 13 of the 75 pts (17.3%). Among the 29 untreated pts reactivation occurred in 2 pts, both developing hepatitis, after >100 m from diagnosis. Eleven (23.9%) reactivations were observed in the 46 treated pts, none had received previous Lamivudine. In 4 pts reactivation, accompanied by hepatitis in 2, was detected during treatment which consisted of: alkilating agents (2 pts), alemtuzumab (A) alone or in combination with CHT (2 pts). In the remaining 7 pts reactivation occurred after a median of 12 m (range 5-36) from the end of therapy and was characterized by hepatitis in 4 pts. Treatment consisted of FAMP alone or in combination with cyclophosphamide (4 cases), A alone or in combination with CHT (2 pts), alkilating agent (1 case). HBV-DNA became undetectable after nucleos(t)ide analogues in all pts, no fatal events hepatitis related were observed. Nucleos(t)ides are recommended in pts HbsAg+ve, nevertheless there is not general agreement on prophylactic treatment in pts HbsAg-ve and anti-Hbc+ve +/- HBsAb+ve. In our series Lamivudine, even administered in a low number of cases, showed to be effective in preventing reactivation. Even though in pts not receiving prophylactic treatment the close monitoring of serological tests, HBV-DNA detection and the prompt commencement of therapy at reactivation determined the rapid disappearance of the viral load and prevented the development of fatal events. Furthermore in anti-Hbc+ve pts a continuous and prolonged surveillance of HBV tests is needed as we observed reactivations in untreated pts and also after a long time from the end of treatment.

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HEPATITIS B VIRUS EXPOSURE AND ITS CORRELATION WITH BIOLOGICAL AND CLINICAL CHARACTERISTICS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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The prevalence of hepatitis B virus (HBV) infection in patients with haematological malignancies is increased in comparison with the general population worldwide. Furthermore patients with lymphoproliferative disorders have a higher risk of HBV reactivation after chemotherapy than other cancer patients probably related to more immunosuppressive treatment. Few studies have investigated the association between HBV infection and CLL. The main objective of the present study was to compare the clinico-pathologic features of CLL in HBV serological positive and negative patients. HBV serological screening [hepatitis B surface antigen (HbsAg), antibody to HBsAg (HBsAb), antibodies to hepatitis B core (HBcAb)] had been performed in an unselected series of 327 CLL pts. PCR test for HBV-DNA was performed in selected cases. Among the 327 pts, 105 (32,1%) presented evidence of HBV exposure. Results of the serological screening in a first group of 89 cases tested positive for anti HbcAb are reported in the Table 1. The remaining 16 pts (4,9%) tested positive for HBsAb but negative for HBcAb. None of them had received previous HBV vaccination and all cases were

negative for PCR HBV-DNA. The clinical and biological characteristics of the HBV exposed pts were compared to the HBV negative pts. A statistical significant difference wasn't detected between the two groups regarding: age, gender distribution, FISH, ZAP 70, CD38, Binet stage, β2 microglobulin, LDH. A statistical significant difference was observed analyzing the IgVH mutational status as HBV negative pts showed predominantly a IgVH mutated status (p=0.045). No prevalence or correlation for the IgVH families was recorded between the group of pts. As regards the clinical outcome we didn't observe any difference between the time elapsing from diagnosis to first treatment among the two groups while a statistical significant better survival (p=0.045) was observed in HBV negative pts. This significance persisted even when pts presenting with HbsAg positivity were excluded from the analysis. In conclusion we observed a high incidence of pts presenting with anti HBcAb in our CLL population. The longer overall survival of pts tested negative for HBV is related to the predominant IgVH mutated status observed in those patients.

Table 1. Patients characteristics.

Anti HBcAb positive n. 89 (27,2%)			
Anti HbsAb positive n. 69 (77,5%)		Anti HBsAb negative n. 20 (22,5%)	
HbsAg + n. 6 (9%)	HbsAg - n. 63 (91%)	HbsAg + n. 5 (25%)	HbsAg - n. 15 (75%)

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CYTOMEGALOVIRUS (CMV) REACTIVATION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS RECEIVING ALEMTUZUMAB EITHER AS MONOTHERAPY OR IN COMBINATION WITH CHEMOTHERAPY

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Alemtuzumab (A) therapy is associated with profound immunosuppression leading to Cytomegalovirus (CMV) reactivation. This event in chronic lymphocytic leukemia (CLL) ranges from 4-42%. The wide range reflects study design, population and viral detection methods. To evaluate the incidence of CMV reactivation and its correlation with A schedule we analyzed 85 CLL pts. Fifty-six pts received A alone: 3 fludarabine (F) refractory pts at the dose of 90 mg/w for 12 w, 53 F responsive pts at the dose of 30 mg/w for 6-12 w as consolidation. In 29 pts A was associated to either F plus cyclophosphamyde or Bendamustine at the dose of 10 or 20 mg for 3 consecutive days every 4 w. Pts characteristics are listed in the table. CMVpp65 antigenemia and quantitative PCR (10 cases) were evaluated weekly; reactivation was considered an antigenemia ≥10 cells. Overall CMV reactivation occurred in 20 pts (23.5%), 9 cases were symptomatic; none developed CMV disease. Reactivation rate in pts receiving A alone was 28,5%, positive test appeared after a median of 4,5 w (range 1-6) from treatment initiation, after a median A dose of 140 mg (range 60-180). In pts receiving A in combination the reactivation rate was 13.8%; median time from treatment initiation to reactivation was 3 w (range 3-16) after a median of 60 mg (range 60-240) of A.

Table 1. Patients characteristics.

	Alemtuzumab alone	Alemtuzumab in combination
Sex M/F %	73/27	52/48
Age y median	55	59
IgVH status mutated %	55	34
ZAP70 positive %	59	34
previous lines of treatment median (range)	0 [0-3]	1 [0-5]
Total dose previous F mg median (range)	1280 [480-3240]	1000 [0-3840]
DT previous CHT median month	5 [2-27]	8 [0-90]

Pre-emptive treatment with ganciclovir was administered to 17 pts while 3 asymptomatic pts were treated with Valaciclovir as they presented 10 positive cells with a rapid negativization. None of the characteristics listed in the table showed a statistical significance. In conclusion there is a trend (p=0.117) of a higher reactivation rate in pts treated with A alone without a statistical significance probably due to the unbalanced and small size of the studied sample. Interestingly in previously treated pts the probability of CMV reactivation decreases from previous therapy discontinuation of 2.5% per week (p=0.310). Further investigations in larger series are warranted to confirm the impact of disease characteristics and treatment schedule on CMV reactivation thus to ameliorate prophylactic schedule and better define the timing of A therapy in sequential strategies.

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PHENOTYPIC AND FUNCTIONAL FEATURES OF VGAMMA9/VDELTA2 T CELLS CORRELATE WITH THE MUTATIONAL STATUS OF THE TUMOR IMMUNOGLOBULINE AND WITH DISEASE AGGRESSIVENESS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The Vγ9/Vδ2 (Vγ9/Vδ2) subset of γδ (γδ) T lymphocytes recognizes the tumor cells-derived phosphoantigene isopentenyl pyrophosphate (IPP), which is produced via the mevalonate (Mev) pathway. Zoledronic acid (ZA), the most potent Aminobisphosphonate, specifically inhibit the Mev pathway enzyme farnesyl pyrophosphate (FPP) synthase, thus leading to the accumulation of IPP. Stimulation with ZA and low doses of interleukin-2 (IL-2) normally leads to the expansion of Vγ9/Vδ2 T cells in vitro. We have investigated the proliferative response of Vγ9/Vδ2 T cells to ZA and IL-2 in 87 patients with chronic lymphocytic leukemia (CLL) at diagnosis. Proliferation of gd T cells was observed in 43 patients (49%)(responders, R), whereas 44 patients (51%) were non-responders (NR). Vγ9/Vδ2 T-cell subset distribution was well balanced in R patients, whereas effectors subsets [i.e., effector memory (TEM), and terminally differentiated effector memory (TEMRA)] were largely predominant in NR patients. TEMRA of NR patients expressed higher levels of the inhibitory receptor ILT2 and tended to have lower levels of the costimulatory molecule NKG2D, as compared to TEMRA of R patients. The proliferative response of Vγ9/Vδ2 T cells was significantly associated with the mutational status of the tumor immunoglobulin heavy chain variable region (IgVH), which is a well known prognostic factor in CLL. Indeed, 82% of R patients had mutated (M) IgVH, whereas 77% of unmutated (UM) patients were NR (p<0.001). Given this association, we evaluated the rate of activity of the Mev pathway in tumor cells of M and UM patients by 1) analysis of gene profiling data 2) quantification of the metabolites FPP and IPP, and of the final product cholesterol. These analysis showed that the pathway is significantly more active in UM than in M tumor cells. The higher amount of IPP produced by UM cells most likely lead to the *in vivo* chronic stimulation of gd T cells and to their differentiation into functionally impaired ILT2-positive TEMRA cells. Given the tight association between the R/NR status and the IgVH mutational status we also analyzed the independent prognostic impact of R/NR status in multivariate Cox analysis. NR patients had a significantly shorter time to first treatment thus pointing to the R/NR status as a new independent prognostic factor. Therefore, our data define a novel mechanism of immune escape which can contribute to determine disease aggressiveness in CLL patients.

P360

THE PROTEIN CONTENT IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: A MALDI-TOF MS PROFILING STUDY

Bossio S., Cutrona G.,[1] Gentile M., Vigna E., Lucia E., Mazzone C., Franzese S., Matis S., Servillo P., Gigliotti V., Le Pera M.2, Urso E.,[2] Fabris S.,[1] Recchia A.G., Cavalcanti S., Neri A.,[3] Ferrarini M.,[1] Qualtieri A.,[2] Morabito F.

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Different clinical course maybe experienced by CLL patients. Several markers are currently utilized as powerful prognostic tools. Proteomic studies are used to investigate the proteome complexity of CLL through the identification of new potential markers. MALDI-TOF MS profiling were utilized to examine the protein content of purified B-cells selected by specific antibodies linked to surface magnetic beads (Dynalbeads). B-cells were lysed and sub-fractionated in nuclear, microsomal and water-soluble components. The latter fraction underwent a desalting/concentration step over ZipTip C18 and peptide/protein profiles were analyzed using a VoyagerDE PRO MALDI-TOF mass spectrometer (PerSeptive-Biosystems). Separate spectra were obtained for a restricted mass-to charge (m/z) range (1000-25000 Da) in linear mode geometry, by applying an acceleration voltage of 25 kV.

Figure 1. Cluster analysis of 25 patient affected, comprising the CD38 values with 14 differential expressed ion signals with statistical significance (p<0.05) between two groups.

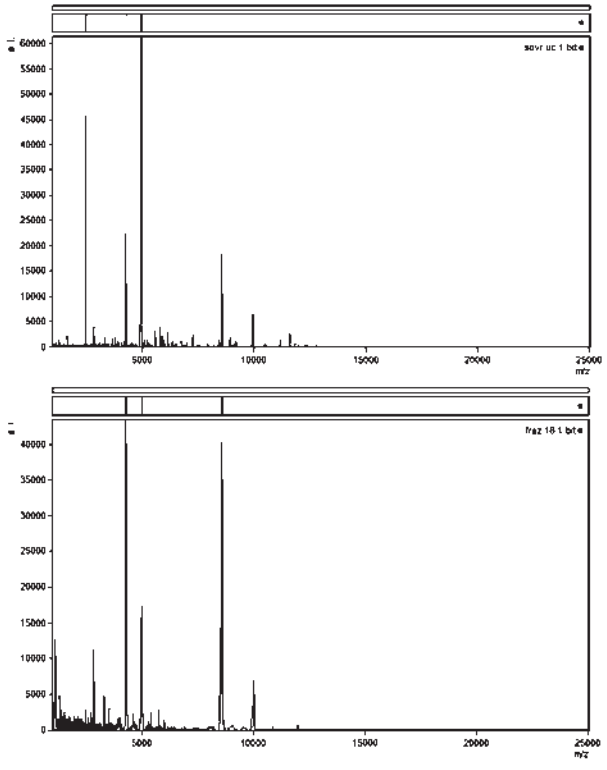

Figure 2. Representative mass spectra of: (A) cytosolic extract from lymphoblastoid cells; (B) HPLC fraction #17 from lymphoblastoid cytosolic extract. For each spectrum it is shown also a gel like representation (top panels).

The acquired spectra, assayed in duplicate, were then processed for automated advanced baseline correction and noise. The peak area of each signal was normalized by presenting as a percentage of the total peak area (individual peak area/total peak area per cent). For the characterization of peptide/proteins differentially expressed we used the extracts from lymphoblastoid cells immortalised by Epstein-Barr Virus subsequently subjected to cell lysis and finally sub-fractionation of nuclear, microsomal and water-soluble components.

The proteins from cytosolic extract, were separated by reverse phase high-performance liquid chromatografy (HPLC) and collected on 40 fractions. The relative fractions were finally analyzed using a VoyagerDE PRO MALDI-TOF mass spectrometer (PerSeptiveBiosystems). A hierarchical cluster analysis, with Pearson correlation as similarity metrics, and an average linkage as a cluster method, was also performed on the dataset and CD38 values using Cluster 3 and TreView software. The results obtained by cluster analysis separated all patients examined in two distinct branches. A student's t test applied to these groups allowed the identification of 14 differentially expressed ion signals with statistical significance (p<0.05, Figure 1). Figure 2 illustrates mass spectra of cytosolic components from lymphoblastoid extract (Figure 2A) and of a fraction (#17) after HPLC separation (Figure 2B). Although the purification process in still suboptimal, some ion signals of potential interest can be observed. These preliminary results demonstrated that CLL may be studied on the basis of their protein/peptide content using the MALDI-TOF protein profiling analysis.

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LOW DENSITY LIPOPROTEIN RECEPTOR PROTEIN 4 (LRP4) RS2306029 SINGLE NUCLEOTIDE POLYMORPHISM PREDISPOSES TO TRANSFORMATION OF CHRONIC LYMPHOCYTIC LEUKEMIA TO RICHTER SYNDROME

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Background. The rationale of the study stems from two considerations:

i) mechanisms and risk factors of CLL transformation to Richter syndrome (RS) are largely unknown; ii) the identification of a CD38 single nucleotide polymorphism (SNP) as RS risk factor suggests that the host genetic background may be relevant for RS development (Aydin S et al. Blood 2008;111:5646). *Aims*. To explore the role of the host genetic background in RS transformation. Methods. The study was based on a consecutive series of 331 CLL, of which 21 had transformed to RS. Using an educated guess approach, SNPs were selected according to the following criteria: i) reported association with CLL prognosis; ii) minor allele frequency >5% in Caucasians. Accordingly, 44 SNPs from 44 genes were genotyped on germline DNA extracted from peripheral blood granulocytes by SNP-minisequencing. Primary endpoint of the study was cumulative risk of transformation measured from date of CLL diagnosis to date of biopsy showing that RS transformation had occurred, death or last follow-up. The association between SNPs and risk of RS transformation was actuarially assessed by univariate log-rank analysis considering the minor allele as acting either in a dominant or a in a recessive fashion. Cox proportional hazard regression was used to build multivariate models for survival analysis. False discovery rate (FDR) was used to control for multiple statistical testing. Results. Univariate log-rank analysis identified LRP4 rs2306029, a SNP affecting the low density lipoprotein receptor protein 4 gene, as the sole SNP associated with RS transformation. CLL who carried LRP4 rs2306029 TT variant genotype displayed a higher risk of transformation (5-year risk: 14.1%) compared to patients carrying the LRP4 rs2306029 CT/CC genotypes that contained the wild type allele (5-year risk: 4.7%) (HR:4.08; p<0.001). The association between LRP4 rs2306029 and RS transformation remained significant also after correction for multiple comparisons by FDR (q=.040). Other variables at CLL diagnosis associated with an increased risk of RS were advanced Binet stage (p<0.001), lymph node size >3 cm, LDH elevation (p<0.001), CD38 expression (p=.010), ZAP70 expression (p=0.017), unfavorable FISH karyotype (p<0.001), IGHV homology >98% (p<0.001), usage of IGHV4-39 (p<0.001), and stereotyped HCDR3 (p<0.001).

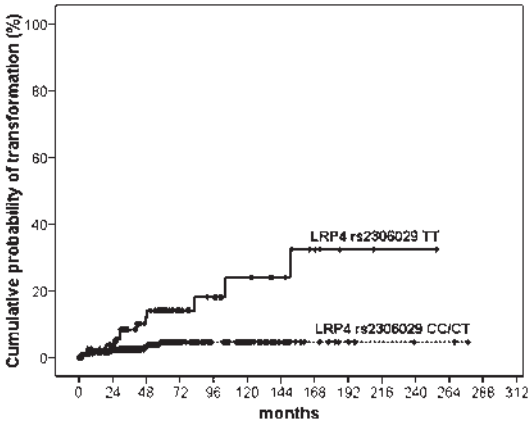

Figure.

None of the clinico-biological categories associated with an increased risk of RS was enriched with the LRP4 rs2306029 TT variant genotype (p>0.050 in all instances), suggesting that LRP4 rs2306029 TT is not a surrogate of other RS risk factors. Multivariate analysis selected LRP4 rs2306029 TT as an independent predictor of RS (HR:3.20; p=.024), along with stereotyped HCDR3 (HR:3.13; p=.033), IGHV4-39 usage (HR:5.22; p=.008) and unfavorable FISH karyotype (HR:3.18; p=.033). LRP4 rs2306029 is a non-synonymous SNP mapping to exon 31 of LRP4 and leading to Ser1554Gly amino acid substitution. *In silico* analysis with PupaSuite (*http://pupasuite.bioinfo.cipf.es/*), PolyPhen (*http://genetics.bwh.harvard.edu/pph/*) and SNPeffect (*http://snpeffect.vib.be/*) algorithms predicted LRP4 rs2306029 T variant allele to be dysfunctional and lead to altered LRP4 protein. Conclusions. rs2306029 non-synonymous SNP affecting LRP4 may predispose to CLL transformation to RS. Since LRP4 is involved in Wnt signalling and is expressed in CLL, the gene variant may have pathogenetic relevance for RS development.

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SERUM LEVEL OF CD26 PREDICTS TIME TO FIRST TREATMENT IN EARLY B-CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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We analyzed the correlation between well-established biological parameters of prognostic relevance in B-cell chronic lymphocytic leukemia [CLL] (i.e, mutational status of the immunoglobulin heavy chain variable region [IgVH], ZAP-70- and CD38-expression) and serum levels of CD26 (dipeptidyl peptidase IV, DPP IV) by evaluating the impact of these variables on the time to first treatment [TFT] in a series of 69 previously untreated Binet stage A B-cell CLL patients. By using a commercial ELISA (R &D Systems, USA) we found that with exception of a borderline significance for ZAP-70 (p=0.07) and CD38 (p=0.08), circulating levels of CD26 did not correlate with either Rai substages (p=0.520) or other biomarker (2-microglobulin [p=0.933], LDH [p=0.101], mutational status of IgVH [p=0.320]). Maximally selected logrank statistic plots identified a CD26 serum concentration of 371 ng/mL as the best cut-off. This threshold allowed the identification of two subsets of patients with CD26 serum levels higher and lower that 371 ng/mL, respectively, whose clinical outcome was different with respect to TFT (i.e., 46% and 71% at 5 years, respectively; p=0.005). Along with higher serum levels of CD26, the univariate Cox proportional hazard model identified absence of mutation in IgVH (p<0.0001) as predictor of shorter TFT. Since in multivariate analysis all these parameters maintained their discriminating power (mutational status of IgVH , p<0.0001; soluble CD26, p=0.02) their combined effect on clinical outcome was assessed. When 3 groups were considered: 1. Low-risk group (n=31), patients with concordant IgVHmut and low level of soluble CD26; 2, intermediate risk group (n=26), patients with discordant pattern; 3, high-risk group (n=12), patients with concordant IgVHunmut and high level of soluble CD26, differences in the TFT were statistically significant, with a TFT at 5 years of respectively 88% , 51% and 43% (p<0.0001). Our results indicate that in early B-cell CLL biological profile including among other parameters soluble CD26 may provide a useful insight into the complex interrelationship of prognostic variables. Furthermore, CD26 along with mutational status of IgVH can be adequately used to predict clinical behaviour of patients with low risk disease.

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EARLY ACTIVATION AND B-CELL RECEPTOR HYPERSTIMULATION DETECTED BY CD69 AND CD79B OVEREXPRESSION IDENTIFY POOR RISK PATIENTS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. B-cell chronic lymphocytic leukemia (B-CLL) exhibits features of activated and antigen-experienced B-lymphocytes and CD69 up-regulation resembles B cells at an earlier and greater state of activation. Moreover, high B-cell receptor (BCR) signaling detected by CD79b overexepression may increase cell survival and cycle progression. Therefore, a hyperactivated and hyperstimulated B-CLL phenotype enhances intracellular signaling intermediates such as cyclin D2 and phosphorylated STAT1, STAT3 and ZAP-70 proteins, thus identifying patients (pts) with a more aggressive disease. *Aims*. The primary endpoints of our study were: (i) to determine progression free survival (PFS) and overall survival (OS) upon CD69 and CD79b expression; (ii) whether CD69 and CD79b expression had additive prognostic value and finally 3)whether CD69 and CD79b were independent prognostic factors.

Patients and Methods. We investigated 412 pts, median age 65 years, 219 males and 193 females. With regard to modified Rai stages, 124 had a low stage, 270 an intermediate stage and 18 a high stage. CD69 and CD79b were determined by multicolor flow cytometry, fixing a cut-off value of 30%. *Results*. $CD69^+$ and $CD79b^+$ B-CLL pts were 88/334 (26%) and 208/402 (52%), respectively. There were significant correlations between Ig V gene mutational status and CD69 (226 cases, p=0.0007) or CD79b (253 cases, p<0.00001). Equally, significant associations were found between ZAP-70 and CD79b (p<0.0001) or CD69 (p=0.008). With regard to clinical outcome, both a shorter PFS and OS were observed in $CD79b^+$ pts (10% *vs.* 56% and 40% *vs.* 93% at 14 years, p<0.00001) as well as in $CD69^+$ pts (3% *vs.* 49% at 14 years, p<0.00001 and 44% *vs.* 67% at 14 years, p=0.00004). Noteworthy, CD79b and CD69 showed additive prognostic properties, since CD79b < 30% plus CD69 < 30% identified a B-CLL subset at better prognosis with regard to PFS (77% vs 2% at 14 years; p<0.00001) and OS (95% *vs.* 31% at 14 years; p<0.00001). The two discordant subsets ($CD69^+CD79b^-$ and $CD69^-CD79b^+$) showed an intermediate outcome (Figure). In multivariate analysis of PFS and OS, CD69 (p=0.008 and p=0.002) and CD79b (p=0.0003 and p=0.0006) resulted to be independent prognostic factors. Conclusion. CD69 and CD79b antigens, determined by flow cytometry, should be considered novel important prognostic parameters in B-CLL. Their easy and rapid laboratory evaluation could allow us to identify early progressive pts and to take timely therapeutic decisions.

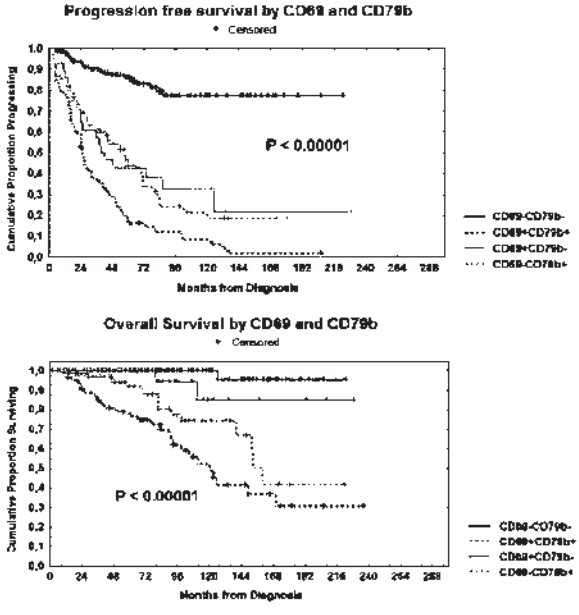

Figure.

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THE PROGNOSIS OF CLINICAL MONOCLONAL B CELL LYMPHOCYTOSIS DIFFERS FROM PROGNOSIS OF RAI 0 CHRONIC LYMPHOCYTIC LEUKAEMIA AND IS RECAPITULATED BY BIOLOGICAL RISK FACTORS

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Monoclonal B-cell lymphocytosis (MBL) is an asymptomatic monoclonal expansion of <5.0x10^9/L circulating CLL-phenotype B-cells. Since the introduction of the new IWCLL guidelines, it has been a matter of debate whether the 5.0x10^9/L CLL-phenotype cell cut-off for CLL diagnosis has a clinical rationale for the definition of MBL. The relationship between MBL and Rai 0 CLL, as well as the impact of biological risk factors on MBL prognosis, are unknown. Out of 460 B-cell expansions with CLL-phenotype, 123 clinical MBL (cMBL) were compared to 146 Rai 0 CLL for clinical and biological profile and outcome, in order to identify the features that distinguish cMBL from Rai 0 CLL; and to identify the clinical or biological variables that best predict the risk of evolution of cMBL to CLL requiring treatment. We found that cMBL had better humoral immune capacity and lower infection risk, lower prevalence of del11q22-q23/del17p13 and TP53 mutations, slower lymphocyte doubling time, and longer treatment-free survival. Also, cMBL diagnosis was a protective factor for treatment risk. Despite these favourable features, all cMBL were projected to progress, and lymphocytes <1.2x10^9/L and >3.7x10^9/L were the best thresholds predicting the lowest and highest risk of progression to CLL. Although IGHV status, CD38 and CD49d expression, and FISH karyotype individually predicted treatment-free survival, multivariate analysis identified the presence of +12 or del17p13 as the sole independent predictor of treatment requirement in cMBL (HR: 5.39, 95% CI 1.98-14.44, p=.001). In this study we confirm that the 5.0x10^9/L cell cut-off can be used to distinguish cMBL from CLL. More importantly, however, we document that: i) cMBL and Rai 0 CLL differ in some biological features and outcome; and ii) cMBL who are destined to progress to symptomatic CLL/SLL requiring treatment can be identified by biological risk factors. Overall, these data show that cMBL has a more favourable clinical course than Rai 0 CLL. Since the biological profile can predict treatment requirement, stratification based on biological prognosticators may be helpful for cMBL management.

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THE COMBINATION OF SERUM THYMIDINE KINASE, B-2-MICROGLOBULIN AND SOLUBLE CD23 AT DIAGNOSIS IS PREDICTIVE FOR PROGRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA, INDEPENDENTLY FROM ZAP-70 EXPRESSION

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Thymidine kinase (TK), β-2-microglobulin (β2M) and soluble CD23 (sCD23) are among the most widely used soluble prognostic factors (sPFs) in patients with B-cell chronic lymphocytic leukemia (B-CLL). Aim of our study was the retrospective evaluation of a prognostic score modeled on their combination. We analysed the levels of these three sPFs in B-CLL patients, using available sera sampled at diagnosis and stored at –20°C. Of 254 patients, 150 were males (59%), median age at diagnosis was 66 years (range: 40-85) and Binet stage was A in 208 cases (82%), B in 36 (14%), and C in 10 (4%). Median follow-up was 77 months (14-259). TK activity was measured by a radioenzymatic assay by Immunotech (Beckman-Coulter): the mean value of 80 healthy donors was 3.7 U/L +/- 2 (SD). sCD23 levels were measured by ELISA (Biosource International, Camarilla, CA, USA): the mean value of 30 healthy donors was 1.24 U/ml +/- 0.34 (SD). We set at 15 U/L the cut-off for significance for TK, at 85 U/ml that for sCD23 and at 3 mg/dl the one for b2M. In 156 patients we could also evaluate the expression of ZAP-70 by B-CLL cells by immunohistochemistry (clone 2F3.2, Upstate, Lake Placid NY, USA).

Table 1. Effect of prognostic factors on Time to Progression in 156 CLL patients.

Variable	*Unadjusted HR (95% CI)*	*p*	*Adjusted HR (95% CI)*	*p*
Age >65	1.03 (0.57-6.54)	*ns*	0.79 (0.50-1.24)	*ns*
Sex (Male)	1.33 (0.92-1.94)	*ns*	1.04 (0.66-1.63)	*ns*
Ly >30x10^9/L	5.07 (3.36-7.75)	*<0.0001*	1.71 (0.97-3.00)	*ns*
Binet (stage B or C)	8.87 (5.91-13.33)	*<0.0001*	3.57 (1.91-6.40)	*<0.0001*
ZAP-70 positivity	4.1 (2.58-6.54)	*<0.0001*	2.85 (1.73-4.60)	*<0.0001*
sPF Score=2	6.02 (4.11-8.83)	*<0.0001*	1.82 (1.05-3.16)	*0.03*

Finally, we defined a score on the basis of the presence of an elevated value (0 = no factors; 1 = one elevated sPF; 2 = two or more elevated sPFs). All sPFs were associated with shorter TTP: TK>15 U/L: hazard ratio (HR) 3.3; 95% CI: 2.3-4.8; sCD23>85 U/mL: HR 6.11; 95% CI: 4.1-9.0; B2M>3 mg/dL: 4.1; 95% CI: 2.8-5.9. Nevertheless, none of them was independent from most of other well-known risk factors, including ZAP-

70 expression. 167 patients resulted score=0, 43 score=1, and 44 score=2. At the multivariate, score=2 proved independent from ZAP-70 expression, advanced stage, age, sex and lymphocyte count in predicting earlier progression (HR 1.82; 95% CI: 1.05-3.16) (Table 1). Score=2 also identified a subgroup at very high risk of progression among stage A, ZAP-70 negative patients, with median TTP of only 10 months (HR 2.56; 95% CI: 1.18-5.52). Score=2 was significantly associated with poorer OS only at the univariate analysis. The combined evaluation of TK, sCD23 and b2M levels is a simple and effective way to predict prognosis in B-CLL patients independently from other factors, including stage and ZAP-70. *Funding. Supported by: Regione Veneto "Ricerca Sanitaria Finalizzata"; "Fondazione G. Berlucchi per la Ricerca sul Cancro"; AIRC - Associazione Italiana Ricerca sul Cancro; Fondazione CARIVERONA.*

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PHARMACOKINETIC (PK) OF ALEMTUZUMAB AFTER SUBCUTANEOUS (SC) ADMINISTRATION FOR CONSOLIDATION IN PATIENTS WITH CLL

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The pharmacokinetic approach appears to be an important tool for the clinician to select patients who can be benefit from more individualized administration schedules and in designing new therapeutic regimens. The objective of this study is to describe the PK properties of Alemtuzumab in 29 patients with CLL receiving the MoAb consolidation treatment, 10 mg three times/week for six weeks by subcutaneous administration and possible evaluation of correlation between serum levels with clinical response.Serum samples were collected from the patients on days 1, 3, 5, 15, 17, 22, and 31, immediately before sc administration. Therefore, after the last dose on day 40, samples were collected about weekly up to day 101. On day 15 or on day 22, was evaluated total systemic exposure to Alemtuzumab (AUC 0-12hors). Serum concentrations were evaluated by an ELISA, previously developed and validated in our laboratory. Alemtuzumab serum concentration quantified before each sc administration increased gradually during the first week and then faster during weeks 2 and 3, and then approached the steady state. The accumulation ratio was 25-fold higher during week 3 (day 3 vs day 17) and over 75-fold higher during week 5 (day 3 vs day 31). AUC0-12hours values differ significantly from day 15 to day 22 (median values: 8.73 micrograms*hours/microliters and 9.43 micrograms *hours/microliters, respectively). The median Cpre-dose of all samples in the first 31 days was 0.63 micrograms/microliters and the median Ctrough exceeded 1.0 micrograms/microliters (1.14 micrograms/microliters) only after the last dose: we can hypothesize that Alemtuzumab, continues to be slowly absorbed through tissues for about 2-3 weeks after the last administration, providing steady serum levels, and thereafter starts to decrease. Complete response rates expressed as the percentage of patients with AUC0-12hours values correlating with effective treatment, increased with higher levels of systemic exposure to Alemtuzumab. The Alemtuzumab disposition parameters, after sc administration, reported in this study characterized a pharmacokinetic model that can be challenged with new doses and/or dosage schedule. A detailed pharmacokinetic profile may lead to a more rapid and effective treatment regimen.

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PROGNOSTIC VALUE OF ABDOMINAL ULTRASONOGRAPHY IN THE INITIAL ASSESSMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Abnormal abdominal computed tomography has been proven to be a significant predictor of disease progression (DP) in early-stage Chronic Lymphocytic Leukemia (CLL). The purpose of this study was to define the prognostic significance of abdominal ultrasonography (US) in CLL. An abdominal US has been included in the initial work-up of 400 CLL patients diagnosed at a single Institution. The median follow-up of the patients is 89 months. Stage assessment according to Rai classification was: stage 0, 60% of patients; stage I, 14.5%; stage II, 21% and stages 3-4, 4.5%. Enlarged abdominal nodes were detected at diagnosis by abdominal US in 11% of patients (44 cases). The rate of positive cases increased significantly with the stage as follows: stage 0, 3% of patients; stages I-II, 21% and stages 3-4, 39% ($p<0.0001$). When abdominal US was repeated at the time of DP, the rate of positive cases increased to 68.5% (100 cases). DP was more frequent among patients presenting with abdominal adenomegalies than in those with no detectable nodes at diagnosis (75% *vs.* 32%; $p<0.0001$). Overall, patients with enlarged abdominal nodes showed a significantly shorter time to treatment (TTT) compared to those with no abdominal adenomegalies (at 4 yrs: 77% vs 24%; $p<0.0001$). The detection of abdominal enlarged nodes maintained an unfavorable effect on TTT within all stages. However, the difference in TTT reached statistical significance for stages I-II only (at 4 years: 82% vs 35%; $p<0.00001$), while for patients with stages III-IV a trend to statistical significance was observed (p.08). In multivariate analysis, including age, stage, and abdominal enlarged nodes, stage and the evidence of lymphadenopathies by abdominal US emerged as independent prognostic factors for TTT. When response to first line therapy was analyzed, a significantly higher responses rate was recorded in patients with no enlarged nodes (81% vs 20%; $p=0.02$). Moreover, the evidence of abdominal adenomegalies was associated with a significantly lower overall survival (OS) probability ($p=0.0013$). In a large series of CLL patients, the recognition at diagnosis of enlarged abdominal nodes by US emerged as a significant unfavorable prognostic factor for DP, TTT, response to first line therapy and OS. Considering its prognostic value, abdominal US, a non-invasive and cost-effective imaging technique, should be included in the initial work-up of CLL patients.

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A LONG TERM OUTCOME OF 121 HAIRY CELL LEUKEMIA (HCL) PATIENTS WITH A MEDIAN-LONG FOLLOW-UP: BOLOGNA EXPERIENCE

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Background. In the past, the first choice of treatments of HCL were splenectomy and α-interferon. In 1990s, purine analogs changed radically the treatment modality inducing a high number of complete and durable responses. New biologic agents such as monoclonal antibodies offered a well tolerated, complementary treatment, particularly for patients with refractory disease. *Patients and Methods*. We retrospectively reviewed 129 HCL patients. Of these, 121 were included in the study. We analyzed the outcome of the different lines of therapy in 121 HCL patients followed in our institute from 1984 to 2008 with a median follow-up of 101 months. They were divided in subgroups according to the number of treatments. Group A included 64 patients undergoing to a single line of therapy; group B was represented by 23 patients treated with two lines of treatment; group C had 16 patients undergoing to three lines; group D included 10 patients with four lines of therapy; and group E was represented by 8 patients treated five times. *Results.* Of the 121 patients, 72 received cladribine, 10 pentostatin, 36 α-interferon, and 3 splenectomy obtaining 77% of complete

response (CR), 18% of partial response (PR) and 5% of non responders (NR). 53 patients relapsed with a median disease-free survival of 3 years. They were retreated with four different lines of therapy: 41 with cladribine, 5 with pentostatin, 5 with α-interferon and 2 with rituximab achieving 72% of CR, 17% of PR, and 11% of NR with a median disease-free survival of 2.8 years. Of them, 30 patients received the third-line therapy: 23 patients received cladribine, 5 rituximab, 1 splenectomy and 1 α-interferon, achieving 80% of CR, 13.5% of PR, and 6.5% of NR. Fourteen patients relapsed with a median disease-free survival of 2.5 years. They were retreated with four different lines of therapy: 11 with cladribine, 2 with pentostatin, and 1 with rituximab achieving 67% of CR, 27% of PR and 6% of NR; seven patients relapsed with a median disease-free survival of 3,6 years. These 7 patients received the fifth-line therapy and only one relapsed after treatment (3 cladribine, 3 rituximab and 1 splenectomy) with a disease-free survival of 1,3 years. *Conclusions.* Our study confirms the high risk (almost 50% of all patients) of retreatment of HCL patients and the need to maximize primary response including the possibility to combine purine analogs with rituximab or other monoclonal antibodies.

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RITUXIMAB-CONTAINING CHEMOTHERAPY FOR AUTOIMMUNE HEMOLYTIC ANEMIA ASSOCIATED WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is an entity complicated by autoimmune hemolytic anemia (AIHA), a potentially life-threatening event; a series of large studies suggested that AIHA occurs in the course of CCL approximately in 10% to 26% of cases. Treatment of autoimmune complications of CLL is primarily based on immunosuppression with drugs such as glucocorticoids. Besides splenectomy, there are not many known, effective and established alternatives for patients failing steroid therapy, although intravenous immunoglobulin and immunosuppressive drugs have been used with varying degrees of success. Rituximab, an active agent against B cell malignancies, effectively target lymphocytes and inhibits autoimmune processes. Here, we report the safety and efficacy of a rituximab-containing regimen in a CLL patient with steroid-refractory AIHA. At the diagnosis the blood chemistry showed severe anemia (Hb value 8.0 grams/decilitres), high white blood cell count ($163x10^9$/litres), altered hemolysis markers and direct antiglobulin test (DAT) was positive for both complement and IgG. The schedule of RCD regimen include the following drugs: Rituximab 375 milligrams/m^2 i.v. infusion given on day 1, cyclophosphamide 750 milligrams/m^2 i.v. on day 2 and dexamethasone 20 milligrams was given i.v. on D-1, D-2 and orally from D-3 to D-7. Four cycles, repeated every 4 weeks, were administered. No Rituximab-related side effects were recorded. At the end of treatment hemoglobin level was 13.9 grams/decilitres, DAT became negative and was noted a marked reduction of the lymphocytosis ($8x10^9$/litres). Median duration of response was 12 months. The patient had active CLL at the time of development of AIHA and, after receiving treatment with RCD, achieved the complete response according to NCI criteria both to AIHA and CLL, indicating a close relationship between the activity of CLL and AIHA. This may be explained by the fact that $CD5^+$ lymphocytes are involved in both CLL and the autoimmune phenomenon complicating CLL. Our results confirm that a rituximab-based combination regimen (RCD) is highly effective and safe in treating steroid-refractory CLL-related AIHA.

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NOVEL SURROGATE MARKERS OF IGHV MUTATIONAL STATUS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease with some patients exhibiting an indolent course and surviving for many years without treatment, and others developing an aggressive and rapidly progressive disease. Although the IGHV mutational status analysis represents a very reliable predictor of clinical outcome, it is expensive and beyond the capacities of most diagnostic laboratories. To identify surrogate markers we performed a gene expression profiling analysis of CD19+ purified cells from 80 Binet stage A B-CLL patients by means of Affymetrix HG-U133A arrays. The comparison of 46 IGHV-unmutated versus 34 mutated samples using the PAM software identified 59 well-characterized genes: 43 genes had a higher and 16 genes a lower average expression in the IGHV-unmutated group. These genes are involved in cellular functions, including cell cycle regulation (*SEPT7, SEPT10, CDK2AP1*), cell proliferation (*SLAMF1, LDOC1*), apoptosis (*CD63, IFT57, P2RX1, RNF130, TNFRSF1B*), cell adhesion (*CNTNAP2, C1orf38, PCDH9*), immune response (*ZAP70, IFI44*), signal transduction (*AKAP13, RASGRP1, USP6NL, TGFBR3, AKAP12*), lipid metabolism and fatty-acid degradation (*FADS3, LPL, LASS6*), cell-cell signalling (*FCRL2*), phospholipid biosynthetic process (*AYTL2*), regulation of circadian rhythm (*EGR3, CRY1, OPN3*), DNA-dependent regulation of transcription (*MYBL1, NR4A2, NRIP1, ZBTB20*), muscle development (*VAMP5, SRI, DMD*). The expression signature was successfully validated by a meta-analysis of a publicly available gene expression dataset of 100 B-CLL (*Haslinger et al., 2005*). The expression levels of 11 genes (*LPL, ZBTB20, ZAP70, CRY1, COBLL1, SEPT10, LDOC1, TNFRSF1B, DMD, SRI, NRIP1*) was validated by real-time PCR (Q-RT-PCR) in 40/80 samples. The prognostic impact for Time To Treatment (TTT) of the 59 candidate classifier genes was investigated in 77/80 patients, 49 (36.4%) of whom received treatment after a median follow up of 4 years. As expected, patients with unmutated IGHV genes had a risk of therapy requirement that was about 3 times higher (HR: 3.1,95% C.I. 1.6-5.8, $p<0.0001$) than those with mutated IGHV. Based on combined microarray and Q-RT-PCR expression analyses, 4 candidate genes (*ZAP-70, LPL, TNFRSF1B* and CRY1) significantly predicted TTT. All but one gene (*TNFRSF1B*) are already known to be have a prognostic role as predictor of disease outcome. The predictive power of the novel putative surrogate marker for the IgVH mutational status, *TNFRSF1B*, is currently under investigation in our Institutions.

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APRIL SERUM LEVELS PREDICT TIME TO TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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In chronic lymphocityc leukemia (B-CLL) B lymphocytes accumulate and survive due to apoptosis resistance. Molecular and cellular interactions occurring in tissue microenvironment may support the survival of B-CLL cells. APRIL (a proliferation-inducing ligand), a TNF superfamily member released both by microenvironment cells and by malignant lymphocytes themselves, play a role in protecting B-CLL cells against spon-

taneous apoptosis through paracrine and autocrine pathways. We retrospectively evaluated by ELISA serum samples from 83 B-CLL patients referred to our Institution from 1993 to 2008. Sera were collected at diagnosis and before any treatment upon patients consent. Sera from 25 age and sex matched healthy donors were used as control. The following characteristics were considered: gender (51 male; 32 female); age (47 <65 years; 36 >65 years); classification according to Rai (73 stage 0-2; 10 stage 3-4) and Binet (56 stage A; 27 stage B-C); lymphocytes count (41<13000/m^3; 42 >13000/m^3); development of autoimmune cytopenia (AIC) (34 with AIC, 49 without AIC); ZAP-70 (24 negative; 50 positive) and CD38 expression (28 negative; 37 positive); IgVH genes status (12 mutated; 26 unmutated); sCD23 (37<60 U/L; 24 >60 U/L), Thymidine Kinase (30<9.14 U/L; 31 >9.14 U/L) and β2 microglobulin levels (25<2 mg/L; 47 >2 mg/L); cytogenetic analysis (23 with normal or favourable karyotype, 9 with unfavourable karyotype). We found significantly higher levels of APRIL in the sera of B-CLL patients as compared to normal donors (17.37±3.773 *vs.* 4.186±0,683 ng/mL; Mann-Whitney test, $p<0.0001$). The mean APRIL serum level was increased in patients with unfavorable karyotype as compared to those with favourable karyotype (17.22±7.629 *vs.* 5.68±0.623 ng/mL, $p=0.032$). No other significant associations were found between APRIL levels and the other markers evaluated. We also divided patients according to median APRIL serum level (<7.08 ng/mL vs >7.08 ng/mL). The two groups did not significantly differ regarding overall survival and other clinical-biological parameters, but interestingly patients with APRIL serum level below the median level showed a significantly higher time to treatment as compared to patients with APRIL level above this value (34.40 months±5.45 vs 10.8 months±4.279) ($p=0.021$). Although based on a limited number of patients, our results suggest that APRIL is increased in B-CLL patients and may predict time to treatment.

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TWO CASES OF LYMPHOPROLIFERATIVE SYNDROME AND CONCOMITANT MYELOPROLIFERATIVE CHRONIC SYNDROME

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Lymphoproliferative and myeloproliferative disease in the same patient is a very uncommon event, rarely reported in literature. We report two cases observed in our institution. The first patient is a 75 years old male affected by Essential Thrombocythaemia (ET) from 1998, treated with hidroxyurea. On October 2006 he developed a papular skin lesion. The histological picture documented a Diffuse Large B Cell Lymphoma (DLBCL) (CD20 and Bcl2 positive, CD30 and Bcl6 negative, Ki67 >80%). In the bone marrow coexisted myeloproliferative pictures, with abnormal proliferation of megacariocytic and myeloid progenitors, infiltration of lymphocytic cells with a nodular pattern and the same immunohistochemical findings of the skin lesion was documented. The Jak2 mutational status was negative. The patient underwent six R-CHOP regimen cycles. He stopped hidroxyurea and started treatement with Anagrelide. A complete remission (CR) of lymphoma occurred with bone marrow lacking in lymphoid infiltration only with bright myeloproliferative pictures. At last follow-up the patient is still in CR of his lymphoma and is carring on his ET in treatment with Anagrelide with a good response. The second case is related to a 74 years old female who referred to our institution with a six months history of isolated lymphocitosis. A diagnosis of typical B-CLL Stage A Binet/0 Rai was made. Subsequently the patient developed a progressive increase in platelet count associated to iron deficiency. She was treated with orally iron supply and 100 mg daily Aspirin. After nine months the platelet count increased without iron deficiency. The patient repeated a bone marrow biopsy. The immunohistochemical analysis showed nodular B-CLL lymphocytes infiltration associated to a hypercellular marrow with megakaryocytic hyperplasia (Figure 1). The peripheral blood smears showed 70% lymphocytes, Gumprecht nuclear shadows and a great number of platelets. The JAK2 analysis was negative and the cariotype was normal. According to WHO criteria a diagnosis of ET was made. The coexistence of myeloproliferative and lymphoproliferative disease in the same patient is well known after exposure to chemo or radiotherapy, but is a rare event prior to therapy. Some authors suppose that there are two independent proliferation as reaction to the same antigenic event and is unknown the role of Jak2 mutation and translocation or deletion of 8, 14, 17, 12 and 13 chromosomes. In the first case a possible role of hidroxyurea treatment for several years has to be underlined, the second case probably represents a synchronous biological manifestation of mielo and lymphoproliferative disease without previous idroxicarbamide exposure and without chromosomal aberrations. Both our patients are alive, in good clinical conditions and none had thrombotic events. Further investigation are necessary to explain the pathogenesis, molecular basis and prognosis of these rare cases.

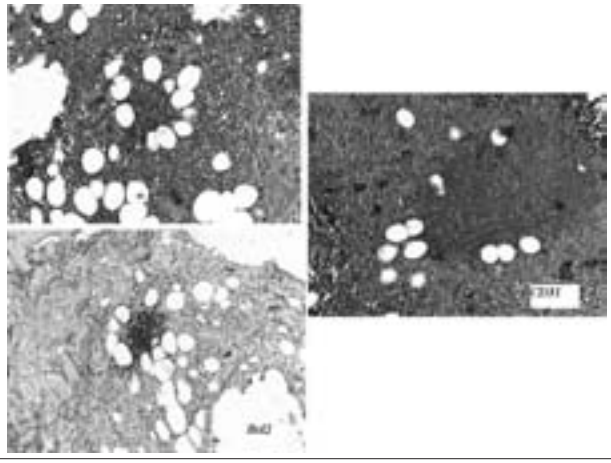

Figure 1.

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POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: ANALYSIS OF EARLY AND LATE CASES IN 105 PATIENTS RECEIVING SOLID ORGANS

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Post-transplant lymphoproliferative disorders (PTLDs) are a spectrum of lymphoid proliferations, occurring in solid organ of immunosuppressed subjects or in bone marrow transplant recipients. PTLDs most commonly derive from B-cell lineage and are associated with Epstein-Barr virus(EBV) infection. We retrospectively analyzed six cases of PTLDs among a consecutive series of 105 solid organ transplanted patients. Five were males, one female, their mean age was 55 years (range: 15-72). Transplanted organs were heart (3 cases), kidney (2 cases), and liver (1 case). EBV DNA load was performed by quantitative polymerase chain reaction. Treatment consisted for all patients in an initial immunosuppressive therapy; among which: one patient also received adjuvant immunochemotherapy (R-CEOP), while another one received immunotherapy (Rituximab) alone. We labelled as early PTLD which occurred within 12 months from transplant. In our patients, the median time from transplant to diagnosis of PTLD was 9 (early) and 135 (late) months. Histological and immunohistochemical analysis revealed diffuse large B-cell lymphoma in three subjects, anaplastic large cell lymphoma in one, multiple myeloma in another and lymphoplasmacytic lymphoma in the last patient. EBV serology was positive in five cases before transplant and in six after it. Median viral load was 2400 copies/mL (range: 400-10000 copies/mL). At today, two patients are in complete remission, two in partial remission, and two patients are died. A review of the literature shows that more than 90% of EBV-related PTLDs commonly arise within one year from transplant. It is remarkable that in our experience we observed a late PTLD onset in 4/6 cases. The two patients who developed PTLD within one year from transplantation were the two ones with the higher EBV-DNA load (median: 6000 copies/mL). In the other four patients, with PTLD late onset, a lower viral load was detected (median: 550 copies/mL). It has been reported that patients with undetectable or low EBV viral load for the first six months after transplant do not develop PTLDs, while a chronic high EBV load is a predictor of *de novo* or recurrent PTLDs. We think that the PTLD late onset in our patients, could be related to their low EBV viral load. To date, it is not clear which threshold value of EBV-DNA can be considered predictive of PTLD development. High viral load or a rising trend could define higher risk patients, so that frequent EBV load monitoring is mandatory.

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FLUDARABINE AND CYCLOPHOSPHAMIDE AS FRONT-LINE CHEMOTHERAPY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. THE IMPACT OF BIOLOGICAL PARAMETERS ON CLINICAL OUTCOME

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We assessed the influence of immunoglobulin variable region heavy chain (IgVH) gene mutation status, interphase cytogenetic abnormalities, expression of ZAP70 and CD38 on clinical outcome after front-line therapy with fludarabine and cyclophosphamide in 64 patients with chronic lymphocytic leukemia (CLL). Thirty seven patients received oral fludarabine and oral cyclophosphamide for three consecutive days every 4 weeks for six cycles. Twenty seven patients received fludarabine and cyclophosphamide intravenously for three days. Fifthy patients were evaluable for IgVH status; thirty two patients had unmutated and 18 had mutated IgVH genes. Fifthy patients were evaluable for interphase cytogenetic abnormality del(11q22.3), del(17p13.1), del(13q34), tris(12). Fifteen patients had the *high risk* chromosomal aberrations del(11q22.3) or del(17p13.1). Twenty-eight patients were ZAP70-positive and twenty two patients were CD38-positive. Among the 57 valuable patients, 30 patients (52,5%) obtained a complete remission and 18 (31.5%) a partial response. The median progression-free survival (PFS) was 38 months and median time to re-treatment (TTR) was 42 months, while median overall survival (OS) was 118 months. A significantly lower overall response rate (53% *vs.* 86%, χ^2 p=0.018) was noticed in the high risk cytogenetic abnormalities group; no statistical differences were detected for the IgVH, ZAP70 and CD38 categories. Biological parameters were not significant predictors for PFS or OS. A significantly shorter TTR was noticed in the high risk cytogenetic group (22 vs 85 months, p=0.004) and TTR was also shorter in IgVH-unmutated than in IgVH mutated patients (36 *vs.* 85 months, p=0.027). In multivariate analysis (Cox regression analysis) the response to treatment (HR 0,062; CI 0,017-0,223; p<0.001), the high risk cytogenetic abnormality (HR 3,242; CI 1,105-9,512; p=0.032), and unmutated IgVH genes (HR 3,431; CI 1,09-10,8; p=0.035), were indipendent predictors of TTR; there were no significant differences between subgroups by age, stage, sex, expression of ZAP70 and CD38. These results underline the importance of biological stratifications in the front-line treatment of CLL patients. Confirming our previous published data, the combination of fludarabine and cyclophosphamide is an effective regimen that would be appropriate especially in patients with low risk biological parameters (absence of unmutated IgVH genes and *high risk* cytogenetic abnormalities).

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CLONAL T-CELL CHRONIC LYMPHOCYTIC DISORDER IN A POLYCYTHEMIA VERA PATIENT

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The association of chronic myeloproliferative and lymphoproliferative disorder has been rarely reported in the English literature. We here report a patient with Polycythemia Vera and T-cell chronic lymphoproliferative disorder. A 65-year-old Caucasian man presented erythrocytosis. He had not previous clinical history of any illness. Physical examination showed only mild splenomegaly. Laboratory work-up detected an oxygen saturation of 97.5 per cent, red blood cell mass of 40 milliliters/kilogram, serum erythropoietin levels of 10.7 International Units/Liter, and normal lymphocyte count in the peripheral blood. An increased number of erythroid cells was detected in the bone marrow. The cytogenetic analysis revealed a normal karyotype, while the RT-PCR did not show the presence of BCR/ABL fusion gene or Jak-2 gene V617F mutation. Polycythemia Vera diagnosis was made. The patient was treated with intermittent administration of hydroxyurea. Four years later, the patient showed a constant lymphocytosis in the peripheral blood (lymphocytes: 6000/μL) with $CD3^+$; $CD4^+$; $CD45^+$ phenotype. In the bone marrow, a 46 per cent of lymphocytes was detected. These cells were $CD3^+$; $CD5^+$; $CD4^+$; $CD7^-$; $CD8^-$ and the PCR showed a T-cell receptor gamma clonal rearrangement. The karyotype was normal. Total body CT-scan did not show lymphadenopathy and/or organ involvement. The final diagnosis was T-cell monoclonal lymphoproliferative disease. The clinical course remained continuously stable, with no increase in the bone marrow infiltration or peripheral blood lymphocytosis, and persisting negative CT-scan. To date, 2 years after the diagnosis, no specific treatment for the lymphoproliferative disease has been started. The patient still continue his therapy with hydroxyurea for the Polycythemia Vera. Although the possible evolution of Polycythemia Vera in Acute Myeloid Leukemia is known, the occurrence of a metachronous chronic T-cell lymphoproliferative disorder has not been previously described. Our hypothesis is that the demonstrated apoptotic effect of hydroxyurea on the lymphocytes, could explain the non-progression of the clonal T-cell lymphoproliferative chronic disorder in our patient.

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HEPATITIS C VIRUS-POSITIVE CHRONIC LYMPHOCYTIC LEUKEMIA

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The association of hepatitis C virus (HCV) and B-cell chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) is not well established. Some epidemiological studies tend to rule out such a relationship. Again, little is known about the clinic-biological features and outcome of HCV-positive patients with CLL. We retrospectively evaluated clinico-hematological characteristics of 23 HCV-positive CLL patients compared with 189 HCV-negative CLL patients seen at our Institutions aiming to evaluate clinico-biological features and outcome of HCV infected patients with CLL at diagnosis compared to HCV negative CLL patients. No differences were found with respect to sex distribution and age. The HCV genotype was known only in 4 patients: in 3 cases was found 2a/2c, in one case 1b. No difference was also found in the absolute lymphocyte count, hemoglobin level, platelet count, Rai and Binet clinical stage at diagnosis, lymphocyte doubling time. Mutational status of IgVH, CD38 expression, and ZAP-70 expression did not show differences among the two groups of patients. The major cytogenetic abnormalities, detected by means of FISH in 13 HCV-positive patients, showed 7 cases of normal karyotype,3 cases of trisomy 12, 2 cases of deletion 13q14, 1 case of deletion of 11q22.3 (ATM). Finally, overall survivals of HCV infected patients and HCV-negative patients did not show any significant difference (p 0.1). In conclusion, HCV-positive patients with B-cell CLL seem to not differ from other patients for presentation and clinical outcome as well. However, preliminary results need to be confirmed on a large cohort of patients.

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MODIFIED DHAP SCHEDULE CONTAINING OXALIPLATIN (OX-DHA) IS EFFECTIVE AND WIDELY APPLICABLE IN CHRONIC LYMPHOCYTIC LEUKEMIA AND WALDESTROM DISEASE

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Introduction. The DHAP regimen is a well established chemotherapy schedule, commonly employed in the salvage treatment of high-risk lymphoma. In spite of its high efficacy, the use of DHAP has some restraint due to the potential renal toxicity of Cisplatin. In order to widen the applicability of the regimen, a new schedule has been developed, with Cisplatin replaced by its analogue Oxaliplatin, which has low if any renal toxicity. The inclusion of Oxaliplatin into the original DHAP regimen (Ox-DHA) markedly improves the tolerability and widens the regimen applicability. Aim To evaluate feasibility and efficacy of the Ox-DHA regimen in non-follicular low-grade lymphoma patients. Patients and Methods The Ox-DHA schedule includes: Oxaliplatin 100 mg/sqm

on day 1, Cytarabine 2 g/m^2 (3-hr i.v. infusion) on day 2, 3 p.m., and on day 3, 8 a.m. (the time schedule has been arranged in order to shorten the interval between the two Ara-C doses), Dexamethasone 40 mg days 1-4. Rituximab (375 mg/m^2) was added in 22 patients. Variable dose reductions (25-50%) were used in patients aged over 70 yrs. Between 2002 and 2008, 174 Ox-DHA courses were administered in an outpatient setting to 55 low-grade lymphoma patients; their median age was 59 yrs. (range: 41-84), 36 were male, 19 female; 45 patients had Chronic Lymphocytic Leukemia (CLL), 10 had Waldestrom disease (WD); 39 patients received Ox-DHA for relapsed/refractory disease. Results Ox-DHA had hematological toxicity analogous to that commonly observed with the original DHAP schedule; few patients required short hospitalization for infectious complications. The program was discontinued in 4 patients (3 for disease progression and 1 for autoimmune hemolytic anemia). There were no severe liver or renal toxicities. Among 48 patients assessable for response, the overall response (OR) rate was 87.5%; in particular, all patients receiving Ox-DHA at diagnosis reached a complete remission (CR) or a very good partial remission (VGPR), while the OR rate was 82 % in patients treated for refractory/relapsed disease, with 56% of them achieving CR/VGPR (68% and 40% for patients at 1st and 2nd relapse, respectively) and 26% achieving partial remission (PR). Among 10 WD patients, 4 obtained a VGPR, 4 a PR and 2 had a stable disease. *Conclusions*. The Ox-DHA is a well tolerated outpatient regimen, highly effective both front-line and in the rescue for relapsed/refractory disease in non-follicular low-grade lymphoma patients

Myelodysplastic Syndrome II

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HIGH-DOSE RHUEPO FOR THE TREATMENT OF PATIENTS WITH LOW-RISK MYELODYSPLASTIC SYNDROMES

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Recombinant human erythropoietin (rHuEpo) is effective in about 30% of anemic patients with myelodysplastic syndrome (MDS). Recently, it has been suggested that adequate doses may increase response rates. We performed a prospective non-randomized Phase II study to evaluate the efficacy in terms of erythroid response of high dose rHuEpo. Patients were included if Hb <11 g/dL and previously untreated. Patients received rHuEpo 40,000 U s.c. twice weekly until erythroid response, as evaluated at 3 months. Once weekly dosing was considered for patients with Hb increase ≥2 g/dL within the first 2 weeks of therapy or in patients reaching Hb = 12 g/dL. Responders continued to receive treatment at dose adjusted to maintain Hb level not exceeding 12 g/dL until loss of response, while non responders at 3 months went off the study. Ninety-two patients [41M/51F, median age 74.1 years, interquartile range (IR) 63.7-80.6] were included. Of the 61 patients with evaluable cytogenetics, 37 were normal, 7 had del(5q) as a single abnormality, 2 had trisomy 8, 6 had other single abnormalities, 7 had double abnormalities [including 4 with del(5q) associated to other abnormalities] and 2 had complex karyotypes; IPSS risk score among these 61 evaluable patients was low in 27, Int-1 in 31 and Int-2 in the remaining 3. Median disease duration was 9.5 months (IR 2.4-28.6). Serum Epo levels were available in 52 patients, with median value being 80.1 Mu/mL (IR 40.7-224). Mean Hb in 49 transfusion-free (TF) patients was 9.0±SD 0.87 g/dL; 43 transfusion-dependent (TD) patients required a median number of monthly transfusions of 3 (interquartile range 2-4). Two patients are too early for response evaluation; 46/90 evaluable patients (51.1%) had an erythroid response in a median time of 7 weeks (IR 5-9). Of the 47 evaluable TF patients, 31 (65.9%) had a response, with a mean Hb increase of 2.2±0.1 g/dL; 11 of them reached the target Hb 12 g/dL requiring dose reduction. Fifteen out of 43 TD patients (35%) responded and 12 of them became transfusion-free. Median duration of response was 19 (95% CI 15-22) months. At multivariate Cox regression analysis, by controlling for MDS duration, IPSS, transfusion-dependence, serum Epo and Hb levels, factors associated with response and time to response were lower serum Epo levels (p=0.002) and higher Hb levels (p=0.001). Adequate doses of rHuEpo show a good response rate compared to previous results reported with lower dosing schedules. Randomized studies comparing different dosing regimens are thus required to confirm these findings.

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A SYSTEMATIC REVIEW AND META-ANALYSIS OF THALIDOMIDE THERAPY AS SINGLE AGENT IN MYELODYSPLASTIC SYNDROMES

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Thalidomide has been used to improve the cytopenia of myelodysplastic syndromes (MDS). However, due to the heterogeneity of patients populations treated and the variability of doses/schedules adopted in the different studies so far performed, the real efficacy of thalidomide in MDS remains controversial. Furthermore, some concerns also exist about its tolerability. Indeed, no individual clinical trial has been sufficiently extensive to provide a basis for a decision model to use thalidomide in MDS. In order to better define the role of thalidomide in MDS, we performed a systematic review of all major published articles on this topic by using a Pub-Med web-site methodology. Ten phase I-II stud-

ies, including a total of 419 MDS patients who had received thalidomide as single agent, were identified and examined. Neither phase III trials, nor previous meta-analyses were found. Thalidomide doses widely varied in the different studies, ranging from 50 to 1000 mg/d. The response criteria and the characteristics of patients also were not uniform. Overall, average response rate was 29% (range 9-56%) and 43% (range 16-88%) on intention-to-treat analysis or considering only people able to receive the drug for at least 12 weeks, respectively. The large majority of responses were erythroid in nature (mostly resulting in transfusion-independence) and were achieved within 2-3 months, without a clear evidence of a dose-response effect. Responses were more frequently observed in patients with lower IPSS risk score and with a recent diagnosis at treatment (< 1 year). There was no evidence of a correlation between response to thalidomide and baseline levels of endogenous erythropoietin, transfusion support or prior treatment with epoetins. Cytogenetic response or changes in marrow morphology were only occasionally reported. The duration of response was highly variable, ranging from 3 months to more than 6 years in single patients. Side effects, mainly peripheral neuropathy, sedation, constipation, and skin rash, were frequent, determining a very high and often early drop-out (mean 45%, range 15-67%), even in responders and especially in elderly patients where thalidomide doses > 200 mg/d were employed. Despite the fact that no specific prophylaxis was generally adopted, thrombotic events were very rare and exclusively associated with higher doses of the drug. To conclude, based on available evidences, thalidomide remains a possible therapeutic option for selected MDS patients, if appropriately employed and managed.

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PROLIFERATIVE AND APOPTOTIC SIGNALLING IN BONE MARROW CELL SUBPOPULATIONS OF MYELODYSPLASTIC SYNDROMES PATIENTS USING FLOW-CYTOMETRY TECHNIQUE

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Development of effective treatments for MDS has been impaired by limited insights into MDS pathogenesis and alterations of intracellular mechanisms. We analyzed intracellular signal transduction pathways of MDS bone marrow cellular diverse subpopulations using multiparameter flow-cytometry. We obtained optimal results by fixing cells with formaldehyde (BD Cytofix buffer) then, after permeabilization, staining with APC anti-human CD34, PE anti-human CD71, PerCP anti-human CD45 and Alexa-Fluor488 anti-Stat5 (pY694), Alexa-Fluor488 anti-ERK1/2 (pT202/pY204), Alexa-Fluor488 anti-p38 (pT180/pY182) and Alexa-Fluor488 anti-cleaved caspase-3 (D175). Samples were analysed on a FacsCanto cytometer (BD) with 6 colour beams. Activation of MAP kinases ERK1/2 and p38 was evaluated in parallel to activation of STAT5 and caspase-3 in bone marrow mononuclear cell subpopulations CD34 positive, CD45 positive and CD71 positive obtained from 60 MDS patients of all FAB subtypes at diagnosis and 6 normal donors. Moreover, we analysed the same parameters after erythropoietin (EPO) and granulocyte colony stimulating factor (G-CSF) stimulation in 30 MDS. Basal activation of MAPK phospho-proteins, STAT5 and caspase-3 varies among primary MDS cells and it is different among the cellular subsets. G-CSF is activating STAT5 in CD34 positive MDS cells. EPO stimulation fails to induce STAT5 activation in 22/30 CD71 positive MDS cells while is active in normal and in 8/30 CD71 positive MDS cells. In 20/30 MDS patients it has been possible to analyse EPO response *in vivo* (evaluated as an increase in Hb of more than 20g/l without transfusion after 8 weeks of EPO treatment) and we found out that in 18/20 cases it correlates with EPO dependent STAT5 activation.

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MONOCENTRIC EVALUATION OF RESPONSE AND TOLERABILITY OF SUBCUTANEOUS AZACITIDINE IN ELDERLY MDS PATIENTS WITH COMORBIDITIES

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Myelodysplastic syndromes (MDS) are affecting mainly elderly patients, and age is considered per se a negative prognostic factor. The AZA-001 trail demonstrated that MDS patients aged > 75 yrs treated with azacitidine have a significantly longer OS respect to best supportive care treated patients (Seymour, ASH 2008). We wanted to verify whether elderly and very elderly patients were less prone to respond to azacitidine or presented more side effects related to therapy. We analyzed 36 elderly MDS patients (IPSS INT-1 13/36 and INT-2/high 23/36) treated in our Center with subcutaneous Azacitidine 75 mg/kg/day for 7 days every 28. Mean number of cycles was 11 (range: 42-2). Mean age was 70,39 yrs (60-82); 33% of patients (12/36) were >= 75 yrs; 41% (5/12) >= 80 yrs. Overall response rate according IWG criteria 2006 was 44% , stable disease 36.6%. We demonstrated by Fisher test that the type of IWG response (CR, PR, HI, SD, DP) did not correlate with age. We also evaluated Charlson comorbidity index in relation to age and to hematological response and no correlation was showed. Hematological and non hematological adverse events were mostly mild (grade 1/ 2: 77,8 %, 22,2% grade 3 / 4: 61,5 %, 38,5%) and uniformly distributed independently from age. Median overall survival (OS) of our patient cohort was comparable to that obtained in AZA-001 trial (20,5 months vs 24.6). Median OS in patients < 75yrs and >= 75 yrs was not significantly different (p value > 0.7). In conclusion, azacitidine is safe and effective in very elderly MDS patients.

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SEQUENTIAL KARYOTIPIC ANALYSIS IN 79 PATIENTS WITH MYELODYSPLASTIC SYNDROME: CLINICAL CORRELATION AND IMPACT ON PROGNOSIS

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Background. To analyse the usefulness of adding karyotypic studies to the clinical and marrow cytological follow-up of myelodysplastic syndromes (MDS). *Methods*. In 79 MDS pts aged 68 ys (range 15-91), F/M 31/48, a karyotypic study was performed whenever pts underwent marrow morphological reevaluation during a median follow-up of 13 months (range 0-97). Treatment included supportive measures +/- growth factors, corticosteroids, and low-dose hydroxyurea in 4 pts with CMML; pts treated with aggressive chemotherapy were excluded. MDS were defined according to FAB classification and karyotypes according to IPSS risk classes. *Results*. A total of 119 combined morphological/cytogenetic analyses were performed. At diagnosis 46 cases were RA, 4 RARS, 38 RAEB, 10 RAEB-T, 10 CMML and 11 MDS not specified. Distribution among IPSS karyotype groups was: 66 good, 26 intermediate and 27 poor. At baseline no significant correlation was found between FAB morphology and IPSS cytogenetic risk class. FAB morphology remained "stable" in 49 cases (41%); it regressed to a lower risk FAB class in 11 cases ("better morphology": 9%). Morphological evolution occurred in 59 cases (50%), respectively to a higher risk MDS FAB class in 19 ("worse MDS morphology": 16%), or to acute leukaemia in 40 ("leukaemic evolution": 34%). IPSS karyotype risk class was stable in 84 cases (70%). It regressed to a lower IPSS risk in 14 ("better karyotype": 12%) and progressed to a higher IPSS risk in 21 ("worse karyotype": 18%). No significant correlations were found between the morphological and cytogenetic variations. The incidence of both favourable (IPSS good) or unfavourable karyotipe (IPSS intermediate+poor) progressively decrease with increasing marrow blasts percentage:respectively, for RA, RARS, CMML, NAS: 59% favourable vs 58,5% unfavourable; for RAEB 32% vs 34% and for RAEB-T: 9% vs 7,5%. Morphological changes during follow-up had a significant impact on median survival, which was 12 months (range: 0-68) in pts both with "better" and "stable" morphology, 5 months (0.5-97) in pts with "worse MDS" and 3 months in pts with "leukaemic evolution" (p<0.009). Conversely no significant effect of karyotypic changes was demonstrated on survival, which was 5, 6.5 and 7 months for pts with *better*, *stable* and *worse*

karyotype, respectively (p=0.9). (Figure 1). *Conclusions*. Karyotypic changes are frequent in MDS, but cytogenetic reanalysis during the morphological follow-up of patients with MDS did not add useful prognostic informations and cannot be recommended on a routine basis. However the usefulness of cytogenetic monitoring in subgroups with specific chromosomal.

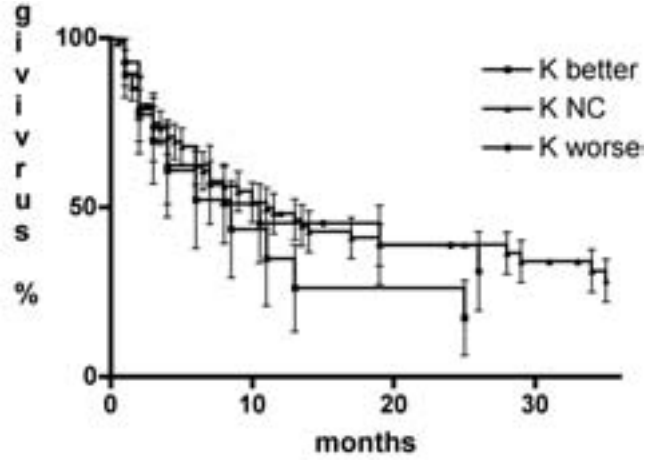

Figure 1. Effect of karyotype change on surviva.

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ROLE OF PHOSPHOINOSITIDE-PHOSPHOLIPASE C (PI-PLC) β1 IN THE PROGRESSION OF MYELODYSPLASTIC SYNDROMES: GENETIC AND EPIGENETIC MECHANISMS

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Myelodysplastic syndromes (MDS) are a heterogeneous group of hematological malignancies characterized by an increased although variable risk of evolution in acute myeloid leukemia (AML). Lipid signalling pathways are involved in many important processes, such as cell growth, differentiation and apoptosis (*Faenza I et al, Front Biosci 2008*). Namely, nuclear phosphoinositide-phospholipase C (PI-PLC) β1 appears as one of the main players of the signal transduction pathways. In this study we investigated the role of PI-PLCβ1 in the MDS progression towards AML, by analyzing the role of either the genetic and the epigenetic processes. In fact, in a recent study we also observed that demethylating agents, such as azacitidine, induce an increase in PI-PLCβ1 mRNAs accompanied by a down-regulation of activated Akt (*Follo MY et al, Leukemia 2008*), prompted us to investigate the epigenetic mechanisms involving PI-PLCbβ1. As for the genetic investigations, fluorescent in situ hybridization analyses demonstrated that PI-PLCβ1 undergoes an interstitial mono-allelic deletion, as 35/80 (43.75%) of the MDS patients analyzed showed this cytogenetic alteration. Interestingly, 23/35 (65.7%) of the MDS patients bearing the PI-PLCβ1 mono-allelic deletion evolved into AML, retaining a statistically higher significance as a prognostic factor of evolution into AML. On the other hand, the structure of PI-PLCβ1 promoter, displaying two CpG Islands, as well as the degree of PI-PLCβ1 methylation during azacitidine administration, showed that also epigenetic mechanisms could be involved in the MDS progression. Here we report for the first time not only that PI-PLC β1 is indeed hypermethylated, but also that the amount of PI-PLCβ1 is linked to azacitidine responsiveness in MDS patients. Following treatment, and in correlation with the clinical status of the patients, PI-PLCβ1 expression increased, whereas PI-PLCβ1 methylation was reduced. Taken together, our findings suggest that PI-PLCβ1 could be directly involved in the MDS progression towards AML, via genetic and epigenetic mechanisms, and strengthen the importance of lipid signalling in MDS, indicating a promising new prognostic and therapeutic approach.

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PATIENTS WITH MYELODYSPLASTIC SYNDROMES SHOW INCREASED FREQUENCY OF REGULATORY T-CELLS

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Regulatory T-cells (Treg) represents a small fraction of peripheral $CD4^+$ T-cells which plays a crucial role in the maintenance of immune tolerance, thus influencing the pathophysiology of several neoplastic and autoimmune diseases. Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal haematologic diseases, characterised by a marked immune dysregulation, as shown by the frequent occurrence of autoimmune manifestations as well as by the specific involvement of T-cell clones in the functional inhibition of haematopoietic precursors. We analysed the frequencies of Treg in a cohort of patients with MDS, especially focusing on their possible impact on the disease progression. The frequency of Treg was determined on the peripheral blood of 35 MDS patients (7 RA, 5 RARS, 18 RCMD, 4 RAEB and one 5q- syndrome) and 35 normal controls by flow-cytometry. Treg were identified by considering the $CD4^+$ cell fraction characterised by a very high (>2 log) expression of CD25 and by a very low (<2 log) expression of CD127, as well as by determining the expression of FoxP3 and CD152. MDS patients overall showed a higher frequency of Treg than normal controls (1,51% vs. 1,14%, p<0.05). We then compared Treg frequencies in patients belonging to different WHO subclasses, demonstrating a clear trend towards a higher frequency of Treg in high risk (RCMD and RAEB) than in low risk (RA, RARS and 5q- syndrome) patients (1,71% vs. 1,20%, p=0.056). When we stratified patients by WPSS, cytogenetics, blood counts and coexistence of autoimmune phenomena, we could not detect any statistically significant difference. Only transfusion dependence was associated to a reduced frequency of Treg (1,79% vs. 1,12%, p<0.05). Our data show that patients with MDS display an increased frequency of $CD4^+CD25^{high}$ $Foxp3^+$ Treg, which is even more pronounced in high risk patients. Such a difference between different patient subgroups may imply an involvement of Treg in modulating disease evolution, thus suggesting that their expansion could favour a progression of MDS towards more aggressive entities. Moreover we may speculate that the lower frequency of Treg in transfusion dependent patients may mirror a reduced control of possible autoreactive T-cells, which are known to be responsible of the inhibition of haematopoietic precursors and, therefore, of an increased need of transfusions.

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MYELODYSPLASTIC SYNDROMES: MICROVASCULAR DENSITY AND IMMUNOHISTOCHEMICAL EXPRESSION OF THE PROTEINS CONTROLLIN ANGIOGENESIS

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Introduction. Vascular endothelial growth factor (VEGF), the main pro-angiogenetic factor, has three tyrosin-kinase receptors (VEGFRs): VEGFR-1 and VEGFR-2 stimulate angiogenesis, and VEGFR-3 lymph-angiogenesis. In bone marrow (BM), the VEGF/VEGFR system regulates angiogenesis by means of a paracrine mechanism and hemopoiesis mainly by means of an autocrine process. In particular, there is an abnormal angiogenesis in myelodysplastic syndromes (MDS). *Materials and Methods* We evaluated microvascular density (MVD) and the expression of VEGF, VEGFR-1 and VEGFR-2 in hemopoietic cells and marrow stromal elements in BM trephine biopsies of 91 patients with MDS (20 refractory anemia, 23 with refractory cytopenia with multilinear dysplasia, six with 5q syndrome, 31 with refractory anemia with excess blasts type 1, and 11 with refractory anemia with excess blasts type 2), six patients with acute myeloid leukemia evolving from MDS (sAML), and 20 normal controls. MVD was evaluated after immunohistochemical CD34 staining by calculating the average number of vessels in five high magnification microscopic fields (HPFs) in "hot spots" areas. We recorded the percentage of all nucleated BM cells that were positive for VEGF

and VEGFR-1 and, because of their limited number, the average number of VEGFR-2 immunoreactive cells in five HPFs. *Results*. MVD was greater in the patients with MDS (13.17±7.6) and less in those with sAML (4.93±2.1) than in normal controls (7.00±3.4) (p<0.01). Immunohistochemistry showed that VEGF and its receptors were co-expressed in different cell types. VEGF expression was higher in the patients with MDS (63.02±12.80) and sAML (75.00±17.61) than in normal controls (26.5± 8.12) (p<0.0001). Furthermore, it was higher in the MDS patients with >5% blasts than in those with <5% blasts (66.43±12.06 vs 60.58 ±2.01)(p<0.05), and in patients in the poor prognosis IPSS risk categories (int-2 + high vs low + int-1) (68.75±9.23 vs 60.97±13.2) (p<0.002). The immunohistochemical expression of VEGFR-1 was higher in MDS >5% blasts vs those with <5% (51.28±13.46 vs 43.67±12.99) (p<0.01). The expression of VEGFR-2 was lower in the MDS patients than in normal controls (22.42±27.69 vs 59.50±12.53) (p<0.01). *Conclusions*. A higher MVD and increased VEGF and VEGFR-1 expression were observed in cases of more advanced MDS, and increased VEGF and VEGFR-1 expression in those with leukemic evolution, thus suggesting a possible role of VEGF/VEGFR-1 interactions in these MDS groups.

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CLINICAL AND BIOLOGICAL EFFECTS OF 5-AZACITIDINE FIVE DAYS/MONTHLY SCHEDULE IN SYMPTOMATIC LOW-RISK (IPSS: 0-1) MYELODISPLASTIC PATIENT

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Background. Nucleoside 5-Azacitidine (5-Aza) administered at a dose of 75 mg/mq/day subcutaneously for 7 days, every 28 days, induces high hematologic response rates and reduces progression to acute myeloid leukaemia (AML) in the high risk MDS patients. *Aim*. The use of 5-Aza in the earlier phases of MDS could reduce the proliferative advantage of MDS clone and favours the regrowth of normal hematopoiesis. In the setting of low-risk MDS patients lower doses of 5-Aza could be enough to induce hematologic responses. We attempted to use an alternative schedule, 75 mg/mq subcutaneous daily for 5 consecutive days every 28 days, for a total of 8 courses, to evaluate its efficacy and tolerability in low risk MDS patients. Pharmacogenomic studies (the gene expression profile and single nucleotide polymorphism analysis), and the determination of cytokine's pattern, before and after 5-Aza treatment, were planned to identify new biological markers to predict the response. Methods. Between May and December 2008 we enrolled 15 patients in the multicentric clinical trial. According to WHO criteria 6 patients had refractory anemia (RA), 5 patients refractory cytopenia with multilineage dysplasia (RCMD) and 4 patient refractory anemia with excess blasts-1 (RAEB-1); all patients were classified as Low Risk (IPSS score 0-1). Age at diagnosis ranged between 56 and 82 years. All patients failed EPO therapy and were in chronic red blood cell (RBC) supportive care with a median transfusions requirement of 4 units/monthly. *Results*. The response treatment criteria was according to IWG 2006. 5 out of 15 patients completed the 8 courses of therapy; 4 patients obtained an hematologic improvement (HI) with an erythroid response whereas 1 patient maintained a stable disease. The others 10 patients are ongoing. The drug was very well tolerated. Hematologic toxicity consisted in neutropenia (WHO grade III) and thrombocytopenia (WHO grade II) in one patient but it was transitory and no delay of treatment was necessary. *Conclusion*. Our preliminary results show that the 5-Aza five days/monthly schedule is very well tolerated and it appears to have an efficacy similar to the seven days/monthly schedule, at least in low-risk MDS setting. Considering that the optimal schedule and duration for demethylating agents has not yet been established, further MDS patients recruitment is warranted to confirm the efficacy of this alternative 5-Aza low dose regimen. Biological studies will be performed after the clinical study and they will be correlated to the treatment response. The results could be useful to elucidate the genetic bases for individual susceptibility to the beneficial (or adverse) effect of 5-AZA, in order to optimise the therapy.

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PROGNOSTIC VALUE OF BONE MARROW FLOW CYTOMETRY IN INT-2/HIGH RISK MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes are malignant diseases of bone-marrow stem-cells, characterised by ineffective haemopoiesis leading to peripheral-blood cytopenias.The prognostic instrument established for myelodysplastic syndromes (MDS) is the International Prognostic Scoring System (IPSS), which is based on medullary blast cell count, number of cytopenias, and cytogenetics. Flow cytometry of bone marrow might add significantly to diagnostic and prognostic criteria. *Aim*. The aim of this study is the identification of prognostic subgroups in bone marrow cells phenotype in patients with INT-2/High risk MDS. *Methods*. We evaluated 15 patients with MDS scored as int-2/HIGH IPSS risk. We analized bone marrow cells phenotype by flow cytometry (CD13, CD33, CD34, CD14, CD61, CD117, CD56, glycophorin) at diagnosis, and during treatment with 5 Azacitidine. Patients had a median age of 66 years (range 50-83) and according to WHO classification there were 5 RCMD, 3 AREB 1, 7 AREB2. All patients were treated with 5 azacitidine 75 mg/mq s.c. for 7 day every 4 weeks and completed at least 4 cycles. Complete Response and partial response occured in 13,3% and 26,6% of patients, respectively. Stable or progressive disease was observed in 30% and 26% ofpatients. *Results*. We observed higher expression of $CD34^{+/}CD56^{+/}$ by flow-cytometry performed on bone-marrow samples at diagnosis and during therapy with 5-AZA in patients with stable or progressive disease and all of these patients were transfusion-dependent before and during treatment. *Conclusions*. Flow-cytometry could be useful to identify a subset of patients with Int2-2/High risk SDM that could profit of alternative therapies.

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FUNCTIONAL *IN VITRO* EVALUATION OF BACTERICIDAL AND FUNGICIDAL ACTIVITY OF NEUTROPHILS FROM PATIENTS SUFFERING FROM MYELODYSPLASIA

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Aims. To evaluate the *in vitro* killing activity against Gram-negative (*Escherichia coli)* and Gram-positive (*Lactococcus lacti*) bacteria and yeast (Candida albicans) of neutrophils from patients (pts) suffering from myelodysplasia (dPMN) compared to bactericidal and fungicidal function of normal neutrophils. *Methods*. dPMN obtained from peripheral blood of healthy volunteers and pts with myelodysplasia were isolated through dextran sedimentation and centrifugation over Fycoll; in all cases the cell population consisted of >95% neutrophils as determined by May-Grunwald Giemsa staining. PMN obtained were subsequently incubated with bacteria and yeast colonies (10^9 CFU/mL) and the time killing both of dPMN and normal neutrophils was evaluated trough the bacteria and yeast intra- and extracellular counts at 8, 24, 48 and 72 hours of incubation. *Results*. Neutrophils from 16 pts with myelodysplasia were collected (Table 1) and the following data emerged: a) a killing activity of dPMN against *E.coli* significantly reduced at 8 (p<0.0138), 48 (p<0,0009) and 72 hours (0,0002) compared to controls; b) a killing activity of dPMN against *L. lacti* similar between the 2 groups at 8 hours (p 0.65) and significantly reduced at 24, 48 e 72 h (p-value 0.005, 0.004, 0.0002 respectively); c) a killing activity of dPMN against C. albicans similar between the 2 groups at 8 hours (p 0.11) but significantly reduced at 24 (p 0.0008), 48 e 72 hours (p<0.0001 each). Comparing killing activity of dPMN against the various microorganisms, a difference in microbicidal properties emerged, resulting the killing activity against *E.coli* higher than against *C. albicans* [p <0.0001 at 24,48 e 72 hours] and *L.lacti* [at 24 p 0.03, at 48 p 0.02, at 72 p 0.005]; between *C. albicans* and *L.lacti* a superior killing activity for the latter was evidenced [at 24 and 48 h p-value 0.003, a 72 ore p-value 0.001]. *Conclusions.* Dysplastic PMN show a significantly reduced function against bacteria and yeast respect to nor-

mal neutrophils, justifying the high susceptibility to infections of pts suffering from myelodysplasia. Fungicidal activity results mainly impaired, followed by anti-gram-positive activity. Additional functional studies with phenotypic and molecular evaluation of dPMN needed for a complete assessment of infective risk of this kind of pts.

Table 1. Clinical features of 16 patients studied.

Patients (n°)	16
Median age (range)	70 (38-82)
Sex (M/F)	10/6
MDS subtype (WHO):	
RA/ RARS	8/1
CMML	5
RAEB	2
IPSS category:	
Low	2
Intermediate-low	8
Intermediate-high	4
High	2
Median PMN value (range)	2,48 x 10^9/L (1,2-61,4)
Previous infective episodes:	
Recurrent pneumonia	4
Recurrent cutaneous abscess	1

RA= refractory anemia; RARS: refractory anemia with ring sideroblasts; CMML= chronic myelomonocytic leukemia; RAEB= refractory anemia with excess of blasts.

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INEFFECTIVE HEMATOPOIESIS AND MARKERS OF ANGIOGENEISIS IN MYELODYSPLASTIC SYNDROMES

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Ineffective hematopoiesis is one of the main features of myelodysplastic syndromes (MDS). Recent evidence underlines the role of dysregulated cytokine production in promoting disturbance in marrow stromal microenviroment. Moreover, immunomodulatory and anti-angiogenic therapies such as lenalidomide have been found active in this setting. Circulating endothelial cells (CECs) correlates well with *in vivo* and in vitro models of angiogenesis and are found increased in different settings. More recently, different subsets of BM-derived cell expressing angiopoietin or VEGF receptors have been characterized as important in angiogenesis. To assess the role of CEC and BM-derived cells in this setting, we evaluated the level of CECs, Tie2 and VEGFR-1 expressing circulating cells in a cohort of MDS patients. We additionally evaluate a group of cytokines related to angiogenesis. We analyzed 28 MDS patients classified as follows: RCMD (9), RA (5), RARS (6), AREB1-2 (3), 5q- syndrome (5) and 15 controls. CECs ($CD146^+/CD31^+/CD45$-), $VEGR1^+/CD45^+$ and $TIE2^+/CD45^+$ cells were evaluated by flow cytometry. Serum concentration of VEGF, Ang-1, Ang-2 and bFGF were assessed by commercial ELISA kits. The data were statistically analysed using the Mann-Whitney U test for between-group comparisons. $p<0.05$ was taken as a cut-off point for statistical significance. MDS patients were characterized by elevated CECs ($p=.02$) while $VEGFR1^+/CD45^+$ ($p=.02$) and Ang-1 ($p=.0006$) were decreased. No differences were found regarding the other cytokines evaluated. Stratifying patients into IPSS groups we found that higher risks (INT-1 and 2, n=12) are characterized by markedly decreased $VEGFR1^+/CD45^+$, $TIE2^+/CD45^+$, VEGF, bFGF and Ang-1 in comparison to both controls and low risk group ($p<.05$). Similar results were found dividing patients according to WPSS. Patients who did not respond to EPO showed lower VEGF and Ang1 in respect to EPO responders ($p<.003$). While increased angiogenesis is an hallmark of hematologic malignancies, an unusual amount of endothelial and BM-derived cell is present in MDS patients. Moreover, cytokine and cellular disturbances characterize higher risk MDS, in which ineffective hematopoiesis is prominent. These preliminary data shows that dysregulated pattern of cell and cytokine production may have a role in the pathogenesis of MDS.

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HYPOXIA AND INEFFECTIVE ERYTHROPOYESIS BUT NOT IRON REGULATE HEPCIDIN LEVELS IN LOW-RISK UNTRANSFUSED MYELODYSPLASTIC PATIENTS

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Hepcidin, the key hormone of iron homeostasis, is influenced by different stimuli such as inflammation or iron overload that both increase its expression while iron deficiency and hypoxia have an opposite effect. Growth differentiation factor 15 (GDF15), a member of the transforming growth factor-β family, has been described as a potential hepcidin inhibitor in conditions characterized by ineffective erythropoiesis. Little is known about iron regulation in myelodysplastic syndromes (MDS) in which anemia is common and ineffective erythropoiesis coexists with chronic inflammation or iron overload with opposite effects on hepcidin regulation. To evaluate iron parameters and erythroid activity in a cohort of low-risk MDS patients at diagnosis or never transfused. 60 serum samples were collected from 48 low-risk MDS patients after written informed consent. In 12 out of 48 patients, samples were collected also after 4 months of EPO treatment. Serum GFD15 evaluation was performed by ELISA while hepcidin evaluation by SELDI-TOF-MS technique. The results were compared to 30 matched healthy controls and 30 transfusion-dependent thalassemic patients. Statistical analysis was performed by SPSS software. Median serum ferritin was 178 ng/mL (range 19-1959), median transferrin saturation 28% (4-90%), median hemoglobin (Hb) value was 11,15 g/dL (7,4-14,6). Serum GDF15 was marked increased in patients with erythroid dysplasia compared to healthy controls (median value 2537 pg/mL versus 206, p>0,001 by Mann-Whitney test). Serum hepcidin was significantly increased respect to healthy controls (median value of 7,740 nM/L, range 0,55-41,54) considering the whole population. Only in patients with Hb below or equal to 12 g/dL (25 out of 48 patients) GDF15 and hepcidin show a significant negative correlation. No significant correlations was found between iron parameters and neither GDF15 nor hepcidin levels whereas there was a significant correlation between sTFR and both parameters. Hepcidin levels are higher in MDS patients than in controls probably for the predominance of the inflammation pathway. Only in anemic patients GDF15 and hepcidin show a significant correlation, probably for either hypoxia or ineffective erythropoiesis. The iron pathway is not crucial in hepcidin regulation: very few patients show laboratory findings of iron overload and there is no relation between iron and neither GDF15 nor hepcidin levels.

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CLINICAL AND BIOLOGICAL FEATURES OF MYELODYSPLASTIC SYNDROMES WITH BONE MARROW FIBROSIS

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Background. The clinical heterogeneity of myelodysplastic syndromes (MDS) still cause clinical decision-making to be a matter of debate. The aim of improving our ability to evaluate prognosis in MDS patients has driven the search for unifying features,that would stratify patients into distinct subgroups. Among the different parameters evaluated bone marrow histology is emerging as having an important prognostic significance,by influencing marrow insufficiency and outcome. *Methods.* We retrospectively collected clinical and hematological data,including bone marrow fibrosis (MF), of 158 consecutive patients with diagnosis of MDS.All patients were reclassified according to the WHO criteria; 43 patients were excluded,since having been

classified as affected by CMML (23) and AML with more than 20% marrow blasts (20). The grading of MF was established according to the European Consensus guidelines. Cytogenetic analysis was successful in 90 (78.3%) out of 115 patients;therefore the IPSS and the WPSS at the diagnosis have been assessed in these 90 patients. *Results.* Out of the 115 evaluated patients, 43 (37.4%) showed a grade 0 fibrosis,while 72 (62.6%) showed pictures of marrow fibrosis,ranging from mild (grade 1) to moderate (grade 2),respectively in 44 (38.3%) and 28 (24.3%) patients;none of the patients had MF grade 3.There were no significant differences between patients without MF and those with grade 1 MF, regarding clinical characteristics,karyotype and outcome;so we unified patients with grade 0 and grade 1 fibrosis in a single subgroup (75.7% of patients) and matched their clinical findings and outcome with those of the grade 2 subgroup (24.3%). Among these two populations we found MF is significantly related to more severe thrombocytopenia, poor-risk cytogenetic, bi- and trilineage dysplasia, higher risk of leukemic evolution and poor O.S. *Conclusions.* Beside the main changes in hematopoiesis and in cytogenetic pattern,other cellular and mesenchymal marrow components not involved in the neoplastic clone are emerging as having a role in marrow insufficiency and in leukemic transformation. Evaluation of marrow histology has becoming an essential tool in predicting prognosis in MDS patients, the presence of MF being an independent unfavorable prognostic factor. Moreover, the evaluation of MF in the setting of the new therapeutic strategies (antiangiogenetic, epigenetic) probably will be able to give new insights into their biologic activity and impact on the clinical outcome.

Table 1. Clinical characteristics at diagnosis of the 36 patients enrolled in the study.

Parameters	*Number of patients (%)*
Age, median (range)	
67 (54-83) years	
≤60 years	6 (17%)
≥60 years	30 (83%)
Gender	
Male	24 (67%
Female	12 (33%)
WHO classification	
RA	9 (25%)
RARS	7 (19%)
RCMD	5 (14%)
RCMD-RS	2 (6%)
5q-syndrome	1 (3%)
RAEB-1	0 (0%)
RAEB-2	5 (14%)
MDS/MPD (MMCL)	7 (19%)
Bone marrow blasts (%), median (range)	2 (0-19)
0-4.9	29 (80%)
5-9.9	1 (3%)
10-19.9	6 (17%)
Cytogenetic risk	
Good	31 (86%)
Intermediate	4 (11%)
Poor	1 (3%)
Absolute neutrophil count (x109/L), median (range)	2.37 (0.3-32)
Hemoglobin (g/dL), median (range)	10.7 (7.5-15.5)
Platelet count (x109/L), median (range)	200 (27-681)
Ferritin (ng/mL), median (range)	318 (34-1915)
Endogenous epoietin (mIU/mL), median (range)	66 (18-312)
LDH (U/L), median (range)	355 (99-589)
Splenomegaly	
No	22 (61%)
Yes	14 (39%)
Transfusion dependence	
No	25 (69%)
Yes	11 (31%)
IPSS risk group	
Low	28 (78%)
Intermediate-1	3 (8%)
Intermediate-2	4 (11%)
High	1 (3%)
WPSS risk group	
Very low	20 (55%)
Low	10 (28%)
Intermediate	1 (3%)
High	2 (6%)
Very high	3 (8%)

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PLATELET-DERIVED GROWTH FACTOR β RECEPTOR (PDGFRB) GENE IS REARRANGED IN A SIGNIFICANT PERCENTAGE OF MYELODYSPLASTIC SYNDROMES WITH NORMAL KARYOTYPE

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PDGFRB, the gene codifying for the platelet-derived growth factor receptor β, is located on chromosome 5q33, and in chronic myelomonocytic leukemia (CMML) its activation is more frequently caused by the t(5;12)(q33;p13) translocation, associated with the *ETV6-PDGFRB* fusion gene. Nevertheless, other partners of *PDGFRB* are been described. Rapid, complete, and long-lasting responses have been reported after treatment with Glivec in patients with t(5;12), t(5;14), t(5;10), t(5;15), t(5;17) and t(1;5), all classified as affected by CMML, or myeloproliferative disorders associated with eosinophilia. We started to investigate by fluorescence *in situ* hybridization (FISH) technique the eventual disruption of the PDGFRB signal (resulting from the rearrangement of *PDGFRB*) in bone marrow samples from 36 patients (29 with MDS, and 7 with CMML), all without translocations involving chromosome 5 after conventional Q-banding analysis. FISH was performed by using *PDGFRB* (5q32) Break Dual-labelling probe (Resnova, Genzano di Roma, Italy). Only in one case aberration of chromosome 5 was detected after conventional Q-banding; in the remaining 35 cases, no translocations involving chromosome 5 were observed. In 31 cases karyotype was normal. In 21 patients, WT1 gene was measured by quantitative RT-PCR; in 5 out of these cases (24%), WT1 resulted over-expressed (in respect of values reported for healthy subjects). Two of these patients were affected by RA, one by RCMD-RS, and other two presented with RAEB-2. When the 36 patients were evaluated by FISH, a *PDGFRB* rearrangement was identified in 11 cases (30%). Three of these PDGFRB-rearranged patients were classified as affected by RA, 3 by RARS, 2 by CMML, and 3 by RAEB-2. Two patients were resistant to epoietins, 4 to azacitidine, 2 to hydroxyurea and 6-mercaptopurine. The observation that in our series 7 of 29 patients affected by *de novo* MDS (24%) and 2 of 7 cases of CMML (28%), all with normal karyotype, showed PDGFRB rearrangement would be very relevant. Given these therapeutic implications, our findings stress the need of investigating all MDS and MDS/MPD cases also for PDGFRB rearrangement. The fluorescent *in situ* hybridization, a quite simple and rapid technique, would represent the method of choice for this molecular screening.

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IRON CHELATION WITH DEFERASIROX IN MYELODYSPLASTIC SYNDROMES AND MYELOFIBROSIS WITH TRANSFUSIONAL IRON OVERLOAD. RETROSPECTIVE STUDY OF 21 PATIENTS

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Iron chelating therapy (ICT) has been historically underutilized in myelodysplastic syndromes (MDS) owing to the difficulty to administer deferoxamine to elderly MDS patients (pts). Recently a new oral iron chelator (deferasirox) has become available, and has proven effective for treating transfusional iron overload. From July 2003, in our Institution, 21 pts (12 males), median age: 74 (46-85) yrs, with transfusional iron overload, underwent ICT with deferasirox. 15 pts were affected by MDS (IPSS risk: low: 8 pts; int-1: 7 pts; int-2: 4 pts) and 2 pts by primary myelofibrosis (PMF). All pts had a normal pre-treatment value of serum creatinine and glomerular filtration rate (GFR). The median time from diagnosis to the start of ICT was of 48 (12-180) months. At baseline, the pts had received a median number of 63 (21-504) packed red cell (PRC) transfusion, and in the last 12 weeks they had received a median number of 9 (6-24) units of PRC. Median serum ferritin (SF) level at baseline was of 3.353 (833-5.476) ng/mL. The starting dose of deferasirox was of 10 mg/Kg/day (5 patients) or 20 mg/Kg/day (13 pts), and was established on the basis of : a) severity of pre-treatment iron overload (established by SF and number of previous transfusions); b) transfusion pace at baseline. For 3 pts the starting dose was lower (5 mg/Kg/day) because of advanced age and/or clinical condition. The effectiveness of ICT was estimated on the basis of monthly assessment of SF. The median duration of ICT was of 11 (3-69) months. Overall, the median SF significantly decreased after ICT : 2.303 (725-5.657) ng/mL. In 9 pts the decrease of SF was > 500 ng/mL, 3 pts showed a minor decrease of SF (< 500 ng/mL), 4 pts anyhow maintained a stable iron load (SF substantially unchanged), in spite of continuation of PRC transfusions, and the 5 remaining pts showed a further increase of SF. Adverse events occurred in 11 pts: in 7 pts a > 33% rise of serum creatinine above baseline was detected: in 6 pts the dose of ICT was reduced, and in only 1 case ICT had to be permanently discontinued. 2 pts complained of transient gastrointestinal side effects, and 2 pts showed a mild and transient skin rash. The dose of ICT was reduced in 3 pts because of SF level < 500 ng/mL, and was increased in 2 pts (in one case up to 30 mg/Kg/day) because of increasing SF. One pt showed a moderate decrease of transfusion pace after ICT.

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HPA-1 POLYMORPHISMS IN PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (PH-MPN) DOES NOT INCREASE THE RISK OF THROMBOTIC COMPLICATIONS

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Background. Somatic point mutation, which constitutively activated signal transduction is present in almost all polycytemia vera (PV) and in about half essential thrombocythemia (ET) patients. JAK2 mutated ET patients develop more thromboses than WT cases. In caucasian population, the rare HPA-1 b allele seems to be related to a high frequency of thrombosis. The aim of this study is to evaluate if HPA-1 b allele is common in patients with Ph-MPN and thrombotic complications. *Patients*. We report 97 Ph-MPN patients (29 males and 68 females, mean age 64±19 y). 29 patients (9.2%) suffered for thrombotic complications (8 arterial and 21 venous). 200 healthy blood donors comparable for sex and age without prothrombotic risk factors and no thrombotic events were used as controls. *Material and methods*. HPA-1 polymorphisms were searched in genomic DNA from whole blood using RT- PCR with TaqMan probes. JAK2V617F mutation was searched in peripheral blood granulocytes DNA with allele-specific PCR. Pearson's χ^2 test (two by two table) was used to compare categorical variables. *Results*. In Ph-MPN patients, HPA-1 b allele (28.9% a/b, 3% b/b) was as frequent as in healthy donors (24% a/b; 2% b/b). No statistical difference in thrombotic occurrence was observed in PV comparing those carrying or not JAK2V617F mutation or HPA-1 b allele. In contrast, ET JAK2 mutated patients had higher incidence of thrombosis compared to WT. No statistical correlation was found between MPN carrying HPA-1 b allele compared to WT. *Conclusions.* The frequency of HPA-1 b allele is similar in Ph-MPN patients and in healthy controls. We confirm that JAK2V617F ET have a higher risk of thrombotic complications than ET WT. The presence of heterozygous or homozygous HPA-1 b allele does not favour thrombotic risk.

Table.

		*JAK2*V617F / WT	p	HPA-1 b / WT	p
PV (32)	Thrombosis (15)	13/2	NS	5/10	NS
	No thrombosis (17)	12/5		6/11	
ET (65)	Thrombosis (14)	13/1	0.002	4/10	NS
	No thrombosis (51)	28/23		16/35	
tot	Thrombosis(29)	26/3	0.006	9/20	NS
	No thrombosis (68)	40/28		22/46	
	Healthy controls (200)	0/200		52/148	

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JAK2V617F DETECTION AND QUANTIFICATION – EXPERIENCE IN THE MOLECULAR BIOLOGY LABORATORY IN PESCARA

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In the suspicion of a myeloproliferative disorder, JAK2V617F detection simplify the approch to clinical diagnosis and represent a possible target for specific JAK2 inhibitor drugs. JAK2V617F in most of patients with polycitemia vera (PV) and primary myelofibrosis (PMF) is present in a homozygous state because of a mitotic recombination. On the contrary this event occurs rarely in essential thrombocytemia (ET). Therefore, JAK2V617F burden may be useful (but not sufficient) to guide diagnosis among PV, PMF and ET. Besides, numerical aberrations of JAK2 have been previously described in lymphomas and myeloprolipherative neoplasms (MPN), where clinical and diagnostic associations are

not yet clear. We report here technical and scientific considerations based on our experience on JAK2V617F detection and quantification since 2007, when we introduced real time polymerase chain reaction-based allelic discrimination assay as the technique to screen DNA samples from patients with clinical evidence suggestive of MPN. Allele frequency was calculated as described by Soren and co-workers (*Gen.Res., 2000*). For each DNA sample a control gene was amplified to test DNA amount and identifie JAK2 quantitative alterations. When a commercial kit for JAK2V617F quantification was available, the two methods were compared to assess the best procedure to adopt. Finally, to support diagnostic decision of particular cases, we evaluated JAK2V617F zygosity analyzing single picked erithroid colonies derived from peripheral blood. 1577 samples from 1210 subjects have been analyzed for JAK2V617F detection. The results on patients with known diagnosis based on WHO criteria, agree with currently reported data on mutational frequency of JAK2V617F in PV, ET and PMF. We found a decreasing continuous scale with the highest JAK2V617F burden in patients with PV and PMF followed by ET. 113 out of 655 subjects with JAK2WT showed quantitative alterations (64 jak2 gene deletions and 49 amplifications). Preliminary data on MPN patients show that JAK2 amplification might be associated to JAK2 overexpression in erithroid progenitors but not in granulocytes. The comparison between our method and the JAK2V617F quantification commercial kit, even if based on theoretical and technical differences, showed comparable results. Therefore, we consider suitable to screen samples with the allelic discrimination test and reserve the kit for specific application such monitoring response to therapy.

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MOLECULAR PROFILE OF CD34+ STEM/PROGENITOR CELLS ACCORDING TO JAK2V617F MUTATION STATUS IN ESSENTIAL THROMBOCYTHEMIA

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JAK2V617F mutation has been reported in about 40-60% of Essential Thrombocythemia (ET) patients. However, little is known about specific molecular abnormalities of the hematopoietic stem cell compartment of ET according to JAK2 mutation. The aim of this study was to evaluate whether JAK2V617F mutation causes altered gene expression profile in the $CD34^+$ stem/progenitor cell compartment of ET as compared to that of JAK2V617F-negative patients to identify differentially expressed genes. Total RNA from bone marrow $CD34^+$ cells of 8 JAK2V617F-positive and 8 JAK2V617F-negative ET patients was extracted and two-cycle target labeling assays, as well as the Affymetrix HG-U133A GeneChip arrays hybridization, staining, and scanning, were performed, using Affymetrix standard protocols. Our results demonstrate that CD34+ stem/progenitor cells of a subset of ET patients harbor JAK2V617F mutation. Moreover, we show that the gene expression profile of ET is not significantly altered in JAK2V617F-positive $CD34^+$ stem cells as compared to JAK2V617F-negative counterparts. By using Real-Time Quantitative RT-PCR, we also demonstrate that the expression of target genes of the JAK2/STAT pathway (BCL2L1, MYC, PIM1, SOCS1, SOCS2) is not significantly different among the two groups of patients. Consistently, when the pattern of gene expression identified in normal bone marrow $CD34^+$ stem/progenitor cells was compared with that of ET patients (JAK2V617F-positive and negative), we found differentially expressed genes but we did not observe significant differences in expression for any of the target genes of the JAK-STAT pathway. Therefore, our findings suggest that JAK2V617F mutation has no significant influence on gene expression profile of stem/progenitor cells in ET.

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PROGNOSTIC IMPLICATIONS OF THE EUROPEAN CONSENSUS FOR GRADING OF BONE MARROW FIBROSIS IN CHRONIC IDIOPATHIC MYELOFIBROSIS

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Introduction. Various clinical prognostic scoring systemsl have been suggested as means of selecting high-risk chronic idiopathic myelofibrosis (CIMF) patients at diagnosis. The WHO has recently proposed strict diagnostic criteria for CIMF, and the European consensus for bone marrow fibrosis (BMF) grading recommends four classes. It has been suggested that BMF grading may play a prognostic role in CIMF. *Materials and Methods* We tested a prognostic model for overall survival (OS) based on the WHO criteria and BMF grading in 230 consecutive patients with chronic myeloproliferative disorders (205 with CIMF and 25 with post-polycythemic myelofibrosis, post-PV MF), (123 males and 107 females, M/F ratio 1.1; median age 67 years, range 27-86), who were diagnosed between 1996 and 2008 (median follow-up 24 months, range 1-150), and classified on the basis of the WHO criteria. On the basis of the European consensus on BMF grading, 80 of the CIMF cases were classified as CIMF-0, 57 as CIMF-1, 38 as CIMF-2, 14 as CIMF-3, and 16 as CIMF unclassifiable. Clinical data were available for all of the patients, all of whom were Philadelphia chromosome-negative (Ph-). The OS curves were calculated according to Kaplan-Meier and compared by means of the log-rank test. A p value of <0.05 was considered significant. *Results*: The results showed that our model is significantly associated with different OS and clearly discriminates the OS of CIMF-0 + CIMF-1 vs CIMF-2 + CIMF-3 vs post-PV MF patients (Kaplan-Meier, $p<0.0003$). *Conclusions*. We found that the WHO classification and the European consensus for BMF grading, used as a prognostic parameter in CIMF, were significantly associated with different survival in our series of patients. If validated in larger series, the results of this analysis may have a significant impact on current CIMF prognostication.

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BONE MARROW ANGIOGENESIS IN PATIENTS WITH PH-NEGATIVE MYELOPROLIFERATIVE DISORDERS: IMMUNOHISTOCHEMICAL ANALISIS

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Background. Increased bone marrow angiogenesis evaluated as bone marrow microvessel density (MVD) or as immunohistochemical expression of angiogenic factors in bone marrow biopsy has been demonstrated in a variety of haematological disorder including Polycythemia Vera (PV), Essential Trombocythemia (ET) and Primary Myelofibrosis (PMF). Aims. To evaluate bone marrow angiogenesis with immunohistochemical expression of the following antibodies : CD34, Matrix metalloproteinase 9 (MMP-9), VEGF, VEGF receptor (KDR/flk-1). *Method*. Bone marrow paraffin embedded biopsies from 30 patients with Ph negative myeloproliferative disorders were studied (10 PV, 10 ET, 10 PMF) and compared with five controls. The main clinical features of the patients were: median age 52 years; 7 male, 5 female. Splenomegaly was observed in 10 patients, 8 patients showed increased white blood count, EPO levels was high only in two patients, JAK2 mutation was positive in 9 patients (all PV). To identify BM microvessel, we use anti-CD34 staining, and, to identify the areas with hot spots, all section was scanned at 100x magnification. On that basis, we selected three areas with the highest number of microvessel, then microvessel were counted at 400x magnification in each of these spots. VEGF, MMP-9 and KDR expression was evaluated as percentage of positive cells in a total of eight consecutive areas at 400x magnification. *Results*. Bone marrow biopsy specimens

from patients with CIMF showed a increased vascularity compared to PV and TE. Median number of microvessels (visual count) was 70 in CIMF, 25 in PV and 48 in TE. VEGF expression was higher in PV patients (median expression 53%), then TE (27.5%) and PMF (30%), even if in these casis the vascularisation was higher. In all patients MMP-9 percentage expression was <15%, while KDR median expression was higher in PMF rather then PV and TE (10% vs 5%, respectively). *Conclusion*. Our results suggests a higher grade of vascularisation in PMF, with a higher KDR percentage respect to TE and PV, without a proportional increased of VEGF. In PV and TE we have a reduced grade of vascularisation, with a low expression of KDR and MMP-9. More cases are necessary to correlate our data with clinical characteristic and clinical outcome of negative myeloproliferative disorders .

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ACCELERATED TELOMERE SHORTENING IN PH - NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS: COMPARISON WITH THE HEALTHY POPULATION AND CORRELATIONS WITH TREATMENT RECEIVED

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Introduction. Telomeres are reliable indicators of previous cell proliferation and cell ageing. Moreover, a marked though variable loss of telomere length (TL) has been observed in several neoplasias, including hematological malignancies. This supports the possible influence of TL in the development of many lymphoid and myeloid disorders. Furthermore, several studies suggest the prognostic relevance of TL in these disorders. Aim of the present study was to evaluate TL dynamics in patients with Ph-negative Chronic Myeloproliferative Neoplasms (Ph-neg-CMNs). *Patients and Methods.* Peripheral blood (PB) samples were obtained from 62 Ph-neg-CMNs patients (median age 66 yrs, range 38-89). Forty-nine patients had Polycythemia Vera (PV), 10 had Essential Thombocythemia (TE), 2 had Idiopathic Myelofibrosis and 1 had an Unclassified CMNs. Forty-five patients had been exposed to various treatments for at least one year. Among them, 33 received Hydroxyurea, 3 Busulfan, 2 Pipobroman, 2 Anagrelide and 1 the Hystone Deacetylase Inhibitor ITF2357. Seventeen patients had never been exposed to cytotoxic drugs. Samples were obtained either at diagnosis or during follow-up. As a control, PB samples from 54 healthy age-matched subjects (median age 62 yrs, range 31-94) were analyzed as well. TL was assessed by Southern blot analysis, according to standard procedures (TeloTAGGG Telomere Length Assay Kit, Roche Diagnostic, Mannheim, Germany). Results: Ph-neg-CMNs patients showed individual TL that correlated with age, with progressive shortening as observed in the healthy population. However, TL was significantly shortened compared to healthy age-matched individuals (p=0.0001) both in PB mononuclear cells (median TL: 5,070 bp, range: 3,990-8,890) and PB granulocytes (median TL: 5,230 bp, range: 3730-9570). There was no statistical difference in TL between PV and TE (p=0.4416). Patients receiving cytotoxic treatments showed a PB median TL of 4,910 bp (range: 3,730-8,890) lower than that of patients never exposed to cytoreduction (median TL 5,640 bp, range: 3,990-9,570). However, this difference was not statistically significant. Conclusion: accelerated telomere shortening is present both in lymphoid and myeloid PB cells of Ph-neg-CMNs. The loss is particularly pronounced in those patients who have been exposed to chronic cytotoxic drugs. These results prompt further studies to verify possible correlations between TL, type of treatment received and clinical outcome.

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CLARKSON'S SYNDROME: A RARE SYSTEMIC DISEASE MIMICKING HAEMATOLOGICAL DISORDERS

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In the last 4 years we had the opportunity of evaluating a 56 years-old man who came to the Emergency Room with hypotension, loss of conscience, and haematological parameters compatible with the diagnosis of polycythaemia: RB =7.71x10^9/liter; Hb = 22,7 grams/deciliter; hematocrit = 67%; leukocitosis with neutrophilia (WBC: 23.7 x 10(e)6/liter; Neutrophils: 20.0x10^6/liter). After the acute phase, treated by hydration and phlebotomy, generalized oedemas appeared with a severe increase in body weight, while all blood values returned in the normal range. In consideration of the rapid change of the clinical and haematological features, the previous hypothesis was excluded, and an increased vascular permeability syndrome was suspected. One year later, an IgG kappa monoclonal gammopathy was found. The clinical features, the laboratory findings and the association of a monoclonal gammopathy led to diagnosis of Clarkson Syndrome, a rare clinical condition described for the first time in 1962, characterized by an increased capillary permeability, extravasation of fluids, hypotension, peripheral oedemas, hemoconcentration with hematocrit even over 60% , and , in most cases, by the presence of a monoclonal gammopathy (without a demonstrated pathogenetic role). We investigated the haematological aspects of the syndrome, by three bone marrow examinations, studying morphological features, caryotype, molecular biology, immunophenotype. Plasma cells were 2-3% in every evaluation. Cariotype was normal, with FISH negativity. PCR for rearranged IgH (CDR3) was positive in 2006, negative in 2007, positive in 2009. PCR for JAK2(V617F) was always negative. The immunophenotype analysis, performed by a BD FACSCanto II with a six fluorescence analysis, detected: lymphocytes 8%; CD3: 81%; CD4: 44%; CD8: 31%; CD5: 81%; CD19: 11%; NK: 6%; plasma cells: 1%, identified by plasma cell markers CD138/CD38. 20% of this population showed normal phenotype (CD19$^+$CD45$^+$), while 80% of the plasma cell population showed abnormal phenotype CD19$^-$/ CD45$^-$/CD56$^+$/CD117$^+$. According to the European Myeloma Network, this pattern is suggestive of MGUS. We underline the role of the haematologist, who may often be the first specialist involved in evaluating a syndrome which may mimic a haematological disease (either a myeloproliferative disease, or a plasma cell dyscrasia). His contribution may be determinant if he keeps in mind to include Clarkson's Syndrome into differential diagnosis.

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ATYPICAL MYELOPROLIFERATIVE DISORDER PRESENTING FIP1L1-PDGFRA REARRANGEMENT

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Atypical myeloproliferative disorders (MPD) include systemic mastocytosis (SM), hypereosinophilic syndrome (HES), chronic eosinophilic leukemia (CEL), chronic neutrophilic leukemia (CNL), chronic myelomonocytic leukemia (CMML) and unclassified MPD(UMPD). Recent studies have shown the association between rearrangements involving platelet–derived growth factor receptors A and B (PDGFRA and PDGFRB) and clonal eosinophilia that characterize a subset of HES, SM, CEL, CMML and UMPD patients. The detection of these mutations in the absence of eosinophilia is very unusual. Here we describe a 69 year old woman referred at our hospital for leukocytosis (192x10^3/microliter WBC), thrombocytopenia (24x10^3/microliter PLTS), and severe anemia (Hb 5.4 g/dL). Differential leukocyte count showed: neutrophils 55%, lymphocytes 2%, metamyelocytes 20%, myelocytes 20%, blasts 3%. Bone-marrow examination showed marked granuloblastic hyperplasia with a blast count <5%. The patient presented with mild splenomegaly and showed a normal karyotype; molecular investigations excluded BCR/ABL, AML1/ETO and inv(16) rearrangement as well as JAK2V617F, FLT3 and NPM1 mutations. Nested RT-PCR showed a positive signal for a reciprocal FIP1L1-PDGFRA fusion transcript, despite no evidence for clinical and laboratory phenotype of HES, SM or CEL was present. The presence of reciprocal fusion transcript suggests a molecular rearrangement due to t(4;4) rather than to interstitial deletion 4q12 or more complex rearrangements. To assess this hypothesis, molecular genomic investigations and comparative genomic hybridization (CGH) are in progress. The patient was started on imatinib mesilate treatment with already evident haematological improvement after 15 days.

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MEASUREMENT OF BONE MINERAL DENSITY WITH DUAL-ENERGY X-RAABSORPTIOMETRY (DXA) IN PATIENTS AFFECTED BY MASTOCYTOSIS

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Mastocytosis is a clonal myeloproliferative disorder characterised by proliferation and accumulation of mast cells (MC) within various organs, most commonly the skin and the bone marrow. Osteoporosis, lytic lesions and, less frequently, osteosclerosis have been observed in patients with mastocytosis. MC may have a pathogenetic role in the development of bone lesions, either because of neoplastic infiltration itself or as an effect of MC mediators, like histamine, heparin, tryptase, and cytokines. To date, single cases or very small groups of patients with mastocytosis and bone involvement have been reported, and informations about the actual prevalence of osteoporosis in mastocytosis are lacking. Moreover, only a minority of these patients was studied with the current "gold standard" for diagnosing osteoporosis: Dual energy X-ray Absorptiometry (DXA) at spine and hip.

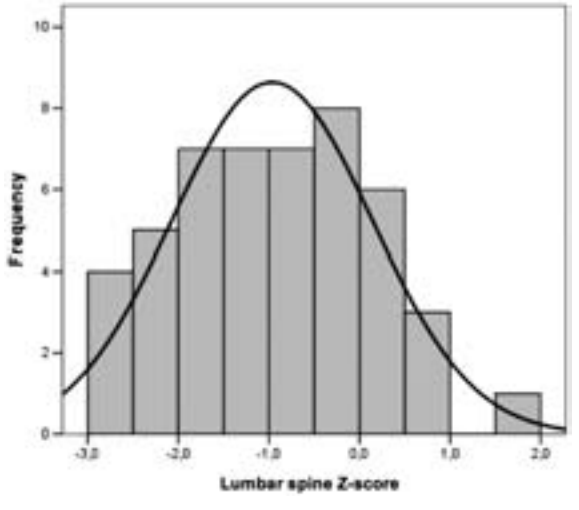

Figure 1.

We assessed the bone mineral density (BMD) using central DXA in our series of patients with clonal mast cell disorders (MCD) diagnosed according to WHO criteria. Patients with an history of anaphylaxis and abnormal MC immunophenotype or detection of a KIT mutation, but non fulfilling all criteria for diagnosis of Systemic Mastocytosis (SM), were classified as having Monoclonal Mast Cell Activation Syndrome (MMAS). We evaluated 53 patients (36 males, 17 females), median age 45 years (range 19-77), 44 with indolent SM (ISM) and 9 with MMAS. Five patients with lumbar spondylosis were excluded from spine analysis. BMD of lumbar spine (L1-L4) was reduced in patients with clonal MCD as compared to age- and sex-matched healthy subjects (mean Z-score -0.92±1.12 and -1.14±1.09 SD for ISM and MMAS, respectively), while BMD at total hip or femoral neck was not significantly affected in both subgroups. However, there was a wide variation of Z-score at lumbar spine among all patients, ranging from -3 and +1.9 (Figure 1). Osteoporosis (T-score < -2.5) was present in 15% of patients at lumbar spine (12% and 18% in subjects < or ≥45 years, respectively), and in 7% at femoral neck. Median Z-score of patients with lumbar osteoporosis was -2.4 (range -1.5 - -3), indicating a severe reduction of BMD. These results support the usefulness of DXA examination in all patients with clonal MCD and suggest that osteoporosis is not infrequent, in particular at vertebral site. Further studies are needed to elucidate the clinical and bioumoral parameters associated with the risk of osteopenia and fractures.

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PROLIFERATIVE VARIANT OF CMML EMERGING DURING TREATMENT WITH IMATINIB IN A PH+ CML PATIENT IN COMPLETE CYTOGENETIC REMISSION: A CASE STUDY POINTING ON THE ROLE OF RAS MUTATIONS ACQUISITION

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We report the case of a 73-year-old man who died from CMML while being in cytogenetic complete remission (CCyR) of a previously diagnosed Ph+ CML On 04/2004, the pt was diagnosed with chronic phase CML, intermediate risk. After 1 month of standard dose Imatinib (IM) he achieved hematological CR. After 6 mos CCyR was documented but the CBC count showed 24x10^9/L WBC with 21% monocytes, pointing to a diagnosis of CMML, proliferative variant (MP-CMML). IM was maintained and a major molecular response was achieved in 12 mos. In the following 24 months WBC remained stable and CCyR was repeatedly confirmed. In 01/2007 a molecular remission was documented but an increase of WBC was noted. No change in therapy was made until 02/2008, when the WBC further raised up to 70x10^9/L and cutaneous leukemic lesions appeared. A resolution of the lesions and a normalization of the WBC count was obtained in 4 weeks with the addition of HU, eventually stopped 2 months later. On 06/2008 the pt was hospitalized due to renal failure, fever and bone pain. A spine MRI showed the presence of para-vertebral masses. HU was resumed, achieving complete resolution of symptoms and a significant reduction of the spine lesions. However, a few weeks later the pt progressed to AML, and eventually died 3 weeks later from MOF. In 10/2007, direct sequencing of the RAS genes was performed from a PB sample, identifying a NRAS G12R mutation. Hence, we retrospectively analyzed different PB samples to establish when the mutation firstly appeared. No mutation was detected at the time of CML diagnosis. Unexpectedly, the presence of a different NRAS mutation (G13V) was unveiled on a sample from 07/2004 and confirmed up to 10/2007, when this mutation was replaced by the G12R. The G12R allele was found also in the DNA from the cutaneous lesions. To investigate further, we set up an allele-specific PCR (ASP) for each of the 2 mutations identified, and rescreened all samples. Apart from confirming the sequencing results, ASP unveiled the presence of the G12R 30 months before sequencing, and allowed the detection of the G13V up to 10/2007, when sequencing showed only the G12R. This is the first report of a case of CMML emerged during treatment with IM in a CML pt in CCyR and possibly driven by the occurrence of a NRAS mutation. Our molecular analyses allowed to detect a second mutated clone, with higher proliferative feature, that gradually became predominant preceding transformation to acute leukemia.

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ROLE OF THE TYROSINE KINASE PROTEIN C-ROS IN THE PATHOGENESIS OF CHRONIC MYELOMONOCYTIC LEUKEMIA

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The abnormal activation of tyrosine kinases are a common finding in chronic myeloproliferative disorders. c-Ros is an orphan RTK displaying

transforming activity whose role has been established in the development of neuronal neoplasia. The aim of this study was to evaluate the involvement of c-Ros in the pathogenesis of chronic myelomonocytic leukemia (CMML) and to establish the effects of c-Ros activation. c-Ros expression was evaluated by RQ-PCR in 133 samples collected from 96 CMML at diagnosis (96 BM and 37 PB) and 60 healthy donors (30 PB and 30 BM). The protein amount and localization was analyzed by westen blot and immunofluorescence assay. To establish the effects of c-Ros activation we generated a chimeric receptor containing the extracellular domain derived from epidermal growth factor receptor (EGFR) and the transmembrane and cytoplasmic domains from c-ros (ER). The chimeric receptor was then transfected in NIH3T3 and HEK293T cells. Transfected and control cells were then stimulated with 100 nM EGF ligand and proliferation and apoptosis evaluated by incorporation of 3H timidine and MTT assay and by annexin V measurement. Ros is undetectable in healthy subjects but it is overexpressed in CMML (p<0.0001) in both BM and PB cells with a median value of 2- Ct in BM of 380 (range 10-63303) and 212 in PB (range 6-30012). WB confirmed the presence of c-Ros protein in CMML cells but not in normal controls. Immunofluorescence localized the protein in the cytoplasm. ROS is highly expressed in $CD34^+$ cells and monocytes from CMML patients but not in their normal counterparts. Sequence analysis revealed the absence of mutations of c-Ros promoter. SNPs analysis exclude the presence of duplications or deletions of the gene. Moreover we found that the EGF induced activation of c-Ros affects proliferation by increasing of 3.5 folds the proliferation rate as compared to cells transfected with the empty vector and stimulated with EGF under the same conditions. Furthermore cell adhesion was 4 folds decreased as compared to control. By contrast apoptosis is not affected by c-ROS activity (p=0.2). This study demonstrates that c-Ros is abnormally expressed in patients with CMML. The abnormal activation of c-Ros is responsible for loss of adhesion and increased proliferation. In conclusion, we identified a new tyrosine kinase which may be responsible for the proliferation defect typical of CMML cells and could represent a target for molecular therapies.

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GENE EXPRESSION ANALYSIS OF THE KINOME AND PHOSPHATOME IN CHRONIC MYELOPROLIFERATIVE DISEASES

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Background. Ph negative Chronic Myeloproliferative Disorders (CMPD) are likely characterized by a deregulated tyrosine kinase (TK) activity which is well identified and characterized only in a small subset of patients. The possibility to identify additional TK involved in the pathogenesis of CMPD and in CML progression possesses a relevant clinical significance since it offers the possibility to design new molecules able to inhibit in a selective manner the specific target, giving the patient the possibility to be treated with a new molecular approach. Furthermore, the involvement of tyrosine phosphatases is becoming more evident. *Aim*. The aim of the present study was to identify the presence of activated TKs or deregulated phosphateas in CMPD and in CML progression through a gene expression analysis based on Real Time PCR of the kinome and phosphatome. Methods: We analysed the expression level of 95 genes coding for TK and 15 phosphatases in 30 patients affected by Ph- CMPD, 20 CML in chronic phase and blast crisis and 10 BM samples obtained from healthy volunteers. The quantitative analysis was performed using the ABI Prism 7900 (Applied Byosystems) using the micro fluidic card and the assays-on-demand system. The series of the patients studied included CMML, chronic eosinophilic leukemias(CEL), primary myelofibrosis and CML patients in both chronic phase and blast crisis. The final value of expression has been calculated using the software SDS 2.1. *Results*. This study allowed to identify 30 different genes which are aberrantly expressed in CMPD. The majority of them were upregulated and few of them significantly downmodulated. The selected genes have been then clustered based on their biological significance. A further analysis allowed us to select 6 TK genes which appeared to be of particular interest in different subgroups of patients. In addition 4 phosphates have been identified as involved in CMPD or 3 in CML progression. These genes have been further studied in an enlarged series of patients and their role established by *gain and loss of function* experiments. *Conclusions*. This study allowed us to establish a pattern of expression of TK and phosphatases and to identify genes involved in the pathogenesis of CMPD and in CML progression.

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T/NK LYMPHOCYTOSIS IN CML PH+ PATIENTS TREATED WITH DASATINIB

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Background. Some authors reported that Natural Killer (NK) cells from CML patients are defective in NK cell activity and NK cell numbers decrease as the disease progresses to the advanced phase and probably the abnormal BCR/ABL gene causes abnormal NK cell differentiation.TK inhibitors reduce BCR/ABL transcription and could restore NK cell numbers and/or function. Moreover Dasatinib,by the blockade of SRC Kinases,could affect the development of NK cells as well as T-lymphocytes.Our aim was to verify the impact of Dasatinib treatment on T $CD8^+$ and NK cells modulation. *Methods*. We evaluated 21 patients with CML resistant/intolerant to Imatinib and treated with Dasatinib at a starting dose of 70 mg/BID or 100 mg/QD.Blood count was monitored; lymphocytosis has been definited by an increased number of peripheral blood lymphocyte counts > $3.0x10^9$/L and by the predominance of LGLs in peripheral blood smear. Immunophenotyping was done with flow-cytometry using antibodies against the following antigens:CD2,CD3,CD4,CD5,CD7,CD8,CD16,CD56. Results.With a median of 19 mo. of Dasatinib therapy (range 3-43),13/21 cases(62%) developed peripheral blood lymphocytosis. Median onset of lymphocytosis was 3 months after the start of Dasatinib therapy (range 1-12) and duration was 14 months (range 6-40). Lymphocytosis was $CD3^+$/$CD8^+$/Cytotoxic T Cell in 8 patients (61.5%) and $CD3^-$/$CD16^+$/$CD56^+$/NK Cell in 5 patients (38.5%). In all 13 patients no symptoms or signs suggestive of LGL leukemia or viral infections were documented.There was no significant difference in terms of the frequency of severe adverse events, including pleural effusion between patients with and without lymphocytosis. 9(69.2%) of the 13 patients who developed lymphocytosis achieved MMolR and 4/9 presented Bcr/Abl mutation at the time of pre-dasatinib treatment (F317L,E255K,F359V,E255K),whereas only 3(37.5%) of the 8 patients without lymphocytosis achieved MMolR and 1/3 presented Bcr/Abl mutation (F359V). Moreover molecular response was earlier in the group of patients with lymphocytosis (8vs12 mo.). *Conclusions*.The development of lymphocytosis in our patients seems to be associated to an improved response to dasatinib in terms of molecular response and in terms of timing to response. The assessment of higher frequency lymphocytosis requires further analysis;a larger cohort of patients is needed to explore the biological role of lymphocytosis and the impact on the long term outcome in patients treated with dasatinib.

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REDUCED SURVIVAL IN PATIENTS WITH PRIMARY MYELOFIBROSIS IS PREDICTED BY A LOW BURDEN OF JAK2V617 MUTATED ALLELE

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The clinical relevance of JAK2V617F mutation in patients (pts) with primary myelofibrosis (PMF) is still under debate. Aiming to evaluate the impact of JAK2 V617F on disease progression and outcome in PMF, we measured V617F allele burden by real-time PCR in granulocyte DNA from of 186 PMF patients who were evaluated at the time of diagnosis, before any treatment. JAK2V617F mutation was found in 68.2% of the pts; median allele burden was 54% (range 5-100%). Frequency of patient according to V617F burden was 13.3% in the first quartile, 33.8% in the second, 24.4% in the third, and 28.3% in the fourth. JAK2V617F-mutated pts had significantly higher leukocyte (p=0.009) or platelet (p=0.02) count and hemoglobin level (p<0.0001) compared to wild-type (WT); anemic pts (hemoglobin <10g/dL) were significantly less among JAK2V617-mutated (22% versus 44%, p<0.003). JAK2V617F mutated

pts were preferentially found in the Dupriez low risk category (p=0.017), while there was no difference according to risk categories of Cervantes score. Time to anemia and leukopenia was significantly longer in JAK2V617F-mutated patients compared to WT. Time to both anemia and leukopenia was significantly shorter in mutated pts belonging to the lower quartile as compared to patients in upper quartiles (p<0.02) as well as to WT pts (p<0.017). and conversely, time to large splenomegaly was longer compared to upper quartiles (p=0.02). After a median follow-up of 17.2 months, 23 pts (12.3%) had died, 15 of whom because of leukemia. A JAK2V617F mutated genotype did not impact on leukemia transformation or OS. In multivariate analysis OS was predicted only by age (p<0.0001) and blasts >1% (p=0.02). However, OS was significantly reduced in patients in the lower V617F quartile as compared to ether mutated pts with >25% allele or WT ones. In multivariate analysis that included patient stratification according to V617F allele burden, factors significantly associated with reduced survival were age (p=0.001), a blast count >1% (p=0.006) and a JAK2V617F burden <25% (p=0.002). Causes of death in the 1-25% quartile were represented by systemic infections or sepsis (n= 5) and multi-organ failure (n= 1), while no case of leukemia transformation was observed. In conclusions these data indicate that a low JAK2V617F allele burden in PMF represents an independent factor associated with shorter survival possibly because of the consequences of bone marrow failure rather than leukemia transformation.

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LEUKOCYTOSIS AND THROMBOSIS IN MPNS: A RETROSPECTIVE SINGLE CENTER STUDY ON 203 PATIENTS

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The Ph negative myeloproliferative neoplasms (MPNs) are a heterogeneous group of disorders characterized by excessive production of blood cells by hematopoietic precursors. The major cause of mortality and morbidity are thromboembolic complications, which occur more frequently in older subjects or in those with previous thrombosis. Identification of new risk factors for venous and arterial thrombosis is an enduring theme. Age and previous history of thrombosis are the two well established risk factors and more recently leukocytosis has been identified as a new predictor on thrombosis development. We aimed to define if leukocytosis at diagnosis is associated with an increased incidence of thrombosis in a cohort of 203 patients (pts) with Ph negative MPNs. We retrospectively evaluated 203 pts diagnosed according to WHO criteria (17 idiopathic myelofibrosis (IMF), 159 thrombocythemia (ET), 27 unclassifiable myeloproliferative neoplasms (MPNs-U). The mean age was 68 17 years, the mean follow up was 45.8 4.9 months. At diagnosis the pts were classified as intermediate-low (LIR) or high (HR) thrombotic risk according to standard risk factors (age 60 years and/or a previous major thrombotic event). LIR pts were managed only with antiplatelet drugs while HR started both cytoreductive and antiplatelet drugs. 63 LIR and 96 HR ET, 4 LIR and 13 HR IMF, 16 LIR and 11 HR MPNs-U pts, showed the following haematological data in IMF, ET and MPNs-U respectively: Ht: 41.4% 5.8; 43.6% 4.5; 43.9% 4.1; WBC 9,2 2,4x10^9/L; 8.7 2,4x10^9/L; 8.6 2,5x10^9/L; Plt 818.4 430x10^9/L; 707.1 210x10^9/L; 784 244x10^9/L. The mean WBC count in 73 pts with major thrombosis at diagnosis or during follow up was 9.2 2.6x10^9/L. In the remaining 130 pts the WBC count was 8.4 2.3x10^9/L. On univariate analysis performed on whole cohort, the WBC count was not associated with an higher thrombotic risk (p=0.0389; 95%CI: 0.007525 to 0.2773). We found a significant correlation between WBC count over 9.2 x10^9/L and frequency of thrombosis only in the LIR group (p<0.002, 95%CI 2.04 to 18.4, OR 6.13 vs. p 0.35, 95%CI 0.71 to 3.00, OR 1.46). Finally, the correlation between WBC count and thrombosis on univariable analysis was retained in LIR ET subgroup (p<0.0003, 95%CI: 0.0089 to 0.09) but not in HR ET patients, IMF and MPNs-U ones. Our data highlight the correlation between WBC count at diagnosis and thrombotic risk especially in low and intermediate risk pts.

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FLUORO-CYTOMETRIC VALUATION OF PLATELET MEMBRANE GLYCOPROTEINS IN PATIENTS AFFECTED BY CHRONIC MYELOPROLIFERATIVE DISORDERS

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Incoming from the consideration that emorragic and thrombotic events represent the most frequent complications, with greater impact to morbidity and mortality for patients affected by CMD (Chronic Myeloproliferative Disorders), we have focus our attention on platelets, in particular on the expression of some specific structural elements of theyr plasmatic membrane, that is components IIb and IIIa of the glicoproteic complex IIb/IIIa and component Ib of glicoproteic complex IX/Ib /Ib /V. These two glicoproteic complexes are very important for platelet activity: the first is a fibrinogen receptor (GP IIb/IIIa), the second is a von Willebrand's factor receptor (GP IX/Ib /Ib /V). We used flow cytometry to determinate the expression of these glicoproteins, through monoclonal antibodies specifically directed to these ones. Moreover we have determined platelets activation state through a monoclonal antibody directed to P-Selectine (CD 62p). In the analysis of results we have considered an eventual diversity of expression linked to the presence of Jak2 V617F mutation, found through Multiplex PCR. Significative differences between Essential Thrombocytemia (ET) and True Policytemia (TP) patients and controls (Table 1A) in the expression of these glicoproteic components are not found. From our studies doesn't neither results a direct correlation between Jak2 mutation and structural alterations of these membrane components (Table 1B). We signale a tendency to a decrease of the expression of these three glicoproteins in patients affected by CML rather than ET and TP patients, probably provoking a lower frequency of thrombotic manifestations in CML rather than ET and TP. Myelofibrosis patients have a similar behaviour to CML patients. About P-Selectine, patients result frequently negative; only in one of the few positive cases, P-selectine expression was associated to important thrombotic events. According to collected data, the result of platelet membrane glicoproteins expression in ET and TP would justify thrombotic risk in patients affected by these pathologies. Lower values in CML, that would suggest minor frequence of thrombotic risk, instead need a more extensive valuation.

Table 1a.

	CONTROLLI	CML	TE	TP	MI
%GP IIb	87,8	79,68	88	89,6	76
%GPIb,	71,4	61,62	74,8	69,9	67,5
%GPIIIa	87,9	81,12	85,3	90,1	74,2

Table 1b.

GLICOPROTEINE	TE JAK2 NEGATIVE	TE JAK2 POSITIVE
%GP IIb	88,68	87,5
%GPIb,	75,4	70,15
%GPIIIa	87	86,52

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CLINICO-PATHOLOGICAL EVALUATION OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE: PRELIMINARY RESULTS.

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Background. in the natural history of essential trombocythemia (ET) a myelofibrotic evolution occurs in a small percentage of cases. The use of cytoreductive molecules could have a further pathogenetic effect in increasing myelofibrosis. *Objectives*. to evaluate the main clinico-pathological parameters, with particular interest for the picture of bone marrow biopsy (BMB), in a series of ET patients treated with anagrelide (ANA). *Material and Methods*. a series of 180 ET patients of the RIT, treated with ANA and with available specimens of BMB at diagnosis, were considered for this retrospective study. Additionally, the available specimens of BMB performed before, during and after ANA treatment were obtained. The diagnosis validation following the WHO 2008 criteria has been performed till now in 80 cases diagnosed before the year 2003 according to the PVSG criteria. The Panel of Pathologists and Clinicians of the RIT revised the specimens of BMB at diagnosis and, in nine of them, also of BMB done before, during and after ANA therapy. *Results*: the 80 analysed patients, 34 males and 46 females with a mean age of 37 years (range 13-82), had a mean platelet (PLT) count at diagnosis of $1097x10^9$/L. The patients were reclassified as follows: 24 (30%) ET, 41 (51.3%) initial PMF (primary myelofibrosis) of which 28 with grade 0 fibrosis, 5 (6.2%) overt PMF, and 10 MPD-U (myeloproliferative disorder-unclassifiable). The nine patients with sequential BMB were validated as ET in six cases and as initial PMF in the other three cases. At distance from diagnosis of 0-224 months (median 96) they started a treatment with ANA, whose duration was 4-93 months (median 48). The BMB picture at baseline and after 4-57 months (median 29) of ANA treatment was the same in the six cases of ET, while in the three cases of initial PMF the BMB picture became that of overt PMF, with evident clinical myelofibrotic evolution in one case. *Conclusion*. It is confirmed that ET diagnosis done according the PVSG criteria is confirmed on the basis of the WHO criteria in 1/3 of cases, while a bone marrow picture of initial PMF is observed in about fifty percent of cases. Moreover, a possible relationship between myelofibrotic evolution and a treatment with cytotoxic drugs as ANA, IFN, or HU needs to be evaluated having that as primary endpoint of a controlled randomized study.

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FAMILIAL CHRONIC MYELOPROLIFERATIVE DISORDERS

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Chronic myeloproliferative neoplasms (CMNs), which include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), have a sporadic occurrence . Small studies of familial clusters of CMNs have been reported. Familial chronic myeloproliferative neoplasms are defined when in the same pedigree at least two relatives have a chronic myeloproliferative neoplasms. Familial CMNs should be distinguished from inherited disorders because of Mendelian transmission, high penetrance and polyclonal hematopoiesis, the latter are named "hereditary erythrocytosis" and "hereditary thrombocytosis". Recently, it has been reported a 5-to 7-fold higher risk of MPN among first-degree relatives of patients with MPNs. These findings give support to the results of small studies which are in favor of familial clustering in MPNs. The analysis of mutations (V617F), exon 12 of JAK2 and MPL improves our ability to discriminate among these conditions. In our series, among 290 patients with sporadic CMNs and 80 chronic myeloid leukemia, the prevalence of familial cases was 3% With 8 pedigrees, 16 patients (6%) were identified with two relatives affected.Allocated to familial CMNs were 5 PV, 6 ET, 5PMF, 1 CML as compared to 75 PV, 114 ET, 101 PMF, 80 CML sporadic cases. Regarding the clinical phenotype within the familial clusters, only 3 of 8 families showed a homogeneous pattern, which means the same disease in all affected relatives (PV1 family and ET 2 families). Fourteen families exhibited a mixed phenotype among PV, ET and PMF. According to JAK2 (V617F) mutational status within families, 2 families (2 pedigrees PV and 2 pedigrees TE) were positive pattern. 6 families showed a heterogeneous pattern; they included both JAK2 (V617F)-positive and JAK2 (V617F)-negative patients. Among the 16 patients JAK2 (V617F) mutational status was present in the 100% of PV, 60% of TE and 75% of PMF; homozigosity was present in PV only. If pedigree was in female clinical phenotype at diagnosis had prevalence for PV, while prevalence for TE was present in mix pedigree. Finally, we describe one family with chronic myeloid leukemia (CML) and PMF JAK2 positive among two brothers and one family with PMF and PV in mother and son. In our series, clinical presentation, therapeutic approach and complications were similar in familial and sporadic cases. This study suggests the importance of family history as part of the initial work-up of patients with CMDs and in this setting biological studies, single nucleotide polymorphism (SNP) screening can be indicated.

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THE SUBJECTS WITH APPARENT ERYTHROCYTOSIS IN JAK2 ERA MAY BE QUALIFIED AS VOLUNTEERS BLOOD DONORS?

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Introduction of molecular biology methods in the clinical practice defined the new diagnostic criteria for the polycythemia vera (PV). The diagnostic work-up, which in the past was very difficult according to minor and major criteria, was simplified with the identification of JAK2 mutation in the exon 12 and 14 (WHO 2008): now this marker allows the identification of patients with PV. Objective of this study is to evaluate clinical and biological characteristics in patients with erythrocytosis and without criteria for PV diagnosis, namely apparent erythrocytosis (EA). We excluded from this cohort JAK2 negative patients, who are affected by familial chronic myeloproliferative neoplasms (CMNs) and secondary erythrovytosis. We studied 102 patients, 61 with PV in accordance both to PVSG criteria and its modifications (Person 1996 e P.J. Campbell ASH 2005), and to WHO 2008, and 41 with EA. Follow-up at three years of JAK2 negative patients with EA was performed and none of these patients became positive. We evaluated the following parameters: red cells mass, spleen dimension, vitamin B12, WBC count, platelets count, Hct values, bone marrow biopsy, spontaneous growth of erythroid colony, EPO serum levels and JAK2 mutations. The two patients category (PV and EA) had very different clinical and laboratory characteristics. In EA patients red cells mass was lower than in PV patients (mean 28.5±3 ml/Kg vs 42.5±7) (p=0.0001). No EA patient had splenomegaly, leukocytosis or thrombocytosis (p=0.0001). At the diagnosis, the mean Hct values were lower in EA subjects (51±2 vs 58±5) (p=0.0001); like wise spontaneous growth of endogenous erythroid colonies were lower. In accordance, the difference between erytrhopoietin levels was statistically significant (8,6±8 vs 24±14) (p=0.0001). The histopathological examination of the bone marrow biopsy, with respect to hyperplasia and bone marrow cellularity, allowed a clear distinction between PV and EA. No patient, classified as EA, showed JAK2 mutation on the 12 and 14 exon, both in the diagnosis and in the subsequent 3 years follow-up. Patients with PV not treated with chemotherapy and EA subjects, annually performed venesections in aimed to keep an hematocryt value around 45 %; they received a mean of 7 and 4 annual venesections, respectively. We can discuss if EA subjects can be proposed as erythroapheresis blood donors, with a double donation index as compared to normal donors.

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THE LONG-TERM DURABILITY OF MOLECULAR RESPONSES IN PATIENTS WITH FIP1L1-PDGFRA CHRONIC HEOSINOPHILIC LEUKEMIA TREATED WITH IMATINIB: THE ITALIAN HES0203 EXPERIENCE AFTER A 4-YEAR FOLLOW-UP

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Imatinib mesylate is the first line treatment for hyperosinophilic syndrome with FIP1L1-PDGFRα (F/P) fusion gene. Few clinical and molecular data on the outcome of patients with FIP1L1-PDGFRA positive CEL treated with imatinib are available to evaluate the long term follow up of patients and to evaluate the clinical correlation with differente transcripts of fusion gene. A prospective phase 2 multicenter study of the use of imatinib 400 mg/daily in patients with hypereosinophilic syndrome, irrespectably of F/P status was established in 2001. 72 patients were treated with IM 100 to 400 mg daily; the 33 F/P positive patients (F/P+) were regularly monitored with nested RT-PCR. The observation period of F/P+ patients ranges between 23 and 85 months (median 48 months). There were 32 males and one female patient. Organ involvement was recorded in 42% of F/P+. After imatinib therapy all patients achieved a complete hematologic response (CHR) in less than one month, and PCR negativity in a median time of 3 months. They became negative for organ localizations and free of symptoms. All patients who continue imatinib therapy remain in CHR and RT-PCR negative, with a dose of 100 to 400 mg daily. From September 2007 all patients except one (late responder) were treated with 100 mg daily. In six patients IM treatment was discontinued for variable period for different reasons, and in 5 cases the fusion transcript became rapidly detectable. CHR was maintained, other than in one case. The transcript was again undetectable upon treatment resumption, other than in one case. All samples were valuable for molecular analysis. Fusion gene sequencing demonstrate an extreme variability of FIP1L1-PDGFRA junction sequences, but with no correlation with kinetic of molecular response or with the presence at diagnosis of peculiar organ involvement. More complexity of transcript is noted in patients with longer history of disease prior to imatinib therapy. With this large series of patients we can confirm the extremely sensitivity of F/P+ CEL to imatinib therapy, without any significant toxicity after protracted therapy and without acquisition of resistance. The complexity and variability in FIP1L1-PDGFRα transcripts seems to no correlate with phenotype of disease, even though different kinetic of response have been observed. Prolonged clinical and molecular follow-up of these patients is essential to understand the CEL disease.

Autologous Transplantation

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BUSULFAN-MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN AML PATIENTS IN FIRST CR: A "GRUPPO ITALIANO TRAPIANTO DI MIDOLLO OSSEO (GITMO)" RETROSPECTIVE STUDY

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Acute myelogenous leukemia (AML) patients (=129; median age =50 years; range 16-72) in first complete remission (CR) received busulphan and melphalan (Bu/Mel) as conditioning regimen prior autologous stem cell transplantation (ASCT). Eighty two patients (63.6%) received peripheral blood stem cells (PBSCs) and 47 patients (36.4%) received bone marrow (BM) cells. Cytogenetic categories distribution was conventionally defined as favorable (15.5%); intermediate (60.1%) and unfavorable (24.3%). With a median follow-up of 31 months, the 8-years projected overall survival (OS) and disease-free survival (DFS) was 62% and 56% for the whole population, respectively. The relapse rate was 46% and the non-relapse mortality was 4.65%. Although PBSC transplantation led to a faster hematological recovery than BM transplantation, in univariate analysis the stem cell source, cytogenetics and different busulphan formulations did not significantly affect OS and DFS whereas age and the number of post-remission chemotherapy cycles did have significant impact on the clinical outcome. Multivariate analysis identified age < 55 years as the only important independent predictor for OS and DFS. Our data suggest that Bu/Mel is an effective conditioning regimen even for high risk AML patients in first CR undergoing ASCT being associated with a low toxicity profile (mainly mucositis) and mortality.

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A PHASE II MONOCENTER STUDY OF MODIFIED BEAM REGIMEN FOR HODGKIN'S DISEASE AND NON-HODGKIN'S LYMPHOMAS

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The intensive therapy followed by an autologous transplant of hematopoietic stem cells (HSCs) is and has been widely used for the treatment of Hodgkin's and non-Hodgkin's lymphoma, with the intent of increasing the number of patients cured in first line treatment with high risk disease including those non completely responsive to first line treatment or relapsed cases. For over 25 years the therapy most widely used as conditioning regimen for autologous HSCs transplant of lymphomas is the BEAM protocol, which foresees the administration of Nitrumon 300 mg/sm day -6, Cytosine Arabinoside (Ara-C) 200 or 400 mg/sm and Etoposide 200 mg/sm once a day from day -5 to day -2 and Melphalan 140 mg/sm day -1. Ara-C, when used in the scheme of therapy for the treatment of lymphomas it is administered in high doses (1-2 gr/sm) because its known to be more effective. With the intention to increase the efficiency of the BEAM protocol, we have modified the treatment schedule accordingly: Nitrumon 300 mg/sm day -5, Ara-C 2 gr/sm and Etoposide 200 mg/sm once a day for 3 days from -4 to -2 and Melphalan 140 mg/sm day -1. The aim in this first phase of the study was to evaluate the toxicity of the treatment and the resumption of the myelopoiesis. From April 2007 to January 2009 we treated 20 patients

with Hodgkin's disease and non-Hodgkin's lymphoma. All patients received HSCs from peripheral blood with an average of 6,3x10^6 cells/Kg (range 3,6-14,1) and all patients received granulocyte growth factor (Lenograstim) from day +6. Patients characteristics, toxicity and hematological recovery are shown on the table below. The treatment did not cause intense nausea or vomiting, the mucositis and transfusion requirement were not high. A patient with relapsed-resistent high grade non-Hodgkin lymphoma, heavily pre-treated and at high risk of infection for paraplegia and implanted bladder catheter died from sepsis at day +7. The hematological resumption resulted as that of the traditional BEAM protocol. Hospital discharge was possible after 11-17 days after the transplant. The modified BEAM protocol with high dose Ara-C resulted a well tolerated treatment with no higher toxicity respect to traditional BEAM and could be used as conditioning regimen in autologous HSCs transplant for Hodgkin's and non-Hodgkin's lymphomas. The therapeutic result of the treatment would be to verify on a larger group of patients.

Table.

PAT	SEX	DIAG	STATUS	CD34+/KG	G-CSF (days)	FEVER	TRANSF RBC/PLT	MUCOSITIS/ GRADE WHO	PMN>500 (days from ABMT)	PLT>20000 (days from ABMT)	DISCHARGE (days from ABMT)
1	F	NHL	PR	10	6	NO	2/1	YES/I	11	11	12
2	M	NHL	CR	3.8	7	NO	0/2	NO	13	13	13
3	M	HD	PR	8.1	6	NO	0/1	NO	11	9	12
4	M	NHL	PR	14.1	8	NO	0/1	NO	11	9	12
5	F	NHL	CR	4	9	YES	0/1	YES/II	11	10	14
6	F	NHL	PR	6	7	NO	3/1	NO	12	12	14
7	F	NHL	CR	5.8	8	YES	2/1	YES/III	11	11	14
8	M	NHL	CR	6.3	7	YES	0/3	NO	12	NR	13
9	M	NHL	PR	6.3	NV	YES	4/2	YES/I	NE*	NE*	NE*
10	M	NHL	CR	5.7	7	YES	4/3	NO	12	12	14
11	F	NHL	PR	3.7	7	YES	0/2	NO	12	12	17
12	M	NHL	PR	6.3	8	NO	3/2	YES/I	11	11	13
13	M	NHL	CR	8.6	7	YES	1/2	YES/III	10	12	13
14	M	HD	PR	6	9	NO	1/4	YES/I	12	14	14
15	F	NHL	CR	5	6	YES	3/2	YES/I	10	11	16
16	F	NHL	RES	3.6	11	YES	9/10	YES/III	13	15	16
17	M	NHL	CR	4	7	NO	0/1	YES/I	11	11	12
18	M	HD	CR	10.8	7	NO	0/1	NO	12	12	12
19	M	LNH	CR	6.8	7	NO	0/2	YES/I	10	11	11
20	M	LNH	PR	5.5	6	YES	0/2	YES/I	11	11	13

** Died on day +7 from ABMT*

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REINFORCED MINI-BEAM FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN REFRACTORY LYMPHOMAS: A STUDY OF FEASIBILITY AND EFFICACY

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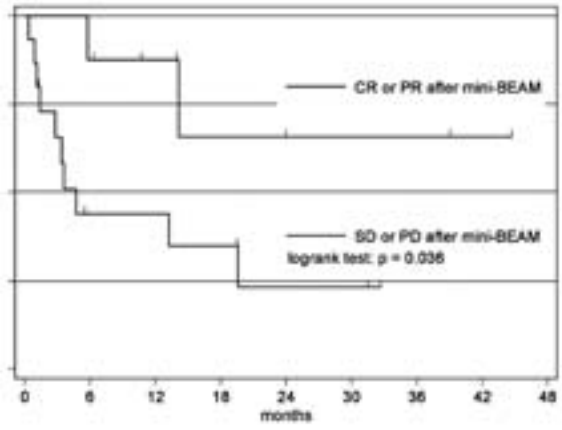
Introduction. Autologous stem cell transplantation (ASCT) is the standard of care for refractory-relapsed lymphoma patients (pts), but high tumor burden at transplant impairs outcome. We tested efficacy and feasibility of reinforced mini-BEAM followed by stem cell infusion (mini-ASCT) as extreme debulking attempt in pts with disappointing response to previous salvage therapy. *Patients and Methods.* Eleven Hodgkin's lymphoma and 15 aggressive non Hodgkin's lymphoma pts received mini-BEAM therapy (BCNU 100 mg/m^2 on d-6, VP-16 75 mg/m^2/d and Ara-C 200 mg/m^2/d from d-5 to d-2, Melphalan 100 mg/m^2 on d-1) followed by autologous stem cell infusion. Median age was 44 years (range 20-67). Eighteen pts (69%) were primary refractory, while 8 pts (31%) relapsed. All 26 pts had an adequate stem cell harvest; after a minimum of two previous salvage regimens, 11 pts (43%) presented with stable disease and 15 pts (57%).with progressive disease (PD). *Results*. Median number of CD34$^+$ cells infused was 6.7 106/kg (range: 1.4-20). Median time for neutrophils and platelets recovery was 9 (range 7-15) and 11 days (range 8-15). Twelve pts (46%) had fever with 2 documented infections. Four pts (15%) experienced grade 2 mucositis. Overall response rate (ORR) to mini-ASCT was 35% (CR 8%, PR 27%), while 10 pts (38%) maintained SD and 7 pts (27%) progressed. Standard ASCT was performed in 14 pts; 12 pts were excluded because of poor conditions or progressive disease. Response to ASCT, evaluable in 12 pts, was CR in 5 pts (42%) and PR in 7 pts (58%). Considering all the 26 pts, after a median follow-up of 11.5 months median overall survival (OS) and event-free survival (EFS) are 17 and 14 months, respectively. Separating mini plus standard ASCT pts from mini-ASCT pts, median OS and EFS are not reached in the first cohort, while are 5 and 3 months respectively in the second cohort. Both differences in OS and EFS are statistically significant (logrank test: p=0.014 and p=0.005). At univariate analysis, response to mini-BEAM is associated with longer EFS (p= 0.036) (Figure 1). Discussion: Reinforced mini-BEAM with stem cell rescue is a safe strategy for pts with refractory lymphoma who failed prior salvage therapies. In this unfavourable setting of pts ORR was encouraging. Survival data need to be confirmed with longer follow-up but indicate an advantage for pts responding to mini-BEAM and subsequently treated with standard ASCT, which must be performed in a tandem-fashion whenever possible.

Figure 1. Event-free survival of patients responding to mini-BEAM versus patients not responding to mini-BEAM.



P417

AUTOLOGOUS STEM CELL TRANSPLANTATION IN OLD MULTIPLE MYELOMA PATIENTS

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Feasibility of autologous stem cell transplant (ASCT) in multiple myeloma (MM) patients older than 65 years is still controversial, while ASCT is considered the standard treatment between 60 and 65 years. To assess the differences in toxicities and survival in these two groups, we analyzed the data of 47 consecutive symptomatic MM patients aged >=60 years who received ASCT in our institution between 2001 and 2008. Overall (OS) and progression free survival (PFS) were analyzed by Kaplan-Meier method, cumulative incidences (CI) by CI method and correlations by Spearman rank test. Response and toxicities are defined as per IMWG criteria and CTCAEv3. Median age was 64 years (range 60-74), 22 patients were >65 years old (47%), 22 were male (47%). Thirty-nine (83%) patients received ASCT as first line, 8 (17%) at relapse. Twenty (43%) patients received an induction with bortezomib, 9 with thalidomide (19%), 18 with VAD (38%). Five patients received one ASCT (11%), 39 (83%) and 3 (6%) received 2 and 3 ASCT respectively. Melphalan dose was 200mg/m^2 in 16 patients (34%), 100 or 140mg/sqm in 27 (57%) and <100 mg/m^2 in 4 patients (9%) according to age and co-morbidities. Median follow-up was 25 months (3-78). Neutrophils >500/mcl and platelets >20000/mcl were achieved at a median of +10 days after ASCT. Four patients (9%) achieved stringent complete response, 12 (26%) complete response, 16 (35%) very good partial response, 7 (15%) partial response and 8 (17%) stable disease. One-year OS was 92%, 2- and 3-year OS were 88%. One-, 2- and 3-year PFS were 72%, 49% and 32%. CI of relapse was 26%, 44% and 65%

at 1, 2 and 3 years. OS, PFS and CI of relapse were not significantly affected by Durie-Salmon stage, bortezomib in induction therapy, and ASCT as first line. Grade (G) 2 toxicity was observed in 14 (15%) and G3 in 25 (27%) of the total 93 ASCT performed. No G4 toxicities were observed. Main G2 toxicities were nausea (5 ASCT, 5%) and mucositis (4 ASCT, 4%). Main G3 toxicities were infection (14 ASCT, 15%) and febrile neutropenia (7 ASCT, 7%). Age>65 years did not significantly impact OS (p=0.88) nor PFS (p=0.64), and older age was not significantly correlated with toxicity (rho=-0.05, p=0.70). In conclusion ASCT with melphalan dose adjusted on the basis of age and co-morbidities is a feasible treatment option for MM patients beyond 65 years. In our cohort of patients a good anti-MM activity was observed and the treatment was not hampered by an increase in toxicity.

P418

SAFETY AND EFFICACY OF ZEVALIN COMBINED WITH HIGH-DOSE BEAM AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR THE TREATMENT OF RELAPSED-REFRACTORY NON HODGKIN LYMPHOMAS: RESULTS OF AN ITALIAN MULTICENTER STUDY

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90Y-Zevalin® combined with high dose therapy and autologous stem cell transplantation (ASCT) is gaining increasing importance for relapsed/refractory non Hodgkin Lymphoma (nHL). We evaluated the feasibility and clinical results of the addition of Zevalin at standard dose to BEAM regimen in patients who failed to achieve complete remission (CR) after first line chemotherapy. Methods. Between October 2005 and April 2009, 63 patients were enrolled in 12 Italian Centers. The treatment plan is shown in figure 1. PBSCs were collected after Rituximab-DHAP and G-CSF. Patients' characteristics are shown in table 1. Results. The median $CD34^+$ cells infused was $5.5x10^6$/Kilograms (range 2.55-34). The median time to platelet and neutrophil counts higher than $20x10^9$/L and $0.5x10^9$/L were 14 (range, 9-60) and 10 days (range, 8-20), respectively. Grade III and IV mucosites occurred in 22 (35%) and 6 (9%) patients, respectively. Febrile neutropenia occurred in 49 patients (78%). Ten pneumonitis and 17 blood stream infections were documented. Two patients developed an atrial fibrillation. Fifty-four of 63 patients were valuable for 90-day response. The 90-day overall response (ORR) was 89% with 78% of CR. Six relapses (14%) and 5 progressions occurred at a median follow-up of 247 days post ASCT (range, 125-818). The potential factor to predict CR was at least Partial Response (PR) before ASCT (p<0.06). Forty-four patients are alive at a median follow-up of 365 days post ASCT (range, 6-1106): 38 CR (60%), 1 PR (2%), 3 progressive disease (PD, 5%), 2 not valuable for response (Figure 2). Ninetheen patients died (30%): 8 early deaths before day 90, 1 for ARDS (+230), 1 TRM post a subsequent RIC allotransplant (+95) and 9 for PD. The Kaplan-Meyer estimated 3y-EFS is 63%. Two statistically risk factors for EFS were documented: the disease status pre ASCT (CR vs nonCR, p<0.05) and infused $CD34^+$cells (<$4x10^6$/Kg; p<0.03). Cox multivariate regression analysis demonstrated infused $CD34^+$cells as the only significant risk factor for EFS (40% vs 70%, p<0,02). Eight early deaths occurred: 3 for septic shock (day +6, +12 and +39), 1 for pneumonia (+22), 1 for BK encephalites (+61), 1 for MOF (+14), 1 for stomach bleeding (+53) and 1 for PD (+28). The Kaplan-Meyer estimated Treatment Related Mortality (TRM) is 10%. Age more than 60 years was the only statistical risk factor for 90-day TRM (5% in younger vs 22% in elderly patients, p<0,03). *Conclusion*. In nHL patients who failed to achieve CR after first line chemotherapy, Zevalin plus transplant is capable to induce 89% ORR, 78% of CR and a 3yEFS of 63%, with sustained engraftment and an acceptable extra-haematological toxicity, unless for patients older than 60 years.

Table 1. Patient characteristics and 90-day response post ASCT

Patient Characteristics	N = 63
Median age, y (range)	52 (18-75)
Histology, no. (%)	
Follicular	19 (30)
Aggressive	44 (70)
III-IV stage at diagnosis, no. (%)	50 (79)
Median number of prior chemotherapy, no. (range)	2 (2-5)
IPI, grade	
0	8 (13)
>1	55 (87)
Bone marrow involvement at diagnosis, no. (%)	25 (40)
Prior rituximab, no. (%)	58 (92)
Status at enrollment	
Relapse	16 (25)
Progression	16 (25)
PR	26 (42)
CR	5 (8)
Status at transplant	
Progressive disease	14 (22)
PR	28 (44)
CR	21 (34)
Median time to HST, months (range)	16 (5-108)
90-day Response Rate	54 valuable cases
CR	42 (78%)
PR	6 (11%)
Progressive disease	6 (11%)
Not Valuable	1

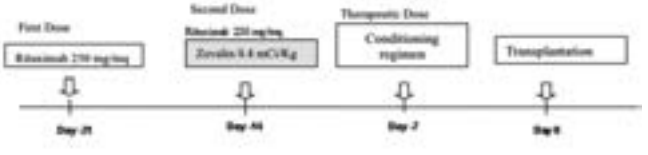

Figure 1. Treatment plan.

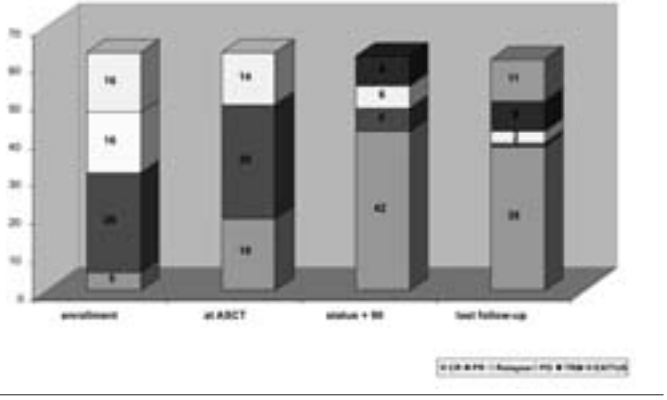

Figure 1. Response rates in the different phases of treatment.

P419

IMPROVING ACCESS TO HIGH DOSE CHEMOTHERAPY (HDC) WITH HEMOPOIETIC STEM CELL (HSC) RESCUE: THE EXPERIENCE OF ONCOLOGY DEPARTMENT OF LECCO

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Background and objectives. HDC with HSC rescue has definite indications in hematologic malignancies but facilities for HSC harvest and storage are not widely available and patients are usually referred to large Oncology/Hematology Department often located in large cities with a significant distress for patients and their families. *Materials and Methods.* Oncology Department of Lecco in collaboration with the Haematology Unit of Niguarda Hospital in Milan started a cooperative protocol of HDC-HSC intending to offer these procedures to patients living in Lecco and in the neighborough with multiple myeloma (MM) or lymphomas (Hodgkin's, HD, or Non Hodgkin's, NHL) for whom indication for HDS-HCS was agreed, underwent remission induction. HSC mobilization and peripheral CD34$^+$ monitoring at Oncology Department of Lecco while harvesting and storage was performed at due time at Haematology Unit of Niguarda Hospital in Milan. HDC with HSC rescue was then performed either at Niguarda Hospital or at Lecco Hospital: in the latter case HSC were transported and reinfused at Lecco Hospital one day following HDC. *Results*. From june 2001 to march 2009, 50 patients (27 male and 23 female), median age 51(range 23-72 years), 23 multiple myeloma (MM), 13 high grade non-Hodgkin lymphoma and 6 Hodgkin's lymphoma (NHL), 7 HD in first relapse and 1 LLC entered the protocol.Mobilization consisted of DCEP for MM, DHAP for NHL and HD, A total of 80 leukapheresis (1-4 per patient, median 1) have been performed and a median 9,3 (4-39,3)x10^6 /Kg of HSC were stored. To date HDC has been delivered to 32 patients (8 MM double transplantation, 15 MM single transplantation and 8 NHL and 6 HD single) either at Niguarda Hospital (myeloablative, 27 procedures) or at Lecco Hospital (8 myeloablative and 10 non-myeloablative procedures). The procedure was well tolerated without unexpected complications and all patients were discharged within 19 days after HDC. *Conclusions*. Based on our experience, HDC with HSC is feasible also in an Oncology department which does not have facilities for HSC harvest and storage provided a strict cooperative protocol with a referral center is agreed. Patients from Lecco area received full HDC with HSC with reduced logistic problems and limited distress. An open cooperation between motivated staff operating at the participating centers was mainstay for this successful work.

P420

CONTINUOUS INFUSION IDARUBICIN AND INTRAVENOUS BUSULPHAN AS ÙCONDITIONING REGIMEN TO AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA

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The current study aimed at the evaluation of efficacy and toxicity of a combination of intravenous (iv) Busulphan (Bu) and continuous infusion (c.i.) Idarubicin (IDA) as a conditioning to autologous stem cell transplantation (ASCT) in patients with acute myeloid leukemia (AML). The protocol included IDA at 20mg/sqm daily as 3 days continuous infusion (from day -13 to -11) and intravenous BU at 12.8 mg/kg daily from day -5 to -2, fractionated in four doses/day . Patients aged over 60 years (n=11, 35%) received a reduced schedule (two days IDA and 3 days BU at the same dose). Thirty-one patients with a median age of 51 years (28-72) were enrolled. All patients received peripheral blood stem cells. The median interval between diagnosis and ASCT was 4 months. The median number of CD34$^+$ cells infused was 5.9x10^6/kg. The median number of days to PMN >500/cmm and platelets >20000/cmm was 10 and 13, respectively. In order to perform a comparison in terms of hematological and non hematological toxicity, a group of 30 patients, who were previously autografted after conditioning with IDA and oral Bu was considered. Selection of factors for a matched pair analysis included median age, percentage of subjects aged over 60 years, median CD34$^+$ cell received, cytogenetic and molecular findings and percent of secondary AML. In both subgroups no occurrence of transplant related toxicity (TRM) was registered; in addition, results in terms of neutrophils and platelet recovery, need of RBC and platelet transfusion were comparable. Finally, no difference was found as far as occurrence of documented infections (either bacterial or fungal) as well as of FUO and liver, cardiac, renal or pulmonary toxic episodes. On the contrary, patients conditioned with iv BU experienced much less frequently grade 3-4 oral mucositis as compared to the control group (12% vs 80%, p:<0.0001). This resulted in less frequent use and shorter duration of TPN (20 % vs. 83%, p<0.0001; 5 days vs. 11 days, p: 0.02, respectively) and shorter duration of hospitalization (27 days vs. 31 days, p: 0.04). Finally, patients conditioned with iv BU had significantly lower need of narcotic analgesics (13% vs 80 %, p:<0.0001). We conclude that replacement of oral with intravenous BU results in a more favorable toxicity profile. A longer follow-up is required to assess a potential advantage in terms of disease free survival.

P421

ROLE OF POSITRON EMISSION TOMOGRAPHY BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION IN MALIGNANT LYMPHOMAS

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High-dose therapy with autologous stem-cell transplantation (ASCT) is a well established salvage treatment in relapsed/refractory Hodgkin's (HL) and non-Hodgkin's lymphomas (NHL) patients (pts). Clinical response evaluation before ASCT is usually assessed with computed tomography (CT), which has been considered predictive of the outcome. Here we present a retrospective analysis investigating the possible role of positron emission tomography (PET) in pts with HL and NHL suitable for ASCT. We evaluated 49 pts, 22 NHL (11 diffuse large B-cell, 8 mantle cell, 2 follicular, 1 Burkitt) and 27 HD. At diagnosis 22 pts presented stage I-II, 27 pts stage III-IV, and 25 pts presented bulky disease; median age was 36 years (range 18-67), and B-symptoms were present in 53% of pts. Eighteen pts received ASCT because of relapse (median time to relapse 14 months (range 2-64)), 23 pts were considered because of primary refractoriness; 6 pts with mantle cell and 2 with diffuse large B-cell NHL received ASCT in first complete remission according to internal guidelines. Median time between PET and transplantation was 15 days (range 2-36). Pre-ASCT PET was negative in 30 pts (61%) and positive in 19 pts (39%); pre-ASCT CT showed a complete or partial response in 32 pts (74%), a stable or progressive disease in 11 pts (26%), 6 pts did not perform a pre-ASCT CT. Overall survival (OS) and progression-free survival (PFS) at 4 years were 69% and 66%, respectively. OS and PFS well correlated with pre-transplantation PET and CT results (OS p.004 and p.006, respectively; PFS p.000 and p.000, respectively) in the entire group of pts examined; the PET positive and negative predictive values (PPV; NPV) for PFS were 63% and 90%, respectively, while TC PPV and NPV were 91% and 87%, respectively. The predictive value of pre-ASCT PET was significantly related with OS (p.0004) and PFS (p.0001) in NHL pts. In HL pts pre-ASCT CT (OS p.036, PFS p.0000) seems to be superior to PET (OS p.6409; PFS p.067). A multivariate analysis showed that pre-ASCT PET could be considered the only prognostic factor for OS. In conclusion, in our retrospective analysis the predictive value of pre-transplantation PET seems to be confirmed at least in NHL pts. A similar role was not observed in HL pts, probably because of the relative small number of pts; the use of PET before ASCT in HL needs further investigations.

P422

PBSC MOBILIZATION AND COLLECTION IN PATIENTS WITH HODGKIN AND NON-HODGKIN LYMPHOMA:COMPARISON OF FILGRASTIM VERSUS PEGFILGRASTIM

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Granulocyte colony stimulating factor (G-CSF) is the elective cytokine for peripheral blood stem cell (PBSC) mobilization in haematological diseases. Pegylated filgastrim (PEG-G-CSF) has been shown to be equivalent to G-CSF in neutrophil recovery post chemotherapy. In this study, we evaluated the efficacy of a single dose of PEG-G-CSF versus daily G-CSF administration for mobilizing PBSC in Hodgkin and non-Hodgkin lymphoma patients. Statistical significance was performed by Mann-Whitney U test. Between March 2007 and October 2008, 24 consecutive patients were mobilized either with different cycles of chemotherapy plus G-CSF (5 males and 7 females) or PEG-G-CSF (4 males and 8 females) at the dose of 5 microgrammi/kg/day up to the collection, or a single 6 mg dose respectively. The average age was 50 yrs in the G-CSF group (32-63) and 43 yrs in the PEG group (20-64). The median weight was 72 kg in the G-CSF group (51-105) and 78 kg in the PEG group (42-100). No significative difference was observed in either group. Precount values are summarized in the following table. In G-CSF and PEG group, the interval from mobilization to the first collection was 8,5 (7-13) and 7,5 (5-11) days respectively (NS). The target dose of 4 $x10^6$/kg CD34 was reached in all patients with 33 PBSC apheresis and in 4 (G-CSF group) versus 5 (PEG-group) 2 procedures were performed. The patient's blood volume processed (G-CSF vs PEG-group) was equivalent to 2,5 vs 2,6 Lt and the CD34$x10^6$/Kg median harvested was 5.7 (1,73-17,4) and 4,8 (1,6-18.8), the CFU-GM $x10^4$/Kg median was 57.6 (17-196) and 65 (19-18.8) and the CD3$x10^6$/Kg median was 59.8 (5-186) and 99 (4-210) respectively. No significative difference was noted in the 2 groups. The results suggest that, although the precount values are slightly higher in the G-CSF group, a single dose of PEG-G-CSF has the similar efficacy in PBSC mobilization of lymphoma patients. Furthermore, a shorter mobilization/collection interval was observed in the PEG group. This characteristic and a single dose of PEG-G-CSF enhanced the compliance of patients in a day hospital regime. These results should be confirmed with a larger number of observations.

Table 1.

	G-CSF-group	PEG-group	p
WBC x 10^6/mL	11,4 (3-19,5)	7,2 (2,8-22)	NS
CD34/microL	62(20-283)	39 (20-132)	NS
PLTx10^9/mL	50 (17-187)	96 (21-244)	NS
GM-CFU x 10^4/mL	8844 (4090-33698)	6314 (2645-27589)	NS

P423

IS AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS A HIGH RISK PROCEDURE?

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High dose chemotherapy followed by autologous previously collected stem cell transplantation has shown to significatively improve the outcome in several hematopoietic malignancies. In advanced Aggressive Non Hodgkin Lymphomas high dose chemotherapy followed by autotransplantation is a widely applied policy. In Multiple Myeloma one or two autologous transplant are considered the golden standard in patients <65 years and several clinical trials have shown that responses are higher and overall survival is better in auto transplanted young patients than in non auto transplanted ones. The role of autologous stem cell transplantation in Acute Myeloid Leukemias is still a concern; several authors suggest that high dose chemotherapy and reinfusion of patient stem cells can be the best consolidation treatment in first remission low risk leukemias. Few studies have so far analyzed the toxicity, feasibility and response in elderly patients. We performed a retrospective analysis on a cohort of consecutive patients affected by haematologic malignancies, submitted to autologous stem cells transplantation at age >65 years in our Institution from 2005 to 2008. The study includes 30 procedures in 26 patients (Multiple Myeloma: n. 12; Non Hodgkin Lymphoma: n. 10; Acute Leukemias: n. 4 ;). The mean age at transplant was 68 years (median 68, range: 65-75 years). They received 1 transplant (n. 22) or 2 transplants (n. 4) depending on stage of disease and diagnosis. Conditioning regimens were BEAM in 8, Alkeran alone in 18, BuCy in 3, Bu Mel in 1. Drug dosages were adjusted considering clinical conditions and previous therapy lines. Mean dosage administered was 75% of that scheduled in adult patients. Early transplant mortality (<1 month) was registered in only 1 patient, transplanted in progression for aggressive refractory non Hodgkin Lymphoma. Response to treatment depended on stage of disease. Hematologic recovery was not affected by the age of the patient at transplant. Toxicity was not different from that registered in younger patients who underwent the same procedures. We conclude that patients>65 years can be submitted to autologous stem cell transplantation especially when autotransplantation is planned in first or second remission.

P424

ANALYSIS OF THE KINETICS OF CD34+ PERIPHERAL BLOOD STEM CELL RECOVERY IN PATIENTS WITH LYMPHOMA OR MYELOMA FAILING MOBILIZATION ATTEMPTS

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Autologous stem cell transplantation is a potentially curative treatment for haematological malignancies. Poor mobilization of peripheral blood stem cells (PBSC) can be a major obstacle to the full completion of the treatment strategy. The kinetic of $CD34^+$ PBSC recovery shows a wide range of variation in different patients. A prompt identification of patients at risk for inadequate harvest could allow to rescue patients by the early addition of novel mobilizing agents. Aim of the present work was to retrospectively identify the patterns of $CD34^+$ PBSC kinetic in 43 cases of mobilization failure (<3$x10^6$ $CD34^+$ PBSC /kg). Nine patients had myeloma and 34 had lymphoma, with Hodgkin's disease (9), particularly in HIV^+ patients (5/9), and T/NK lymphoma (6) being overrepresented. Mobilization regimens consisted of G-CSF in 2 cases, and of chemotherapy + G-CSF in 41 (CTX 3-7g/sqm, high-dose Ara-C; DHAP like). Daily $CD34^+$ PBSC count was performed starting from the expected collection day according to mobilization regimen. $CD34^+$ cells count was performed using a single platform analysis on flow cytometer according to the ISHAGE standards. Stem cell harvest by apheresis was performed when the $CD34^+$ PBSC count was >0.02$x10^9$/L. Three patterns of $CD34^+$ PBSC kinetic were identified: 1) less than 0.010$x10^9$/L on the third day since the start of $CD34^+$ stem cell count (SCCStart) with a WBC count of at least 4.0$x10^9$/L (poor mobilizers): 25 patients (58%); median $CD34^+$ PBSC on the third day after SCCstart: 0.002 $x10^9$/L (range 0-0.008). 2) any decrease in $CD34^+$ PBSC within seven days since SCCStart in patients failing to reach levels of 0.02 $x10^9$/L (insufficient mobilizers): 9 patients (21%); median peak $CD34^+$ PBSC: 0.01 $x10^9$/L (range 0.005-0.012) 3) less than 0.02 $x10^9$/L $CD34^+$ PBSC seven days after SCCStart and WBCs <50.0$x10^9$/L (slow mobilizers): 9 patients (21%); median $CD34^+$ PBSC count on the seventh day after CDCstart: 0.02$x10^9$/L (range 0-0.014). These results should be confirmed in larger studies, which may also identify associations between $CD34^+$ PBSC kinetics and clinical variables and differences between mobilizers and non- mobilizers, with the ultimate goal of planning a rational use of novel agents to reduce the rate of mobilization failures.

P425

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN RESISTANT/RELAPSED FOLLICULAR MALIGNANT LYMPHOMA PATIENTS (PTS) AT THE EUROPEAN INSTITUTE OF ONCOLOGY, MILAN

Bassi S., Cocorocchio E., Rabascio C., Bertazzoni P., Calleri A., Agazzi A., Pastano R., Gigli F., Vanazzi A., Alietti A., Sammassimo S., Nassi L., Laszlo D., Calabrese L., Peccatori F.A., Martinelli G.

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Because of natural history of non-Hodgkin's follicular lymphoma (FL),

high-dose chemotherapy and ASCT represent an alternative treatment able to improve freedom from progression and hint of durable response in selected pts. At our institution from 1997 to 2008 32 pts with pretreteated FL were considered for high-dose chemotherapy and ASCT. The median number of previous treatments was 1 (1-5) and 22 pts never achieved a complete response (CR) to previous lines with a median time of treatment-failure from the last therapy of 8 months (3-18). Before high-dose chemotherapy they received 2 cycles of CHOP-like regimen, followed by Cyclophosphamide 4 g/mq to mobilize peripheral blood stem cells (PBSC). After the collection pts underwent 3 cycles of subcutaneous Cladribine at a daily dose of 0.1 mg/Kg for Day 1-5 monthly. The conditioning regimen was based on Mitoxantrone 60 mg/mq + Melphalan 180 mg/mq, followed by PBSC re-infusion 24h later and G-CSF starting 24h after re-infusion. Twenty-nine pts underwent the ASCT (before the procedure 12 were in CR, 15 in partial response (PR) and 2 in stable disease), while 2 pts did not proceed to ASCT because of progressive disease (PD) and 1 because failed stem cells collection. The engraftment was at a median of 11 days for leucocytes and 13 days for platelets (>20.000/mmc). Although the initial engrafment, 8 pts presented a late recovery of leucocytes and platelets to normal values, in part justified by the low median count of PBSC received ($1,9x10^6$ $CD34^+$/Kg; range 1.1-7.1). Grade 3 mucositis was described in 7 pts. During aplasia a 45% of documented infection rate was reported. One patient in CR presented myelodysplastic syndrome at 18 months from ASCT. After ASCT 26 pts were in CR, 1 in PR and 2 pts died before response assessment. Nine pts in CR showed PD at a median time of 14 months (8-92) from ASCT. With a median follow up of 4 years from ASCT (range 1 month -11 years), 21 pts are alive and 15 (52%) in CR, included 12 pts not responsive to previous treatments. Eight pts died, 3 for PD and 5 for treatment-related causes. Although our chemotherapy regimen included immunosuppressive agents such as the purine analogue Cladribine and Rituximab, it results safe and feasible without an increased incidence of infections. Moreover, the high rate of CR and the sustained freedom from progression up to now especially in pts resistant to previous treatment, confirms such modality of treatment a valid option in pretreated FL pts.

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AUTOLOGOUS STEM CELL TRANSPLANTATION IS FEASIBLE AND LEADS TO DURABLE DISEASE CONTROL IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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High-dose therapy with autologous stem cell support (ASCT) is considered the standard of care for younger newly diagnosed multiple myeloma (MM) patients with less than 65 years of age. Controversies exist concerning the role of this procedure in elderly patients. In this study we analyzed the outcome of 33 patients with a median age of 67 years (range 65-71) who received a single or double ASCT. Seventeen patients were primarily treated with four 28-day cycles of VAD, while the remaining 16 patients received four 28-day cycles of thalidomide (200 mg/d) and dexamethasone (40 mg/d for 4 d, every 28 d) (Thal-Dex, four 28-day cycles) as induction in preparation for ASCT. A median of $6.3x10^6$ $CD34^+$ cells/kg (range 1.25-11.1) were collected following CTX and G-CSF. All patients underwent an ASCT to support high-dose therapy with melphalan 200 mg/m^2; 9 patients received a second ASCT. Median time to recovery of neutrophils and platelets was 12 days (range 10-15) and 13 days (range 10-15), respectively. Cumulative incidence of transplant related mortality (TRM) at 100 days was 0%. On an intention to treat basis, all patients but one achieved at least a partial response (PR) and 50% at least a very good partial response (VGPR), including 20% immunofixation negative complete response (CR). With a median follow up of 46 months (range 18-108), the 5-year projected overall survival (OS) rate was 66%, median duration of response (DOR) was 35 and median duration of event free survival (EFS) was 38 months. No difference in quality of response was reported after ASCT in the two subgroups of patients who received either VAD or Thal-Dex as induction therapy. Similarly, curves of EFS and OS were almost superimposable. In conclusion, this study suggests that up-front ASCT is feasible and well tolerated in fit MM patients aged ≥65 years. Based on favorable outcomes in terms of VGPR/CR rates, EFS and OS, ASCT can be considered a therapeutic option for selected elderly patients with newly diagnosed MM.

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THIOTEPA-CYCLOPHOSPHAMIDE HIGH-DOSE IMMUNOSUPPRESSIVE THERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN AGGRESSIVE FORMS OF MULTIPLE SCLEROSIS: RESULTS IN 9 PATIENTS.

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Background. Autologous stem cell transplantation (ASCT) has been performed for aggressive Multiple Sclerosis (MS) with raising frequency; standard myeloablative chemotherapy (BEAM) +/- *in vivo* T-cell depletion produces long-term remission of disease, with significant morbidity and mortality. We assessed the feasibility of high-dose immunosuppression with Thiotepa and cyclophosphamide (Cy) with ASCT in patients with aggressive forms of MS. *Methods*. From December 2005 to October 2007, 9 patients affected by MS, unresponsive to conventional therapies, with elevated inflammatory disease activity, underwent ASCT in our institution. Five patients had a relapsing-remitting, 3 a secondary progressive and 1 an hyper-acute MS. Patients had a median of 26 years, 6 (range 2.5-8) points in EDSS disability score and 15 enhancing lesions (range 1-67) on MRI before ASCT, with a median of 2 relapses in the 2 years before ASCT. Autologous hematopoietic stem cells were mobilized with Cy 4 gr/mq at day 0 followed by granulocyte colony stimulating factor 5 mcg/Kg/day from day +2 to stem cell harvest. Harvest was not manipulated before cryopreservation. The conditioning regimen before ASCT consisted of Thiotepa 5 mg/Kg bid on day –5 and Cy 50 mg/Kg on days –3 and –2. The median follow-up was 29 months. *Results*. Mobilization was successfull in all cases, with a median of $12.91x10^6$ collected and $5.7x10^6$ infused $CD34^+$ cells/Kg. Hematopoietic recovery was documented safely in 9/9 patients, with a median of 10 days for neutrophil and platelet engraftment. The median time of hospitalization was 24 days. Six patients developed febrile neutropenia, lasting a median of 4.5 days. There were no major adverse events. TRM was 0%. Seven patients were stable or improved at the last follow-up: 5 improved EDSS by at least 0.5 points, 2 were stable. Relapses were observed in 5 patients (4 at 1 year, 1 at 2 years after ASCT) with complete recovery after steroids. The confirmed progression free survival is 100%, the disease activity free survival is 44%. A significative reduction by the 79% of annualized relapse rate and 99,6% of enhancing lesions occurred. *Conclusions*. High-dose immunosuppressive therapy with Thiotepa and Cy followed by ASCT reduced disease activity and disability progression of aggressive MS with safe profile and low toxicities. Larger number of patients and longer follow-up will be necessary to establish the value of this regimen in producing long-term disease remissions.

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EFFICACY OF MYELOMA-LIKE THERAPY IN IMPROVING HAEMATOLOGIC AND ORGAN RESPONSE IN MONOCLONAL IMMUNOGLOBULIN DEPOSITION DISEASE (MIDD)

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MIDD is a rare entity which includes a group of disorders characterised by the deposition in solid organs of monoclonal light chains (LCDD) or heavy chains (HCDD) or both. Management of these disorders is still controversial. In this study we analysed the outcome of 8 patients (7 with LCDD and 1 with HCDD) who received ASCT upfront. Median age of the patients was 47 yrs. 3 patients had a baseline serum creatinine above 2 mg/dL and 2 of them were dialysis dependent. 6 patients received different induction therapies before PBSC collection. ASCT was given to support melphalan at doses ranging between 200 mg/m^2 to 100 mg/m^2 on the basis of renal function. 2 patients received 2 consecutive courses of melphalan 140 mg/m^2 with double ASCT. Toxicity of ASCT was comparable to that observed in MM patients with no major adverse events. TRM was 0%. 7 patients were evaluable for haematologic response according to EBMT criteria. Of these patients, 5 achieved a CR 3 months after ASCT, 1 patient achieved a PR and 1 patient progressed. Organ response was evaluated in 6 patients. Of these, 3 showed an improvement of renal function with a decrease in proteinuria in the range between 60% and 95% at time of

last follow up, and 3 progressed (one of them despite the achievement of haematological CR). Patients in dialysis at the time of diagnosis were considered not evaluable for kidney response. Both achieved a CR following ASCT, and subsequently received a kidney transplant at 2 and 8 years, respectively, without any sign of recurrence of kidney disease. With a median follow up of 24 months (5-120) all patients are alive, 5 without evidence of haematologic recurrence of MIDD. These data support the feasibility and efficacy of ASCT in selected patients with MIDD. Notably, organ response can occur at a later time period than haematologic response. Kidney transplantation is a valuable option for patients with end stage renal failure who achieve a sustained CR after ASCT.

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HISTOLOGY AND RITUXIMAB ADDITION ARE THE MAIN FACTORS INFLUENCING THE LONG-TERM OUTCOME IN HIGH-RISK LYMPHOMA FOLLOWING HIGH-DOSE THERAPY AND AUTOGRAFT: A 20-YR. FOLLOW-UP IN 1,347 PATIENTS BY GITIL (GRUPPO ITALIANO TERAPIE INNOVATIVE NEI LINFOMI)

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Introduction. High-dose (hd) therapy with stem cell autograft is an effective treatment option for high-risk lymphoma patients. The value of autograft is well established in both non-Hodgkin s (NHL) and Hodgkin s Lymphoma (HL). However, the outcome at long-term of patients receiving salvage treatments with autograft has not been fully addressed. Aim of the study. To evaluate the long-term survival in a large series of high-risk lymphoma patients, treated with the hd-sequential (HDS) chemotherapy approach, followed by peripheral blood progenitor cell (PBPC) autograft. *Patients and Methods*. Data have been collected on 1,347 lymphoma patients who have received either the original or modified HDS in the last two decades at 11 Centers, associated to GITIL. The series included 1,024 B-cell NHL, 234 HL and 89 T-cell NHL; there were 695 high-grade, 278 low-grade and 140 mantle-cell lymphoma; median age was 46 yrs; 57% were male. Overall, 640 (47%) patients received HDS front-line, 707 patients as salvage treatment; advanced-stage was documented in 76%, high serum LDH in 51%, an ECOG Performance Status of 2-3 in 36% of treated patients. The original HDS was employed in 49%, while the hd-Ara-C supplemented schedule in 51%; Rituximab was added to HDS (R-HDS) in 523 (51%) B-cell NHL. Results. At a median follow-up of 7 yrs, a significantly better outcome was seen in B-cell NHL compared to HL and to T-cell NHL: their projected Overall Survival (OS) at 10 yrs. are 58%, 51% and 47%, respectively. This resulted in a median survival of 17.7 yrs. for B-cell NHL, 11.1 yrs. for HL and 8.3 yrs. for T-cell NHL, while the median event-free survival (EFS) was of 10.3 yrs. for B-cell NHL, 4.7 yrs. for HL and 7.4 yrs. for T-cell NHL. Among B-cell NHL, those receiving R-HDS had a better outcome compared to those receiving HDS without Rituximab (R-), with a 5 and 10-yr OS projections, respectively of 69% and 62% for R-HDS, and 62% and 55% for R-. R-HDS treated showed a significantly better (p=0.001) 5- and 10-yr. EFS projections (60% and 54%, respectively) compared to R- patients (53% and 46%). *Conclusions*. i. a prolonged survival can be achieved in most high-risk lymphoma patients receiving hd-therapy with autograft; ii. B-cell NHL are those with the most favorable outcome; iii. a long-term EFS can be obtained in approximately half of HL patients; iv. Rituximab addition proved to be effective also in this large series including various B-cell NHL subtypes.

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INTERMEDIATE DOSE ARA-C EFFECTIVELY MOBILIZES PERIPHERAL BLOOD PROGENITORS CELLS IN A PREVIOUS POOR MOBILIZER HAEMATOLOGIC MALIGNANCIES PATIENTS

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Background. High-dose chemotherapy and autologous stem cell rescue is considered a standard strategy for patients with relapsed/refractory hematologic malignancies patients. Use of alchilating agents, several lines of treatments, cisplatin and fludarabine, are all factors adversely affecting PBPCs mobilitation. However the optimal mobilizing regimen has not been defined in poor mobilizer patients. *Aims*. In this study we assessed the efficacy and safety of intermediate dose ARA-C given to mobilize PBSCs. *Patients and Methods*. Twenty-five patients (16 M; 9 F; median age 61 yrs; range 26-70 yrs) with hematologic malignancies: 14 non-Hodgkin's lymphoma (NHL), 10 multiple myeloma (MM) and 1 LAM, who have failed a previous attempt between January 2007 and May 2009, were scheduled to receive intermediate dose ARA-C to mobilize PBSCs. *Methods*. Patients were primed using intermediate dose of ARA-C administered intravenously at a dose of 800 mg/m2 every 12 h for 6 consecutive doses, + rhG-CSF 5 or 10 microgr/Kg subcutaneously. *Results*. A median of 3 chemotherapeutic regimens (range 2-7) were previously given and all patients failed prior harvesting of PBSCs. Three patients with MM were given two consecutive autotransplants with Melphalan 200 mg/mq as conditioning regimen. One patient with NHL received 4 cycles of chemotherapy, one patient underwent Bone Marrow Autotransplant and 2 cycles of Ibritumomab Tiuxetan (Zevalin®). Collection of PBSCs was successfull in 23 out of 25 patients (92%) (14 NHL, 8 MM and 1 LAM). Two patients whit MM were no mobilizers with intermediate dose of ARA-C also (one of them received two consecutive autologous PBSC while one other showed a refractory disease at the time of mobilization). Harvesting of PBSCs was performed at a median time of 14.5 days (range 7-17 days) after ARA-C administration. The median number of subcutaneous injections of rhG-CSF was 7 (range 4-13). The median number of WBC count was 4500/mmc (range 1400-14800) at the time of collection with $CD34^+$ median number of 1.2 % (range 0.3-6). In all mobilizer patients the required number of CD34 + cells were harvested after a single leukapheresis. The median number of CD34 + cells collected was $6x10^6$/Kg (range 2.04-25) with 4 as a median number of cryopreserved bags (range 2-8). All patients experienced neutropenia < 500/microL, and 9 out of 23 had febbrile neutropenia (1 to 4 days). Fourteen patients received a median of 1 packed red cell transfusions (range 1-3) and 23 patients a median of 1 apheretic platelet products (range 1-3). No patients experienced WHO grade III-IV mucositis and diarrhea. *Results*. Our experience, showed that PBSC collection using intermediate-dose of ARA-C + rhG-CSF is safe and effective in poor mobilize patients with haematologic malignancies. Mobilization and collection of PBPCs were found independent from the number and type of previous chemotherapies. Indeed it is surprising that mobilization was also achieved in the patient with LNH who received several chemotherapies, Bone Marrow Autotransplant and two cycles of Zevalin®.

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LONG TERM FOLLOW-UP IN PATIENTS WITH FOLLICULAR LYMPHOMA AFTER A TOTAL BODY IRRADIATION (TBI)- BASED CONDITIONING FOR AUTOLOGOUS STEM CELL TRANSPLANTATION

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Supralethal chemo-radio-therapy can potentially cure follicular lymphoma. However TBI-based conditioning regimen for autologous stem cell transplantation (ASCT)is associated with an increased risk of transplant related mortality(TRM)and second neoplasms with significative

worsening of long term event free survival EFS). Between February 1992 and December 2005, 63 patients (38 males, 25 females, median age 47 years; range 28-60 years;) had ASCT after a conditioning regimen including SF 8 Gy TBI at high dose rate with lung shielding followed by Thiotepa 5 mg/Kg/day for two days. Graft content was as follows: $CD34^+$ cells: $9.1x10^6$/Kg (range 2.0 -$23.5x10^6$); $CD34^+$ cells were purged by positive selection in 23/63 patients. Disease stage at diagnosis was advanced in 53 patients (clinical stage III in 12, IV in 41); B symptoms were present in 15; 47 patients had undergone only first-line therapy before ASCT. 19 patients had received rituximab. At the time of ASCT 34 patients were in complete remission (CR), 20 were in partial remission (PR), 4 had achieved very good PR, 3 were refractory (REFR) to chemotherapy and 2 were in relapse (REL). All patients engrafted with good haematological reconstitution. Short term complications included 2 fatal cases of interstitial pneumonia, 1 case of VOD which quickly resolved, 1 case of renal failure which became chronic. TRM was 3.1%. Major long term complications included one bladder cancer after 2 years and one breast cancer after 6 years with both patients surviving after surgery and chemotherapy. At a median follow-up of 6.9 years (range 0.21 -16.9 years) 52/63 patients survive and 44/63 were in complete remission. Three year overall survival (OS) and EFS rates were respectively 90% and 79%; projected 5-year OS and EFS rates were respectively 85% and 72%. Disease status at ASCT was a significant prognostic factor for EFS (p= 0.03 CR vs REFR/REL, p=0,07 CR vs PR) as was time from diagnosis to ASCT (< 10 mouths p= 0,03). Patients who received rituximab before ASCT tended to have better EFS. Purging by positive selection not influences EFS (p=0,16). SF 8 Gy TBI at high dose rate with lung shielding plus thiotepa as conditioning to ASCT for patients with follicular lymphoma is feasible with low TRM and low incidence of second neoplasms.

In memory of Prof Antonio Tabilio, close friend and colleague, eminent physician and scientist.

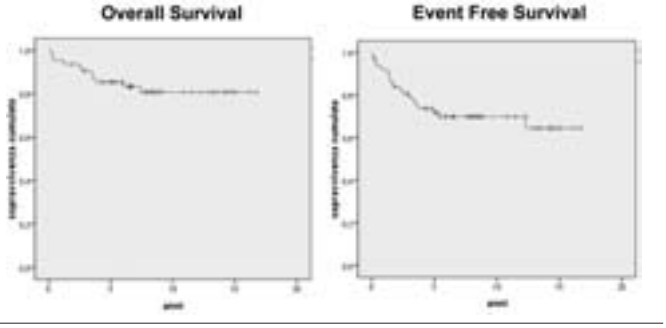

Figure 1.

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PROSPECTIVE STUDY ON THE IMPACT OF A GRAFT CONTAINING MORE THAN $5X10^6$/KG $CD34^+$ CELLS ON IMMUNOLOGICAL RICOSTITUTION

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The absolute lymphocyte count (ALC) recovery of 500 cells/mL or more at day 15 (ALC-15) after autologous peripheral blood hematopoietic stem cell transplantation (ASCT) has been reported as a powerful prognostic indicator of clinical outcomes in hematologic malignancies. This was also demonstrated in our study in which we analized the relationship between ALC with OS and PFS in 144 patients with Non Hodgkin's Lymphoma undergoing ASCT. Stem cell mobilization consisted of chemotherapy associated with G-CSF 5 μg/kg/day. Forty-nine pts (47.1%) were in CR at the transplant time; the others showed an advanced status of disease. The main conditioning regimens were BEAM (78 pts). Variables analyzed with regard to PFS and OS were age, sex, LNH histotypes, previous number of chemotherapy lines, previous treatment with MabThera, disease status at transplant, ALC pre-apheresis, ALC-15. Median number of infused $CD34^+$ cells was $4.8x10^6$/kg (2.0-16.0). After a median follow-up of 28.1 mo. (1-181), 75 (72.1%) and 69 pts (66.3%) were alive and without progression or relapse CR, respectively. Univariate analysis demonstrated a remarkable impact of ALC-15 on OS (0.0051) and PFS (0.0026). Cox regression analysis showed that ALC-15 (p=0.017) and disease status at the time of transplant (p=0.036) were the predictive factors influenced OS. On the basis of these data, we prospectively assessed whether increasing the number of infused $CD34^+$ cells could be related to a more rapid and stable immunological reconstitution (IR) thus improving the PFS and OS. We examined 27 pts, including 14 with lymphoma, 10 with myeloma and 2 with acute leukemia that were infused $CD34^+$ cells >$5x10^6$/Kg, mean 6.6 (5.9-7.7). At day 15 immunophenotyping was performed for the lymphocyte and natural killer cells. We observed that the median number of ALC in this group of patients was 397 /mL and in particular in only ten pts we observed a number of ALC > 500/mL. The median number of NK-15 cells was 89/mL (10-579). It seems there is no correlation between CD34+ peripheral blood progenitor cell dose and early lymphocyte recovery because while increasing the number of infused do not observe an increase in the ALC and does not induce the advance of the IR, as the number of NK-15 is below the normal range 90-590. A threshold number of $CD34^+$ cells should not be the only parameter considered for an adequate PBSC collection and could be required of new drugs to improve the quality of the apheretic product.

Hodgkin's Lymphoma

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VEBEP REGIMEN AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN PATIENTS (PTS) WITH HD AND HIV INFECTION (HD-HIV): FINAL RESULTS OF THE ITALIAN COOPERATIVE GROUP ON AIDS AND TUMORS (GICAT) STUDY

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Background. The outcome of pts with HD-HIV is still poor, because the duration of complete remission (CR) is short. To improve the prognosis of HD-HIV, a feasibility study with the VEBEP regimen and HAART was started in previously untreated HD-HIV pts. *Methods*. CT included epirubicin 30 mg/m^2/day (days 1-3), cyclophosphamide 1000 mg/m^2 (day 1), vinorelbine 25 mg/m^2 (day 1), bleomycin 10 mg/m^2 (day 3) and prednisone 100 mg/m^2/day (days 1-3). Results: Since September 2001, 71 pts have been enrolled. The median age was 41 yrs. The median CD4$^+$ cell count was 248/mm3 and 51% of pts had a detectable HIV viral load. Stage III-IV was present in 50/71 (70%) pts. Histologic subtypes were: MC 70%, NS 20%, LD 4%, LP 2%, unknown 4%. Four toxic deaths were observed (septic shock, PCP, hepatic failure and pneumonia during neutropenia). An absolute neutrophil count <500 was noted in 60% of pts. Grade 3-4 anemia was observed in 38% of pts and severe thrombocytopenia in 22% of pts. Twenty-two per cent of pts had febrile neutropenia with 19 documented infections in 16 pts (4 varicella, 4 bacterial pneumonia, 3 bacterial sepsis, 2 PCP, 1 cerebral toxoplasmosis, 1 esophageal candidiasis, 1 HBV reactivation, 1 HCV reactivation, 1 prostatitis, 1 salmonellosis). CR was obtained in 47/71 pts (66%) and PR in 9/71 pts (13%). With a median follow up of 22 months, only 4 pts have relapsed. OS and TTF at 24 months are 69% and 59%, respectively. *Conclusions*. Our preliminary data demonstrate that VEBEP regimen in combination with HAART is feasible and active in pts with HD-HIV. This study was supported by ISS grants.

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ANALYSIS OF LATE EFFECTS IN HODGKIN'S POPULATION TREATED BETWEEN 1980-2005 IN A SINGLE INSTITUTION

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From 1980 to 2005 in our institution we've diagnosed and treated 361 patients (pts)affected by Hodgkin's disease. At the moment of diagnosis the median age of the patients was 29 years (range 13-69). About 72% of patients were in the stage 1°-2° and 28% were in the stage 3°-4°.More than 54% of all patients received ABVD, 17% received MOOP, 10% MOOP/ABVD, 3% VBM and 1% received Stanford V scheme.Over 70% of all patients received radiotherapy (RT) with median dose of 4500 cGy (range 1000-16210).Over 94% of all patients obtained a Complete remission, however 11% relapsed. 130 patients among 361 are at this moment evaluable for late effects of chemio-radiotherapy.26 pts were treated in the period 1980-84 (25%) ; 6 pts treated 1985-89 (4.6%);21 pts treated in 1990-94 (16,1%); 24 pts treated in 1995-99 (19%) and 35 pts in the period 2000-2005 (27%). 50 pts among 130 (38.5%) present late effects related to the therapy. 24 pts (48%) present heart disease: 13 pts (40%) Valvulopathy, 8 pts (25%) Ischemic heart disease, 7 pts (21%) Congestive heart failure, 4 pts (14%) Pericardic disease.Therapies of these patients were performed: In 1980-89 (11 pts) , 6 pts (25%) received MOOP + RT,2 pts (8,3%) received M/A and 3 pts only RT. In 1990-99 (7 pts), 3 pts(12,5%) received ABVD +RT, 2 pts(8,3%) received MA+RT , 1 pt(4,1%) received ABVD and 1 pt (4.1%) received M/A.In 2000-05 (6 pts), 2 pts (8,3%) received ABVD+RT and 4pts(16,6%) received only ABVD. 18 pts present Endocrinological-disease, in all cases, represented by hypothyroidism; 16 pts among 18 had done Chemioteraphy and Radiotheraphy , while 2 pts received only Chemiotheraphy. Besides 8 pts show both endocrinological and cardiological disease; in these cases 3 pts (37.5) had done ABVD+RT, 4 pts (50%) MOOP+RT and 1 patient (12,5%) only RT. *Conclusions*. In our population 38% of patients had developed secondary late effects. In update of the analysis of factor affecting late effect is ongoing and it could be presented during the meeting. Although there are many variables for the development of a late effect we think that this analysis could lead to try to reduce the late toxicities of treatments.

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FURTHER IMPROVEMENT OF THE VBM CHEMOTHERAPY COMBINED WITH RADIOTHERAPY IN THE TREATMENT OF EARLY, LOW-RISK HODGKIN LYMPHOMA.

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The clinical fortune of the VBM [vinblastine (V), bleomycin (B), methotrexate (M)] chemotherapy and its subsequent modifications has been uncertain. It was originally devised as less toxic chemotherapy for combination with involved-field radiotherapy (IF-RT) in laparotomized, early-stage Hodgkin's patients. Subsequently, several trials successfully tested this combination also in clinically staged patients. The only concerns constantly regarded pulmonary toxicity. Some years ago some of us were able to demonstrate that this toxicity is mainly related to doses and techniques of RT to the mediastinum, especially when sandwiched among chemotherapy courses; moreover, the reduction of the dose of bleomycin after the second cycle, and the start of RT about one month after the end of VBM were able to abate lung toxicity. The sligtly increased recurrence rate outside the irradiated sites observed in the last trial suggested us to strengthen the schedule by introducing a standard dose of cyclophosphamide (C) and a low-dose three-day medication with prednisone (P) to the other unmodified drugs. Moreover the days of administration were changed from 1 and 8 every 21 to 1 and 14 every 28. So, the schedule (CVbMp) used in the present new pilot-study was as follows (doses in mg/sqm): C 650, V 6, B 6 in cycles 1-2 and 4 in cycles 3-6, M 30, all i.v., day 1 and 15, P 40 p.o., days 1-3 and 15-17; cycles had to be from 4 to 6. IF-RT shlould have start at least 3 weeks after the completion of chemotherapy. From January 2004 and December 2008 33 patients were enrolled. Criteria of inclusion were the following: stage IA-IIA, no prior treatments for lymphoma, no bulky mass, no extranodal involvement, no more than 3 involved sites, no hilar involvement, ESR < 40 mm at 1st h, good cardiac, pulmonary, hepatic and renal functions. Mean age was 40 years (range: 16-72, with 5 subjects > 60 years), performance status was good (only one patient with ECOG grade 1), histology was LP in 3 cases, LR in 4, NS in 18, MC in 6, LD in 1, unclassifiable in 1. Patients evaluable for response were 27, with 26 complete remission and one partial reponse. The mean number of cycles administered per patient was 4.5. With a median follow-up of 30 months only one relapse was recorded, after 23 months from the end of therapy. Toxicity was very mild: only 5 cases with grade 3-4 leucopenia and 2 with grade 1-2 hepatotoxicity. No clinical or radiological signs of lung damage were recorded. The CVbMp chemotherapy can be considered a very good refinement of the original VBM schedule, since the correct timing of IF-RT, the reduction of the dose of bleomycin and the brief steroid medication prevent any pulmonary toxicity, the 14-day interval among i.v. administrations further improves the hematological tolerability and the addition of moderate doses of C increases the antitumoral activity without noticeable detriment to tolerability. Early-stage any-age patients (elderly ones included) can get benefit out of this combination.

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QUANTITATIVE CT-PERFUSION MEASUREMENTS IN HODGKIN LYMPHOMA AND COMPARISON WITH FDG-PET: PRELIMINARY EXPERIENCE

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Background. Structural CT criteria such as nodal size and appearance have a poor correlation with the vitality of a lymphoma mass, particu-

larly in Hodgkin Lymphoma. However, differences in vascularity between tumors and normal surrounding tissues offer potential for more precise imaging. Many tumor capillary beds have a higher vessel density than normal, with a larger flow of blood per unit volume, or perfusion; moreover, these capillary beds also have increased permeability. CT-perfusion is an exciting CT technology that allows functional evaluation of tissue vascularity. *Aims*. This study investigates the potential for functional CT-perfusion and permeability measurements in distinguishing between active, recurrent disease and residual scar tissue, also in comparison with 18-Fluoro-deoxyglucose positron emission tomography (FDG-PET) that recently become widely used in the management of patients with malignant lymphomas, providing unique metabolic information about tissue vitality and prognosis of patients. *Methods*. 23 patients with Hodgkin lymphoma underwent imaging with both modalities, CT-perfusion with 64-detector scanner (VCT, GE Healthcare, contrast material 100 ml, 4 ml/sec) and FDG-PET at staging and early assessment(n=2), staging, early assessment and restaging (n=2), or at early assessment and restaging (n=10) and only at restaging (n=9). All 39 CT-perfusion studies were conducted on the most significant lymphoadenopathy and analyzed by using commercially available software (CT Perfusion 3 - Body tumour protocol; GE Healthcare Technologies); blood volume (BV), bloodflow (BF), mean transit time (MTT) and permeability surface (PS) were measured. *Results*. thirtythree out of 39 (85%) CT-perfusion and FDG –PET scan resulted concordant. In particular, all examination were positive at staging, while FDG-PET was positive and TC-perfusion negative in a case at early assessment and the opposite happened in another patient, while the two techniques resulted different in four cases at restaging, in which FDG-PET was negative but TC-perfusion was doubtful/positive. *Conclusions*: CT-perfusion like FDG-PET offers the advantage of functional tissue characterization that is largely independent of morphologic criteria. It can represent a potential alternative non-invasive method in evaluating response to treatment in early assessment of Hodgkin lymphoma and in distinguishing between active, recurrent disease and residual scar tissue after therapy. Further and larger studies are needed to confirm these preliminary data and to extend the prognostic value of FDG-PET to TC-perfusion, particularly when used in the early assessment of response to treatment.

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VEBEP AND LOW-DOSE RADIOTHERAPY: A VINORELBINE-CONTAINING THERAPY FOR NEWLY DIAGNOSED ADVANCED HODGKIN'S LYMPHOMA

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Introduction. In Hodgkin Lymphoma (HL), the 5-year freedom from progression (FFP) rate, ranges from 60% to 70% after 6 to 8 cycles of combined doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD). In this prospective, phase 2 study, the authors tested a new-generation,vinorelbine-containing, intensive regimen (VEBEP) with low-doses radiotherapy (RT) in 98 patients with clinical stage (CS) IIB through IV HL. *Methods*. From November 1997 to February 2004, 98 adult patients with newly diagnosed biopsy-proven HL, classified as stage IIB, III (A and B), and IV (A and B) according to the Ann Arbor criteria, were enrolled into this prospective nonrandomized study. The regimen consisted in epidoxorubicin 30 mg/mq iv day 1-3, cyclophosphamide 1000 mg/mq iv on day 1, vinorelbine 25 mg/mq iv on day 2, bleomycin 10 mg/mq iv on day 3, and prednisone 100 mg iv day 1-3. Treatment plan varied on the basis of Ann Arbor/Cotswold stage: in the first part of the trial, including 83 pts, IIB were given four courses of VEBEP and involved field (IF) RT (30 Gy), whereas III and IV stages were given six courses of VEBEP with RT only on bulky sites. In the last 15 pts treatment plan consist of two additional courses for every stages. *Results*. A total of 79 patients (80.6%) entered complete response (CR) at the end of the treatment program. Toxicity was globally mild, but we observed one toxic-death (septic shock during chemotherapy). With a median follow-up of 68 months (range 6-169), 68 % patients were free from lymphoma progression. A total of 13 patients have died (10 with disease) for an overall survival rate (OS) of 88% at 5 years. Among the 85 patients alive, all but one are disease free, 59 in first CR, and 25 in second or further CR. Among several prognostic factors analyzed, (histology, stage, B-symptoms, bulky-disease, number of cycles) no correlation with FFP or OS emerged. *Conclusions*. The results from this prospective study strongly suggest that 6 to 8 courses of ABVD, a chemotherapy regimen that was designed in 1974, remain the reference treatment for patients with advanced HL. Furthermore the follow-up confirms that much more of 50% of relapsed/resistant patients can be cured with the current salvage therapy.

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HODGKIN LYMPHOMA: POLYARTHRITIS AS UNUSUAL PRESENTING FEATURE IN A PEDIATRIC PATIENT

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Arthritis as a presenting sign of lymphoma is extremely rare. It can be caused by a variety of pathogenetic mechanism, including malignant cell invasion of bone and bone marrow; the spreading to osteoarticular sites is prevalently vascular, but joint paint or swelling due to bone involvement is rarely present in Hodgkin Lymphoma. A 12-year-old girl was admitted to our hospital because of artharlgia and swelling of both ankles and left knee. Physical examination showed supraclavicular lymphoadnopathies. Laboratory values showed a normal complete blood cell count, a mild increment of ESR (35 mm/hr) and elevated LDH value (1200 U/L). Autoantibodies and rheumatoid factor were negative. A lymph node biopsy (supraclavicular) was performed and the histopathological examination was consistent with Hodgkin Lymphoma (HL), nodular sclerosis. CT scan showed mediastinal lymphadenopathies of 2 cm, multiple splenic lesion, bone marrow biopsy resulted negative and clinical stage was IIIsA. The AIEOP-LH-2004 protocol was started. Yet after the first polychemotherapeutic infusion according the COPP/ABV regimen joint pain improvement occurred, with progressive rapid reduction of the swelling and today after two course of COPP/ABV the arthralgia resolved completely. Several recent studies have confirmed that the incidence of non-Hodgkin lymphoma is increased in both adults and children with autoimmune disease such as rheumatoid arthritis. Leukaemia and more rarely other tumours such as lymphomas, may present with musculo-skeletal manifestations; however, true articular signs such as joint swelling have been reported very rarely at the onset of HL. In this case polyarthritis could be due to tumor effects in the form of paraneoplastic syndromes, whose pathogenesis isn't understood. In regard to the effect of antiblastic therapy, the antinflammatory effect of prednisolone included in COPP regimen could itself explain the regression of paraneoplastic or inflammatory polyarthritis. It will be interesting to see if arthritis will disappear forever at the end of HL treatment. Even if rare, lymphoma should be considered in patients with unexplained arthritis especially if pain is out of proportion to the objective finding and if there are lynmphadenopaties.

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PET-FDG BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION IN ADVANCED HODGKIN'S LYMPHOMA

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*Background.*In advanced stage Hodgkin's lymphoma (HL), FDG-PET performed after two courses of ABVD chemotherapy has demonstrated a very high negative predictive value (NPV) and positive predictive value (PPV), and is presently considered the most relevant available prognostic factor in correlation with outcome. *Aims*. To investigate the prognostic role of PET performed before autologous stem cell transplantation (ASCT) in resistant or relapsed HL. *Methods*. From May 2005 to November 2008, 19 patients with resistant or relapsed HL underwent a salvage chemotherapy program consisting of 3 or 4 courses of IEV or IGEV chemotherapy with peripheral blood stem cell collection followed by BEAM-conditioned ASCT. Seven patients were consolidated with the BEACOPP regimen (2 to 4 courses) before transplantation. The median

age was 30 years (22 - 46); 9 patients were males and 10 females. At the time of enrolment, 9 patients were resistant to first-line chemotherapy, 7 were in first relapse (in 2 cases, the relapse occurred within 12 months) and 3 were in second or subsequent relapse. *Results*. Pre-transplant PET evaluation was negative in 11 cases: of these, 7 are currently in continuous complete remission (CCR) after a median follow-up of 23 months (range 9 - 32), while 4 have relapsed after 5, 12, 20 and 31 months from transplant, respectively. Among the 8 patients autografted after a positive PET, 6 relapsed after a median follow-up of 15 months (range 7-28). The NPV of pre-transplant PET was 63.6%; the PPV was 75.0%. The prognostic value of the following parameters at the time of enrolment was evaluated: presence of bulky and/or extranodal disease, number of previous chemotherapy lines, chemoresistance/chemosensitivity to pre-transplant therapy evaluated by CT scan; no statistically significant correlation with the outcome was recorded. *Summary/conclusions*. Our preliminary results show that in resistant or relapsed HL a positive pre-transplant PET predicts progression or relapse in 75% of patients. A negative PET predicts long-term disease control in two thirds of patients. Pre-transplant PET does not have the same high predictive value as interim PET performed during first induction; other prognostic factors need to be considered, although none of those examined showed a statistically significant role. These data need to be further confirmed on a larger number of patients.

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EARLY FDG-PET SCAN CONFIRMS ITS PROGNOSTIC IMPACT ALSO IN LOCALIZED STAGE, ABVD TREATED HODGKIN LYMPHOMA PATIENTS

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We explored the predictive value on therapy outcome of an early evaluation of treatment response by 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) scan performed after two corses of ABVD in pts with localized Hodgkin's disease. From 2002, 163 new localized stage Hodgkin's lymphoma pts were consecutively admitted to nine Italian hematological centers on behalf of Intergruppo Italiano Linfomi. Pts with stage I-IIA according to Ann Arbor stage, independent of presence of bulky disease, were considered for the study. FDG-PET was mandatory at baseline, after two cycles and at the end of therapy. We evaluated the progression free survival of pts starting from the time of diagnosis to relapse or progression of disease or last follow-up. No treatment variation based only on PET-2 results was allowed. The median age was 33 years (16-75), 85 pts were female, 15 pts presented stage I and 148 stage II, bulky was reported in 45 pts. One-hundred and fourty-eight pts were treated with combined modality (CT+RT) and 15 pts were treated with chemotherapy alone. The FDG-PET performed after two cycles (PET2) was positive in 23 pts (14%): 12 (52%) progressed or relapsed and 11 remained in CR. By contrast 130/140 (93%) pts with a negative PET2 remained in CR. Thus the positive predictive value of a PET2 was 52% and the negative predictive value was 93%. The sensitivity and specificity of PET2 were 55% and 92%, respectively. Seventeen pts showed disease progression during therapy or within 12 months after having reached CR, 11/17 (65%) were PET2 positive. Six pts died due to the disease, four were PET2 positive and two were PET2 negative. In univariate analysis negative FDG-PET performed after two cycles (p .0000), absence of bulky disease at diagnosis (.004) were statistically correlated with a better progression free survival. In multivariate analysis only PET2 was independently predictive of relapse/progression probability (p .000). With a median follow-up of 31 months (range 6-87) 154 pts are alive and 141 (92%) are free from progression. The 2-yr FFS probability for PET2 negative and for PET2 positive patients were 94% and 58% respectively. *Conclusion*. This multicentric study confirms that FDG-PET scan performed after two courses of conventional standard dose chemotherapy was able to predict treatment outcome in early stage Hodgkin disease. Due to the large number of false positive PET2 in localized lymphoma we suggest new evaluation methods in this subset of pts.

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CEREBRAL RELAPSE OF HODGKIN LYMPHOMA (HL) SUCCESSFULLY TREATED WITH A COMBINED TREATMENT INCLUDING AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) AND CRANIAL IRRADIATION. ANALYSIS OF ONE CASE WITH REVIEW OF THE LITERATURE

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CNS involvement by HL is rare in patients with systemic HL. We report a case of a patient who developed isolated brain involvement at HL relapse successfully treated with a combined approach. In October 2003 a 24-year old man developed laterocervical lymphnode enlargement with B symptoms. A diagnosis of HL, mixed cellularity, clinical stage IV B, IPS score 5, was made. He received 8 courses of ABVD regimen, obtaining a complete remission (CR) on May 2004. In January 2005 he developed frontal and peri-orbital headache and vomiting. Brain CT and MR scan showed an expanding process with oedema and shift of the middle line structures. The stereotactic biopsy confirmed the diagnosis of HL. Restaging was negative, indicating an early relapse of HL, stage IAE (CNS). Two DHAP cycles (Dexametazone, high-dose Cytarabine and Cisplatin) were administered. Intrathecal therapy with Ara-C and Dexametazone was associated. Peripheral blood stem cells were collected on the 2nd DHAP and ASCT was performed after conditioning by CBV-Mx (Cyclophosphamide, BCNU, Etoposide, Mitoxantrone).The pre-ASCT PET scan showed no FDG-uptake. The brain CT scan at 1 month showed a CR. Total cranial irradiation (30.6 Gy) with a boost of 8 Gy on the right temporal region was performed as consolidation. At 43 months from the end of the therapy, the patient is in CR, with a good quality of life and no neurologic sequelae. There is no clear consensus as to the best treatment of patients with CNS involvement, but radiotherapy has been the standard approach. Unfortunately response is often poor, with a median time from diagnosis to death of 46 months. However, when combined modality treatment is given, including radiation and chemotherapy, prolonged DFS may be achieved. High-dose therapy and ASCT has been widely investigated in patients with refractory or relapsing HL and is associated with higher CR rates and improved progression-free survival. The present case shows that patients with CNS HL should be worked up and treated with curative intent. Our approach consisting of II-line chemotherapy including CNS barrier-crossing agents, intrathecal prophylaxis, and consolidation with ASCT and cranial irradiation proved well tolerated and may become standard for this unusual HL presentation.

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LACK OF HEPATOXICITY BY THE ASSOCIATION OF BORTEZOMIB AND GEMCITABINE IN HODGKIN'S LYMPHOMA (HL): ITALIAN LYMPHOMA INTERGROUP (IIL) EXPERIENCE

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Background. Despite IGEV (ifosfamide, gemcitabine, vinorelbine and prednisone) has demonstrated high complete remission (CR) rate as induction before high dose consolidation , there is the need for enhancing CR rate, which represents the strongest prognostic factor in this setting. Preclinical evidence and clinical data on solid tumors suggest a synergism of action between bortezomib and gemcitabine. Mendler *et al.* (*Ann Oncol 19: 1759, 2008*) have reported an unexpected liver toxicity (tox) by the association of gemcitabine and bortezomib in HL . Herein we report the IIL experience, in terms of toxicity (tox), from an ongoing randomised phase II comparison between IGEV and IGEV + bortezomib at 1.3 mg/mq on day 1,4 & 8 of each course (B-IGEV) in the induction phase of refractory-relapsed HL. *Methods*. Since February 2008 patients (pts) with relapsed/refractory HL after first line chemotherapy are allocated in a 1:1 ratio to receive 4 cycles (cy) of standard IGEV or 4 cy of B-IGEV. Full blood count and complete biochemistry profile is required at

day 1 of each course and at each bortezomib administration as per protocol. *Results*. As of March 2008, of 28 patients accrued, 13 have been allocated to IGEV and 15 to B-IGEV. A total of 74 cy and 174 hepatic biochemistry determinations (det) are evaluable. Results of hepatotoxicity are reported in the Table. Data on transaminases, alkaline phosphatase and bilirubin toxicity are expressed in percent . Grade 3-4 transaminase increase occurred in 2 pts in the IGEV and 2 in the B-IGEV group . Overall, treatment was delayed or given at reduced dose in 7 pts (5 IGEV and 2 B-IGEV). Two courses were delayed (both IGEV) for hematological tox, and 6 were administered at reduced dose: 3 IGEV (1 for hematological tox , 1 for hepatic tox, 1 for medical decision) and 3 B-IGEV (1 for hypotension , 1 for hematological and cutaneous tox, 1 for medical decision). *Conclusions*. in our experience, hepatotoxicity did not represent a major concern in delivering a gemcitabine-bortezomib containing therapy.

Table. Hepatotoxicity of IGEV vs B-IGEV.

	N^ pts	N^ cy	N^ det	N^ det altered	ALT		AST		ALP		Bilirubin	
					G1-2	G 3-4	G 1-2	G 3-4	G 1-2	G 3-4	G 1-2	G 3-4
IGEV	13	34	70	42	48	6	20	2	43	0	0	0
BIGEV	15	40	104	63	50	5	34	0	38	0	0	1

Data of tox grading in percent

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LATE EFFECTS OF TREATMENT FOR EARLY STAGE HODGKIN'S LYMPHOMA: A SINGLE CENTRE EXPERIENCE

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Background. Long term toxicities are the major concern in survivors of early stage Hodgkin's Lymphoma (HL). Current approaches attempt to achieve the higher remission rate, while reducing treatment-related toxicities. Aim. To compare the outcome of early stage HL treated with chemotherapy with or without radiotherapy, we reviewed clinical features, therapy, and long term outcome of 79 patients (pts) treated at our institution from 1999 to 2005. *Methods*. 44 pts were treated with 4-6 cycles of ABVD plus involved field radiotherapy (CT+RT group) and 35 pts with chemotherapy alone, consisting of 3-6 cycles of the ABVD regimen (CT group). Median pts age was 36 years, 53% were males, 72% presented B symptoms and 75% had clinical stage II. The histology was nodular sclerosis in 82% patients. Patients with bulky disease were excluded from the analysis. The two therapeutic groups were statistically comparable for clinical features and median follow-up (60 and 62 months for the CT and CT+RT group respectively). *Results.* In the CT+RT group 88% achieved CR and 12% PR after first line therapy whereas in the CT group 83% achieved CR and 17% PR. High dose chemotherapy was employed as salvage therapy for 5 partial responders in the CT and 4 in the CT+RT group. One relapse occurred in the CT+RT group at 12 months after first-line therapy. At a median follow-up of 60 months the disease-free survival was 88% in the CT group and 89% in the CT+RT group (p: n. s.). In the CT+RT group 3 patients (6.8%) developed long term toxicities (1 case of Chronic Restrictive Pulmonary Disease, 1 case of hypothyroidism and 1 of thyroid cancer at 41, 49 and 53 months of follow-up respectively) whereas 1 patient (2.8%) in the CT group developed a late toxic event consisting in cardio-myopathy at 20 months of follow-up (p: n s). Our retrospective analysis shows that a high response rate was achieved both with chemotherapy alone and with chemoradiotherapy; no difference was observed in terms of relapse rate. Nevertheless, we observed a higher incidence of long term toxicities in the CT+RT group. Multicentric studies and longer follow up are required to individualize tailored strategies, to improve the remission rate and minimize toxicity.

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ROLE OF POSITRON EMISSION TOMOGRAPHY (PET) AFTER 2 AND 4 COURSES OF CHEMOTHERAPY IN PATIENTS WITH HODGKIN'S LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background. Positron emission tomography (PET) is a useful tool for the management of patients (pts) with Hodgkin's Lymphoma (HL),for staging and evaluating response to treatment. Several studies have shown the prognostic value of PET performed after two courses of chemotherapy. *Aims*. The aim of the present study is to investigate the value of PET performed after 2 (PET2) and 4 (PET4) cycles of therapy for the management of pts with HL observed in our institution from September 2006 to September 2008. *Methods*. A total of 26 pts with newly diagnosed HL were staged with: total body CT scan, bone marrow biopsy and whole body PET. All pts received chemotherapy according to ABVD regimen. After two courses PET scan was performed (PET2), and after 4 cycles restaging was performed including CT and PET scan (PET4). All pts with positive PET4 continued the treatment: with two more courses of ABVD or Radiotherapy, and those with progressive disease or with adverse prognostic factors at diagnosis received salvage treatment consisting of: IGEV chemotherapy, stem cells collection, BEAM chemotherapy with autologous stem cells transplantation. *Results*. After two courses of ABVD in 22 (85%) pts PET was negative, but no treatment change was performed on the basis of the PET 2 results. In 20 pts (76%) there was a correspondence between PET2 and PET4: 15 negative and 5 positive; in 3 (12%) pts PET2 was positive and PET4 negative and in 3 (12%) PET2 was negative and PET4 positive. After 4 cycles of therapy 18 (69%) pts were in Complete Remission (CR); 5(19%) were in Partial Remission (PR) and 3 (12%) had progressive disease. The 24 months progression free survival (PFS) rate for pts with negative PET2 is 89 % and with positive PET2 is 12%. For pts with negative PET4 24 months PFS rate is 92% and with positive PET4 is 8%. Comparing the two subset of pts, with positive PET2/PET4 and negative PET2/PET4, the first group shows a worse prognosis. *Conclusion*. In our experience PET2 and PET4 have the same impact on the outcome of pts affected by Hodgkin Lymphoma: positive PET after 2 or 4 cycles of therapy shows a negative predictive value and can identify pts who need a more intensive treatment approach. Further studies are needed to establish the best time to use PET for an early response assessment to the treatment in order to select individually tailored therapeutic strategies.

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PU001

REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR LYMPHOMAS

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We analyzed data on 46 patients with lymphomas, treated with reduced intensity conditioning and allograft between 2002 and 2007 at our institution. Twenty-three patients had HL, seven high-grade lymphomas, twelve low-grade and 4 T-cell lymphomas. Median age was 37.5. 29% patients had a chemosensitive disease and 87% had been treated before with autologous transplantation. 59% received stem cell from peripheral blood and 41% from bone marrow. The donor was a related sibling for 27 patients and unrelated for 19. Conditioning regimen was the association Tiothepa (10 mg/kg), Fludarabine (60 Mg/kg), Endoxan Cyclophosphamide (60 mg/kg) +/- ATG-F (total dose 15 mg/kg) for patients who received a related allograft, and Tiothepa (10 mg/kg), Melphalan (30 Mg/mq), Endoxan (100 mg/mq) + ATG-F Fresenius for the unrelated transplant. All patients, except one who died at day 7 after transplantation, engrafted. Overall survival was 51% at 3 yrs and it was significantly associated with chemoresistant vs chemosensitive disease (31% vs 93%). Transplant related mortality was 23% at 3 yrs, and we observed an association with chemoresistance (33% vs 8% in chemosensitive group). Relapse was 66% at 3 yrs, and significantly higher for patients with chemoresistant disease (74% vs 34%). We also tried to analyze the Graft-versus-Lymphoma effect, looking for association between complete remission (CR) and chronic GVHD: of 24 patients who obtained CR, 17 had experimented cGVHD, but of 13 who did not obtain response only 4 had cGVHD (chi squared=6.072, p=0.048). However, different diseases had different response to the immunological effect: HL and high grade NHL appeared to have a very limited response to GVL effect, but in low grade NHL and peripheral T-cell Lymphomas there was an higher rate of response with a minimal relapse risk at 3 yrs. In conclusion, we can say that allogeneic stem cell transplantation represents an effective therapeutic option for Lymphomas, even if best results are obtained in low grade and peripheral T-cell Lymphomas. More studies will be required to better understand the role of allograft in the treatment of HL and high grade NHL. In addition, the importance of chemosensitivity/ chemoresistance suggests questions about the best timing to subject patients to allo-transplantation.

PU002

SUCCESSFUL COMBINATION TREATMENT WITH THALIDOMIDE AND DONOR LYMPHOCYTE INFUSION AFTER MYELOMA PROGRESSION POST ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction. The relapse rate after of multiple myeloma patients, resistant to induction therapy, is still considerable, also after a tandem program with autologous stem cell transplant (autoSCT) followed by reduced conditioning allogeneic stem cell transplant (RIC-alloSCT). Due to a well documented graft versus myeloma effect, adoptive immunotherapy with donor lymphocyte infusion (DLI) has become a treatment option in this setting. To improve the anti-myloma effect of DLI we associated low-dose of thalidomide in a patients with a myeloma in progression after RIC-alloSCT. *Methods*. A 56 year old patient with multiple myeloma III stage, resistant to induction treatment (consisted of 4 cycle according to VAD regimen; high dose of cyclophosphamide and radiotherapy) was submitted to a tandem transplant program (high dose melphalan and autoSCT followed with RIC alloSCT). *Results*. At 6 months from RICalloSCT a progressive increase of monoclonal IgG/k and of plasmacell bone marrow infiltrate was documented. A program with thalidomide (100 mg/daily) and DLI (CD3-0.5x10^7/kg monthly for the first three doses; then 1.0x10^7/kg/monthly for the other ten doses) was than started. Major toxicity of thalidomide was weakness grade I/II and peripheral neuropathy grade I/II; no acute and chronic GVHD was seen. A progressive clearance of monoclonal IgG/k was documented (from 35 g/L when thalidomide plus DLI were started to 10 g/L on March 2009, at 2 years from transplant, when patient was alive, in major response without any sign of active GVHD and no sign of disease progression). *Conclusion*. Adoptive immunotherapy with low-dose thalidomide and DLI could induce and antimyeloma effect and is well tolerated.

PU003

OUTCOMES AFTER ALLOGENEIC STEM CELL TRANSPLANT FOR REFRACTORY AND RELAPSED ACUTE MYELOID LEUKEMIA

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Introduction. Prognosis of patients with refractory and relapsed acute myeloid leukemia (AML) is poor, with allogeneic stem cell transplantation (alloSCT) being the option with the highest antileukemic potential. It is well known that myeloablative conditioning is associated with a high treatment related mortality (TRM) and that, on the other hand, reduced intensity conditioning (RIC) may be inefficient for long-term disease control. In this regard, we retrospectively compared the outcome of a series of patients with refractory and relapsed AML submitted to conventional or RIC SCT at our Insitute. *Methods*. Between 1996 to 2008, 65 consecutive AML patients were submitted to alloSCT. Forty-eight patients were submitted to myeloablative SCT: median was age 48 years (18-60); 29 (60.5%) patients were transplanted from HLA-identical sibling and 19 (39.5%) from unrelated donors. Seventeen patients were submitted to RIC - SCT: median age was 60 years (23-69); 10 (59.0%) patients were transplanted from HLA-identical sibling and 7 (41.0%) from unrelated donors. *Results*. Myeloablative SCT: acute GVHD grade 0-1 occurred in 26 (54.5%) patients, grade II in 13 (27.0%) and grade III-IV in 9 (18.5%) patients. Chronic GVHD was absent in 20 (52.5%) of 38 valuable patients, limited in 6 (16.0%) and extensive in 12 (31.5%) patients; RIC SCT: acute GVHD grade 0-1 occurred in 14 (82.0%) patients, grade II in 2 (12.0%) and grade III-IV in 1 (6.0%) patients. Chronic GVHD was absent in 8 (66.5%) of 12 valuable patients, limited in 1 (8.5%) and extensive in 3 (25.0%) patients. Survival: median follow-up from transplant was 10 months (1–196) and 4 months (1–50) respectively (myeloablative vs RIC-SCT). One year probability of overall survival was 30.0% and 39.0% respectively (myeloablative vs RIC SCT) – p:0.3; one year probability of disease free survival (DFS) was 25.0% and 38.0% respectively (myeloablative vs RIC SCT) – p=0.08. TRM was 35.5% vs 26.5% (myeloablative vs RIC SCT respectively) – p=0.07. *Conclusion*. Our results suggest that relapsed – resistant AML patients may achieve a DFS higher than 20%, irrespective of intensity of conditioning regimen, with a reduction of TRM in RIC setting, as exceptive.

PU004

NOT PUBLISHED

PU005

CHIMERISM STATUS ANALYSIS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION ON GRANULOCYTES AND MONONUCLEATED CELLS OF PERIPHERAL BLOOD

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Background "Short Tandem Repeats" (STR) analysis evaluates Chimerism Status (CS) after allogeneic haematopoietic stem cell transplantation (HSCT). The high STR polymorphism permits to differentiate donor (D) or recipient (R) origin of the analysed cells. Complete CS (CCS) correlates with a good prognosis, conversely the mixed CS predicts disease relapse or transplant rejection, particularly when R amount

rises along the time (increasing-mixed CS). Patients and methods We evaluated 24 myeloablative HSCT performed on 23 onco-hematologic patients (pts) ,15 from sibling related donor (SRD) and 9 from matched unrelated donor (MUD). All MUD-HSCT and one of SRD-HSCT received Anti-Thymocyte-Globulin (ATG), 14/15 SRD-HSCT did not received ATG nor other T-depletion procedures. At time +30, +60, +90, +120 days after HSCT, CS analysis was conducted separately on granulocytes (GC) and mononucleated cells (MnC). A semi-quantitative method, based on PCR amplification of 16 STR markers, was applied to define the CS. We assumed the CS in GC population as expression of myeloid engraftment. We also correlated CS in MnC, with single different leukocyte subpopulations (lymphocytes, monocytes) in peripheral blood count and with the reconstitution of lymphocyte subpopulations (B, T cells and NK cells) at the same time point of analysis, in order to compare the reciprocal course. *Results*: At time + 30, +60, +90 and +120 after HSCT, we evaluated CS on the transplanted pts. In GC we observed a stable CCS in 21/24 HSCT since the first control. In MnC population 17/24 HSCT presented CCS at the first control, 3/24 evolved to CCS successively. 4/24 HSCT presented increasing-mixed CS either in GC or MnC followed by relapse (2 pts) or rejection. Considering each different leukocyte subpopulations, we observed that MnC amount was constituted for 50% from lymphocytes and 50% from monocytes. Into the analysis of lymphocyte subpopulations, T-lymphocytes were virtually absent (either T4 or T8) till to +120 after HSCT in pts who received ATG. In these ones CS analysis in MnC was virtually performed in monocytes, B and NK cells only. Conversely pts who did not received ATG presented complete and more rapid reconstitution of each lymphocyte subpopulations. *Conclusions*: CS in GC reflect the engraftment of myeloid lineage. Instead CS in MnC must be considered in the context of type of transplant (MUD or SRD, T-depletion or T repletion) and correlated with single peripheral blood subpopulations.

PU006

REDUCED-INTENSITY CONDITIONING ALLOGENEIC TRANSPLANT IN HEAVILY PRE-TREATED CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS: A SINGLE CENTRE EXPERIENCE

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We assessed the feasibility and efficacy of non-myeloablative allogeneic hematopoietic cell transplantation (HCT) in heavily pre-treated patients (pts) affected by Chronic Lymphocytic Leukaemia (CLL). We studied all patients with positron emission tomography (PET)/computed tomography (CT) and patient specific polymerase chain reaction (PCR) assay to detect minimal residual disease during follow-up after transplantation. We retrospectively analyzed 11 pts, affected by refractory or relapsed CLL undergoing HCT with reduced-intensity conditioning (RIC) after a median of 4 lines of chemotherapy, between March 2004 and December 2008. The donor's source for RIC transplantation derived from HLA-matched related or unrelated HLA-matched donors. Prognostic biological parameters, (IgVH mutational status, ZAP-70 and CD38 expression and chromosomal abnormalities by FISH), were studied at baseline. We applied comorbidities index (HCT-CI) according to Sorror score. Four pts achieved complete remission (RC) at median of 12 months (range 12-35) from transplantation and maintained continuous CR at a median of 32 months (range 29-54) from transplantation. Three pts achieved partial remission at median of 16 months from transplant documented by PET/CT scanning. Three pts died, one pt died for transplant related mortality (TRM) at day +39 and the second pt died from disease progression (PD) 12 months after transplant. The third pt achieved PR and relapsed 12 and 20 months from RIC transplant respectively; he died from fungal infection 27 months after RIC transplant. At a median time of 21 months, 8 out of 11 pts are alive: 4 in CR, 1 in PR and 1 in PD. Two pts, are too early for the assessment of disease status. All pts engrafted and reached > 500 granulocytes/mm3 at a median of 14 days (range 8-22) after SCT. Four out of 11 pts required red blood cell transfusions and only 2 pts needed platelet transfusions. The median length of hospitalization was 25 days (range 13-39). One pt experienced Gram-positive sepsis and 2 were developed fever of unknown origin. Six pts experienced CMV reactivation resolved by pre-emptive therapy with oral ganciclovir or valganciclovir. RIC transplant is recommended even in high-risk pre-treated CLL pts in which no other chemo-immunotherapy can be used because of refractory disease and/or poor biological parameters. PET/TC is a useful instrument to monitor the progressive reduction of neoplastic activity and to plan the management of residual disease.

PU007

PEDIATRIC TRANSPLANTATION EXPERIENCE IN CAMPANIA: A REFLECTION ON DATA

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We present the cases observed in the Unit of Bone Marrow Transplantation during the years 1996/2008. In this period 148 transplantation procedures, of which 79 types of autologous and 69 allogenic type (including 4 non-donor family) were performed. Patients were admitted to hospital rooms and a Positive Pressure Laminar flow on the bed, the area filtered with HEPA filters. As for autologous transplantation, the number of patients is 72 (7 were subjected to a tandem of infusion), 46 males, 24 females, age is between 2 and 11 years, the diseases are AML 23, ALL10, NB 16, NHL 5, HL5, Medulloblastoma 5, Ewing Sarcoma 1, Breast Cancer Entodermico1, Pinealoblastoma 1, Peduncles Brain Glioma 1, Rhabdomyosarcoma 1, Glioblastoma 1, Astrocytoma 1, Embryonal Carcinoma 1. TRM is the total of 2.5%. Specifically analyzing the Overall Survival and Disease Free Survival in disease undergoing autologous bone marrow results are the follows: for solid tumors, the OS is 43%, the DFS of 30% for the AML OS is 82%, the DFS of 72%; for HL of the OS is 100%, the DFS of 72% for ALL patients are alive without disease 41%, while the dead are 59%. Children undergoing bone marrow transplantation allogenic were 65 (4 were subjected to a second transplant) distributed as follows: 45 males and 20 females; age between 5 months and 17 years, the diseases are AML 26, ALL18, Hemoglobinopathies 6, NHL 3, Lymphohistiocytosis 3, FA 3, SCID 3, AA 2, Osteopetrosis 1, FAS deficit 1, Blackfan-Diamond 1. The donors was 32 males and 33 females. The TRM was 6%; 3% of patients had a rejection; in 1.5% of cases has developed a second neoplasm, the OS is 77%, the DFS of 62%. In 26% of the cases was presented GVHD: in 8, 6% was acute grade 2-3, in 13% was made in chronic and 4.3% in extended. Assessing disease undergoing transplantation Allogeneic Bone Marrow in LLA the OS is 68%, the DFS 65% in AML, the OS is 83%, the DFS 100%, in non-oncological diseases is of the OS is 88%, the DFS 100%. There were no further significant infectious episodes occurred during periods of hospitalization in a protected environment. Our experience, although on a limited number of patients, leads us to believe that bone marrow transplantation is a technique that can significantly increase life expectancy and mortality with an acceptable toxicity for patients with a good quality of life after the resumption of immunity. Allogeneic transplantation in these areas are significantly affected by GVHD.

PU008

B-CELL IMMUNE RECONSTITUTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION AND ITS ROLE IN THE DEVELOPMENT OF CHRONIC GRAFT-VERSUS-HOST DISEASE

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Conflicting results are reported on the status of B-lymphopoiesis after allogeneic stem cell transplantation (SCT) and particularly in patients with chronic Graft versus Host Disease (cGvHD). Severe B-cell lymphocytopenia and hypogammaglobulinemia are documented, as well as B-hyperactivity is suggested by the production of pathological autoantibodies and by the successful response to Rituximab in certain clinical manifestations of cGvHD. Our study evaluates the kinetics of B cell recovery and engraftment after allogeneic SCT in comparison with T, NK and myeloid cells. The final objective is to identify if there is a correlation between B lymphocytes recovery and the development of cGvHD. Twenty-three consecutive patients, median age 42 years (range 28-67) were prospectively studied. We analysed hematopoietic chimerism at day 90, 120 and 180 on whole PB, on immunomagneti-

cally sorted CD3+ and CD19+ cells and on granulocyte fraction. Full donor chimerism (FDC) was defined as the presence of at least 95% donor cells. We evaluated B, T and NK cells in PB by immonophenotyping at day 30, 90, 120 and 180. Four patients developed cGvHD in median at 5 months after SCT, with at least 2 organs involved and a median score of 3 according the Organ Scoring System (Filipovich et al,BBMT,2005). CGvHD progressed from a pre-existent acute GvHD in all 4 cases. No patient showed autoantibodies. We could not analyzed B cell chimerism at day 90 due to the low number of CD19+, but at day 120 FDC in CD19+ cells was found in all patients, as well as in CD3+ cells and granulocytes. We observed a similar kinetics of CD3+, CD4+ and CD8+ cells recovery in all patients, with the lowest cells concentrations at day 90 and a subsequent progressive increase at day 120 and 180. Patients with cGVHD showed a lower NK cells concentrations at day 90 in comparison with controls. All patients showed B cells concentrations no higher than 100/microliter from 90 to 180 days. However, the group with subsequent cGvHD showed lower B-cell concentrations at day 120 (p=0.03) in comparison with controls. Severe B lymphocytopenia persisted through the first 180 days after SCT. FDC could be observed in B cells of all patients at day 120 after SCT. Patients at risk of cGVHD had a significative reduction of B cells concentrations at day 120. We are planning to extend the follow-up of the patients and to confirm our results in a larger population.

PU009

NOT PUBLISHED

PU010

MYASTHENIA GRAVIS LIKE SYNDROME AS ATYPICAL MANIFESTATION OF CHRONIC GRAFT VERSUS HOST DISEASE

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Myasthenia gravis is a rare manifestation of immune dysregulation of chronic graft versus host disease (cGVHD). This report describes a single case of severe, overwhelming respiratory failure myasthenia like syndrome after allogeneic hematopoietic stem cells transplantation and successfully treated with high dose cyclophosphamide. A 19 years old male was diagnosed of acute myeloid leukemia in November 2006. In February 2007 while in partial remission, he underwent a matched sibling allogeneic stem cells transplantation. Short course Metotrexate, Cyclosporine and Thymoglobulin (ATG) were given as GVHD prophylaxis. Engraftment occurred by day + 12. The post transplant period was complicated by grade II acute GVHD and extended chronic GVHD, involving primarily oral mucosa and skin. Patient was treated with corticosteroids and cyclosporine as first line therapy. In April 2008 a photopheresis program was started (2 days/every other week at the beginning and 2 days/monthly afterwards). The treatment was discontinued after 13 courses with very good response (complete remission). After gradual reduction of the immunosuppressive therapy he started to complain progressive disphagia and dysphonia (brain TC and MRI, Ear Nose Throat examination were negative). In February 2009 the consultant neurologist diagnosed a myasthenia like syndrome (acetylcholine receptor and anti musk antibodies negative) not responding to standard therapy with plasma-exchange (3 courses), cyclosporine and high dose steroids. He developed progressive overwhelming respiratory failure which required urgent ventilatory mechanical support. For the worsening of the clinical conditions and the strong suspicions of an unusual pattern of cGVHD he was treated with a single high doses intravenous cyclophosphamide (4 grams/m^2).The respiratory syndrome quickly improved and patient was extubated after two days from cyclophosphamide infusion. All the neurologic GVHD manifestations regressed. At the time of this writing patient is in good clinical conditions. He is still on cyclosporine and steroids, and monthly high dose intravenous immunoglobulin are also administrated. This particular case is a serious unusual manifestation of cGVHD. Because very few cases are reported in the literature, treatment of these atypical neurologic complications is not standardized. High dose of cyclophosphamide has to be considered when other treatments fail and life-threatening complications occur.

PU011

ATG-GENZYME VERSUS ATG-FRESENIUS IN PATIENTS UNDERGOING ALLOGENEIC HSCT FOR HIGH RISK HEMATOLOGIC MALIGNANCIE; A RETROSPECTIVE EVALUATION

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Objective: Graft-versus-host disease and infections still remain the two major complications of allogeneic HSCT. Many immunosuppressive strategies have been explored and different type of ATG or Monoclonal Antibodies have been used with controversial results. In this report we retrospectively analysed the efficacy of two preparations of ATG (ATG-Genzyme Thymoglobulin, ATG-G) and ATG-Fresenius (ATG-F) in two cohorts of transplanted patients with the aim to evaluate their impact on GVHD, rejection, infections, relapse, and survival. *Patients and methods*: Since July 2005 to March 2009, 50 consecutive patients undergoing myeloablative conditioning from HLA identical or mismatch siblings (n=26) or unrelated donors (n=24), were retrospectively analysed. Underlying diseases were advanced and/or high risk haematologic malignancies Thirty (30) patients received ATG-G at a total dose of 4.5 mg/kg if HLA identical sibling transplants (n = 17) or 6 mg/kg if unrelated transplants (n=13). Twenty (20) patients received ATG-F at a total dose of 15 mg/kg if HLA identical sibling transplants (n=7), or 30 mg/kg if unrelated or mismatched transplants (n=13). Both ATGs were administered on days -3,-2,-1. GVHD prophylaxis was performed with Cyclosporine and sMTX. Groups were comparable as regards age, underlying diseases and conditioning (Table 1). *Results*. Infusion related side effects occurred more frequently in the ATG-G group (80% vs 65%). Time of engraftment was similar. Acute GVHD II-IV occurred in 14% of ATG-G patients versus 20% of ATG-F patients. Two patients rejected in the ATG-G group, none in the ATG-F group. *Conclusions*. In this retrospective analysis, a preliminary observation underlines no statistical difference in the incidence of Acute and Chronic GVHD among patients receiving the two types of ATG. Early post transplant bacterial infections and Cytomegalovirus infections were similar in the two groups. At 21 months post-transplant Overall Survival was 68% for all patients in the two groups . In the same period the probability of relapse was 36% in the ATG-G group vs 20% in the ATG-F group (p=ns). These data must be confirmed in a randomised prospective study and longer follow-up is necessary.

Table 1. Patients characteristics.

	ATG - G	*ATG- F*
Patient number	30	20
Age median (range)	45 (17-64)	50 (17-60)
Male/Female	16/14	11/9
Type of transplant		
Unrelated	13	11
Identical Sibling	17	7
Mismatch Sibling	0	2
Source of HSCs		
PBSC	27	15
BM	3	5

PU012

CYCLOSPORIN MONITORING AT 2 HOURS FROM ORAL INTAKE LEADS TO LESS TOXICITY AND BETTER COMPLIANCE IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELLS TRANSPLANTATION AND IMMUNOSUPPRESSIVE TREATMENT FOR SEVERE APLASTIC ANEMIA

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Nephrotoxicity derived from Cyclosporin treatment is a well known complication and for this reason drug level baseline is usually monitored (before morning oral intake). Recently, data in the literature demonstrated a higher reliability of drug serum level after 2 hours from oral intake as regards to absorption and needed doses in the specific patient. These reports are obtained among Solid Organ Transplant settings. *Aim.* To assess if cyclosporin blood level at 2 h from oral intake is a more reliable

tool than the pre-intake level to prevent or reduce nephrotoxicity in Hematopoietic Stem Cells Transplantation (HSCT)and Severe Aplastic Anemia (SAA) patients, and to tailor the required dose in each patient. *Materials and methods. Patients.* From February to November 2008 we prospectively enrolled 11 patients post HSCT and 4 under Immunosuppressive treatment (IS) for SAA. We monitored serum level of cyclosporin at time 0 and after 2 hours from oral intake, creatinine and urea serum levels, drug toxicity, and Graft versus host disease (GvHD) in HSCT group. Oral dose was then modified according to the 2 hours post cyclosporin results. *Results*. The incidence of hypercreatininemia, renal failure, hypomagnesiemia, headache and tremors was trivial and drug withdrawal was very seldom required. Creatinine serum level was ≥1.5 in 3 patients (20%). Only 2 out of 15 patients (13%) needed cyclosporine withdrawal: one for renal toxicity, the other one for trombotic trombocitopenic purpura; both of them were in HSCT group. Other side effects (headache and tremors) were evident only in 2 (13%) patients, one with SAA, the other one in HSCT group. Cyclosporin level after 2 hours seemed to be independent from the dosage/Kg of body weight, especially in children. In 7 out of 11 HSCT patients (63%) acute GvHD (grade I-II) was evident whereas limited chronic GvHD in 4 out of 11 patients (36%) was found. Both SAA and HSCT patients tolerated cyclosporin with 2 hour level monitoring very well, with apparent less toxicity. The two patients requiring cyclosporin withdrawal were both in the HSCT group. *Conclusions.* Our data suggest that dose adjustment of cyclosporin according to 2-hour level is more accurate than the basal one and more effective to prevent toxicity and side effects. Furthermore, it also provides a better compliance. The scarce number of patients doesn't allow to draw definite and firm conclusions, prompting the need to further prospective studies.

PU013

PERHIPHERAL BLOOD STEM CELL YELD IN 103 NORMAL DONORS MOBILISED WITH GLYCOSYLATED GRANULOCYTE COLONY-STYMULATING FACTOR (G-CSF)

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Background. Mobilised peripheral blood is the main source of stem cells collected from normal healthy donors (HD) in adult allotransplant-settings. We present our experience of mobilising and collecting 103 HD using standardised procedures. Relatively little information is available about the factors predicting for satisfactory stem cell mobilisation and collection from HD like age, sex or donor weight. Material and methods. Peripheral blood stem cells (PBSC) were collected from 103 HD between January 2005 and December 2008, 48 (47%) for related transplant, 55 (53%) for unrelated transplant. All donors were mobilised with glycosylated G-CSF at 10 micrograms/Kg/die s.c. for four days and 1 apheresis was started on day +5 after 9 doses of G-CSF. Further 2 vials of G-CSF were administered when a second apheresis was necessary. Apheresis was performed with Com.Tec, Fresenius, ACD was used as the anticoagulant and the target $CD34^+$ recipient cell dose was $4x10^6$/Kg. *Results*. Most of the related (72%) and unrelated (71%) donors were male, while the unrelated male and female tended to be younger than related donors with median ages of 36 and 41, respectively ($p<0.001$). No significant difference in the weight of the two groups. The $CD34^+$ cell target of $4x10^6$/Kg body recipient was reached after a single apheresis in 90/103 donors (87%). Two donors were considered poor mobilisers (<$2x10^6$/Kg /Kg $CD34^+$ cells after 2 aphereses). Nevertheless in a case we reached the minimum of $2x10^6$/Kg $CD34^+$ cells with three procedures, in the other one a further bone marrow harvest was needed (final count of $2,28x10^6$/Kg $CD34^+$ cells).Male donors achieved significantly greater numbers of $CD34^+$ cells than female donors, both as absolute count and as median range (386 *vs.* 261). Nevertheless if we correct the $CD34^+$ cell yield by median donor body weight (83 kg for the male and 57 Kg for the female), we obtain 4.6 and 4.5 $x10^6$ $CD34^+$/Kg, respectively. Discussion. This single institution study confirms the effectiveness of glycosylated G-CSF mobilisation in a large group of normal HD for allogeneic transplantation with 87% of requested cell yield with a single apheresis and 98% with two apheresis. The impact of age was apparently not statistically important while sex was, because $CD34^+$ absolute number and dose collected were higher in male than in female. This was mainly due to its different body weight, that in the end seems to be the best predictive factor of stem cell yield.

PU014

ROLE OF AB0 INCOMPATIBILITY ON ALLOGENEIC TRANSPLANT OUTCOME OF 140 PATIENTS WITH ACUTE LEUKAEMIA TREATED IN A SINGLE INSTITUTION

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Introduction. Approximately one-third of allogeneic stem cell transplants (allo-SCT) are performed across the AB0 blood group barrier. The impact of AB0-incompatibility on allogeneic transplantation outcome remains a controversial issue. In particular, several studies reported that AB0 incompatibility increases Transplant Related Mortality (TRM) and represents a risk factor in allo-SCT. Methods and Patients. For these reasons, in our study we evaluated, retrospectively, the association between AB0 incompatibility and the incidence and the severity of acute and chronic GvHD, TRM, relapse, overall survival (OS). A total of 140 patients with acute leukaemia who underwent transplantation in a single Institution were studied. The variables known to affect the transplantation outcome, such age and sex, were balanced between AB0 matched and AB0 mismatched transplants. We divided the patients into two groups, AB0 compatible and no AB0 compatible including in the latter group major, minor and bidirectional incompatibilities. The standard procedure for AB0-incompatible transplants was the red blood cell (RBC) depletion and plasma depletion when anti-recipient hemagglutinin were > 1:32. Results. The median follow-up was 17 months (r.0.2-182). Analysis of our data shows no difference in term of acute and chronic GvHD, TRM, relapse and OS in both groups. Other variables considered such as time to reach absolute neutrophil count >$0,5x10^9$/l and to platelet count >$30x10^9$/L have not shown differences in the two examined groups. In multivariate analysis we found that the AB0 incompatibility group had significantly statistically greater transfusion need (p=0,026). This difference may be explained by the reaction of haemolytic transfusion due to immunological incompatibility between donor and recipient. All this led us to use a transfusion policy in AB0 incompatible transplantation using group 0 of RBC until the title of hemagglutinins are undetectable or until the change of blood group. Conclusion. There was no evidence of a substantial effect of AB0 blood group incompatibility on the outcome of bone marrow transplantation among patients with leukemia. However, in our Institution are still being further studies to confirm definitively these results.

PU015

AUTOLOGOUS BONE MARROW TRANSPLANTATION WITH REDUCED INTENSITY OF CHEMOTHERAPY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES INELEGIBLE FOR HIGH DOSE OF CHEMOTHERAPY

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Introduction. Severe regimen-related toxicity often complicates transplant procedure performed in patients with haematological malignancies that have severe comorbidity. Moreover, the severity of the prognosis of these diseases requires a post consolidation treatment. *Aim of the study*. We studied the safety and efficacy of a reduced intensity Fludarabine(Famp) and Melphalan (L-Pam) conditioning regimen in 8 patients ineligible for high dose of chemotherapy. *Patients and Methods*. The median age was 51 years (r. 14-62) and all patients was female but one. Diagnoses included AML (n=7), ALL (n=1). The status at the transplant was first CR (n=7) and relapse (n=1). The median interval between

diagnosis and transplant was 7 months (r 3-12). The dosage of Famp and L-Pam was 90 milligrams/square metre divided for three consecutive days and 140 milligrams/square metre in one day. The reasons for exclusion from a program of high-dose chemotherapy are infectious in 4 cases and poor performance status in remaining cases. Five patients received peripheral hematopoietic progenitor cells (PHPC) with median number of $CD34^+$ infused=$4x10^6$/kilograms (r. 3,5-5,6); 2 patients received both, PHPC and bone marrow cells (BMC), and 1 only BMC. *Results*. One patient is too early and excluded from these assessments. The seven patients evaluated achieved hematopoietic engraftment with a median time to absolute neutrophil count >$0.5x10^9$/L and to platelet count >$30x10^9$/L of 17 and 16 days, respectively. Three patients experienced grade 2 of fever and 4 patients had grade 2 of mucositis that did not require opioid drugs. Only one patient one months after hospital discharge showed lung infection disease. The median duration of hospitalization from the date of the start of chemotherapy was 22 days (r. 21-35). Only one patient died for relapse at 153 days post-transplant. The others patients, after a median follow-up of 14 months (r. 1-42), are alive and still well. Two of them are carrying out maintenance therapy post-transplant. *Conclusion*. The Famp and L-Pam conditioning regimen in this setting of patients revealed a reduced toxicity. It might be able to take in the future an outpatient transplant program. Despite the short median follow-up, we observed encouraging prolonged complete remissions. It's necessary to enrol additional patients to better understand the real impact of this procedure on the haematological malignancies outcome.

PU016

WHAT IS THE BEST CONDITIONING FOR AUTOTRANSPLANT IN AML PATIENTS?

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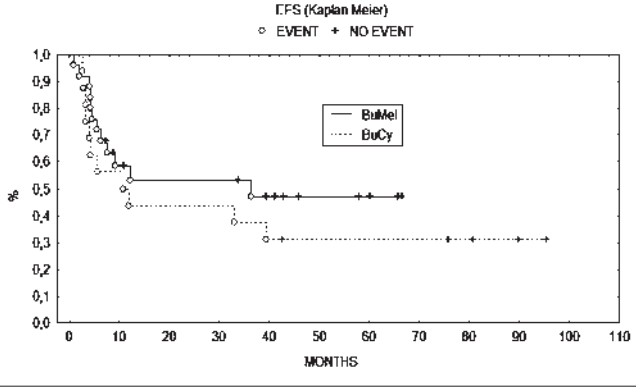
The conditioning regime more used in bone marrow transplantation, in patient with AML, has been the BuCy-2. Such treatment, mieloablative and immunosuppressive, he favours the engraftment of the marrow in the allogeneic transplant, not clear the advantage in the autologous transplant. In the last years, new conditioning regimes to more intense mieloablation has been used in AML autotransplant patients. We have retrospectively evaluated, in terms of efficacy and results, the classical conditioning BuCy-2 with a conditioning with busulfan (4 mg/Kg days -6 -3) and melphalan (140 mg/m^2 day -2) (BuMel). From June 2001 to December 2008 we have autotransplantated 41 patients with AML in first CR (18 males and 23 females; median age: 46 years (range 14-73) subtype FAB: M0: 2; M1: 7; M2: 12; M4: 17; M5: 3). 25 patients have been conditioned with BuMel (12 M and 13 F; median age: 46 years (range 18-73) subtype FAB M1: 5; M2: 8; M4: 9; M5; 3) and 16 with BuCy-2 (6 M and 10 F; median age: 41 years (range 14-59) subtype FAB M0: 2; M1: 2; M2: 4; M4: 8). The factors of risk in the 2 groups are similar. High risk: 8 patients (6 in BuMel and 2 in BuCy group), intermediate risk: 27 (14 in BuCy-2 and 13 in BuMel) and low risk: 6 (4 in BuMel and 2 in BuCy group). The PBSC has been the source of the stem cells in all patients, and the median CD34 infused cells has been of 5,15 and $5x10^6$/Kg in BuCy-2 and BuMel groups respectively. All patients have achieved a full haematological recovery.



Figure

The median days to neutrophil> 1000/mm^3 and platelets> 20000/mm^3 have been of 14 and 12 days in the BuCy-2 and BuMel groups respectively. In the BuMel group one patient is died for mycosis (mucor) at day +27 after transplantation. With median follow-up of 31 months (range 4-92 months), after autotransplant, 14 patients (56%) they are alive (12 in CR) in BuMel group; in the BuCy-2 group the median DFS and OS are 9 and 12 months respectively. The EFS projected to 67 months is 50% and 33% in BuMel and BuCy-2 groups respectively (Figure 1), this difference is not statistically significant (p=0.08, Cox F-test). In conclusion, even if the number of the patients is small, the difference in terms of DFS, OS and EFS doesn't seem significant among the two regimes of conditioning. Is necessary a large cohort and a randomized study to confirm these data.

PU017

SINGLE AND DOUBLE AUTOLOGOUS STEM-CELL TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE

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High dose chemotherapy coupled with an autologous stem cell transplantation (ASCT) is widely accepted as effective therapy for multiple myeloma (MM).We present our experience of 16 patients < 60 ys with newly diagnosed MM treated by single or double ASCT. The aim of this study is to analyse the effect of treatment with high-dose chemotherapy and autologous stem-cell support, single or double, on response, according to IMWG criteria, and survival. From 2005 we have performed 23 ASCT after high-dose chemotherapy with Melphalan 200 mg/m^2 on 16 patients, M/F: 9/7, Median age: 58 years (range 39-60 years), 7 tandem transplantations. The first ASCT was performed after induction therapy consisting in 3-4 courses of VAD in 13 pts, 1 pt after 3 courses of PAD, 2 after respective 13 and 6 months of thalidomide and dexametasone. 3 patients, before ASCT, needed of supplementary therapy for archieving at least PR. The median interval from diagnosis to first ASCT was 6 months (3-22 months). All patients were candidates to carry out the double ASCT. The second ASCT was not performed in 9 patients because 2 for failed PBSCT collection, 2 for withdrawal of the informing consent, 1 for cardiac toxicity, 2 for PD, 1 for B19 parvovirus infection, 1 too early for evaluation. The median interval between the 1st and 2nd ASCT was 4 months (range 3-9 months). Before second ASCT all patient obtained at least VGPR. 9 patients performing only one ASCT obtained 4 CR, 4 VGPR and 1 PR, with median response of 9 month (2-31). 4 patients died, 3 for progressive disease and 1 for fatal infection. The median follow up in the 5 surviving patients was 47 months (11-55). 7 patients after second ASCT obtained 5 CR, 2 VGPR, with median response of 8 month (2-23). 3 patients died all for progressive disease. The median follow up in the 4 surviving patients was 20 months (13-61). No therapy-related mortality occurred. The introduction of high-dose chemotherapy supported by autologous SCT has significant improved the prognosis of patients with active MM. In the evaluation of the survival, the new therapies must play a fundamental role, although, it is unclear if novel agents should be used before transplantation or reserved for relapse. Despite their excellent activity, currently there is no evidence that novel agents such as thalidomide, bortezomib and lenalidomide can be advanced to the high-dose chemotherapy and ASCT.

PU018

BILATERAL ADRENAL NON HODGKIN'S LYMPHOMA: CASE REPORT

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The Non-Hodgkin's lymphoma (NHL) is an eterogeneous group of malignancies from the B lymphocytes with main nodal involvement. The

incidence of NHL is 3-5% among all solid malignant neoplasia and extranodal NHL are 30% of NHL. Primary adrenal lymphoma is a rare extranodal NHL which mainly affect elderly men with bilateral involvement. We reported a case of a female affected by advanced primary bilateral adrenal NHL. Hematological and serum biochemical laboratory data displayed anemia with monocytosis in association to increased level of LDH and moderate hypogammaglobulinemia. The peripheral blood smear showed increased level of monocytes (11%) with dysplasia of neutrophils (secondary cytoplasmatic hypogranulation, abnormal segmentation of chromatin) and dyseritropoiesis with circulant erythroblasts. The clinical evaluation showed a recent history of B symptoms. There weren't lymphadenomegalies at the CT. Adrenal tumor burden was <10 centimeters.

Table.

	On admission	*After 20 days*
White cell count (per mm^3)	7.000	8.600
Neutrophils %	45	65
Lymphocytes %	20	10
Monocytes %	34	24
Eosinophils %	1	1
Hb (g/dL)	10.8	8.4
MCV (fl)	90.9	87.4
Platelet count (per mm^3)	205000	164000
LDH (U/liter)	520	788
Total protein (g/dl)	5.7	n.a.
Calcium (mEq/L)	8.1	7.7
Potassium(mEq/L)	4.0	4.2
Chloride(mEq/L)	106	102
Albumin(mEq/L)	2.4	2.0
Ves (mm/h)	14	2
Beta2microglobulin	n.a	3.8
IgG (mg/dL)	715	817
IgA(mg/dL)	172	240
IgM(mg/dL)	41	34
Elettrophoresis gamma(%)	15.2	n.a
Ferritina (ng/mL)	n.a.	1576
GOT (UI/L)	12	16
GPT (UI/L)	7	5

No sign of adrenal insufficiency were reported. The Adrenal biopsy showed a diffuse infiltrate of large immature lymphocytes. Immunoperoxidase stains on a frozen section specimen revealed staining of the cells for the B-cell antigen CD20, CD79a, bcl-2.The diagnosis were consistent with 'diffuse large B cell lymphoma'. At the morphological examination of bone marrow aspirate, the cellularity appeared increased according to the age of the patient and the megakaryocytes were normal; moderate dysplasia of neutrophils was evidenced, macrophages including emosiderina granules were increased while Pearls staining was normal. Differential count of 400 cells, was performed with low and medium power using X100 oil to evidence cellular details. It showed normal values, except for 7.5% monocytes. Citochemical peroxidase excluded infiltration of atypical lymphocytes. Monocytosis was defined by persistent increased count of monocytes (> 950 per mm^3). differential diagnosis was performed with chronic myelomonocytic leukaemia or secondary to solid tumor, lymphoprolipherative diseases and severe infectious diseases (tubercolosis, endocarditis, sepsis), collagenopaties, granulomatouses disease. The optimal treatment of primary bilateral adrenal lymphoma has not been well established currently. Earlier diagnosis, staging and IPI stratification remain the basis for tailored treatment in such rare case of lymphoma, mainly considering the new target therapy.

PU019

A CHRONIC LYMPHOCYTIC LEUKEMIA CASE ANALYZED BY CONVENTIONAL CYTOGENETICS, FISH AND MULTIPLEX FLUORESCENCE IN SITU HYBRIDIZATION

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Complex chromosomal aberrations (CCAs) can be detected in a substantial proportion of hematologic disorders but many of them could not be delineated by conventional cytogenetic banding techniques. Comprehensive analysis of the chromosomal rearrangements in these CCAs has been hampered by the limitations of conventional cytogenetics (CC). Fluorescent *in situ* hybridazion (FISH) technique helps more in understanding the chromosomal rearrangements even in cryptic cytogenetics aberrations but identifies locus specific abnormalities only. Multiplex fluorescence in situ hybridization (M-FISH) is a new generation FISH technique which allows simultaneous identification of all the 24 human chromosomes. So it is very useful in clarifying CCAs, identifying cryptic interchromosomal rearrangements and characterizing marker chromosomes. But it also has some limitations. We used M-FISH to accurately refine the CCAs revealed by Q-banding CC and a standard FISH panel probes in one patient with chronic lymphocytic leucemia(CLL). A 80-year old female with laterocervical lymph node enlargement was biopsied and a diagnosis of low grade B-cell non Hodgkin lymphoma was made. Morphology and immunophenotyping were consistent with lymphocytic lymphoma well differentiated (CD5$^+$, CD19$^+$, CD23$^+$). Staging at diagnosis was Binet B. CD38 and ZAP70 were negative. Molecular analysis revealed unmutated IgV(H) genes. The karyotype was very complex. The standard FISH panel probes used for CLL (6q23, 11q22.3, 12p13, 13q14, 14q32, and 17p13) revealed only 11q deletion and 14q aberration. M-FISH revealed different chromosomal abnormalities included 13q deletion, 11q translocations with chromosome 2, 4 and 7, these last very rarely involved in chromosomal aberrations of chronic lymphocytic leucemia and 14q involvement. In six months a clinical progression of the disease was observed and death occurred after two cycles of chemotherapy with alkylating agents. Our data suggest that multiplex-fluorescence *in situ* hybridization (M-FISH) is a complement in cytogenetics, particularly for the characterization of complex karyotypes, and can have clinicobiological utility for prognosis assessment.

PU020

MN1-ETV6 REARRANGEMENT IN AML DEVELOPING FROM A 5Q- SYNDROME

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Background. MN1 overexpression in AML with normal karyotype is associated with bad prognosis. In AML, MDS, aCML t(12;22)(p13;q12) produces the MN1-ETV6 fusion protein which has transforming activity in NIH3T3 fibroblasts. MN1-ETV6 induces T-lymphoid tumors in mice with Notch-1, Hes-1, c-Myc and Lmo-2 up-regulation and AML in those with HOXA9 and N-Myc up-regulation. *Aim*. To study AML evolving from a 5q- syndrome. *Materials and methods*. Case history. In a 46 year old woman with macrocytic anemia bone marrow karyotype was: 47, XX, del(5)(q13q33), +21. One year later, in 1996 the patient started transfusion therapy. Morphological and cytogenetic features remained stable. In December 2007 and January 2008 bone marrow aspirates showed progression to AML with 46% of myeloid blasts. Karyotype evolved into a complex 46, XX, del(5q), -7, der(12)(p13), +21. The patient started high-dose chemotherapy but died of cerebral haemorrhage after a few days. FISH studies. 1) To delineate del(5q) endpoints we used 5q13-5q35 genomic clones: RP5-910M8 (HEXA B/5q13), RP11-1089B2 and RP11-885P10 (XRCC4/5q13-q14), RP11-642K17 and RP11-204L7 (SPARC/5q31.3-q32), RP11-946D14 (RPS14/5q33), and RP11-117L6 (NPM1/5q35). 2) To characterize additional abnormalities at the onset of AML, we used LSI D7S486, 7q31 (Vysis), LSI TEL/AML1 ES (Vysis), clones RP11-418C2 and RP11-434C1 encompassing ETV6/12p13 and RP11-345E21 encompassing MN1/22q12. 3) To investigate cryptic abnormalities of putative oncogenes/tumor suppressor genes we used: RP11-888J3 (FHIT/3p14.2), BAC clone for IKAROS/7p13-p11, RP11-380G5 (PTEN/10q23.3), RP1-74J1 (WT1/11p13), LSI ATM 11q23 (Vysis), LSI D13S25 13q14 (Vysis), LSI P53 17p13 (Vysis), RP11-748M14 (MADR2/18q21), LSI D20S108 20q12 (Vysis). *Results*. FISH demonstrated SPARC and RPS14 monoallelic loss at del(5q), confirmed monosomy 7, found no deletions of other putative oncogenes and showed der(12)(p13) was a t(12;22)(p13;q12) disrupting ETV6 and MN1. à. Disease was stable for over 153 months in this case of 5q- syndrome with trisomy 21, a time period overlapping with the median follow-up of 5q-syndrome with isolated del(5q). AML onset correlated with cytogenetic evolution towards a complex karyotype, including a t(12;22) which has never been observed in evolved 5q- syndromes and which appeared to be a very bad prognostic marker.

PU021

THE ACUTE LYMPHOBLASTIC LEUKEMIA CHARACTERIZED BY DER (17)T(?;17)(?;Q21).

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THE T-cell Acute Lymphoblastic Leukemia (T-ALL) is associated with an abnormal karyotype in 80 to 90% of patients. The most commonly recognized abnormality are those involving the T-cell-receptor α/delta locus on chromosome 14q11. Other chromosomal rearrangements are found in many cases of T-ALL, some of these are specific and they constitute an important prognostic factor to the outcome of disease and to the application of new targeted therapies. Here, we describe the clinical, immunphenotyping, cytogenetic and Fluorescence *In Situ* Hybridization (FISH) features findings in a T-cell ALL patient with a complex karyotype including der(17)t(5;17)(q13;q21). A 38 year old woman was admitted to our hospital, for dispnea and recurrent perspiration nocturnal, in december 2008. The white cell count was 83,8x10^9/L, Hg 12,8 g/dL, platelets were 26,0x10^9/L, and there was the presence on blood smear of 80% blast cells; serum lactate dehydrogenase level was increased too(LDH 7,483 U/L). A bone marrow aspirate showed the presence of 63% of blast cells. The immunophenotyping of blasts cells showed positivity for CD2$^+$, CD7$^+$, CD5$^+$, CD9$^+$, CD4$^+$,CD8$^+$, CD30$^+$ and TdT. The cytogenetic examination was performed on 24-hour bone marrow cultures without mitogen. Chromosome analysis on 10 metaphases revealed the following complex karyotype: 46,XX[7]/35-45,XX,+der(17)t(5;17)(q13;q21)[3]. The FISH experiment with commercially available probes (Abbott Molecular Catalog) were performed according to the manufacturer's instructions with combinations of various LSI probes. The LSI EGR1/D5S23,D5S721 Dual Color probe set was added in the experiment with LSI p53 (17p13.1) SpectrumOrange Probe. In a normal cell, hybridized with the mixture of probes, the expected pattern was two orange and two green signals on chromosome 5 and two orange signals on chromosome 17. In a hybridized abnormal cell containing der(17)t(5;17), two orange signals was observed on the derivative chromosome on p arm, but there wasn't the red signals on q arm related to 5q31. After FISH analysis the karyotype was 46,XX[7]/35-45,XX,+der(17)t(?;17)(?;q21). This case report underlines the utility of FISH analysis in understanding and claryfing the cytogenetic abnormalities in AL patients with complex kariotypes, to better recognize the genetic lesions to elucidate the molecular patways involved in the development of distinct subgroups of Acute Leukemia (LA).

PU022

BLOOD DONATION AND THROMBOPHILIA: CASE REPORT

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We report a case of a 58 year-old male without significant past medical history; there was no definitive history of venous thromboembolism in his family; he didn't smoke or drink alcohol, he was overweight, with normal lipidic testing and border line blood plessure. He was a blood donor since 1985 with 80 whole blood donations. In March 2008 he underwent phlebotomy of the left arm for whole blood donation; venipuncture was regular without any pain or bleeding or ecchymosis. Two hours later he developed proximal arm pain and swelling; an ultrasound revealed evidence of an extensive deep venous thrombosis involving the basilic, the omeral, as well as the succlavia and the axillary vein of the left. Laboratory testing demonstrated elevated levels of hematocrit (51.4%) and hemoglobin (17.8 gr/dL). He was anticoagulated with heparin followed by warfarin; he had a good response to treatment and the pain and swelling in the left arm resolved after one month. Sequent ultrasounds demonstrated progressive improvement of radiological status with regular fluxion in basilic, omeral, succlavia and axillary veins and stabilized thrombosis on third distal cephalic left vein. The patient remained on warfarin at INR of 2-3 for 6 months. Further laboratory evaluation was performed one month after the cessation of warfarin and demonstrated: heterozygosity of prothrombin G20210A and mildly elevated levels of homocysteine (over 18 micromol/L) with homozygous carriers of the MTHFR 677T variant. Other thrombofilic tests were normal. JAK2 V617F mutation assay was negative. *Conclusion*. There is no evidence that phlebotomy increases the risk of venous thromboembolism but in thrombofilic patients it's possible to suppose that mechanic local damage caused by venipuncture add on biological individual risk factors or occasional transient risk factors may constitute an efficient trigger for vein thrombosis. Maybe it is absurd and unrealistic to propose generalized thrombofilic screening but it is reasonable to make venipuncture with minimal traumatism and precise medication in order to minimize venous stasis, and it's recommendable to collect personal and familiar anamnesis with particular care on thrombotic events.

PU023

THIRTY YEARS OF IMMUNOSUPPRESSIVE TREATMENT IN IDIOPATHIC THROMBOCYTOPENIC PURPURA: A CASE REPORT

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We report a case of a 24ys old male showing petechiaes, ecchymosis and epistaxis in 1978; he was severely thrombocytopenic (plts 4000/microL). Further evaluation failed to reveal any cause of thrombocytopenia and the patient was diagnosed with ITP. He was treated with steroids 1 mg/Kg achieving CR until 1992 when he was admitted at our hospital with plts 3000/microL and bruising. We performed bone marrow examination and we tested for antiplatelets antibodies and we confirmed the diagnosis of ITP. Patient received steroids 1 mg/Kg but during escalation therapy he relapsed and in 1993 he underwent splenectomy achieving a durable CR until 2001, when he relapsed after a tonsil abscess. We confirmed the diagnosis of ITP and the patient began a new cycle of steroids with a good response. In May 2003 he relapsed (plts 4000/microL) and he was treated with Vincristine 2 mg for 4 weekly doses with CR. In June 2004 platelets were 6000/microL and abdominal TC showed accessory spleen so we chose to do a laparoscopic splenectomy preceded by IVIG; platelets remained more than 100000/microL until November 2004, when he relapsed and received Rituximab 375 mg/m^2 weekly for 4 weeks. He achieved CR and he maintained it until 2006: he received a second course of Rituximab at the same doses but after one year he relapsed with platelets 6000/microL. We decided for IVIG and low dose Rituximab (100 mg weekly dose, for 4 weeks). He responded with platelets more than 50000/microL for three months but in March 2008 he was hospitalized for mucocutaneous bleeding and fever. Blood testing revealed positive CMVea; patient was treated with Foscavir until negativity of CMVea, Azathioprine and Danazol; platelet count increased. At the latest follow up in April 2009 platelet count was 400000/microL; the patient was continuing with Azathioprine and Danazol. *Conclusion.* despite very low platelet count, serious bleeding was rare in our case. Currently recommended therapies are associated with significant side effects. Bleeding and infections due to immunosuppression contributed equally to deaths. Treatment may interfere with quality of life more than illness itself. Treatment can be individualized, taking into account the person's needs and lifestyle as well as bleeding. Rituximab at standard dose or low dose seems to be a useful drug for refractory ITP. Newer agents stimulating thrombopoiesis are showing encouraging results and will represent a very promising new therapeutic schedule for ITP.

PU024

TREATMENT OF A DIC POST-PARTUM WITH THE VIRUS INACTIVATED FRESH FROZEN PLASMA SAFE IN A PATIENT AFFECTED BY ACUTE PROMYELOCYTIC LEUKEMIA(M3): A CASE REPORT

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Disseminated Intravascular Coagulation (DIC) is a disorder of coagulation with thrombotic complications due to the intravascular formation of fibrin, and diffuse hemorrhages. The obstetric pathology represents the main causes of a DIC. The occurrence of Acute Promyelocytic Leukemia (APL-M3) during and after the pregnancy is not a frequent event and the management of these patients requires a careful clinical

case evaluation of maternal risk, coagulation status, and therapeutic options. In the present work we present the case of an hemorrhagic shock complicated by a DIC developed in a nullipara at 39° week of gestation, which the diagnosis of an APL was performed 3 days later the birth. A 33-year-old nullipara was hospitalized by our Gynecology and Obstetrics Division in the 2008, in order to be submitted to caesarean section. The patient was treated in the last two months by acetil-salicylic acid because of an unknown proneness to thrombosis. Furthermore, she showed normal parameters of WBC, RBC and PLT for the whole period of pregnancy. The patient was submitted to an emergency caesarean and developed after 4 hours by the birth a vaginal laceration and uterine atony, accompanied by postpartum hemorrage. The laboratory analyses showed an Hb of 7.2 mg/dL and WBC 20.000/µl; after 2 hours they showed the following results: progressive reduction of the platelets (PLT) <80.000/µL, fibrinogen < 200 mg/dL, very high values of D-Dimer, INR >2, and WBC 30.000/µL. These results lead to a DIC postpartum diagnosis and the patient was transfused by virus inactivated fresh frozen plasma safe (VIFFP) at the dose of 3 unity/die until the normalization of the coagulations parameters that it happened in turn of 3 days. Contemporaneously, the patient showed an increase of WBC> 100.000/µL accompanied by massive presence of blasts in the bone marrow and peripheric blood; the flow cytometry analysis confirmed a diagnosis of M3. The patient received all-trans-retinoic acid (ATRA) and Idarubicin, obtaining a complete remission after 6 months. In this work we report a case of unexpected post-partum APL with a previous DIC of obstetric origin and we show that the VIFFP, remains still today, although the recent use of factors recombinant activated factor VII (FVIIa), a therapeutic treatment of high efficacy and safety in the control of the bleeding post-partum DIC correlated.

PU025

ACQUIRED VON WILLEBRAND DISEASE IN A PATIENTS WITH CRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND MITRAL MECHANICAL VALVULAR PROSTHESIS.

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Patient born in 1936. In 1998 he underwent mitral valvular replacement with mechanical valvular prosthesis without haemorragic complications and started oral anticoagulant therapy (OAT). In 2005 asymptomatic chronic lymphatic leukemia (CLL) was diagnosed. In 2006 CLL progression occurred and patient started chemotherapy with fludarabine 25 mg/sqm and cyclophosphamide 250 mg/m^2 for 3 days (FC) for a total of 4 cycles. OAT was interrupted and substituted by low molecular weight heparin (LMWH) 100 U/kg/d until the end of planned chemotherapy. During LMWH treatment aPTT ratio ranged from 1.6 to 2.06. Patient referred rare epistaxis. In August 2007 CLL relapse occurred so that chemotherapy with FC plus Rituximab (R-FC) was resumed. OAT was interrupted again and LMWH 100 U/kg/d was started. In September 2007 he presented a thoracic hematoma that was referred to thrombocytopenia plus LMWH (aPTT ratio 1,79, plts 70.000/mm^3); LMWH was temporally stopped. and then restarted at lower doses. In December 2007 because of bradycardia with syncope a pace maker was placed. The operative procedure was complicated by severe hematoma in operation site with 2 g/dL hemoglobin loss and need of blood transfusion. Dosage of coagulative factors was performed: VIIF 9%, vWF:Ag 13,6%, vWF:Rco (ristocetin cofactor activity) 8%, vWF:CB (collagen binding activity) 1,1% (vWF:Rco/vWF:Ag=0.58; if <0,7 vWD type 2). The patient underwent vWF/ VIIIF concentrate infusion (2000 U twice/d for 2 days followed by 1500 U/d for 3 days) with aPTT normalization and resolution of hemorrhage. The more exhaustive assay attested that no familiar members had vWF deficiency. Patient results are described in the Table 1.

Table 1.

	VIIIF	*VWF:Ag*	*VWF:RCo*
Basal	9%	13.6%	8%
1:2 dilution *	6%	8%	9,6%
1:10 dilution *	10%	2%	7%
1:20 dilution*	12%	3%	2%
1:100 dilution *	11%	2%	4%

We concluded for acquired von Willebrand disease type 2 at elevated hemorrhagic risk; the patients started steroid treatment and stopped anticoagulant therapy. Replacement of mechanical valve with biological one was excluded due to poor patient clinical conditions.

PU026

FOLLOW UP OF A COHORT OF 6 PATIENTS WITH ACQUIRED HEMOPHILIA: CLINICAL CHARACTERISTICS AND TREATMENT RESPONSE

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Acquired hemophilia is a rare, but often severe bleeding disorder caused by autoantibodies against a coagulation factor. Between 2000 and 2009, six cases of acquired hemophilia (three men and three women with a mean age of 69.5 years) have been diagnosed and treated in our department; all the patients showed hemorrhagic episodes and some of them required a transfusional support. Among the above-mentioned cases, three were secondary to a neoplastic pathology (oesophagus carcinoma, breast carcinoma and kidney carcinoma), two were related to autoimmune diseases (one psoriasis and one SLE with previous lupus glomerulonephritis of class V) and one case was idiopathic. The inhibitor titre ranged from 1.5 to 42.0 BU. At present, follow up is 8.3 months/person; the median is 4 months with a range from 2 to 34 months. Three patients died because of the neoplastic pathology and one because of retroperitoneal hemorrhage, as he refused the treatment of the acquired coagulopathy. The remaining patients received simultaneously an antihemorrhagic treatment, with factor VIII or rFVIIa, depending on the inhibitor titre, and an immunosuppressive treatment. Prednisone and Cyclophosphamide were administrated for the immunosuppressive treatment and Rituximab 375 mg/m^2 was associated for four weeks in the last two cases (idiopathic form and SLE) of our cohort; all patients achieved remission of the inhibitor without side effects. During the follow up, one of these patients had a relapse, which was precociously identified before hemarroagic symptoms and treated again with Rituximab. The new remission was achieved after a single adiministration of a standard dose. Thus, in our experience, Rituximab showed effective both in the primary treatment and in the control of relapses, even in cases already treated with Rituximab: further studies are needed to define the treatment following the second remission.

PU027

AXILLARY ARTERY THROMBOSIS IN A PATIENT TREATED FOR TICLOPIDINE - RELATED THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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A 66 years old man was treated with ticlopidine and low molecular weight heparin following surgery for knee prosthesis. Three weeks later he developed microangiopathic hemolitic anemia, severe thrombocytopenia (6.000/µL), fluctuating neurological ischemic symptoms. A diagnosis of TTP was made and appropriated treatment was started. Five plasma-exchang procedures and fresh frozen plasma administrations were requested to obtain a near complete haematological and clinical normalization; in ten days the patient was discharged from the hospital. Unfortunately he presented antral gastritis and was a carrier of *Helicobacter pilori* so other treatment against platelet aggregation was delayed. Two months later for a sudden pain in his right arm an angiographic study was performed so discovering an axillary artery thrombosis. This required an oral anticoagulant treatment that the patient is still taking and that was successful in obtaining complete recovery from the thrombosis. Only some months later we could obtain an assay of ADAMTS 13 inhibitor that resulted present. In the following three years the patient remained in a good health. This case signals one more time that ticlopidine can induce TTP that is associated with the presence of antibodies to ADAMTS 13. The role of this inhibitor in inducing artery thrombosis, though not yet established, could be important if antiplatelet therapy is not performed.

PU028

EFFICACY OF GLUTEN FREE DIET IN COELIAC PATIENTS WITH ASSOCIATED IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

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Background. Idiopathic thrombocytopenic purpura (ITP) is an autoimmune condition caused by platelet auto antibodies, that leads to increased platelets destruction. The typical presentation in childhood consists in sudden onset of petechiae, bruising and bleeding manifestation in patients otherwise in good health. The estimated incidence of ITP ranges between 10-125 new cases/one million (adults and children) year. ITP is often associated with other autoimmune disorders. Celiac Disease (CD) is a very frequent autoimmune condition due an intolerance of the gluten (estimated incidence in Italy is 1/100-150 people) whose more common clinical manifestations are gastrointestinal symptoms, and/or sideropenic anaemia, and/or failure to thrive. Several published studies has shown associations between ITP and CD with a median range of 1/100.Aims:The aim of this investigation is to evaluate and verify the efficacy of gluten free diet to improve the platelets count in new diagnosed coeliac with associated ITP. *Patients and Methods*. Since December 2007 till December 2008 have been enrolled 17 children (9 M and 8 F) mean age 5yrs, ranged between 8 mts-14 yrs with new diagnosed ITP. All patients were screened for CD determining the anti transglutaminase antibodies (TTG) and anti endomisial antibodies (EMA). All patients were evaluated also for other autoimmune conditions determining serum levels of antinuclear antibodies (ANA), anti smooth muscle antibodies (ASMA), anti mitochondrial antibodies (AMA), C3 and C4, thyroidal function and anti tireoperooxhidase antibodies (AbTPO) and anti tireoglobulin antibodies (AbTg), Direct Coombs test. Serologically positive patients to EMA and TTG were submitted to upper endoscopy with jejunal biopsy. *Results*. 2 patients of 17 (1/8.5) were positive to EMA and TTG. Jejunal biopsy was positive for CD (grade 3rd-4th Marsh Classification), confirming diagnosis of CD. Both patients had no gastrointestinal manifestations and only one of two had a mild sideropenic anaemia (Hb 10.2 gr/dL, MCV 72 fL, iron serum levels 24 µg/dL, ferritin serum levels 10 ng/L).Gluten free diet gave only partial remission of ITP and both patients needed treatment with oral corticosteroids. 1/2 still had low platelet count after 1 year of diet (PLt>50.000) but didn't required other treatment than gluten free diet. Summary and *Conclusions.* Our investigation has shown that gluten free diet alone would not conduce to a complete remission of ITP, but it appears to be able to improve the course of ITP, reducing the use of corticosteroids. Otherwise, in our patients the frequency of the association between ITP and CD in our patients seems to be significantly superior compared with previous literature reports (1/8.5 vs 1/100). Considering the short period of the investigation, and the few number of patients, further studies would be needed

PU029

DO HEMATO-ONCOLOGY PATIENTS UNDERGOING HEMOPOIETIC STEM CELL TRANSPLANTATION NEED THROMBOPROPHILAXIS BEFORE INSERTION OF CENTRAL VENOUS CATHETERS ?

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Venous thromboembolism (VTE) is a complication of central venous catheters (CVC) with the highest frequency in hemato-oncology diseases. In this setting we can consider a systemic pro-thrombotic state that could trigger the thrombosis after CVC insertion or alternatively the trauma due to the surgical procedure. Jansen et al. described an increased D-dimer and Fragment 1+2 levels, after CVC insertion, in patients submitted to allogeneic stem cell transplantation which developed symptomatic subclavian vein thrombosis. In our study we enrolled 38 consecutive patients undergoing hemopoietic stem cell transplantation (HSCT), 23 autologous and 15 allogeneic. In all patients non-tunneled a double lumen CVC (Arrow 14,14 gauge) was inserted before starting conditioning regimen. Table 1 describes the characteristics of patients and their conditioning therapy. Catheter was removed in case of CVC-related infection or irreversible occlusion and at the moment of discharge. In patients with clinical signs of thrombosis a color flow Doppler imaging examination was performed. Blood sample, obtained from a peripheral vein and stored at -80°C before analysis, were collected 6 hours before and 12 hours after the CVC insertion. In all patients were evaluated endothelial, platelet, coagulation and fibrinolitic markers as well as hereditary conditions of thrombophilia (Factor V Leiden, hyperhomocisteinemia, prothrombin G20210A, activate protein C resistance, antithrombin III, protein C, protein S). One patient out of 38 (2.6%) developed a CVC-related thrombosis after 7 days from the insertion. Five patient out of 38 were positive in heterozygosis for factor V Leiden and 6 out of 38 showed hyperhomocisteinemia without any significant correlation with thrombotic incidence. After CVC insertion we observed an increased concentration of β-TG, PF4 and TAT compared to basal value without difference between patients with or without thrombophilic alterations (Table). Our data show that a congenital thrombophilic state does not predispose to thrombosis. Probably, the trauma, due to CVC insertion, is able to generate a platelet and coagulative activation but it is not enough to determine clinical thrombosis. Our results are in contrast with previous studies and it could be due to the small number of cases and/or the use of a non-tunneled double lumen catheter. However, based on our conclusions, in this setting of patients is not necessary to assess thrombophilic tests before CVC insertion and also a thromboprophilaxis is not requested.

Table 1

UTOLOGOUS (n. 23)			LLOGENEIC (n.15)		
Conditioning therapy	n° of patients	Status Pre-SCT	Conditioning therapy	n° of patients	Status Pre-SCT
BU-MEL	2	2 RC	FLU-EDX- TG	1	(S)
BE M	14	10 RC, 4 RP	BU-CY	12	12 RC
MEL	7	7 RP	THIO-FLU-EDX	2	1 RP, 1 RC

Table 2

	-TG	PF4	T T	P P	D-D	F1+2	F	vW: g	vW:R	FVIII	t-P	P I
Pre-CVC	17.5	2.5	2.6	489	406	143	375	179	160	119	9.3	9.4
Post-CVC	24.5	5.0	3.6	444	441	179	373	160	162	107	9.1	9.8
*	**0.004**	**0.0001**	**0.007**	0.18	0.98	0.08	0.9	0.3	0.9	0.69	0.59	0.75

-TG: beta-tromboglobulin; PF4: platelet factor 4; T T: thrombin anti-thrombin complex; P P: plasmin anti-plasmin complex; D-D: D-dimer; F1+2: fragment 1+2; F: fibrinogen; t-P : tissue plasminogen activator; P I: plasminogen activator inhibitor.
*** Mann-Whitney test**

PU030

THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP): A NEW IMMUNOSUPPRESSIVE THERAPEUTIC APPROACH IN REFRACTORY/RELAPSED PATIENTS

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TTP is a rare and severe disease characterized by thrombocytopenia, microangiopathic haemolytic anemia, neurological and renal involvement associated with deficiency of the vWF-cleaving protease, ADAMTS13. Plasma exchange(PEX)is the treatment of choice; although 80% of patients respond to PEX, 30-50% of survivors develop refractory or relapsing disease perhaps because of the persistence of high titers of antiADAMTS13 autoantibodies. Rituximab has shown a favorable benefit-risk ratio in plasma-refractory and relapsing TTP, however, long-term follow-up data are not yet available; moreover a small amount of TTP patients does not even respond to rituximab or relapses after it. Cyclofosfamide therapy is described only in TTP associated with autoimmune disease. Here we report 3 patients diagnosed with TTP at our institute from September 2007 to Jenuary 2009 and treated with cyclophosphamide achieving complete remission(CR). Median age at diagnosis was 25 ys, M/F 1/2; one patient had severe renal impairment treated with dialysis and one patient presented with neurological involvement at diagnosis. Blood tests showed hemolytic anemia, unconjugated hyperbilirubinemia increased lactated dehydrogenase, thrombocytopenia and mild impairment of renal function. ADAMTS13 activity was 0% in 2 patients, 20% in the remaining (normal >40%).

Inhibitors were not performed. Patients were initially treated with PEX, steroids and then aspirin. One patient achieved CR after 14 PEX , but she relapsed 14 days after the last PEX; the remaining 2 patients didn't achieve a CR after PEX; so they all received Rituximab at dose of 375 milligrams/m^2 weekly for an average of 4 doses. After the first 2 doses despite an initial ADAMTS13 increase (52%), the first patient had a rapid decrease of activity and a worsening of general conditions. She underwent iv cyclophosphamide 1,1 grams (fractionated in 3 doses every 10 days) with clinical and laboratory improvement. The second patient did not have a CR within a month from Rituximab and the third one had a neurological worsening with coma after the first dose of rituximab; so we decide to administer iv cyclophosphamide to these 2 patients with a clinical and laboratory improvement too. Disease-free status is still ongoing after 19, 8 and 1 months, respectively. Results demonstrate that cyclophosphamide may be effective in obtaining a CR in patients in whom other treatments failed to limit, perhaps, the production of inhibitors of ADAMTS13.

PU031

ACUTE PROMYELOCYTIC LEUKEMIA: WILM'S TUMOR AND FLT3 INTERNAL TANDEM DUPLICATION EVALUATIONS, PROGNOSTIC INDEX OF RELAPSE?

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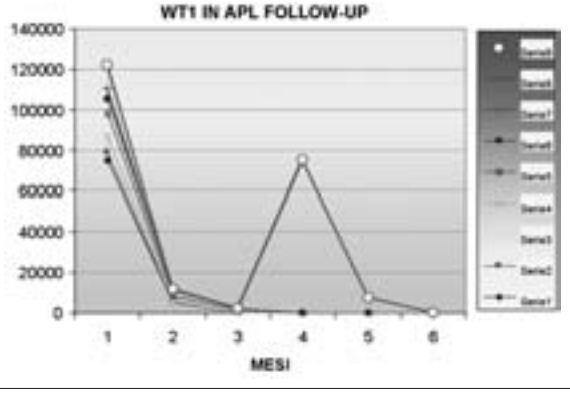
Introduction. The acute Promielocytic Leukemia (APL) is a heterogeneous group of acute Myeloid Leukemia's; it is M3 for the FAB (French-American-British –Cooperative –Group) classification. The APL is clinically characterized from elevate sensibility at the acid trans-retinoic (ATRA) therapy. We have studied prognostic values of Wilm's tumour (WT1) over expression, and FLT3 (FMS Like Kinasi 3) gene mutations in M3 acute myeloid leukemia. *Objective*. To evaluate FLT3/ITD internal tandem duplication and WT1 over expression in acute Promielocytic Leukemia (APL) (exordium and follow-up) in patients from 2007 to 2008 in department of hematology Oncology Hospital of Cagliari. *Material and methods*: We investigated WT1 over expression and FLT3/ITD internal tandem duplication in APL patients, eight new case of acute promyelocytic leukemia's (APL) have been evaluated. seven patients were adults and one pediatric, 26 analyses in follow-up were evaluated. The 8 patients enrolled with t(15,17) rearrangement, 4 were bcr3 isoforms and 4 were bcr1 isoforms. Rna and DNA from Peripheral blood and bone marrow have been utilized. All cases were evaluated with RT-PCR procedure for FLT3(ITD), 1 micrograms of RNA was retro transcript in CDNA , diluted with water to 50 microliter final and 2.5 microliter amplified with 30 Pico moles of each specific primers (Forward and reverse). Polymerase chain reaction was evaluated on 2% agars gel electrophoresis.



Figure

All cases M3 acute myeloid leukemia was evaluated for t(15,17) rearrangement and FLT3/ITD with RT-PCR procedure. WT1 over expression was performed with RT-PCR real time procedure for quantitative determination by taq-man (ABI Prism 7000). *Results*: In 20 % cases of acute promyelocytic leukemia (APL) FLT3/ITD transcript were positives. Wilm' s tumor value was at exordium (3518-74984 range,) and after induction and consolidation reduced at (0-0.001 range). Only in one case, after 4 month from exordium, WT1 was increased (74984), before of clinical relapse, one index prognostic of next relapse. For this case FLT3 internal tandem mutation was' not positive at exordium. *Considerations and conclusions*. Our result show who WT1 quantitative evaluation and FLT3 internal tandem mutation analysis can be a good prognostic marker in APL as early index of relapse, but only WT1 over expression suggest a possible role as index prognostic of next relapse in APL patients.

PU032

USE OF INTRATHECAL DEPOT LIPOSOMAL CYTARABINE (DEPOCYTE) AS TREATMENT OR PROPHYLAXIS OF CENTRAL NERVOUS SYSTEM IN HEMATOLOGICAL MALIGNANCIES: REPORT OF 9 CASES

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DepoCyte is a slow-release formulation of liposomal cytarabine designed for intrathecal administration proven to be useful in clinical trials as a treatment of neoplastic and lymphomatous meningitis both in adult and in child. DepoCyte can be administrated at doses of 50 mg every two weeks because of its pharmacokinetics. Randomized studies compared DepoCyte with standard treatments (as IT administration of non liposomal cytarabine or methotrexate) and showed greater effectiveness and compliance of the depot formulation of cytarabine in the treatment and prophylaxis of neoplastic or lymphomatous meningeal involvement without increase of related toxicity. We report the result of the use of DepoCyte as treatment or prophylaxis in 9 adult patients with hematological malignancies. Of them, 7 patients received DepoCyte as CNS prophylactic therapy of lymphomatous meningitis in association with systemic chemotherapy. Their diagnosis were as follows: 4 with ALL (B- or T-cells) and 3 with Burkitt Lymphoma. These patients obtained a CR and none of them showed clinical signs of meningeal involvement. The other 2 patients, diagnosed with AML (FAB M5) and Follicular Lymphoma, had a CNS relapse after induction systemic therapy. The one with AML had severe neurological signs due to the monoblastic meningeal involvement and died in few days for disease progression. The one with Follicular Lymphoma received IT DepoCyte administrations plus systemic chemotherapy and obtained a good PR. Overall IT depot liposomal cytarabine was well tolerated. In fact headache occured as side effect in 2 patients and mild and reversible arachnoiditis in other 2 patients; the latter easily manageable with steroids. However concurrent dexamethasone therapy was administered with each dose of IT depot liposomal cytarabine to prevent the side effects. Our results show that the IT depot formulation of cytarabine (DepoCyte) is a safe and effective therapy both as treatment of neoplastic or lymphomatous meningeal involvement and as CNS prophylaxis in hematological malignancies.

PU033

THERAPEUTIC REGIMENS INCLUDING CLOFARABINE IN ADULT PATIENTS WITH RELAPSE OR REFRACTORY ACUTE LEUKEMIA

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Background. Clofarabine is a new generation purine nucleoside antimetabolite effective in the treatment of Relapse or Refractory Acute Leukemias either in children and in adults. The aim of our study was to demonstrate the effectiveness and the tolerability of a treatment regimen including Clofarabine or Clofarabine plus Cytarabine in adults with AML and ALL refractory to standard treatment. *Methods*: In 2008 at the Department of Hematology of the University of Pise (Italy) we treated 11 adults with refractory leukemias with chemotherapy regimens Clofarabine based. Patients' diagnosis were as follows: 7 with AML and 4 with ALL. Of them, 4 patients were treated with Clofarabine as second-line therapy, 6 patients as third-line therapy and 1 patient as fourth-line therapy. Clofarabine based regimens were as follows: Cycle A - 7 patients (4 AML, 3 ALL): Clofarabine 20 mg/mg days 1-5; Cycle B - 3 patients (AML): Clofarabine 40 mg/mq days 1-5, Cytarabine 1 gr/mq days 1-5. Cycle A and B were repeated every 3 to 5 weeks based on response to the previous one (CR, PR or non-response) and were start-

ed when hematopoiesis was fully recovered. Patients were allowed to receive a maximum of 2 cycles of induction therapy or until a CR, CR with incomplete platelet recovery (CRp) or partial response (PR) was achieved. A CR required normalization of the marrow (5% blasts) and peripheral counts with no circulating blast cells, a neutrophil count ≥ $1x10^9$/L and platelet counts ≥$100x10^9$/L. A PR consisted of a blood count recovery as for CR, but with persistence of 5% to 25% marrow blasts. A CRp had criteria similar to a CR, but without recovery of platelets ≥ $100 x10^9$/L. 6 patients received just one cycle of therapy and 5 patients received two cycles. During the treatment, transient liver dysfunctions were common in most patients. Myelotoxicity was grade III-IV in all patients; neurotoxicity never occurred. Mucosytis grade III-IV occured in 3 patients and also bacterial sepsis in 3 patients (colonized before the treatment). *Results*. Our results have shown an overall response rate (CR+PR) to Clofarabine higher than 70%: 8 out of 11 patients responded to treatment, one patient was a non-responder and 2 patients died of sepsis before any possible marrow evaluation. Out of 8 responding patients, 3 obtained a CR and 5 a PR. Of the 8 responders, a fully ablative allogenic transplant was performed in 2 CR patients and in 5 PR patients. One is still in CR 6 months far from treatment. *Conclusions*. Our preliminary results show that Clofarabine is a very effective and well tolerated drug for adult patients with Refractory Acute Leukemia and allowed us to obtain good response in high risk patients. Furthermore Clofarabine based regimens provided to obtain response in chemorefractory patients allowing thus to perform subsequent fully ablative allogenic transplant without additional toxicity.

PU034

POLYPLOIDY IN ACUTE PROMYELOCYTIC LEUCEMIA WITHOUT THE 15;17 TRANSLOCATION BUT PML/RAR-α FUSION PROTEIN

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Acute promyelocytic leukemia (APL) is distinguished from other acute myeloid leukemias (AMLs) by cytogenetic, clinical, as well as biological characteristics. In 90% of cases the hallmark of APL is the reciprocal translocation of chromosome 15 to chromosome 17, which leads to the expression of the PML/RAR-α fusion protein. Most cases exhibit a diploid karyotype with a single t(15;17). This abnormality is not detected in approximately 10% of cases with successful karyotype analysis. In the majority of these cases, the PML/RAR-α fusion gene is still formed, resulting from insertion events or more complex rearrangements, revealed by reverse transcription polymerase chain reaction. These cases share the beneficial response to retinoids and favourable prognosis of those with documented t(15;17). Tetraploid or near tetraploid is a rare cytogenetic abnormality in AMLs. To date cases of tetraploid or near tetraploid APL have been described in the literature and all of them presented double t(15,17). We present an unusual case of APL with structurally normal chromosome 15 and 17 and triploid and tetraploid clones found in the karyotype of bone marrow cells, with PML/ RAR-α fusion transcript. A 46-year old male presented for evaluation because of leucopenia (with no blasts at morphological analysis of blood smear) mild anemia and severe thrombocytopenia, associated to mild bleeding into skin. Coagulation tests revealed slightly elevated D-dimer and INR. Bone marrow aspiration showed large, hypergranulated blasts and promyelocytes carrying Auer Rods and infiltrating bone marrow. The immunophenotype was suggestive of APL. A fluorescence *in situ* hybridization (FISH) analysis of marrow blood was urgently requested as soon as the clinical suspect of APL was made, but it resulted in tetraploid/triploid nuclei with no t(15;17) in the 300 nuclei analyzed. Anyway diagnosis of intermediate risk APL was confirmed on the basis of a positive bcr3 PML/RAR-α fusion gene transcript detected by molecular analysis of bone marrow blast cells. Karyotipe analysis performed on 20 metaphases was 92,XXYY[13]/46,XY[7,showing triploid and tetraploid clones and no evidence of t(15;17)as well. To our knowledge this is the second case of APL with impressive blast morphology and unusual cytogenetic findings consisting of polyploidy without any structural alteration detected in metaphase cells or FISH and associated with the presence of the PML/RAR-α transcript.

PU035

FLT3-ITD MUTATION IN HYPERLEUKOCYTIC ACUTE MYELOID LEUKEMIA

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FLT3-ITD is a molecular mutation which represents an important prognostic factor in acute myeloid leukaemia (AML) with negative impact on disease free survival (DFS) and overall survival (OS). The incidence of the mutation reported in literature is 20-27% in AML and 26-30% in AML with normal karyotype. In literature the mutation is associated with high white blood cell (WBC) count. We evaluated the incidence of molecular mutation in a selected cohort with hyperleukocytosis and its influence on outcome.We reviewed 53 consecutive patients with newly hyperleukocytic AML (WBC >$100.000x10^9$/L) admitted to our institution from 1995 to 2009. Median age was 57 years (range 15-80). Karyotype was available in 37 patients: 7 patients had favourable karyotype, 6 patients had unfavourable karyotype and 24 patients had intermediate karyotype. FLT3-ITD was available in 46 patients. Median follow-up was 7.5 months for DFS and 7 months for OS. After induction therapy, complete remission (CR) was achieved in 17 patients (32%). There were 9 early deaths. Median DFS was 7.5 months. Median OS was 9.4 months. The incidence of the mutation was 30% in the cohort and 35% in patients with normal karyotype. Among 6 patients with early deaths available for FLT-ITD there were 4 patients positive (p 0,060).CR was not influenced by the mutation (p=0.738). The median DFS was 6.7 months in the unmutated patients and 11.9 months in the mutated ones (p=0.117). The median OS was 9.6 months in the unmutated patients and 3.5 months in the mutated ones (p=0.555). Among 20 patients with normal karyotype the median DFS was 6.7 months in the unmutated patients and 11.9 months in the mutated ones (p= 0.117) and the median OS was 9.6 months in the unmutated patients and 3.5 months in the mutated ones (p=0.555). In a selected cohort of AML with hyperleukocytosis the incidence of FLT3-ITD was only slightly higher than the reported one in AML in literature. In this selected population CR, DFS and OS were not influenced by the mutation in the total cohort and in patients with normal karyotype. We found a higher incidence of mutation in early deaths but it did not reach statistical significance and a higher number of patients would be necessary.

PU036

COMBINED FLUDARABINE AND CITARABINE REGIMENS IN HIGH RISK AML PATIENTS: A MONOCENTRIC EXPERIENCE

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AML patients older than 60 years or with secondary AML represent an high risk category with poor prognosis. We report thirteen cases of high risk AML patients treated to our institute with induction combined regimens with fludarabine and citarabine (Flu + Ara-c). From November 2005 to March 2009 in our Hematology Unit 13 consecutive patients with high risk myeloid acute leukaemia, elegible for aggressive induction chemotherapy (8 M, 5 F, median age 64, range 49-73) were included: 7 AML de novo with age > 60 yrs, 5 AML secondary to MDS, 1 AML secondary to mitoxantrone treatment for multiple sclerosis. Among these, 6 were treated with FLA regimen (Flu 30 mg/sm day 1-5, Ara-c 2 g/sm day 1-5), 7 with FLAN regimen (Flu 30 mg/sm day 1-5, Ara-c 2 g/sm day 1-5, Mitoxantrone 10 mg/sm day 3-5), 1 of which as rescue treatment after first line chemotherapy resistance. The excluding criteria for mitoxantrone treatment were age > 70 yrs, hearth failure, previous treatment with same drug for multiple sclerosis, high risk of treatment toxicity in MDS s-AML. Five patients treated before 2006 underwent G-CSF (2 FLAG and 3 FLANG). After the induction treatment, 11 patients obtained a CR (11/13, CR 84.6%), 1 patient obtained a CR without platelet recovery, 1 patient resulted resistant (OR 12/13, ORR 92.3%). Consolidation therapy was administered to 9 of 12 CR patients, 6 with fludarabine and citarabine combined regimen, 3 with high dose citarabine alone (3 g/sm bid day 1, 3, 5); 3 patients were not submitted to consolidation because 1 died in CR for infectious event, 1 performed an early allograft and 1 was too early. Among 12 CR patients, 5 are still alive in I CR (41.6%) from 1

to 32 months (median 10.4 months); 4 patients relapsed (33.3%), 2 after graft procedure, allogeneic and autologous respectively, with a duration of remission from 3 to 15 months (median 8.7 months) and 3 patients (25%) died in I CR because of infectious event. Most relevant toxicity was infectious, either in induction or during consolidation. We recorded 7 infectious events of grade 3 or 4: 4 during induction (1 of grade 4 and 3 of grade 3) and 3 during consolidation (2 of grade 4 and 1 of grade 3).In our experience, combining regimens with fludarabine and citarabine represent an efficacious therapeutic option for those AML patients with high risk disease, also for MDS s-AML; unfortunately, infectious toxicity still represents a primary cause of death in this category patients.

PU037

T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA OR T-CELL LYMPHOBLASTIC LYMPHOMA? THIS IS THE QUESTION. A CASE REPORT

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T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are often considered to be different manifestations of the same disease. They are morphologically indistinguishable and by convention, the term lymphoma is used when the process is confined to a mass lesion with no or minimal evidence of peripheral blood (PB) and bone marrow (BM) involvement. If a mass lesion and BM blasts are both present, the distinction between leukemia and lymphoma is arbitrary. Most cases of T-LBL present with advanced disease, especially in children, and may arbitrarily be classified as T-ALL when there are more than 25% BM blasts. For many treatment protocols, a figure > 25% BM blasts is used as the threshold for defined leukemia. An 8-year-old Caucasian female presented with dyspnea, peripheral lymphoadenomegaly and cutaneous lesions on scalp. PB count and smear evaluation were normal. Chest-abdominal CT scan disclosed mediastinal and abdominal adenopathies and hepatic lesions. T lymphoblastic leukemia/lymphoma was diagnosed by BM, cutaneous and sovraclavear lymphonodal biopsy. There was no evidence of CNS involvement. Karyotype and PCR assay were normal. The AIEOP-ALL 2000 protocol was started. At day 8 PB smear evaluation was obviously normal, while the chest radiography showed no improvement. The restaging after the induction phase showed partial hematological response and a minor radiological response, therefore she underwent HR consolidation therapy of the same protocol. After 2 courses she achieved hematological CR despite the radiological findings was not modified. A second line therapy with idarubicin and high-dose of ARA-C achieved a good radiological response. Unfortunately the response was only temporary and a salvage chemotherapy using FLAN scheme was started without success. CNS localization was discovered and the child died of progressive disease 5 months after diagnosis. Our patient's course matched the predictable very poor prognosis despite aggressive chemotherapy. Compared with the B-ALL, T-ALL is generally associated with more unfavorable clinical features, such as a high white-blood-cell count, bulky adenopathy and CNS involvement. Although distinguishing T-LBL with BM involvement from T-ALL may not have therapeutic implications, there are distinct clinicomorphologic and perhaps prognostic differences. With rare exception, cytogenetic findings do not distinguish T-LBL from T-ALL. While both are characterized by similar cytogenetic alteration, they are distinguished by whole genome expression profiling suggesting differences in growth regulatory pathways. A better understanding of different oncogenetic profile may lead to a better characterization of these diseases and hopefully to new specific target therapy.

PU038

THE IMPACT OF FLT3 - ITD IN ADULT ACUTE MYELOID LEUKAEMIA (AML) PATIENTS: A SINGLE CENTER RETROSPECTIVE ANALYSIS

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FLT3-ITD represents one of the most frequent genetic aberrations of AML as they occur in the 20-30% of cases with normal karyotype. Several studies recognized this mutation as a negative prognostic factor although its role in elderly patients is less clear .We have retrospectively reviewed the frequency and prognostic impact of FLT3-ITD mutation in a consecutive series of adult and elderly (>65y) patients with AML series diagnosed and treated at a single Institution.Between January 2007 and January 2009 among 60 consecutive AML patients with median age 69yrs were observed at our center. A FLT3-ITD was detected in 11(18,3 %) cases. Of these, 9 had intermediate and 2 unfavourable risk karyotype and only 1 patient had concomitant NPM1 mutation. Initial Hb median value was 8.5 gr/dL (range 6.5-12.4 g/dL), median WBC count 43x10^9/L (range 4.- 264 x10^9/L), median PLT count was 51.x 10^9/L (range 15.-312.x109/L). Five patients were treated according to the EORTC-GIMEMA AML12 protocol, while the remaining 6, who were unfit or not eligible for intensive treatment, received differentiating (ATRA+LoDAC) or supportive therapy (HU).Of the 11 FLT-ITD+ cases , 2 are too early, 3 (27%) achieved 1st CR, 6 died during induction. Among patients died during induction, 3 were older than 60y (60,72,80y), and 3 had WBC >/= 40.x10^9/L (42.2, 202., 264x 10^9/L). As of April 2009, 5 patients are alive, with a median OS of 3 months(range 1- 18 mo); 2 are in 1st CCR for 2.5+ and 4+ months, respectively; while a 3rd CR patient (a 72y-old woman), who received as 1st induction ATRA+LoDAC relapsed 14 months after CR achievement, and died during 2nd intensive induction. CR rate were disappointingly low in this small series of FLT3-ITD pts, most likely due to concomitant high risk factors (elderly age and hyperleucocytosis). As recently highlighted in a large German study (Buchner *et al*., 2009) also in our series age, high WBC count and NPM1-gerline status were additional factors negatively influencing outcome. However, differentiating treatment allowed to obtain better survival with respect to intensive chemotherapy. If confirmed in larger series, this observation may suggest that non intensive treatment associated with novel targetd agents represents a valid therapeutic option for elderly FLT3-ITD AML patients.

PU039

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM (BPDCN): REPORT OF A CASE ASSOCIATED WITH MYELOMONOCYTIC LEUKEMIA

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Introduction. Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, aggressive systemic neoplasm originated from plasmacytoid dendritic cells (PDC), a hematopoietic-derived cell implicated in the regulation of innate and adaptive immunity. The relationship of PDC and monocytes is debated. We describe the clinical and biological characteristics of a case of BPDCN associated with myelomonocytic leukemia. *Methods*. A 73 years old woman presented with cervical lymphadenopathy and nodular erythematous cutaneous lesions, associated with mild anemia (Hb 11.6 g/dL) and thrombocytopenia (120000/mm^3), white blood cell (WBC) count and LDH were normal. On histology, both skin, lymph nodes and bone marrow were infiltrated by blastic PDC as defined according to their phenotype (CD4+CD56+CD123+BDCA2+ TCL1+CD2AP+ BCL11a+, with coexpression of CD2 and CD7, but negativity for B-, T- and myelo-monocytic markers). Diagnosis of BPDCN was made. Cytofluorometric peripheral blood (PB) assay showed 8% of cells expressing CD2, CD4, CD7, CD56, and HLA-DR. Because of rapid progression with fever, organomegaly, disseminated cutaneous lesions and pleural effusion, aggressive leukemia-oriented combination chemotherapy according to MICE protocol (mitoxantrone, etoposide and cytarabine) was started. In spite of resolution of Pseudomonas Aeruginosa sepsis and pneumonia developed during aplasia the patient remained febrile, with progressively increasing interstitial-alveolar infiltrates and respiratory failure. During the haematologic recovery she developed significant PB monocytosis (21.496/ mm^3) with 25% of atypical BM monocytoid precursors and 3% of BM blasts. PB and BM cytofluorometry and histology showed cells of monocytic lineage expressing CD4+CD56+CD11c+CD14+, as well as CD123 and CD2AP, but negative for other BPDCN markers. BM dysplastic changes were also obvious. After steroid therapy the clinical conditions improved rapidly and monocytosis resolved, but one month later central nervous system relapse with marked liquoral blast cell infiltration occurred and the patient died. *Discussion*. In about 15%-20% of cases BPDCN has been reported

to be associated with or develops into a myelomonocytic leukemia or acute myeloid leukemia; this study shows that partial sharing of markers between the two neoplastic populations may occur, thus suggesting that the two diseases are more than coincidental and may have a common cellular origin.

PU040

ACUTE LYMPHOBLASTIC LEUKEMIA AND PREGNANCY: A CASE REPORT

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In August 2008 a 19 years old girl was referred to our institution because of superficial lymph nodes without systemic symptoms. The peripheral blood showed leucocytosis, anemia and thrombocytopenia (WBC 21800/m^3, Hb 9.9 g/dL, PLT 99000/m^3). The patient was at the 16th week of pregnancy. A lymph node biopsy was compatible with pheripheral T cell Lymphoma, while the bone marrow analysis by morphology and multiparametric flow cytometry were compatible with diagnosis of T Acute Lymphoblastic Leukemia (cyCD3+, cyTdT+, cy79a+, CD7+, CD5+, CD4+, CD2+, CD10+) with blast counts superior to 75%; the cytogenetic analysis showed a complex cariotype: 46,XX, add(2)(q?33), del(6)(q?31), del(10)(q?24). Our aim was to cure leukaemia possibly avoiding any hindrance against pregnancy. After informed consent the patient received induction chemotherapy with Adriamycin (30 mg/sqm iv) plus Oncovin (1,4 mg/sqm iv) days 8,15,22,29 and Prednisone 60 mg/sqm/d for 30 consecutive days. Prophylaxis on CNS was performed with intrathecal administration of liposomal Cytarabine days 1,15,30,45. A bone marrow biopsy performed at the fourth week of therapy showed a CR either morphological either at flow cytometry, the cariotype was normal too. The pregnancy was uneventful and the child was growing correctly. The second course of induction therapy consisted of Cytarabine (75 mg/sqm/d iv for 4 days/weekly for 4 cycles). At the 34th week of pregnancy an healty newborn baby was delivered by caesarum partum with a weight of 2100 g. After one month the patient received consolidation therapy with HAM regimen and collection of pheripheral blood stem cells. In Febrary 2009 she underwent autologous stem cell transplantation. Conditioning regimen consisted of Thiotepa (10 mg/Kg iv day -5) plus Cyclophosphamide (60 mg/Kg/d iv from day -3 to -2) followed by reinfusion PBSC for 9.75x10^6/Kg $CD34^+$ cells. She achieved haematological recovery at day +10. The extra-haematological toxicity during phase of cytopenia was mild and transient. In April 2009 she started maintance therapy according scheduled ALL GIMEMA protocol. The patient is still in CR and her little baby grows normally without apparent deformities. In conclusion the cure of ALL during pregnancy is possible safeguarding the foetus and its maturation. A reasonable therapeutic strategy should be warranted in order to avoid main problems due to single chemotherapy agents.

PU041

SERUM FERRITIN PREDICTS SURVIVAL IN ADULT ACUTE MYELOID LEUKEMIA

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Background. Recent studies have suggested an association between iron overload and posttransplantation liver toxicity, susceptibility to infections, and even survival in patients undergoing hematopoietic stem cell transplantation for hematological malignancies. Aims. To date, there are no data to show that iron availability can and does play a critical role in adult acute myeloid leukemia (AML). We report a study of the role of pretreatment serum ferritin as a prognostic factor in adult AML. Methods. We studied 65 consecutive adult de novo AML patients. In each patient the serum ferritin level was determined at disease onset. The median age of patients was 59 years (range 17 y.rs to 77 y.rs); M3 FAB subtype cases were not considered in this analysis. Thirty-five patients were <60 y.rs and and 30 >60 y.rs. In our series of AML patients, 61 were treated with standard induction therapy and 4 cases died early. AML patients were subdivided into two groups according to ferritin serum value (< 800 versus > 800 ng/mL) and age (< 60 versus >60 y.rs). Results. Among patients < 60 y.rs, 20 (57%) cases showed a ferritin serum value <800 ng/mL. Compared with the > 800 ng/mL group, patients with serum ferritin <800 ng/mL had a longer overall survival (OS) (657 vs 235 days, p =0.01). Moreover, patients with serum ferritin > 800 ng/mL showed a trend toward a higher frequency of documented infections during induction treatment (33% vs 5%, p=0.06) and a higher incidence of relapse (50% vs 23%). Among patients >60 y.rs, 14 (47%) cases showed ferritin serum values <800 ng/mL. Compared with the > 800 ng/ml group, patients with serum ferritin <800 ng/ml had a longer OS (234 vs 66 days, p =0.004). There was no association between serum ferritin values and complete remission, documented infections and frequency of relapse. It is noteworthy that the 4 patients who died early (2 in the < 60 and 2 in the > 60 y.rs group) had ferritin serum values > 1500 ng/mL. *Conclusions*. The results of our study demonstrate a link between serum ferritin and OS of AML patients. Further studies are required in a large series of AML patients to explore the association of serum ferritin with other events (infections, complete remission, disease relapse).

PU042

A RARE CASE OF ACUTE LEUKEMIA OF DENDRITIC CELL LINEAGE

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Plasmacytoid dendritic cell leukemia is a rare entity representing <1% of acute leukemia cases and 0-7% of cutaneous lymphomas. The diagnosis of plasmacytoid dendritic cell leukemia (pDCL) is based on the immunophenotypic profile: CD4+ve, CD56 +ve, CD45+/RO-ve, CD11c-ve, CD116 low,CD123+ve, CD34-ve , CD36+ve,HLA-DR+ve. Few cases of pDCL expressing CD56 but not CD4 are also reported.We describe here the case of a 55 year-old man with a picture of acute leukemia without skin lesions diagnosed as pDCL. The patient was admitted to the Hematology Unit in october 2008 with anemia, disseminated involvement of palpable and deep lymph nodes, enlargement of liver and spleen, pleuro-pericardial effusion and renal failure. Lymph node biopsy showed monomorphous infiltrates with blastic appearance carriing the following immunophenotype: TCL1+ve, CD56+ve, CD68R+ve, CD45+ve, bcl2+ve, TdT +ve/-ve, Ki67 100%, p53 50%. Bone marrow aspiration smears showed a picture of diffuse infiltration (60%) by undifferentiated monomorphous population of medium –to large size blastic cells with CD4-ve, CD56+ve, HLA-DR+ve immunophenotype .The patient. received a chemotherapy course with FLAG-ida (fludarabine 30 mg/m^2 day one-five, Ara-C 2 g/m^2 day one-five, idarubicin 10 mg/m^2 day one and three and G-CSF). The patient achieved a good partial remission after the first cycle of chemoterapy. Then after twenty days the patient received another cycle of chemoterapy FLAG-ida with complete bone marrow blasts disappareance. On January 2009 there was a leukemic relapse with blood, lymph nodes, meningeal and skin involvement. Besides skin lesions as multiple erythematous papules on the trunk, lower arms and head apparead with typical histology showing dermal proliferation of blastoid cells with a grenz zone between epidermis and neoplastic infiltration expressing CD4+ve/CD56 +ve immunophenotype. The patient underwent chemoterapy according to the MACOP-B regimen. At the moment the patient is alive but with persistence of disease. Excellent initial chemosensitivity, but early relapse and rapidly fatal outcome characterize neoplasms of dendritic cell lineage . Our report describes a case with typical clinical and morphological features but showing a negative reaction for CD4 on initial lymph node biopsy and bone marrow aspirate. Only at the relapse time CD4 reaction was positive on skin and blood, a very atypical immunophenotypic course despite the literature reports.

PU043

UNUSUAL MANIFESTATION OF ACUTE MONOBLASTIC LEUKAEMIA. A CASE REPORT.

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Acute myeloid leukemias (AML) are aggressive haematopoietic neoplasms that, if untreated, can lead to death within days. Up to 40% of presenting patients show evidence of extramedullary involvement (EMI) at

diagnosis. EMI is reportedly most prevalent in myelo-monoblastic subtypes of AML and can present as leukaemic infiltrates in many sites including gingival enlargement and mucosal and skin nodules. We report a case of a patient who presented with peripheral paralysis facial nerve and skin manifestation secondary to leukemic infiltration. On April 2009, a 33-years-old male presented to our hospital as having Bell's pulsy with facial disfigurement, difficulties with communication, eating and drinking, multiple infiltrative nodules on his body. In previous months the patient showed central paralysis facial nerve, so he performed cranial and spinal cord MRI which appeared negative. At that time blood tests were normal and the patient, treated with high dose steroids and immunoglobulins, presented in thefollowing days diffuse subcutaneous nodules. For worsening of the clinical signs he arrived at our attention. His peripheral blood count showed mild thrombocytopenia (Plt 133.000/mmc), mild leukocytosis with lymphocytosis (WBC 19.000/mmc, L 10.000/mmc) while biochemical tests emphasized only high LDH. Bone marrow revealed AML M5b subtype. Histologically, cutaneous nodules were formed by monoblast cells showing acute monoblastic leukemia. Cerebrospinal fluid was positive for blast cells. He started chemotherapy including high dose ARA-C, daunorubicin and ethoposide. On account of the certain cranial nerve involvement intrathecal metotrexate was administrated. Today the patient is in aplastic period post induction therapy.AML have a wide variety of clinical manifestations and, specially FAB subtypes of AML-M4 and M5, are commonly associated with extramedullary involvement even if the basic mechanism as to why some leukemic cells infiltrate other tissues is not well understood. Moreover patients with these disease manifestations have usually aggressive clinical course and are associated with a poor prognosis. Rarely extranodal disease is present before leukemia can be detected in the peripheral blood as in our case. So we pay attention to all patient's clinical signs and, if they persist, we must take into account the possibility of typical AML diagnosis.

PU044

DO ANTI-EPILEPTIC DRUGS TRIGGER LEUKEMIA?

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Anti-epileptic drugs such as valproic acid are generally used for their sedative and anti-seizures properties. However previous studies [Piel CJ et al 2001]have shown that valproic acid has a histone deacetylase inhibitory action and therefore could cause hyperacetylation of chromatin and relaxation of chromatin structure [*Coyle TE et al 2005*]. Furthemore, pharmacological studies have shown that lamotragine potentiate valproic acid actions [*De Sarro G et al 1996*]. Case description A 42 years old Caucasian woman with history o epilepsy since the age of 7 years. Patient presented in January 2007 with progressively increasing MCV values, with folate and B12 levels within normal ranges. The other only finding was decreasing white cells count. In July 2008 neutropenia (ANC <0.5x10^9/µL), hyporigenerative anaemia (Hb 8.4 g/dL) with normal platelet count, were first seen. Interesting a pharmacological study conducted in October 2008 documented high lamotragine levesl (14 µg/mL; nv 0.5-4.0) and valproic acid levels (107 µg/mL; nv 50-100), Around this time clinical diabetes insipidus, hyper prolactine level (25.9 ng/mL; nv 4.8-23.3) and diplopia were also documented. In January 2009, a bone marrow aspirate showed 40% blasts FAB=M6. Cytogenetic analysis shown normal female karyotype 46 XX. A detailed past medical history documented that the patient was treated with valproic acid and carbamazepine over 25 years, however since the last 3 years carbamazepine was replaced with lamotragine, which was also increased in July 2008 before the onset of the neutropenia. Patient was treated with induction chemotherapy as per schedule HDS-2 with ARA-C and idarubicin and is currently undergoing consolidation cycle. Valproic acid has been gradually discontinued. *Discussion.* Although valproic acid has already been associated with trilineage haematological toxicities, this study suggests that in association with lamotragine and outside therapeutical ranges these drug could be strongly linked with MDS and leukaemia manifestations. Previous similar studies on leukaemia have also shown that when these drugs are discontinued malignant clones disappear [*Bottom KS et al 1996*]. The finding of diabetes insipidus and hyper-prolactinemia in close association with raised levels of lamotragine and valproic acid is quite intriguing. Hypophisis damage and diabetes insipidus have been already reported as early signs of erythroleukaemia [*Piccin A et al 2007*]. Taken together these data suggests that raised values of anti-epileptics drugs were directly associated with disease progression. Further studies are now warranted to validate these findings. Newly diagnosed leukaemia patients on treatment with valproic acid should be monitored carefully and where possible other anti-seizures drugs should be considered.

PU045

SUCCESSFUL ALEMTUZUMAB THERAPY FOR SEVERE AUTOIMMUNE COMPLICATIONS IN A PATIENT WITH EARLY STAGE OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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A 59 year old woman was admitted to our hospital in March 2002 for a mild lymphocitosis (WBC 10.3x10^3/mmc; Ly 5.6x10^3/mmc). Bone marrow biopsy (BMB) revealed prevalent erythropoiesis and small interstitial and nodular lymphocytes CD5$^+$, CD23$^+$, CD19$^+$, sIgM k$^+$. Organomegaly and limphoadenomegaly were absent. FISH analyses recorded normal karyotype. A diagnosis of B-CLL at Binet stage A was made. After few months a severe warm antibody-autoimmune haemolytic anemia (AHA) (Hb 7.7gr/dL) occourred. Direct antiglobulin test (DAT) was strongly positive (IgG 1:1024; C3d 1:64). Unusually reticulocyte count (82.000 /microl), total bilirubin, dehydrogenase lactic and aptoglobin levels were normal. The patient received standard treatment with methilprednisone and danazol reaching partial remission. In March 2003 she developed recurrence of AHA (Hb 7,3 gr/dL) without CLL progression. Because of refractoriness to steroid therapy, the patient underwent therapy with weekly Rituximab (375 mg/m^2) for 4 doses and rapidly obtained Hb>12gr/dL. After five months anemia relapsed and patient failed response either to Rituximab or cyclophosphamide bolus and plasmaexchange. Three months later, Hb dropped to 4,5 gr/dl and absolute reticulocytes fell to 13.000/microl. BMB revealed a reduced erytropoiesis (<5%) and a stable lymphocytic infiltration (15-20%), suitable with diagnosis of pure red cell aplasia (PCRA). Therefore, subcutaneus Alemtuzumab (15 mg/die x 3 times/ weekly for 8-12 weeks) was administered, but therapy was stopped after 6 weeks for CMV reactivation. However, complete response (CR) was achieved 6 weeks after Alemtuzumab discontinuation with stable haematologic values for 34 months. From July 2007 she presented two new haemolitic events, retreated both with 8 weeks Alemtuzumab courses with CR of 8 and 12 months respectively. In March 2009 she developed fourth AHA relapse; retreatment with Alemtuzumab is ongoing and CLL was stable. AHA is the most common autoimmune disorder in CLL. Pathogenesis of autoimmune complications in CLL involves both T and B cells. Our case is characterized by severe AHA with reticulocytopenia, later on complicated by PCRA, as the only clinical features of CLL. Reticulocytopenia in AHA may be explained by increased apoptosis of erythroid progenitors as described in literature. The efficacy of Alemtuzumab on both autoimmune complications can be explained by its immunosuppressive action on both B and T cells. Frequent relapses show the need for therapy immunosuppressive maintenance.

PU046

MEGALOBLASTIC ANEMIA ACCOMPANIED BY BASOPHILIA: A CASE REPORT

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An 87-years-old woman was admitted to our hospital in November 2008 presenting fatigue, peripheral edema, mild dispnea. She reported to have hypertension, type 2 diabetes mellitus and macrocytic hipochromic anemia identified two years before and treated with folic acid, cobalamin and iron supplementation. Chest x-ray revealed bilateral pleuric effusion, abdominal ultrasound was negative. Echocardiogram showed hypertensive cardiopathy with mild systolic dysfunction. Laboratory examinations revealed: haemoglobin 7.7 grams per deciliter, mean cell volume 108 femtoliters, mean cell hemoglobin 32.4 picograms, mean cell contenet 30.1 grams per deciliter, white blood cell 5000 leukocytes per millimeter cubic, neutrophils 59 per cent, basophils 11 per cent, eosinophils 3 per cent, monocytes 9 per cent, lymphocytes 20 per cent, platelets count 388000 per millimeter cubic. Serum vitamin B12, folic

acid, β2 microglobulin, serum lactate dehydrogenase, gammaglobulines, iron, ferritin, transferring were normal. Hepatitis A,B,C and HIV serology were negative. Immunophenotypic characterization was negative for excess myeloid blast cells but positive for predominantly basophils. Peripheral smear showed anisopoikilocytosis and teardrop erythrocytes. Bone marrow biopsy showed cellular composition of 40 per cent, accumulation of macrophages, rare ringed sideroblasts, dyserythropoiesis and dysmyelopoiesis in a significant proportion of marrow, basophilia; blast cell < 5 per cent, CD34+ 5-10 per cent such as myelodysplasia unclassificable. Blood marrow cytogenetic test (fluorescent in situ hybridization) was positive for deletion 5q in 83 per cent of cells but 2 of 13 marrow metaphases containing a standard philadelphia translocation involving chromosomes 9 and 22 with cytogenetic conventional test and this result was confirmed with quantitative and qualitative RT-PCR molecular test (BCR/ABL; p 210), 3 contaning Philadelphia chromosome and deletion 5q-, 2 contaning only deletion 5q- the other metaphases were normals and JAK-2 was negative (blood marrow). Conclusion: myelodysplastic syndrome and chronic myeloid leukemia. In December 2008 Imatinib 400 milligrams tables day and trasfusional supplement therapy was started; the patient continued also cardiac failure specific therapy. Lenalidomide was not proposed because myelodysplastic syndrome with chromosome 5q deletion is a disorder with a good prognosis, chronic course and unusual evolution in acute leukaemia, while chronic myeloid leukemia is related with high risk of acute trasformation. The patient refused epoetina treatment. During the three months of therapy the patient was trasfused with three units of red blood cells; no were observed complications imatinib related; the genetic control test repeated itself in April. Myelodysplastic syndrome associated with chronic myeloid leukemia is uncommon. Three cases has been reported after treatment with interferon or radiotherapy for myelodysplastic syndrome 5q deletion, chronic myeloid leukemia or gastric diffuse large cell B non Hodgkin lymphoma. In our case the diagnosis was made in the same time. Treatment decision are based primarly on the dominant hematopathologic features.

PU047

A COMPLEX TRANSLOCATION T(6;9;22) IN A CHRONIC MYELOID LEUKEMIA YOUNG PATIENT

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Chronic myeloid leukemia (CML) is generally characterized by the traslocation of chromosome 9 and 22, t(9;22)(q34;q11) which results in the fusion of BCR/ABL gene called Philadelphia (Ph'). About 5-8% of patients showing Philadelphia positive in conventional cytogenetic present various complex translocation involving a third chromosome (4,5,6,7) in addition to chromosome 9 and 22. In our report we discuss one case of a forty years old woman with CML in chronic phase referred at our centre (high risk for CML Sokal score, platelets 1.614x10^9/liters, white blood cells 89x10^9/liters, blasts 1%, basophils 9%, eosinophils 2%, spleen size 2 centimeters below costal margin). At the time of diagnosis her cytogenetic study revealed a complex traslocation involving the chromosome 6, 46, XX, t(9;22;6) (q34;q11.2;q21) in 100% of cells. This variant Ph' rearrangement was confirmed by FISH study using LSI BCR/ABL dual color dual fusion (DF) traslocation probe, chomosome 6,9 and 22. The patient began the Tyrosine Kinase (TK) inhibitors, imatinib mesylate(Gleevec), and achieved a complete cytogenetic response after 3 months and molecular after 6 months. Actually, after 12 months, the patient is still in complete cytogenetic and molecular response. This case report suggests that this variant Philadelphia chromosome is not unfavourable. This could be attributed to the reciprocal translocation with no loss or gain of genetic material.

PU048

INNOVATIVE PHASE I-II STUDY OF CONCOMITANT AND CONSECUTIVE TREATMENT WITH DASATINIB AND MK-0457 IN REFRACTORY PH+ CML AND ALL PATIENTS

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Background. MK-0457 is a pan-aurora kinase inhibitor with demonstrated activity against wild-type and mutated BCR-ABL, including the T315I form, as well as FLT3 and JAK-2. It is a promising molecule for the management of Ph+ leukemias, in which the emergence of mutations in the ABL kinase domain still represents the main mechanism of resistance to TK inhibitors. *Aim*. We conducted an innovative and proof of concept Phase I clinical study of sequential and concomitant treatment with Dasatinib, previously administered for three months, and MK-0457. This combined activity suggests that MK-0457, in association with Dasatinib, would suppress the emergence of T315I and other resistant clone, improving upon the response rate for Dasatinib and the durability of response. The trial investigated two schedules of therapy: patients who achieved and maintained a major hematologic response (MHR) after three months of therapy with Dasatinib (70 mg twice daily) received a 6-hour biweekly infusion of MK-0457 at 64 mg/m^2/hr, whereas patients who failed to achieve a MHR received a 5-days continuous infusion of MK-0457 at 10 mg/m^2/hr, every 4 weeks. Results. Two patients with Ph+ ALL and one patient with CML in myeloid blast crisis, previously unsuccessfully treated with imatinib, were enrolled in the protocol. The first two patients, both in hematologic response after three months of treatment with Dasatinib, subsequently received the 6-hour biweekly schedule, maintaining the haematological response. No haematological toxicity was described. The third patient, enrolled in progression disease, received the 5 days MK-0457 schedule of treatment. His peripheral blood count was consistent with a severe pancytopenia, which required frequent platelets and red blood cells transfusions. His bad clinical performance status was compromised by a severe hemorrhagic pleural effusion, responsible for moderate dyspnoea and severe asthenia. After one cycle of MK-0457, a complete recovery of the pulmonary disease and a complete hematologic response were obtained. In all three patients an allogeneic bone marrow transplantation of savage from HLA identical donor could be performed. *Conclusions*. The sequential and concomitant administration of Dasatinib and MK-0457 represents a promising therapeutic strategy for refractory Ph+ CML and ALL. *Funding: supported by European LeukemiaNet, AIL, AIRC, FIRB 2006, Fondazione del Monte di Bologna e Ravenna, Strategico di Ateneo, PIO 2007 Project, Merck Sharp & Dohme.*

PU049

DASATINIB OVERCOMES IMATINIB SECONDARY RESISTANCE INDUCED BY TWO DIFFERENT BCR-ABL MUTATIONS.

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A 43-year old caucasian man with marked leukocytosis and thrombocytosis, moderate anemia and severe splenomegaly was diagnosed with chronic myeloid leukemia in chronic phase (CML-CP), high and intermediate risk according to the Sokal and Euro score respectively. He was monitored through conventional cytogenetic, FISH analysis with a dual-fusion BCR-ABL probe and an automated FISH imaging system set to score 2500 nuclei for rare cell events (BioView-Duet). Real time-polymerase chain reaction (RT-PCR) and RT quantitative-PCR (RQ-PCR) for BCR-ABL transcript were performed. Mutational screening analyses of BCR/ABL kinase domains (KD) were obtained sequencing nested RT-PCR products. At diagnosis, cytogenetic analysis showed the t(9;22) associated with an inv(7)(p22q32) in 100% Ph+ metaphases. First treatment was hydroxyurea, briefly followed by imatinib 400 mg daily. A complete haematological response (CHR) was achieved within 6 weeks and a complete cytogenetic response (CCgR) within 3 months. At 8 months, an increasing leukocytosis and thrombocytosis (2500x 10^3/microl) occurred. The CCgR was lost and the inv(7)(p22q32) again was present in 100% Ph+ metaphases. The molecular monitoring for BCR-ABL KD revaled the presence of 2 mutations, L248V and L387M and the patient was switched to dasatinib (140 mg daily). He rapidly achieved a CHR together with a gradual reduction in BCR/ABL transcript. After 6 months the CCgR, with disappearance of inv(7), and the MMolR were achieved, which both are still lasting at the last follow up, 12 months of dasatinib. The leukemic clone was strictly characterized at diagnosis by a specific genetic marker, i.e. inv(7)(p22q32). It constitutes one of additional chromosome abnormalities (ACAs), which are rare in earlyCP and become more frequent over time and with disease progres-

sion. Often they have a bad prognostic significance. In this case the biologic significance of inv(7) for the resistance of the patient to imatinib and the responsiveness to dasatinib is not clear. Resistance to imatinib is mainly mediated by point mutations of BCR/ABL KD. L248V and L387M mutations are located respectively in the p-loop and in the activation loop within ABL KD. L248V mutation itself is generally moderately resistant even to dasatinib besides imatinib therapy. The role of L387M in mediating resistance to imatinib is less clear; it demonstrates a 2-fold increase over wild type in both biochemical and cellular IC50 values. Dasatinib is a dual specific SRC and ABL inhibitor, able to bind and inhibit both the active and inactive conformations of ABL; its activity results 300-fold higher compared to imatinib. In our patient it was very quickly able to overcome the secondary imatinib resistance given by two different mutations, one of which partially resistant to it. The identification of clinically significant mutations facilitates selection of alternative approaches to therapy such as dose escalation, second generation TKIs or allogeneic stem cell transplant, if eligible, at an early stage, tailoring patient specific approaches to therapy.

PU050

DASATINIB AS SAFE TREATMENT IN A PATIENT WITH CML AND RETINAL OEDEMA DUE TO IMATINIB

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Imatinib, a Bcr-Abl inhibitor, is the standard therapy in CML. It is not a selective inhibitor but also inhibits ABL,PDGFR and , c-kit. This last activity underlie the collateral effect of imatinib. Superficial oedema is the most common ocular adverse effect described with the use of imatinib. We report a patient who developed retinal oedema during treatment with imatinib. From January 2004 to January 2009 we treated 30 Ph+ CML patients with imatinib. During treatment we performed a complete ophthalmic examination in 7 patients suffering ocular side effects other than simple mild-moderate edema. 6 patients noted moderate epiphora due to conjunctival chemosis and 1 patient suffered for mild visual deterioration with reduction in visual acuity. We report this case. A 64 year old female was diagnosed with chronic phase CML in June 2006 and started imatinib 400 mg/day. A complete haematological response was achieved after one month. In September 2006, she noted mild visual deterioration with reduction in visual acuity. Funduscopic examination showed retinal oedema in both eyes. We started acetazolamide without visual improvement and so imatinib was ceased. The patient's vision showed rapid improvement with resolution of retinal oedema. Because improving of symptom we decided to restart imatinib, but the retinal oedema reappeared without improvement after reduction of imatinib. In November 2007 with the commercialization of dasatinib the patient started dasatinib 100 mg/day. She had not side effects with the use of dasatinib. She maintain CCyR and MMolR and she has preserved visual acuity with no retinal oedema. Retinal oedema is ocular side effect only rarely described with imatinib's use. The mechanism by which imatinib could produce retinal oedema may consist in the inhibition action of imatinib on PDGFR express in the retina.

PU051

NOT PUBLISHED

PU052

THE SCREEN PROJECT: A CML DATA REGISTRY IN SICILY AND CALABRIA

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The introduction of Imatinib Mesylate (IM) has produced major advances in the treatment of Chronic Myeloid Leukemia (CML). However, despite this high clinical efficacy, residual and/or resistant disease is still an issue for several IM-treated patients and appropriate clinical guidelines have been proposed and applied (ELN recommendations). IM-therapy has also revolutionized the lab monitoring approach to CML, since this innovative molecular drug requires an accurate molecular analysis. Given this evidence, we established a CML registry and planned to perform a centralized molecular monitoring by RTQ-PCR of the BCR-ABL transcript expressed by all newly diagnosed CML patients treated with IM. This initial CML data registry begun recruiting patients in April of 2005, with the following aims: (i) to establish a registry of all newly diagnosed Sicilian patients with CML, (ii) to standardize the molecular technology required to analyze the BCR-ABL transcript, (iii) to identify CML individuals that are resistant to IM, (iv) to characterize the molecular mechanisms underlying IM-resistance. The first step was to organize a web site with an electronic clinical database, while all clinical samples (PB and BM) were centralized in a single lab-institution for molecular analysis. The registry was initially designed to create a regional network able to perform the molecular monitoring of IM-treated CML patients and to assure them the same standard of care. Recently, the registry has been expanded to include patients from both Sicily and Calabria and under it's current name (SCREEN: Sicilia and Calabria CML Regional Enterprise) accounts for 210 CML patients. Complete data sets are available for 160 patients. They are 88 males and 72 females, with a median age of 54 years (24-90), and all received conventional IM-treatment (i.e. 400 mg/die). All critical data points are being evaluated according to ELN recommendations. We believe that the SCREEN project will have a significant impact on Sicilian and Calabria CML patients, strengthen clinical integration and cooperation also in the context of the national working group.

PU053

STABLE RESPONSE TO IMATINIB MESILATE IN CHRONIC MYELOID LEUKEMIA THIRTY TWO MONTHS AFTER DISCONTINUATION OF THERAPY

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The possibility of stopping imatinib mesilate therapy in patients achieving a complete haematological, cytogenetic and molecular response is still an object of debate. In February 2002 we saw a 76 years old woman presenting a chronic myeloid leukemia in accelerated phase. Her leukocytes were 191.000 per mm^3 with percentages of 37 neutrophils , 42 neutrophil precursors and 4 blasts. She had the typical finding of Philadelphia chromosome translocation and the resulting BCR-ABL fusion gene. One month later a therapy with imatinib mesilate at a daily dose of 600 mg was started. Hematological, cytogenetic and molecular response was easily obtained in twelve months though a cell line with +8 chromosome appeared. In 2005 she began to experience abdominal pain , nausea and repeated vomiting episodes that usual therapy and the reduction of imatinib dosage could not improve. A progressive body weight loss was present but the patient refused an endoscopic examination and decided to stop imatinib therapy in August 2006. Her symptoms briefly regressed and the patient refused starting again imatinib therapy. Subsequently , in October 2006, she developed a breast cancer and in October 2007 a right colon cancer was discovered. For these two diseases she underwent surgery. Thirty two months after having stopped the therapy the peripheral blood counts still remain in the normal values with 7,600 leukocytes per mm3 and normal differential counts. On cytogenetic analysis trisomy of chromosome 8 is still persistent. On quantitative polymerase chain reaction-based assay BCR-ABL m RNA is not detectable so proving that a complete molecular response is still present.

PU054

GENE EXPRESSION PROFILE (GEP) ANALYSIS AS A TOOL TO CONTROL THE EFFICACY OF DASATINIB, IN RELAPSED IMATINIB-RESISTANT CML PATIENTS PREVIOUSLY UNDERGONE TO ALLOGENEIC STEM CELL TRANSPLANTATION. PRELIMINARY DATA OF A SINGLE INSTITUTION

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Herein, we present results of a pilot study concerning a group of 3 patients that were both resistance to Imatinib and unresponsiveness to cellular therapy. We hypothesized that Dasatinib could potentially

improove the disease by immunomodulatory action followed by Donor Lymphocyte Induction (DLI). We investigated Dasatinib ability to achieve modulatory effects by monitoring immune-transcriptsome. Clinical effects were examined by conventional diagnostic parameters. Patients received Dasatinib 70 mg twice daily (140 mg total daily dose). Dose modifications were allowed for the management of toxicity. Treatment was performed until complete cytogenetic, molecular response and haematological full donor chimerism. *Materials and Methods*. To investigate the immunological changes, we used a TaqMan® Low Density Array, based on comparative CTdd CT method. Relative quantification was performed on cDNA derived from peripheral venous blood specimens harvested after DLI, before and after starting Dasatinib therapy. Assumed that normal control values of all transcripts were = 1, we evaluated over or down-regulation of gene expression profile (GEP) of a panel of 47 genes involved in immune response. *Results*: clinical changes after third month of dasatinib therapy. Dasatinib caused early haematological toxicity in one patient that maintained a low level of donor T cells with presence of Philadelphia chromosome associated to elevated p210 molecular signal. This patient was no evalutable because brief treatment (only 1 month). In two evalutable cases, we observed a fast down-regulation either Th1/Th2 responses and early growth mediators such as EGR2, EGR1. By contrast, multiple pro-inflammatory factors were up-expressed: IFN-α, IL-17, IL-7. Regards to T regulatory cells, Foxp3 was strongly up-regulated in only one patient. Noterworthy, only this patient showed persistent high Foxp3 expression achieving a complete haematological response. Infact, after 1 year cytogenetic analysis did not reveal any Ph chromosome, chimerism showed a 100% donor profile and the BCR-ABL1 transcript was absent. On the contrary, patient with down-expression of Foxp3 no achieved haematological response. *Conclusion*: We think that Dasatinib represent a possibility of cure for CML pts relapsed after A-HSCT and unresponsive to alternative treatments. The present results strongly emphasize the importance of immune response control to achieve the desired clinical effects.

PU055

THE SYNERGISTIC ACTION OF GRAFT VERSUS LEUKEMIA, TYROSIN KINASE INHIBITOR AND CYTOSTATICS DRUGS IN MULTI-RESISTANT CHRONIC MYELOID LEUKEMIA DURING BLASTIC CRISIS

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Introduction. The recent introduction of tyrosine kinase inhibitors (TK-I) for the treatment of Chronic myeloid leukemia has become a valid alternative to standard chemotherapy regimens and bone marrow trasplantation. However a small percentage of these patients do not respond to TK-I. In these cases allogeneic bone marrow transplantation is a valid option. We report on a case of a young patient who developed multiple TK-I resistance post sibling allogeneic HSCT, but within few days disease relapse was found. Multidrugs treatment and Graft versus Leukaemia (GvL) allowed to control disease progression. Case description A 39 years old gentleman of north african ethnicity was first diagnosed in June 2008 with LMC Ph+. Patient was immediately treated with STI-571(Glivec ©) with partial response and two months later he underwent open splenectomy because of splenic rupture due to massive blasts infiltration. In September 2008 a first chemotherapy regimen with idarubicin, ARA-C, etoposide followed by a consolidation cycle with idarubicin and ARA-C were administered. However bone marrow aspirate revealed blats >50%(myeloid type). Patient was started on dasatinib (Spyrcel ©) and within 2 weeks of treatment complete morphological remission was achieved. In February 2009 the patient showed high drug toxicity including bilateral pleural effusion and severe esophagitis and therefore dasatinib was discontinued. Within 10 days patient entered in blast crisis with WCC >56.000x10^9/μL mutational analysis revealed presence of E255K and T315I mutations. As a macthed sibling donor was available, HSCT was performed using a debulking step with Amsacrine and Topotecan, followed by a conditioning regimen with Treosulphan and Fludarabine, GvHD prophylaxis with CSA only. Donor engraftment was achieved on day +19. However on day +23 WCC >65.000x10^9/μL with 10% of blasts. To enhance GvL effect, CSA was withheld and dasatinib recommenced. Also low dose hydroxyurea 500 mg/day and mercaptopurine 50 mg/day were added. Gradually the count decreased to a plateau level of 15.000x10^9/μL with 10% blasts . Skin GvHD grade I was consistently presented. The patient remains on dasatinib maintenance dose and low dose hydroxyurea, is currently alive (day+45) and his performance status is WHO=2. *Conclusions*. This case demonstrates that the association of TK-I and low dose cytostatic drugs should be considered to control accelerated blastic crise post allogeneic HSCT and may act as a valid "bridge" while waiting GvL response. These findings should encourage research and use of TK-I in blastic crises when mutational analysis documents possible drug-efficacy. The TK-I action should be now studied in association with DLI therapy. Larger studies are now warranted to support these findings.

PU056

OPPORTUNISTIC INFECTION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING IMATINIB

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Imatinib front-line treatment of chronic myeloid leukemia (CML) showed a high rate of response. The reported grade 3-4 adverse events were about 10% and their frequency decreased, with statistical significance, after 4 years of therapy. In this report we describe an unusual infection occurred in two patients suffering from chronic myeloid leukemia during treatment with imatinib. Case 1. A 70 years old woman with chronic myeloid leukemia, high risk Sokal, received, as initial therapy, imatinib. A major cytogenetic response was achieved at 6th month. This response was confirmed at 12th and 24th month. The dose increase of imatinib wasn't tolerated by the patient. After 28 months of the therapy, the patient showed abdominal pain, fever and grade 3 diarrhoea with a weight loss of 7 kilos in a week. She was admitted to the hospital, cultural exam of faecium and endoscopic exam of bowel with biopsy showed infection bowel from Candida Albicans. The treatment with Fluconazolo (400 mg per day) obtained resolution of infection. The patient has been receiving therapy with imatinib. Case 2. A 62 years old man, was treated with imatinib for CML, low risk Sokal. He obtained a complete cytogenetic and major molecular response. 65 months after the diagnosis underwent hypocondrial right pain, asthenia and middle fever. The exams showed deranged liver function tests and small diffused liver lesions demonstrated with ultrasound and CT. The liver histology identified Candida in the specimen. The specific therapy with Fluconazolo i.v. obtained a resolution of liver infection. After 12 months the patient is alive in complete cytogenetic and molecular response. *Conclusion*: the long-term administration of imatinib might affect immunity in some patient and predispose to opportunistic infections, as previously observed. In a recent report, it has been described an inhibitor effect of imatinib on specific receptor of CD8+ T-lymphocytes, that is important for cytochines releas

PU057

PHASE II PILOT STUDY OF THE SAFETY AND EFFICACY OF A COMBINATION OF NON-PEGYLATED LIPOSOMAL DOXORUBICIN, CARBOPLATIN, ARACYTIN AND METILPREDNISOLON IN RELAPSED/REFRACTORY AGGRESSIVE NON HODGKIN'S LYMPHOMA IN ELDERLY PATIENTS

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Older patients (pts) with relapsed/refractory aggressive non Hodgkin's lymphoma (NHL) have a dismal prognosis. Very few studies focused on outcomes of these pts, and the optimal therapy for them is unknown. Doxorubicin-containing regimens are very effective in aggressive NHL, but anthracycline-induced cardiotoxicity limits their use in the treatment of relapsed/refractory pts. Because of the significantly less cardiotoxicity of non-pegylated liposomal doxorubicin, we conducted a phase II pilot study of non-pegylated liposomal doxorubicin (Myocet) in combination with carboplatin, aracytin and metilprednisolon (MYCAP) in elderly pts affected by relapsed/refractory NHL who were pretreated with doxorubicin-containing regimens. To prevent opportunistic infections and febrile neutropenia (FN), prophylactic therapy with trimeto-

prim-sulfametaxazole, acyclovir, nistatine and peg-G-CSF have been applied. Ten pts affected by DLBCL (4), MCL (3), FL III° (1), peripheral T cell NHL (1), Anaplastic CD30+Alk- NHL (1), have been enrolled from January 2006 to December 2007. All pts subscribed informed consent. Seven pts were male and 3 female, with a median age of 70 years (60-79). Six pts were in relapse and 4 pts were refractory at study entry. Six pts have been pretreated with Rituximab (R), and 3 pts received 2 prior treatment lines. MYCAP (non-pegylated liposomal doxorubicin 50 mg/m2 day 1, carboplatin 100 mg/m^2 days 1-4, metilprednisolon 500 mg/m^2 days 1-4, and aracytin 2000 mg/m^2 day 5, plus R 375 mg/m^2 day 1 in the 2 R-naive pts) was planned every 3 weeks for 4-6 courses on an outpatient basis. After a median number of 4 courses of MYCAP, there was no treatment-related mortality. Grade 3 and 4 toxicities were mainly hematologic. Overall, we recorded 1 event of FN and 1 Herpes Zoster Virus infection. No significant decreases in ejection fraction greater than 6% occurred. No additional toxicities were found by adding R to chemotherapy. Overall response rate (ORR) was 50% with 40% complete responses (CR). After a median follow-up of 18 months, overall survival (OS) was 30%, with 3 pts alive and 2 in CR. Considering only the B cell NHL, ORR was 63%, CR 50% and OS 37%. In particular, CR rate was 60% in the subgroup of DLBCL/FL pts, with a median duration of response of 20 months (3-24). MYCAP regimen seems ineffective in refractory T cell NHL. Instead, an encouraging efficacy profile was observed in B cell NHL, especially in DLBCL and FL. We obtained CR with MYCAP regimen both with (2 pts) and without R (2 pts). However, by adding the R in all B cell NHL pts we could probably get better results even in pts who had previously received this drug. In conclusion, we demonstrated that non-pegylated liposomal doxorubicin in combination with other anticancer drugs has a manageable safety profile and a fair efficacy in anthracycline-pretreated elderly pts. In particular, no significant signs of cardiotoxicity were recorded. On the basis of our experience, we think that further studies exploring the impact of non-pegylated liposomal doxorubicin, in combination with new active agents, are advisable in elderly pts with aggressive NHL.

PU058

FB-R (FLUDARABINE, BORTEZOMIB, RITUXIMAB) IN MULTI-RELAPSED INDOLENT NON HODGKIN LYMPHOMAS IN ELDERLY: A SINGLE CENTER EXPERIENCE

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Despite remaining an incurable disease, overall survival improvements have been noted in patients with advanced-stage follicular lymphoma. The combination of fludarabine, cyclophosphamide, and rituximab (FC-R) shows significant synergism and may improve patient outcome, so that in same studies, in patients with follicular lymphoma, the respective OR and CR rates were 100% and 80% when used as first line therapy, and 80% and 60% as salvage therapy. Unfortunately many patients show multiple relapses and the response rate is reduced progressively. Moreover some patients show clinical conditions that prevent use of conventional drugs in advantage stage of disease. Proteasome inhibitors have demonstrated clinical potential as novel therapies for non-Hodgkin lymphoma (NHL). Bortezomib, a peptide that inhibits the proteasome by binding directly to its active sites, is the most extensively studied agent in the clinical setting. Single-agent bortezomib is effective in several lymphoid malignancies, and is recommended for second-line treatment of mantle-cell lymphoma (MCL). We have treated six patients with advanced (stage III and IV) indolent (grade I or II) non Hodgkin lymphomas (two patients with follicular grade I and II, one patient with WM, three patients with "small lymphocytic lymphoma"): the median age of the patients was 72 years, 2 male and 4 female. All patients had received a median number of 3 prior therapies (CVP, FC, FC-R, R-CHOP, oral alkilants, etc). All patients completed therapy after a median of 4 cycles of FB-R (fludarabine at a dose of 25 mg/m^2 intravenously on days 1-3, bortezomib at a dose of 1 mg/m^2 i.v. on days 1, 4, 8, 11, and rituximab at a dose of 375 mg/m^2 on Day 1. The overall response rate was 66%, (2 patients with complete remission and 2 patients with partial remission, 2 patients with stable disease) and at a median of 9 months follow-up, the median time to progression had not yet. Grade 3 toxicities included thrombocytopenia (1 patient), neutropenia (1 patient), and peripheral neuropathy (1 patient). Future clinical trials are needed to determine the clinical effectiveness of bortezomib plus chemo/immunotherapy in refractory NHL, particularly in terms of overall survival. However, for patients with limited treatment options, FB-R may provide an alternative

PU059

SUCCESSFUL TREATMENT OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA ASSOCIATED TO ESSENTIAL THROMBOCYTHEMIA AND CHRONIC RENAL FAILURE WITH NON-PEGYLATED LIPOSOMAL DOXORUBICIN-CONTAINING REGIMEN IN A PATIENT AFFECTED BY SEVERE STENOSIS OF RIGHT CORONARIC ARTERY.

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We report what we believe is the first case of angioimmunoblastic lymphoma (AITL) combined with simultaneous essential thrombocythemia (ET), chronic renal failure (CRF) and ischemic cardiopathy. A 45-year-old woman presented with history of fever and progressive multiple cervical lymph node enlargements. There was also a long history of CRF recently treated with peritoneal dialysis; moreover a previous diagnosis of ET requiring only platelet antiaggregants was made in 2001. On examination she had multiple large lymph nodal swellings in the cervical and supraclavear region. Histologic examination of cervical node biopsy detected an AITL. Whole body CT scan revealed cervical, axillar, mediastinal, abdominal and inguinal nodal enlargements. Bone marrow (BM) biopsy showed lymphoma infiltration in the context of a myeloproliferative neoplasm. JAK2 mutation and BCR/ABL rearrangement were absent. No abnormalities were detected at karyotype analysis. Hematologic values were: Hct 32%; Hb 10.5 g/dL; RBC 3.53 x10^6/microL; WBC 14.7x10^3/microL, platelets 593x10^3/microL; CD34 cells 2.306/mL; ESR 108; LDH 349 U/L; serum creatinine 5.54 mg/dL; azotemy 87 mg/dL. Finally, an IgG κ-chain monoclonal gammapaty and an occult HBV carrier status were found. On the basis of these findings, the patient was allocated in Ann Arbor stage IVB. Before starting lymphoma treatment, the patient suffered from a double episode of acute coronary syndrome due to the occlusion of stenotic right coronaric artery. For this reason, the patient received percutaneous transluminal coronary angioplasty with stent placement. Because of this serious cardiopathy, a non-pegylated liposomal doxorubicin-containing regimen was selected for lymphoma treatment (COMP: cyclophosphamide, vincristine, non-pegylated liposomal doxorubicin and prednisone). COMP regimen was administered every 21 days for 8 courses in an outpatient basis. Because of the frailty of the patient and in order to prevent febrile neutropenia and thromboembolic events, the patient was co-treated with pegylated G-CSF and clopidogrel plus ASA. Just prior to the start of chemotherapy, cardiac ejection fraction was 49%. During chemotherapy administration platelet counts ranged from 220 to 774 x10(e)3/microL. Planned treatment was completed without grade 3-4 toxicities. At restaging, (18)FDG-PET/CT revealed complete regression of nodal enlargements. BM biopsy showed the disappearance of lymphoma infiltration and the persistence of myeloproliferative neoplasm. After treatment conclusion, cardiac ejection fraction was 65% and 67% in two consecutive evaluations. The coexistence of myeloproliferative and lymphoproliferative neoplasms is detected more frequently after cytotoxic drug or radiation exposure, while the simultaneous diagnosis of the 2 neoplasms is certainly rare. Actually it is unknown whether common pathogenetic pathways are involved in both neoplastic processes. Probably the myeloid and lymphoid clone independently occurred. We believe that this case is very interesting because of its clinical complexity due to the coexistence of serious comorbidity and to the frailty of our young patient. Our treatment choice was addressed by the prevailing clinical aggressiveness of AITL and the presence of severe cardiopathy. With regard to this latter aspect, we conclude that non-pegylated liposomal doxorubicin-containing regimen was highly active in treating AITL without cardiac toxicity in our patient with concomitant severe cardiac disorder.

PU060

THE LYMPHOMATOID PAPULOSIS: A CONTROVERSIAL DISEASE; BENIGN DISORDER OR LYMPHOMA? A CASE REPORT AND REVIEW OF LICTERATURE

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Introduction: Lymphomatoid papulosis (LyP) is a lymphoproliferative disease characterized by recurrent papules, nodules or plaque, defined in the WHO classification as a $CD30^+$ cutaneous lymphoproliferative lymphoma (CLPD) and regarded as a condition of uncertain malignant potential. Despite having a histologically malignant-appearing infiltrate, patients with LyP have a clinically benign course. It is correlated with malignant lymphomas in 5-20% of case. The aberrant cell is now generally accepted to be an active T helper phenotype. The expression of Ki-1 (CD30) on a significant portion of the infiltrating cells characterizes LyP relates this disorder with Hodgkin's disease, mycosis fungoides and anaplastic T cell lymphoma. The categorization of this disease as a benign disorder versus lymphoma remains controversial. Studies of T cell receptor gene rearrangement demonstrate clonality in many cases. Recents opinions consider that LyP and Ki-1 (CD30) lymphomas are different parts of a clinical and histological spectrum constituted by cutaneous Ki-1 lymphoid infiltrates. *Case report*: We describe the case of a man 37 years old who had for many years a widespread eruption of the limbs, the trunk and buttocks injuries papules-nodularity interpreted as chronic folliculitis and wrongly treated with oral tetracycline and isotreonina. At our hospital he was subjected to biopsy of the skin of the region left of the elbow and examination showed histological infiltrate of lymphocytes and hystiocites organized to V in the dermis, with accentuation around a hair follicle, with the presence in perivascular/interstitial of numerous large and atypical cells, clearly positive for CD 30 and MUM -1 and showed a high proliferation. The picture placed on a histological LyP. Both clinical and radiological examination, the patient had no hepatosplenomegaly and not lymphoadenomegaly. The patient was treated with oral methotrexate, at dose of 5 mg weekly, and has obtained a partial remission greater than 75% of skin lesions. *Discussion*: LyP is a self-healing eruption in the spectrum of $CD30^+$ lymphoproliferative disorders. From the point of view is different histological type A, which is reminiscent of Hodgkin's disease, a type B reminiscent of mycosis fungoides and a C-type reminiscent of the lymphoma to anaplastic large cell $CD30^+$. Non cutaneous CD30-positive anaplastic large cell lymphoma (ALCL) seems to be a biologically distinct entity. ALCL represents a generally recognized group of large cell lymphomas. With the use of molecular and clinical criteria, three entities of ALCL have been identified: primary systemic anaplastic lymphoma kinase (ALK)(+) ALCL, primary systemic ALK^- ALCL, and primary cutaneous ALCL. The knowledge of the existence of these variants is essential in establishing a correct diagnosis. The morphology and the immunophenotype of primary cutaneous ALCL show an overlap with that of LyP. At present there are not treatments that can change the natural history of the disease. The drugs used in controlling short-term symptoms include systemic steroids, the PUVA therapy and oral methotrexate in low doses weekly. Conclusion: Long-term surveillance is essential in all cases of LyP as accurate predictors for the development of malignant lymphoma in these individuals are still lacking. A multidisciplinary approach between dermatologist, oncologist and pathologist is essential for the optimal management of these complex conditions.

PU061

PRIMARY BRONCHIAL-ASSOCIATED LYMPHOID TISSUE LYMPHOMA: A DOUBLE CENTER EXPERIENCE

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Primary bronchial-associated lymphoid tissue lymphoma (BALToma) is a rare extranodal lymphoma arising from mucosa associated lymphoid tissue (MALT) of the bronchus. BALToma represents roughly 3% of extranodal lymphomas and 0.4% of all lymphomas non-Hodgkin (NHL). Unlike some other extranodal marginal zone lymphomas, its pathogenesis seems not closely related to chronic infection. Clinically, BALToma tend to have an indolent course, with localized disease (stage IE-IIE) in the 60-70% of patients. At the time of diagnosis, patients are asymptomatic or have pulmonary symptoms with or without systemic symptoms. Because of their favourable clinical course, BALToma were generally managed conservatively in the past, with either observation or limited surgical resection or low toxicity chemotherapy. Moreover, successful treatment with anti-CD20 monoclonal antibodies and radioimmunotherapy has been reported more recently. We retrospectively evaluated clinical characteristics and outcome of 9 patients with BALToma diagnosed between January 1996 and December 2008. The diagnosis of suspected pulmonary neoplasm was made with chest X-ray and computed tomography (CT) scan. All patients have undergone lung biopsy for histological diagnosis. Overall, one patient was male and 8 female, with a median age of 68 years (range 24-73). At the time of diagnosis 2 patients had stage IE, 1 patient IIE and 6 patients had stage IV. Pulmonary lesions were single in 3 patients and multiple bilateral in 6 patients. Extrapulmonary localizations were recorded in 4 patients, including local or disseminated limphoadenopathy in 3 and bone marrow involvement in 1. Two patients had B symptoms and 1 had bulky mass. FLIPI was 1 in 4 cases, 2 in 4 cases and 3 in 1. Occult hepatitis B virus infection was recorded in one patient and chronic HCV infection in 2. Finally, 3 patients showed IgM monoclonal gammopathy and 1 double monoclonal gammopathy (IgG and IgM). Treatment approach varied widely according to the period of observation of each patient, the extent of disease and the individual needs of patients. A watch and wait approach was used in 2 patients. All 7 remaining patients received mono- or polychemotherapy regimens (clorambucil, CHOP, CVP) in association to rituximab in 5 cases. After first-line treatment, overall response rate was 43% (1 complete response and 2 partial responses), while 4 patients showed stable disease. After second-line treatment with clorambucil (1), R-CHOP (2) and fludarabine based regimen (2), of the 5 patients who received it, 1 obtained a complete response, 3 a partial response and 1 remained with stable disease. Today, after a median follow-up of 25 months (range 6-144), 8 patients are alive, while 1 died of secondary cancer. Our results seem to show that CHOP or CVP combinations with or without Rituximab are not useful when compared to rituximab alone or combined with clorambucil. In effect, our assumption is confirmed by recent data showing that rituximab and 90Y ibritumomab tiuxetan are safe and effective in patients with extranodal marginal-zone lymphoma. Nevertheless, optimal management of BALToma is still not established. Given the indolent behavior of BALToma and the lack of prospective clinical trials, the treatment choice should be tailored for each patient, considering clinical presentation and the expectancy of the patient.

PU062

LOW DOSE INVOLVED FIELD RADIOTHERAPY FOR RECURRENT AND/OR CHEMOTHERAPHY REFRACTORY NHL PATIENTS

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Introduction. Despite the improvement of the NHL patients prognosis, a substantial rate of them (20-30%) showed chemorefractory disease and were undergone to palliative approach. Several authors reported clinical responses after low dose involved field radiotherapy (LDIFRT) in these patients. We report three cases of recurrent and chemotherapy refractory NHL patients who present clinical response after LDIFRT. *Materials and methods*. We treated 3 patients from January 2008 to May 2008. All patients were female with median age 79 years (range 77-81 years.) They have had diagnosis of high grade NHL and were considered having chemorefractory disease after 2-3 types of chemotherapy regimens. All patients showed a bulky disease with clinical symptoms and they received Involved Field (GTV plus 1-2 cm) radiotherapy with a dose of 2 Gy/day to alternate days (total dose 4 Gy). One patient received RT on 3 disease sites (parasternal, breast, suvraclavear limphnodes), one patients was treated on inferior left abdominal region and one patient on right inguinal region. All patients were at the same time treated with steroids plus low-dose oral cyclophosphamide. Response was clinically and radiologically evaluated. Results. We recorded a clinical response (2 CR and 1 PR) and a clinical benefit in all patients at a median time of 14 days from the beginning of radiotherapy. One patient was undergone to re-treatment with standard fractionation RT course for in-field progression after

2 months from response. All patients died for progressive disease at a mean time of 7 months (4-10 months) from RT. Conclusions. LDIFRT seems to be an effective treatment in the palliative management of recurrent and chemotherapy refractory high grade NHL patients.

PU063

LYMPHOPROLIPHERATIVE MALIGNANCIES IN THE ABRUZZO. FIRST REPORT FROM THE GRUPPO ABRUZZESE LINFOMI

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The Gruppo Abruzzese Linfomi (GAL) was founded in February 25, 2009, with the aim of making uniform the diagnostic and therapeutic approaches to Hodgkin and non-Hodgkin lymphomas and conducting epidemiologic studies in the Abruzzo. Obviously, the first step consisted of the launching of an epidemiological registry of lymphoproliferative malignancies in non-pediatric population, starting retrospectively from January 2008. Data were collected from the hematology units of Pescara, L'Aquila, Sulmona, Avezzano, Popoli, Teramo and Vasto. Between January 1, 2008 and December 31, 2008, 240 new cases of lymphoproliferative neoplasms were recorded. Overall, 126 patients were male and 114 female (M/F ratio: 1,105). Median age was 67 years (range 14-89) and 147 patients (61%) are aged 60 years or older. As regards histologic type, 47 were Hodgkin Lymphoma (HL), 40 were Chronic Leukemias (Chronic Lymphocytic Leukemia, Hairy Cell Leukemia and Large Granular Lymphocytic Leukemia) and 153 were non-Hodgkin Lymphomas (NHL). In particular, 61 patients (32%) were diagnosed as having aggressive NHL and 132 (68%) as having indolent NHL. T-cell lymphomas were very rare, representing only 3% of all NHL. Diffuse Large B Cell Lymphoma (DLBCL) (27%), Follicular Lymphoma (22%) and Marginal Zone Lymphoma (18%), including both nodal and extranodal forms were the most common reported histologies. Extranodal lymphomas accounted for 17% of all cases, including 8 patients with gastric Malt lymphoma, 2 with Balt lymphoma, 4 with gastric DLBCL, 3 with primary CNS-DLBCL and 4 with Cutaneous B-cell Lymphomas. Analyzing the living environments of all patients, 110 of them came from rural areas, while 130 from urban or suburban areas. Considering the places of residence, the 2008 incidence rates of lymphoproliferative malignancies were higher in the provinces of Pescara, Chieti and L'Aquila (19/100.000, 18/100.000, 24/100.000, respectively) than in the province of Teramo (10/100000). Overall, given a population of 1.332.536 people in the Abruzzo, our registry show an annual incidence rate of 3.5/100.000 cases for HL and 14.5/100.000 cases for NHL. Analyzing these data, we are surprised by an apparently low incidence rate of NHL in our Region. Most likely, some of these patients are treated by oncology groups and, especially elderly patients, are not addressed in hematology units. Thereby, investigations involving departments of oncology and internal medicine are advisable.

PU064

SAFETY OF LIPOSOMAL CYTARABINE (DEPOCYTE®) IN THE CENTRAL NERVOUS SYSTEM (CNS) PROPHYLAXIS OF PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA (NHL) AND ACUTE LYMPHOBLASTIC LEUKAEMIA: RESULTS OF A SINGLE-CENTER EXPERIENCE

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CNS involvement in acute lymphoblastic leukaemia is a well-recognized risk and CNS prophylaxis is considered mandatory. In contrast, the overall risk of central nervous system (CNS) relapse in aggressive non-Hodgkin lymphomas (NHL) is approximately 5%. Several large retrospective studies would suggest that prophylaxis of CNS relapse in aggressive NHL should be performed in patients with involvement of specific extranodal sites or in patients presenting with a high-intermediate/high IPI score. Flow cytometry (FCM) may provide a method to diagnose CNS involvement early it remains unclear as to whether a negative FCM result in a patient with clear clinical risk factors should receive prophylaxis or if a positive FCM result should be treated with CNS directed therapy at all. It seems therefore reasonable to investigate the safety and efficacy of sustained-release liposomal cytarabine (DepoCyte®). Intrathecal (IT) liposomal cytarabine is distributed widely throughout the CSF and has an extended half-life allowing an administration once every 2–4 weeks. Concerns have recently been expressed on an increased risk of severe neurotoxicity in patients with ALL receiving intrathecal liposomal cytarabine with the Hyper-CVAD regimen. The findings of these studies suggest that liposomal cytarabine should not be given prior to or during treatment with high-dose systemic cytarabine.Since February 2007 we have evaluated the safety and efficacy of liposomal cytarabine in CNS prophylaxis in 8 consecutive patients mostly > 70 years of age with aggressive NHL and ALL. PZ1: DLBCL in stage IA, with IPI 1 and testicular involvement, Karnofsky 75, systemic treatment with R-COMP 21 and RT on controlateral testicular. PZ2: DLBCL in stage IVA with IPI 2 plus paranasal sinus bone and bone marrow involvement; HBV positive; Karnofsky 75, systemic treatment four cycles of R-COMP 21 in a 50% reduced dose because of age + 2 rituximab 375ng/m2 for maintenance. PZ3: DLBCL in stage III E; IPI 2; Karnofsky70; with tonsil and bone marrow involvement, received six cycles R-COM-21 in a 50% reduced dose for age and co-morbidity (without steroid because of diabetes). PZ4: DLBCL in stage IVA; IPI 3; Karnofsky 80; received fourth line treatment with two cycles bendamustine day 1,2 and bortezomib day 1,8,15,22 and rituximab day 1 every 28 days. PZ5: MCL stage IIIA; IPI 1; Karnofsky 60; received six cycles R-COMP 21 with a 25% reduced anthracyline dose because of decreased of the ejection fraction) achieved RC. After five months the patient relapsed with lymphonodes in the neck, abdomen and involvement gland tonsils, his IPI was 1 and Karnofsky was 100; he received five cycles R-GIFOX. PZ6: DLBCL in stage IVA and bone marrow involvement; IPI 1; Karnosky 100; received six cycles R-COMP 21. PZ7: ALL- B; Karnofsky 50; received first-line treatment GMALL 01/81 (Hoelzer 1984–88) in reduced doses for toxicity. PZ8: ALL-B, L2 (BCR-ABL, TEL-AML1, E2A-PBX1, MLL-AF4 negative); Karnofsky 100; received induction ALL Gimema 0904 Daunoblastina, vincristina, prendisolone escalation doses and asparaginasi (3700 UI), two cycles of consolidation therapy with four doses Ara-C 2gr/mq (total dose 3 gr) every 12 h and day 1,2,3 VP150mg/mq (total dose 225 mg); then the patient has undergone allogeneic bone marrow transplantation from related donor. All patients received CNS prophylaxis with IT liposomal cytarabine 50 mg followed by systemic injection of steroids for preventing arachnoiditis. Flow cytometry and cytospin analysis was performed in all patients on CSF samples obtained at every lumbar puncture. The intrathecal injections were given the day before systemic chemotherapy in NHL cases for a total of 4 administrations. Prophylaxis in ALL was given every 3 weeks during induction for a total of 4 doses. *Results*. Seven patients (4 DBLCL, 1 MCL, 2 ALL) achieved a complete response and only one a partial response. At a median follow-up of fourteen months, seven patients were alive and five in continuous CR, one in PR and only one presented a relapse of systemic disease. One patient died in CR because of a cardiac arrest. Isolated relapse of leukaemia or lymphoma in the CNS was not seen. *Conclusions*. Liposomal cytarabine is safe in the prophylaxis of CNS relapse in patients with aggressive NHL or ALL. No drug-related neurological or hematological toxicities were recorded. Liposomal cytarabine could be the drug of choice for CNS prophylaxis, particularly in elderly patients, and should be further investigated in clinical trials.

PU065

PRIMARY LIVER LYMPHOMA: A CASE REPORT WITH REVIEW OF LITERATURE

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Primary non-Hodgkin's lymphoma of the liver is exceedingly rare; until now only 70 cases have been described in the past 40 years in the literature and only 16 patients of them have been treated surgically so far. We report a patient with primary large B-cell non-Hodgkin's lymphoma of the liver who was treated by systemic chemotherapy (R-COMP protocol). *Case report*. A 76 year-old white man was referred for evaluation of an epigastric mass, for lethargy, anorexia, and a 6.5 kg weight loss. The patient denied experiencing fevers, chills, night sweats, nausea or vomiting. The physical examination revealed hepatomegaly with large pal-

pable, smooth, nontender mass in the right hypochondrium. The spleen was not palpable, and there were no other abdominal masses or lymphadenopathy. The remainder of the examination was normal. Laboratory studies showed the following: serum glutamic oxaloacetic transaminase (SGOT) 3O IU/L, lactic dehydrogenase 2104 IU/L, bilirubin direct 0.25 mg/dL, bilirubin indirect 1.13 mg/dL, albumin 3.5 g/dL, prothrombin time 70%, α-fetoprotein 3.8 ng/ dL, and carcinoembryonic antigen 1.7 ug/L, Ferritin 437 ng/ml , β 2 microglobulin 4353 ng/ml. The other laboratory results were normal. A computed tomography (CT) scan of the abdomen disclosed a large mass, 12 cm, replacing the right lobe of the liver with no other abdominal masses or adenopathy. The mass did not extend into the vena cava or the portal vein . A chest CT scan was normal. A Positron Emission Tomography showed the hypercaptation of hepatic lesion, SUV max 16,1. Liver biopsy was performed and the histological diagnosis was large B-cell (CD-20+, LCA +, MUM-1 +, BCL2 +). Tests for CD-34, carcinoembryonic antigen , cytokeratin 7, α-fetoprotein, neurospecific actin, and enolase were negative. After the final pathologic diagnosis, bone marrow and cerebral spinal fluid were examined and found to be free of disease. Because of the age of the patient and for his refusal for surgery and for coexistence of cirrhosis, a reduced systemic chemotherapy with R-COMP protocol (Liposomal doxorubicin-Myocet®, cyclophosphamide , vincristine, prednisone and rituximab) was performed. The patient at today received three courses with partial remission (reduction of the mass to 10 cm). *Discussion*. The first report of primary hepatic lymphoma was by Ata and Kamal in 1965. Primary hepatic lymphoma has been reported in about 70 patients and occurs in a wide age range (7 to 84 years) mainly in male patients; it is an aggressive tumors, notably in those with pre-existing chronic infective hepatitis The predominant histological description of these lesions includes B-cell lymphomas of the so-called histiocytic type or the larged differentiated cell. Microscopically, the liver was composed of an infiltrative diffuse lymphoreticular neoplasia with a uniform population of lymphoid cells of large size with many mitotic figures immunostained positively for leukocyte common antigen and for B-cell markers including CD 20, CD45, RO marked lymphocytes. The primary treatment of primary hepatic lymphoma is the surgery followed by chemotherapy. This diagnosis should not be overlooked in those patients presenting with hepatic disease or mass lesions in the liver.

PU066

R-COMP 21 FOR PATIENTS WITH GASTROINTESTINAL AGGRESSIVE B-CELL NON-HODGKIN LYMPHOMA

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R-CHOP is the established treatment for aggressive diffuse large B-cell non-Hodgkin lymphoma (DLBCL). Although no trial has been specifically designed for DLBCL affecting the gastrointestinal tract, R-CHOP is also extensively used in this setting. The non-pegylated liposomal doxorubicin Myocet (Cephalon, USA) has been shown to be effective and to significantly reduce the cardiac and gastrointestinal toxicity in metastatic breast cancer and aggressive non-Hodgkin lymphoma patients. Pharmacokinetics studies have reported an interesting different biodistribution of the drug, with preferential distribution in the lymphoid tissues (liver, spleen, bone marrow, lymphatics) compared with conventional doxorubicin, making Myocet an attractive option for lymphoid malignancies. Here we report the results of R-COMP 21 (Myocet 50 mg/m^2, cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2, rituximab 375 mg/m^2 on day 1, prednisone 100 mg orally on day 1-5) in 5 patients with gastrointestinal DLBCL (Table 1).

Table 1. Patients characteristics.

	NHL site	*Age*	*Stage*	*IPI*	*PS*	*Follow-up (months)*
Patient 1	Gastric	38	IE	0	0	4
Patient 2	Gastric	83	IE	1	0	22
Patient 3	Gastric	65	IV	3	1	25
Patient 4	Small bowel	79	IV	3	0	16
Patient 5	Small bowel	75	IV	5	3	11

IPI: International Prognostic Index; PS: Performance Status

Chemotherapy has been administered every 21 days for 6 cycles. Three patients had primitive gastric DLBCL, 2 patients had primitive small bowel DLBCL. Mean age was 68 years (range 38-83). Two patients were in stage IE, three patients in stage IV. IPI scores were 0,1,3,3,5. All patients achieved a complete remission and all patients are alive and disease-free after a median follow-up of 16 months (range 4-25). Grade 3 neutropenia was observed only in one case. No febrile neutropenia and no case of heart failure were observed. In conclusion R-COMP has proven to be feasible, effective and with a very limited toxicity in this small series of patients with gastrointestinal aggressive non-Hodgkin lymphoma.

PU067

GREY ZONE LYMPHOMA: CASE REPORT

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A 64-year old man was admitted to our Institution after a 3 months history of irregular fever, sweating, pruritus, bilateral cervical lymph nodes enlargement, weight loss and a recent episode of syncope in the absence of parenchymal alterations at cerebral CT scan, that, however, disclosed a right parapharyngeal obliteration as an occasional finding. Cervical echography confirmed the presence of bilateral adenopaties with an average diameter of 3.5 cm. Examination of the oral cavity confirmed a rhinofaringeal mass which was biopsied. Microscopic analysis showed a sub-epithelial heterogeneous infiltrate made of small atypical lymphocytes, plasma cells, eosinophils and large atypical mononucleated and polynucleated lymphoid elements morphologically suggestive of Hodgkin's (H) and Reed-Stemberg's (R-S) cells, respectively. Importantly, area of relative lymphocyte depletion with predominance of anaplastic H and R-S like cells were observed. Immunohistochemical characterization showed that the large cell population expressed CD20, CD30 and CD15 whereas LCA and LMP1 antigens were absent. Because of substantial difficulties in the differential diagnosis between Hodgkin and large cell non-Hodgkin lymphoma, a further immunohistochemical characterization was performed documenting that the large anaplastic cell population expressed CD79a, IRF4, BOB1+ and OCT2 in the absence of BCL6. These findings are strongly in favour of the diagnosis of unclassificable lymphoma with intermediate characteristics between diffuse large B cells lymphoma and classic Hodgkin disease. Total body CT and PET scan revealed small mediastinal lymphadenopaties. A bilateral bone marrow biopsy did not show atypical lymphoid infiltrates leading to the definition of stage IIb lymphoma. The IPI scored 1. We decided to treat the patient with two courses of ABVD in association with Rituximab followed by short term PET scan evaluation to ascertain whether the treatment option could be switched to R-CHOP or other active regimens for aggressive NHL.

PU068

VERY LATE EXTRANODAL RELAPSE OF HIGH GRADE NON HODGKIN LYMPHOMA. HOW LONG MUST WE CONTINUE TO OBSERVE?

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Diffuse large cell lymphomas (DLCL) are the most common non Hodgkin aggressive lymphomas. Most investigators have shown that practically all patient who are able to achieve and maintain complete remission for about 24 months are cured, because late relapses seldom occur after this period of follow up. However relapses can occur later after remission and the characteristics of patients who relapse have rarely been studied especially for localized disease. We present a case of a very late relapse in a patient with a previous diagnosis of DLCL after nine years of complete remission. In march 2009, a 58 years old man was admitted to our hospital presenting right lateral down abdomen zone and right light pain. He had a previous history of splenic DLCL made in 2000 and treated with splenectomy and MACOP-B schedule, achieving CR in September 2000. He underwent periodical controls for lym-

phomas. Total body CT scan, made 12 months before our observation, was negative. He referred fever and mild weight loss. At the admission a total body CT scan showed a mass of 7 centimetres of diameter interesting the right iliac wing and infiltrating the surronding muscles. A CT scan guided biopsy was performed and a diagnoses of DLC was made. Bone marrow biopsy was normal. The patient was staged as IVEB and RCHOP schedule CT has been started. For this patient, the program is a schedule of 6 RCHOP and HDCT plus PBSC and then RT. Although the majority of patient with high grade lymphomas achieve CR, many of them eventually will relapse. It is known that late relapsing patient have a better prognosis than those in early relapse. However the choice of therapy remain uncertain. Several reports show that patients with a very late relapse, defined as occurring in the fifth year or later from the time of first diagnosis following a period of CCR have a poorer prognosis that justifies the use of more aggressive treatment approach such as HDCT and PBSCT, rather than conventional chemotherapy regimens. The role of novel therapeutic approaches with the addition of rituximab to chemotherapy with or without radiotherapy in preventing other relapses is awaited. But, if a patient is considered cured after five years of CCR, while we observe a rate of relapse, to whom must we continue the controls? Have we indicators to select patients with aggressive lymphomas who are at risk of relapse after five years of CCR?

PU069

AUTOLOGOUS BONE MARROW TRANSPLANTATION WITH PURGING *IN VIVO* IN BURKITT AND BURKITT LIKE LYMPHOMA

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Burkitt's and Burkitt like lymphoma is a highly aggressive lymphoma. In recent years, efforts have focused on improving therapy for this rapidly proliferating neoplasm while minimizing, to the extent possible, treatment-associated toxicity. These efforts have led to the development of high-intensity, short-duration combination chemotherapy that has proven extremely effective for a high proportion of these lymphoma patients. From 2003 in our division have been treated, the patients with these lymphomas, with aggressive treatments, rituximab and auto-transplant with purging *in vivo* From April 2003 to December 2008 we have treated with Autologous stem cell transplantation, purged *in vivo* with monoclonal antibodies, 7 patients (1 F; 6 M median age: 37 years) with Burkitt (5 patients) and Burkitt like (2 patients) lymphoma. In all patients treatment has been effected according to the scheme R-CODOX-M for 2 cycles alternated to R-IVAC for 2 cycles with intratecal medication with ARA-C and MTX. After the last cycle of R-IVAC the patients have been mobilizzed and harvest the PBSC.

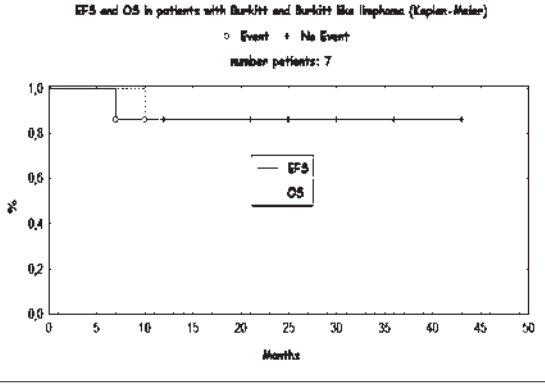

Figure 1.

To the transplantation all patients were in CR. All patients have harvest (median CD34: $4x10^6$/Kg) and minimal residual disease in the harvest has been not detectable to immunophenotype. All the patients have been conditioned with BEAM-R ((with infusion of rituximab to the day +1) and the graft are documented in 7/7 patients (any complication before and after treatment)) with recovery of neutrophils> 1000 in media to day + 11 (range 8-12 days). After transplantation all patients were in CR. With a median follow-up of 40 months after transplantation (range 16-56 months) 6/7 (86%) patients are in CR (1 patients have relapsed with burkitt lymphoma (is relapsed extra-nodular at months +8 and died for disease a months + 10 after transplantation). The EFS and OS projected at 56 months are of the 86% (Graph 1). In conclusion the use of the rituximab and autologous transplantation in this cohort of patients has allowed obtaining very good results with minimal toxicity. Occurred a more number of patients for to confirm such data

PU070

ONLY IMMUNOTHERAPY TREATMENT IN AUTO-TRANSPLANTED RELAPSED FOLLICULAR LYMPHOMA

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In the last years the follicular lymphoma has benefited of transplantation procedures. The problem is the management of the relapse of the disease in post-transplant, were as patients highly treated. From January 2005 we have auto-transplanted, in our Division, 10 follicular lymphomas. 6/10 patients have relapsed by a median PFS of 18 months (range 9-36).All patients received close follow-up with CT and PET and were treated early. The 6 patients relapsed were treated with 4 weekly doses of rituximab 375 mg / sqm for 1 month and then re-evaluated, if CR have started maintenance with rituximab 375 mg / m every 2 months for 2 years. All patients revalued after 4 weekly doses have documented the CR and began rituximab maintenance. With a median follow-up of 20 months all patients are in CR and were not documented complications related to treatment. In conclusion for patients with follicular lymphoma is a close follow-up to document the early relapse of the disease because in our experience for patients relapsed, after the autologous transplantation, the only immunotherapy may be sufficient to obtain a new CR consolidated by maintenance cycles every 2 months. We need a larger cohort and a follow-up longer to confirm these data.

PU071

EFFICAY OF RITUXIMAB, AS SINGLE-AGENT TREATMENT, IN PRIMARY CUTANEOUS INDOLENT B-CELL LYMPHOMA

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Rituximab (anti-CD20 chimeric monoclonal antibody) has been demonstrated to have significant activity in nodal B-cell lymphomas, without significant associated adverse effects. Although primary cutaneous B-cell lymphomas (PCBCL) express CD20 antigen, very few groups reported the use of rituximab in PCBCL. Here we describe the efficacy of rituximab in three patients with primary indolent cutaneous B-cell lymphoma. The patients, with multiple (or single?) skin localizations, have been treated with the following schedule as only therapeutic strategy: 375 mg/m^2/week intravenously for 4 weeks. All of them obtained a fast, complete and long-lasting response. No adverse effects were observed. We conclude that rituximab may represent an effective therapeutic option also for PCBCL.

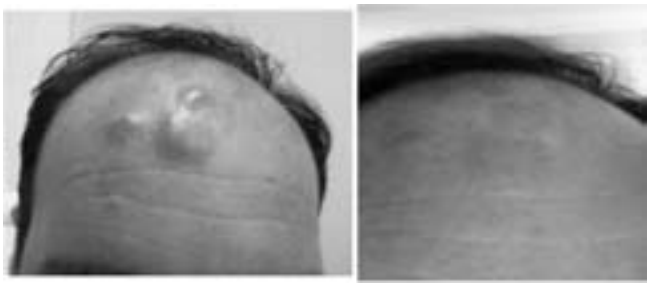

Figure.

PU072

RITUXIMAB CHEMOTHERAPY IN GASTRIC B LYMPHOMA PATIENTS NO UNDERWENT TO SURGERY.

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Background. The treatment of choice for non Hodgkin lymphomas is combination of Rituximab plus chemotherapy (Coiffier et al. NEJM, 2002). The role of surgery in the management of gastric B lymphoma is controversial and it is not yet known if Rituximab combined with chemotherapy, in gastric B lymphomas, improves the activity and efficacy of chemotherapy alone in patients no underwent to gastric surgery. The aim of this retrospective clinical study is the evaluation of the therapy with Rituximab-chemotherapy in gastric B lymphoma patients no underwent to surgery. *Methods*. 40 patients (27 M, 13 F, median age: 58 years. Range age 19-76 years) with an histological confirmed diagnosis of B gastric non Hodgkin lymphomas, treated at our Institutions between 1985 and 2008, were selected by stage (IE-IVE, Ann Arbor classification), ECOG performance status= 0-2; histological features (high grade B-cell: 33 pts; low grade MALT-like Lymphoma:7 pts) and treatment strategies: Chemotherapy alone (Group A) (schedules as CHOP and CHOP-like); Chemotherapy combined to Rituximab (Group B). Primary objectives of study were: response rate (complete and partial response), disease free survival (DFS) at 5 years and overall survival (OS) at 5 years. *Results*. Median follow-up was 77 months. In Group A (19 pts) a complete remission rate of 78,9 %, DFS at 5 years of 73,6%; while in Group B (21 pts) a complete remission rate of 100,0 % and DFS at 5 years of 100% we have observed, respectively. At to date, 14 patients are alive in group A with 5 died (4 causes lymphoma-related; 1 to brain metastatic melanoma) while all patients are alive in group B. *Conclusions*. Rituximab in combination with chemotherapy improves complete remission rate, DFS and overall survival. Future prospective trials are needed to confirm our preliminary results.

PU073

UN UNUSUAL CASE REPORT OF HBV-DNA RIACTIVATION IN A PATIENT AFFECTED BY INDOLENT NHL-HCV RELATED

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We report a case of a 81 years old man affected by marginal lymphoma of bone marrow (50%) and spleen (18 centimeters) with initial peripheric pancitopenia caused by hypersplenism, HBV+ (HBsAg negative, HBcAb positive) and HCV+ (2a/2c genotype) with elevated HCV-RNA. He received α-IFN therapy – without ribavirine because of anemia – with major clinic response: reduction of spleen diameter, HCV-RNA negativity, improved pancitopenia. Because of HbsAg negativity, the patient didn't received prophilaxis with lamivudine. In april 2008 the patient was admitted to hospital with fever, asthenia and legs oedemas. Blood tests demonstrated elevated AST, ALT, Bilirubin and LDH; a total body TC scan had warned in chest, abdomen and retroperitoneum multiple lymphnodes as well as enlargement of liver and spleen. Bacterial, micotic or viral causes of fever were ruled out by blood tests; HCV was persistently negative, but HBV-DNA was highly positive with HBsAg positivity. Lamivudine therapy was immediately started, then the patient was submitted to chemotherapy with EDX 1 grammo first and third day/28 days. This case report of indolent lymphoma HCV+, with major clinic response to initial treatment to IFN, demonstrates as induced HBV-hepatitis is usually immunodepression-related . In NHL-HCV related prevalence of HBV+ is very high (the HCV core protein in vitro inhibits HBV replication): antiviral prophylaxis may be useful in HBcAb + cases, even if no conventional or monoclonal therapy is considered.

PU074

EFFICACY OF A MONTHLY MAINTENANCE WITH RITUXIMAB IN PATIENTS WITH MARGINAL ZONE LYMPHOMA

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After a clinical response post conventional chemotherapy rituximab in maintenance addicts a vantage in all indolent lymphomas and especially in follicular lymphomas, but little is known in a specific subset as marginal zone lymphomas Here we report eight cases of marginal zone lymphoma who obtained response after induction therapy and were maintained with a monthly rituximab schedule. At diagnosis the characteristics of the patients were: median age 65 (range 48-71); male/female ratio 3/5; histology splenic and nodal marginal zone respectively in 7 and 1 patient; bone marrow involvement in all patients; splenomegaly in all cases, mean longitudinal spleen diameter 213 mm (range 300-138); adenopathies only in nodal marginal zone lymphoma; elevated β 2 microglobulin in 6/8 cases, elevated LDH in 3/8 case. Induction therapy includes: R-CHOP in 2 patients, FCR, R-mini CEOP ,VCP, FC, VCMP. Rituximab monthly maintenance was administered for 12 months in 4 patients, for 9 months in 1, for 7 months in 2, and for 6 months in 1 patient. Only in two cases rituximab maintenance were administered for the first time respectively as second and third therapeutic line. After induction 1/8 and 7/8 achieved respectively complete and partial remission. After the maintenance 3/7 patients with partial remission obtained a complete remission, while the others improved their partial response because of the progressive reduction of spleen diameters and bone marrow infiltration. At the moment all the observed patients maintain the same response. At a median follow up of 35 months (range 22-87), the median event free survival from the maintenance stop is 13 months (range 5-40). Adverse effects occurred only in one patient: bronchopneumonia during maintenance, herpes zoster and HBV reactivation after maintenance stop. Here we have shown: after induction therapy all patients but one obtained only a partial response; during maintenance therapy in these partial responders spleen and bone marrow involvement continued to decrease and particularly 3/7 achieved a complete remission; at the moment all patients maintain their response with a median EFS of 13 months from the maintenance stop. In conclusion we take these data to indicate that a monthly rituximab schedule is effective in the control of the disease and in the prevention of progression for its continuative anti-tumour effect.

PU075

EVOLUTION OF NODAL MARGINAL ZONE LYMPHOMA WITH PLASMACYTIC DIFFERENTIATION TO MGUS AFTER MAINTENANCE TREATMENT WITH RITUXIMAB: A CASE REPORT

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Plasmacytic differentiation in splenic, nodal and extranodal marginal zone lymphoma is observed in a variable proportion of cases. Here we report a case of nodal MZL with plasmacytic differentiation in whom treatment with rituximab eliminated lymphoadenopathies albeit the persistence of an increasing monoclonal antibody component. A 65 year old woman was diagnosed as nodal marginal zone lymphoma following a cervical lymphobiopsy. The bone marrow biopsy showed a nodular and interstitial infiltration of CD 20 small lymphocytes mixed to mature monotypic plasmacells (10%), negative for CD 20. At diagnosis she had a serum IgG paraprotein equal to 3,7 g/dl and elevated β2 microglobulin, while the blood cell count and the other serological tests were normal. CT scan revealed the presence of cervical, axillary, lomboaortic and iliac adenopathies and splenomegaly. She was treated according CEOP protocol for six cycles every 21 days plus sequential weekly rituximab for four doses, achieving only a partial reduction of splenomegaly, lymphonodal masses and monoclonal peak. Then she begun IFN maintenance that was stopped after five months because of marked progression with autoimmune haemolytic anemia and increasing of adenopathies, splenomegaly and serum paraprotein. Therefore chemotherapy according VCMP protocol was administered for six cycles obtaining the resolution of anemia albeit a slight reduction on monoclonal component and a poor control of masses. Then she was treated with rituximab for four weekly doses and following for twelve monthly doses. After rituximab maintenance her CT scan was negative for

splenomegaly and lymphoadenopathies, her bone marrow immunophenotype showed an infiltration of CD 20 positive cells inferior to 0,5%, while her monoclonal peak was equal to 1.3 g/dL. After 24 months from the maintenance stop, at her last follow up, the patient showed no lymphonodal masses, a slight splenomegaly and a monoclonal peak equal to 1,6 g/dL. In the nodal marginal zone lymphoma, originating from the marginal zone cell, with plasmacytic differentiation the neoplastic population, is constituted by a mixture of CD 20 variably positive cells in different maturative phases that lead to the CD 20 negative plasmacell as final step. Rituximab exerts exclusively on CD 20 positive cells without any influence on the plasmacells. Therefore in this particular case the rituximab causes an histologic transformation of lymphoma towards a monoclonal gammopathy.

PU076

HIGH GRADE B-NHL IN FRAIL PATIENTS: MANAGEMENT AND RESULTS OF A SINGLE INSTITUTION

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The fate of those patients with high grade NHL, who do not enter in specific protocols because the very old age and the presence of comorbidities, is generally not known or not extensively dealt with. In fact a proportion of patients with NHL of about 20% are so called frail because the age or clinical problems out of the lymphoma. We present our experience on 68 patients with B-HGNHL defined frail and observed over 4 years, since 2002 to 2006. The median age was 68y (61-81) with a prevalence of males (31/27). Symptoms B were present in 35(53%) and advanced stage (III-IV) was recognized in 50(73%); histology was DLCBL in 63(94%) and MCL in 5. PS was >2 in 62(93%). Comorbidities were inclusive of heart problems (52%), diabetes (28%), renal failure (12%), liver elevation of enzymes or liver failure (18%); 65 pts (96%) had almost 2 comorbidities. The therapy was patient related by avoiding Antracyclines in case of myocardial problems, avoiding glucocorticoids in unregulated diabetes and avoiding intensive or great associations in those with renal or liver falure. Schemes included VNCOP-B (16 pts), CHOP (7pts), CHOP/R (9pts), CHOP-like with liposomal Doxorubicin and Rituximab (5 pts), CVP/R (13 pts), Vincristine and Cyclophosphamide low dose (18 pts). 70% of pts reduced or discontinued the therapy and 30% stopped definitely the treatment. Supportive care consisted of transfusion or growth factors as needed. The overall response rate inclusive of CR and good PR was 30% in those patients who completed the therapy not inclusive of the Antracyclin, with rituximab or not, and 55% in those inclusive of Antracyclin, either liposomal or not, and Rituximab; other patients treated by alternative schemes had mild or transient responses. Side effects diabetes disregulation (98%), infections (13%), heart falure (15%), renal falure (20%). Deaths due to therapy were 2 in those treated by Antracyclin. The fate of patients who obtained remission is to maintain CR in 60% of those who did Rituximab and Antracyclin and 20% of those who did not. The cause of death was the progression of lymphoma in 80% of those who did not obtained remission and comorbidity related in 20% of the same patients. The relapsed patients had mostly a progression of disease. We conclude that the therapy of frail patients with HG-BNHL should include Antracyclin (liposomal?) and Rituximab in order to obtain more and durable remissions, although the risk of related deaths is increased with this approach.

PU077

INTRAVASCULAR B-CELL LYMPHOMA WITH ATYPICAL PRESENTATION: EXCLUSIVE BONE MARROW INVOLVEMENT WITH LEUKEMIC PHASE

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Background. We describe a case of intravascular large B-cell lymphoma (IVLBCL), presenting only with bone marrow involvement and leukemic phase. *Case report*. A 76 years-old man comes to our attention, presenting with moderate pancytopenia, fatigue and dyspnoea, with absence of systemic symptoms, superficial lymphoadenopaty and hepatosplenomegaly. Blood smear shows few vacuolated blast-like cells (3%). Bone marrow biopsy shows diffuse infiltration of blastic elements with oval or round nucleus, scattered chromatin, prominent nucleoli and scanty cytoplasm, characterized by a predominant intravascular growth. Immuno-histochemical analysis shows CD20/CD79a +, CD3-, TdT-,CD34-, MPO-, CD68PGM1-, BCL1-, BCL2+, BCL6+, CD10+, CD5-, high MIB1. Diagnosis of intravascular large B-cell lymphoma is done. CT-total body doesn't show no anomaly. PET show aspecific captations. We decide for immuno-polichemotherapy according R-CHOP schedule. Patient dies after acute respiratory failure before beginning therapy, maybe for pulmonary progression. *Discussion.* IVLBCL is a rare subtype of extranodal diffuse large B-cell lymphoma (DLBCL), characterized by growth of neoplastic lymphocyte in the lumina of small vessels of various tissues. IVLBCL is rapidly progressive and often disseminated (*Ponzoni et al J Clin Oncol 2007; 25:3168-73*). Clinical course is characterized by fever, skin lesions, dementia, progressive multisystem failure. Exclusive bone marrow and blood involvement in IVLBCL is very rare (*Estalilla et al Am J Clin Pathol 1999; 112:248-55*). At moment rituximab-containing chemotherapy is best therapeutic option for this lymphoma (*Shimada et al, J Clin Oncol 2008; 26:3189-95*). *Conclusions*. Diagnosis of IVLBCL may be difficult for absence of marked lymphoadenomegaly, splenomegaly or evident extranodal involvement. Formerly, for this reason, most of IVLBCLs was diagnosed post-mortem. Early random skin biopsies or repetitive bone marrow biopsies can be useful for an accurate and timely diagnosis.

PU078

CHEMOTHERAPY WITH ALTERNATING REGIMEN MICMA/IGEV IN ELDERLY PATIENTS WITH REFRACTORY DLBCL: A FIGHT AGAINST WINDMILLS?

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Background. In Europe, more than 50% of the lymphomas arise in patients older than 60 years. Now R-CHOP regimen is the gold standard in treatment of aggressive lymphomas in this patient subset. Salvage stategies are needed for patients older than 60 years who are not cured with first line therapy. In fact high dose chemotherapy is not suitable for most patients over the age of 60 yo. 2nd line therapy with DHAP produces scarces results. *Aim*. To evaluate safety, feasibility, efficacy of 2nd line treatment in patients older than 60 yo with combined chemotherapy with MICMA – IGEV.

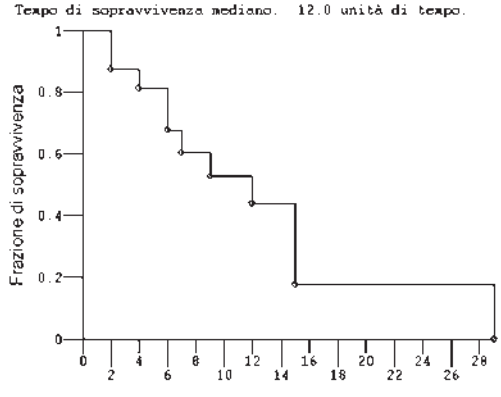

Figure 1.

Results. We treated 20 patients with resistent diffuse large B cell lymphoma (DLBCL) with MICMA-IGEV alternating chemotherapy cycles (MICMA: methylprednisolone 500mg/mq gg1-5, mitoxantrone 10 mg/mq gg1, cytarabine 2 g/mq gg5, carboplatynum 100 mg/mq gg1-4; IGEV: hyphosphamide 2 g/mq gg1-4; gemcytabine 800 mg/mq gg1 and 4). M/F was 12/8, median age was 72.5 years (R63-84). Chemotherapy cycles were administered every 21 days only if complete hematopoietic recovery was occurred. 15 patients (75%) received 4 chemotherapy cycles, 1 patient (5%) received 6 cycles, 4 patients (20%) received 2 cycles. Median cycles administered were 4 (R2-4). At 29 months all patients were dead. Median survival was 12 months. At 24 months survival was 17.6%. 6 patients (30%) showed comorbidities. *Discussion*. In literature patients of all age resistent/relapsed after 1st line chemotherapy and treated with 3 ESHAP or 2 DHAP cycles had a 5 years survival of 23%. Elderly patients (94) with more of 60 years with relapsed/resistent DLBCL and treated with 2-4 cycles of DHAP showed

a median survival of 9 months. Our patients treated with 4 MICMA/IGEV alternating cycles showed a median surviaval of 12 months (R1-29 months). In our study 10 patients (50%) showed non-severe, non life-threatening therapy-related adverse effect (mainly delayed hematopoietic recovery). *Conclusions.* 4 cycles of alternating MICMA/IGEV chemotherapy seems to be safe, feasible and effective in patients with more of 60 yo and with comordidities. These data need of confirmiation on a large coohort of patients.

PU079

USE OF DEFERASIROX IN BLOOD TRANSFUSION-DEPENDENT PATIENTS AFFECTED BY MYELODISPLASTIC SYNDROME: A SINGLE UNIT EXPERIENCE

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Blood transfusion is the only supportive therapeutic chance in MDS patients refratory to other treatments. Repeated transfusions always cause an iron overload with an elevated associated comorbidity and mortality risk independently from their primitive hematological disease. Several studies have demonstrated that patients with "good prognosis" (Refractory Anemia, Acquired Idiopathic Sideroblastic Anemia, and 5q- Syndrome) have an elevated morbidity and mortality risk after the transfusion of more than 100 units of blood red cells. On the basis of these results the use of iron chelators could reduce or prevent the iron overload damage. Deferasirox is a new, convenient, once-daily oral iron chelator that has demonstrated in various clinical trials good efficacy and acceptable safety profile in adult and pediatric patients affected by transfusion-dependent thalassemia major and by different chronic anemias. We have treated 10 patients affected by MDS with deferasirox (4 AISA, 4 RA, 2 5q- Syndrome) refractory to any treatment modality and blood transfusion dependent form at least 1 year. All patients (6 male and 4 female, median age 69 years) showed before the beginning of the iron chelator treatment more than 2000 ng/mL of ferritinemia and a mean blood transfusion request of 1 unit of red blood cells every week in order to maintain Hgb levels higher than 8 g/dL. All patients received deferasirox 10mg/kg p.o. once-a-day. A dose escalation to 20mg/kg p.o. once-a-day was performed after one month from the beginning of the treatment. After one month from the beginning of the therapy with deferasirox all the patients showed a reduction of ferritinemia (an about 15% decrease, r: 8-25%). Interestingly, after 6 months from the beginning of deferasirox therapy, a reduction of the transfusion request (50%) was recorded in four out of ten patients. Until to-day (13 months after the beginning of the therapy) we have not recorded either toxicity or adverse events. Our results confirm the effectiveness of the therapy with deferasirox in reducing the iron overload in polytrasfused MDS patients with acceptable toxicity profile. Moreover, recent studies seem to suggest a therapeutic role of deferasirox in MDS, independently of its iron chelating action: deferasirox seems to act as a potent NFkB inhibitor and this property could explain the activity in MDS, which results in the improvement of the Hgb level.

PU080

HIGHER DOSE OF LENALIDOMIDE IN PROGRESSIVE 5Q- SYNDROME

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Lenalidomide is the golden standard in the treatment of 5q- Syndrome. However, its mechanism of action, the relation between cytogenetic and erythroid response, the duration of treatment and the appropriate posology, are still not defined. In October 2004, a 66 year old man with del 5q31 in 23% metaphases, high transfusion requirement and relevant thrombocytosis was diagnosed. In January 2007 Lenalidomide treatment started with 10 mg/daily for 21 days each month.After 4 weeks, transfusion independence and normalization of platelet count was reached (Hemoglobin >12 gr/dL) without any relevant side effects. Complete cytogenetic response was documented in April 2007 with the disappearance of del 5q31 in 100% of metaphases. In December 2007 complete cytogenetic response was lost with a progressive increase in 5q- positive cells count. In April 2008, the occurrence of thrombocytosis and anaemia with an occasional transfusional support demonstrated the disease's progression. Lenalidomide treatment of 15 mg/daily for 21 days each month started in August 2008 and after 4 weeks transfusion independence was reached with the haemoglobin level at 10 gr/dl and normalization of the platelets count. Hematological response was obtained in contrast with cytogenetic data that showed a progressive increase of 5q- clone up to 90% of metaphases. To date the patient is still responding without any relevant side effects. In several studies giving Lenalidomide for the 5q- Syndrome, the direct cytotoxic effect of this drug on the neoplastic clone is closely correlated with cytogenetic remission and erythroid response. An early surrogate marker of clonal suppression is Lenalidomide-induced thrombocytopenia. In the case reported above, we note that, Lenalidomide 15 mg given to the patient re-induced a response despite the progression of malignant clone. In fact, Lenalidomide related trombocytopenia was less than 50%. In this case, higher doses of the drug could give a stronger modulation of bone marrow microenvironment and play a central role in stimulating residual normal hematopoiesis. A panel of experts recommended to find other treatment strategies including transplantation, in case of a loss of response to Lenalidomide. In our opinion , in selected patients, a higher dose of Lenalidomide may be effective in re-inducing haematological response in progressive 5q- Syndrome.

PU081

ALLOGENEIC STEM CELL TRANSPLANTATION IN LOWER RISK MYELODYSPLASTIC SYNDROMES: SELECTION OF PATIENTS AND CHOICE OF TIMING

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Recent studies reported that in low-intermediate I myelodysplastic patients, transfusion rate is an independent negative prognostic factor for overall survival and evolution in acute myeloid leukaemia. In this setting allogeneic stem cell transplantation should be considered as a disease altering treatment. We reported a case of a 40 years old female who was diagnosed in February 1992 of Myelodysplastic syndrome, refractory anaemia with ringed sideroblastic (RARS) according with the FAB classification. In hindsight she was evaluated for very low risk WHO prognostic score system (WPSS). Because of the not severe anaemia we decide for a watch and wait attitude. In June 2003, a progressive decrease of haemoglobin was observed and patient became transfusion dependent. After a year she had received 13 packed red blood cells (PRBC) unit, echocardiographic evaluation showed an initial cardiac failure (low – moderated mitralic insufficiency, atrials enlargement, low pulmonary ipertension). As the international guidelines we decided for a therapy with erythropoietin growth factor 40.000UI/week. No response was obtained. In April 2005, after receiving 30 PRBC unit, she started iron chelating therapy with deferoxamine 1000 milligrams/day subcutaneous (ferritin was 2000 nanograms/mL). The indirect measurement of cardiac iron overload magnetic resonance imaging (MRI) showed a standard overload (T2*37,7 millisecond). She became low WPSS risk. Because of transfusion dependence, the cardiac and hepatic iron overload, she was treated with Deferasirox 20 milligrams/Kg/day, but she decreased the iron chelator dose (10 milligrams/Kg/day) for a not good tolerance. The progressive worsening of transfusion rate (4-6 PRBC unit/month, 128 PRBC unit from 2003), the changed in intermediate WPSS, the appearance of recurrent atrials fibrillations and the mild cardiac iron overload (T2*13.5 millisecond) have we lead to propose her the stem cells transplantation. In June 2007 she underwent a myeloablative matched sibling allogeneic stem cells transplantation with treosulfan – fludarabine as conditional regimen. Engraftment occurred by day + 12. No acute GVHD was observed. The post transplant period was complicated by several atrial fibrillation episodes. She was treated with deferoxamine 40mg/Kg intravenous during the peritranspland period. Two years after transplant patient is alive, transfusion free (median haemoglobin is 13 ng/mL) and in complete remission. In November 2008 a new echocardiographic evaluation described a strong improvement of the cardiac failure manifestation, in particular right atrial enlargement and fibrillations are regressed. Cardiac iron overload was still detectable (T2* 9.4 millisecond). She was treated with deferoxamine 10/mg/kg/day (ferritin is 1.300 nanograms/ml) until December 2008 when we decided to start Deferasirox 10/mg/kg/day.

PU082

CASE REPORT OF A PATIENT WITH UNEXPECTED RESPONSE

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In January 2007, at our Institution, a 57-year-old man was diagnosed with RAEB 1 MDS, IPSS Int-1,WPSS High.Since diagnosis the patient was transfusion-dependent and received two units of packed RBC every 4 weeks.Treatment with azacytidine (AZA) at the dosage of 75 mg/m2 daily for 7 days every 28 days started in July 2007 in association with gentuzumab ozagamicin (GO) at fixed dosage of 5 mg at day 8. After 6 months, the treatment was interrupted because the patient experienced reactivation of HBV-related hepatitis, from which he was already affected (hepatotoxicity of grade 3). The response to AZA and GO was unsatisfying due to both the persistence of transfusional requirement (3-4 units/month) and the mild increase of bone marrow blastosis not higher than 10%. From April 2008, the patient was enrolled in an experimental treatment program with Lenalidomide at the dosage of 10 mg/daily for 21 days/month and iron chelant therapy with deferroxamine (DFX) due to a persistent ferritin blood level higher than 1000 mug/L. Subsequenlty , no substantial modification of the clinical conditions were observed, and, particularly, neither both the bone marrow blastosis and the transfusional requirement were found changed. From July 2008 due to an intolerance to DFX, the patient was treated with deferasirox(EXJ) 10 mg/kg/day without any signicant side effects. After 4 weeks, the patient showed an hematological improvement and in October 2008 he reached the transfusion independence in association with the decrease of the bone marrow blastosis <5%. From discontinuation of Lenalidomide-therapy (October 2008), the treatment continues with EXJ as single agent:both the erythroid and marrow response are still mantained. To date, the patient is undergoing MUD transplantation. *Discussion.* In our experience, the patient reported no benefits or significant modification of the clinical condition from both the treatment with AZA and Lenalidomide. EXJ, in association with Lenalidomide before, and then as single agent, could have been effective to induce both a hematological response and an important decrease of the bone marrow blastosis. However, we are aware that this unexpected response represents only an isolated case in the cohort of patients affected by MDS. Nevertheless, the recent demonstration that this molecule is a strong NF-κ-B inhibitor with an antiprolipherative effect on myeloid leukemia cells could give access to an innovative use of EXJ.

PU083

MDS (MYELODYSPLASTIC SYNDROME): MAINTENANCE WITH 5-AZA S.C. X 7 DAYS, EVERY EIGHT WEEKS. PERSISTENCE OF CHR AFTER 28 MONTHS.

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Female patient aged 74 with 10-year history of type 2 Diabetes Mellitus under treatment with oral antidiabetics and dyslipidaemia. July 2006 occasional occurrence of pancytopaenia: Neutropenia (WBC 1,600 mm , N 19% mm , L 77%, Mo 1%), Anemia (Hgb 9.2 gr/dL, MCV 105) and thrombocytopaenia (PLT 76,000 mm).PM: BL 16%. Karyotype normal. Diagnosis of AREB (FAB), AREB2 (WHO), IPSS Int. 2 (BL 16%, cytopaenia n). Treatment with Valproic acid 600 mg/day in combination with 5-AZA 75 mg/m^2/day s.c. x 7 days every 28 days, n° 8 cycles (9-10-2006/27-4-2007). After eight months (May 2007): PM BL 4% , Karyotype normal, Complete Haematological Remission (WBC 5,400 mm , N 57%, L 33%, Mo 8%, Hgb 13.1 gr/dl, PLT 202,000 mm). The patient is continuing, at the same doses, treatment with Valproic acid every day and 5-AZA every 6 weeks, for another 4 cycles (7-6-2007/19-10-2007). After twelve months (November 2007): PM BL 3% , Karyotype normal, Complete Haematological Remission persisting (WBC 5,400 mm , N 57%, L 33%, Mo 8%, Hgb 13.1 gr/dl, PLT 202,000 mm) The treatment with Valproic acid 600 mg/day and 5-AZA 75 mg/m^2/day s.c. x 7 days, every 8 weeks, will be continued for a further 11 cycles (14-12-2007/2-4-2009). After 28 months (May 2009): PM RC, Cytogenetics normal, Complete Hematological Remission (WBC 5,200 mm , N 52%, L 34%, Mo 5%, Hgb 13.3 gr/dL, PLT 242,000 mm). *Conclusions*. 5-AZA 75 mg/m^2/day s.c. x 7 days, administered every 8 weeks, combined with Valproic acid 600 mg/day, is well tolerated, causes no significant side effects and is able to maintain Complete Haematological Response for a long period in patients with MDS: AREB (FAB), AREB2 (WHO), IPSS Int. 2

PU084

CASE REPORT: EXTRAMEDULLARY PROGRESSION OF MULTIPLE MYELOMA UNDER BORTEZOMID THERAPY

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Progression of multiple myeloma (MM) from intramedullary to extramedullary sites during the course of disease is not frequent and heralds an aggressive phase of the disease. We report the case of a patient treated with immunomodulatory drugs and chemotherapy and that later developed extramedullary progression at various body sites. A seventy one years old man, with an IgG/kappa MM diagnosticated in third (osteolysis) stage according with Durie and Salmon classification, was repeatedly treated (two cicles Vincristine-Adriamicine-Dexamethasone, Desamethasone and Thalidomide, three cicles of Cyclophosphamide, four cicles Melphalan and Prednison, two cicles Cyclophosphamide) with stable disease. He failed in two occasions the harvest of stem cells from periferal blood for failure of the mobilization. Al last he was treated with four cicles of Bortezomid. Three weeks after the last cicle of Bortezomid the patient developed a significant dyspnea. The cardiac ultrasound showed a normal cardiac pump function. It was documented a progression of MM with doubling the serum M-protein 1000 mg/dL with positive immunofixation with detectable M-spike at serum and urine electrophoresis, with the presence of the plasma cells in the peripheral blood and with a pleuric left effusion, relapsing after pleuric evacuation. The cytofluorometric analysis on pleural fluid noted a 86% of CD138-CD38 positive cells. The renal function and sieric calcaemia were normal. The bone marrow biopsy noted a 90% plasma cells infiltration. It was performed a CT scan that showed an extramedullary involvement with a sternal mass (4 cm), with a mass (8x11 cm) located at left anterior-lower chest wall with extension above and below the costal plan, with a solid nodule (1.5 cm) in left axillary, with a mass (3 cm) in epigastrium and with multiple nodules in the adipose retrosternal tissue in a radiologically suspicion of disease's progression. The patient was evaluated from the surgery but it was not possible to perform a biopsy of the mass. Because of the multiple sites of disease it was not performed radiotherapy. The patient was treated with high dose dexamethasone and with two cicles of cyclophosphamide with no response. He died few mounths later for the progression of the disease.

PU085

BORTEZOMIB IN COMBINATION WITH MELPHALAN AND PREDNISONE FOR THE TREATMENT OF ELDERLY PATIENTS AFFECTED BY MULTIPLE MYELOMA

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Multiple myeloma (MM) is a neoplastic disease especially affecting elder patients even if in recent years it has been also observed in younger patients. The use of the proteasome inhibitor bortezomib has been recently introduced in the treatment of relapsed and/or refractory MM. In fact, bortezomib has proven to be safe and effective in MM patients not only as monotherapy but also given in combination with cytotoxic agents. Bortezomib-based combination regimens have induced clinical benefits with manageable toxicities and may ultimately lead to improvement in the duration of response and survival of patients in the first-line setting. The objective of study was to evaluate the efficiency and safety of bortezomib in combination with melphalan and prednisone (MPV) as a starting regimen for the treatment of elderly patients affected by MM. In our institution we are following 12 elderly patients with stage II/III MM (8 F and 4 M, median age: 75 years, r.: 69-85 years). All patients had, at diagnosis, one or more comorbidity, so they were not eligible for aggressive treatment protocols. As first-line treatment all patients received Melphalan and Prednisone plus Bortezomib chemotherapy (Melphalan 8 mg/sqm p.o. d. 1, 2, 3, 4; Prednisone 75mg p.o. d. 1, 2, 3, 4; Bortezomib 1,3 mg/sqm i.v. d. 1, 8, 15, 22 every 36 days). At a clinical re-staging performed after four courses from the beginning of mel-

phalan-prednisone-bortezomib combined administration a partial remission (reduction of M-component > 50-75%) was recorded in 10 out of 12 patients while the remaining was in steady disease (SD). Thereafter all patients received further four courses of therapy. At one month from the end of treatment 2 out of 12 patients achieved a complete remission (negative immunofixation) and the remaining showed a partial remission (PR) or a very good partial remission (VGPR). At the present, (month +6) only one patient shows a progression disease, while two patients are in CR and the remaining in PR or VGPR. Our results suggest that the combination of melphalan-prednisone-bortezomib is effective and well tolerated in the treatment of MM in elderly "frail" patients. Although there are several published data on the activity of the therapy based on the combination melphalan-prednisone-bortezomib, little is still known about the improvement in the duration of response and survival of elderly patients in the first or second line therapies.

PU086

LENALIDOMIDE AS MAINTENANCE THERAPY IN ELDERLY MULTIPLE MYELOMA PATIENTS

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Lenalidomide is an oral immunomodulatory drug with a structure similar to thalidomide, but it shows a more potent biologic activity and a different safety profile. It represents an important contemporary treatment option for patients with multiple myeloma. In combination with known or new drugs, lenalidomide seems to contribute to survival for myeloma patients with resistant or relapsing disease. In this way lenalidomide increases the available treatment options. According to several studies which evaluated activity of lenalidomide in resistant or relapsed multiple myeloma, in our department lenalidomide was administered as maintenance therapy in elderly myeloma patients with stable disease (partial remission) after induction therapy or more lines of chemotherapy. We treated 5 patients (3M and 2F) with median age of 70 years (range 66-80). These patients completed 12 months of therapy with variable doses of lenalidomide (5-25mg/die p.o., according to tolerability of each patient, for 21 days every one month) in association with dexamethasone (20 mg/die p.o. d. 1, 2, 3, 4). We used Enoxaparin for prophylaxis of venous thromboembolisms. At a clinical re-staging performed after three, six and twelve months from the end of therapy one patient achieved a complete remission (negative immunofixation), two patients showed a very good partial remission and the remaining two patients maintained the partial remission. At the present (month +13) we don't observe any progression of disease. In all patients, the therapy was well tolerated and were not found significant adverse events. Our results seem to suggest a role for lenalidomide, together with dexamethasone, as consolidation/maintenance therapy for previously treated elderly myeloma patients. This therapy seems to lead to an improvement in the prognosis of these patients, without causing severe complications.

PU087

SAFETY AND EFFICACY OF A COMBINATION THERAPY WITH MODIFIED REVLIMID, ADRIAMYCIN AND DEXAMETHASONE (RAD) REGIMEN IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM): A SINGLE CENTRE EXPERIENCE

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Despite the introduction of novel agents, the optimal therapy of relapsed/refractory MM remains controversial. Lenalidomide is an oral immunomodulatory drug with significant activity in this setting and has been approved by EMEA in association with dexamethasone for the treatment of pts with at least one prior therapy. To improve response rate and long term outcome of relapsed/refractory MM pts. Knopp et al. recently reported that RAD association induces high response rate with an acceptable toxicity profile in pre-treated MM pts. The aim of this study was to evaluate the efficacy and safety of a modified RAD regimen. Between May and December 2008, 10 pts (4 M/6 F) with relapsed/refractory MM received six 28-days RAD cycles. Main pretreatment characteristics were the following: median age 69 years (range 50-78); WHO performance status 0-3; median Hb 8.9 gr/dl (range 8.3-12.1); median β2M 4.4mg/L (range 2.5-9.1); median bone marrow plasma cell infiltration 40% (range 5-80). Median time from MM diagnosis to treatment was 33 months (range 11-130). All pts had received more than two chemotherapy regimens (range 1-6). Five pts were previously treated with autologous stem cell transplantation. The RAD modified regimen was designed as follows: Revlimid 25 mg days 1-21, Adriamycin 40 mg/sq.m i.v. days 1,4, Dexamethasone 40 mg p.o. days 1–4. Peg filgrastim was administrated on day 6 and Aspirin 100 mg/die was given as prophylaxis for thrombosis. The IMWG criteria were used for definition of response, while toxicity was graded according to NCI-CTC criteria. Two out 10 pts enrolled were not evaluable for response because of early withdrawal due to progression of disease. Of the 5 pts who completed the planned therapy, 1 achieved CR, 2 VGPR and 2 PR after a median follow-up of 4.5 months (range 2-6). Two pts had a progression of the disease. One patient died after the second cycle for pneumonia. Myelotoxicity was the major side effect leading to a dose reduction in 1 patient. No relevant neurological symptoms were found. In conclusion, these preliminary data show that a modified RAD was feasible, well tolerated and effective in pts with refractory/relapsed MM. Further studies and a long term follow up are warranted to evaluate the duration of responses and the effect on survival.

PU088

SPONTANEOUS SPLENIC ARTERY RUPTURE IN A CASE OF PLASMA CELL LEUKEMIA

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Introduction. We report on a patient (pt) with plasma cell leukemia who developed spontaneous splenic artery rupture. Case report: A 45-year-old black man was admitted, in October 2006, to another institution with a two week history of generalized malaise, nausea, vomiting, myalgia and asthenia. Laboratory data revealed hypercalcemia, hyperuricemia, renal failure, anaemia, leukocytosis, increased serum lactate dehydrogenase and a urine light k chain M component; peripheral blood smear differential blood count revealed 32% plasma cells. Plasma cell infiltrate replacing normal cellularity was found on bone marrow aspirate. FISH analysis showed monosomy 13q14 and presence of t(11;14). A skeletal survey showed multiple osteolytic lesions of skull, dorsal and lumbar spine (vertebral fracture of L4), ribs, pelvis, femoral heads and homers. The pt received emergency treatment with i.v. fluids and electrolytes supplementation, bisphosphonates and corticosteroids and he started haemodialysis. The pt was transferred to our institution and chemotherapy (CT) was started according to the VAD regimen (i.v. Vincristine, Adriamycin and Dexamethasone).

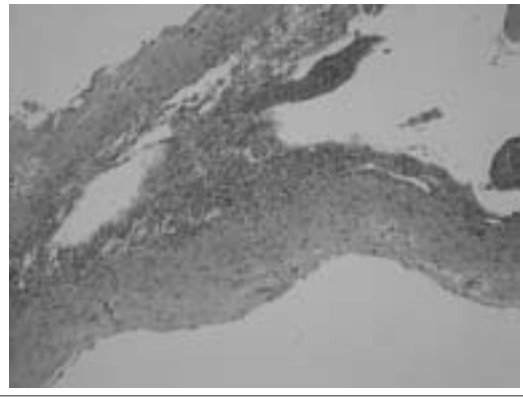

Figure 1

On day +6 from CT, sudden severe hypotension associated with transient loss of consciousness and severe anaemia (haemoglobin: 5.2 g/dL) occurred. Emergency abdominal ultrasonography and computerized tomography were diagnostic for hemoperitoneum with flushing from the splenic artery. Embolization of the splenic artery was attempted, but unsuccessful rendering emergency laparotomic splenectomy necessary. On gross pathological examination, the spleen was enlarged (13 x 15.5x5

cm), weighted 200 g and a 7 cm-long laceration over the hilum was evident. On microscopic examination, massive replacement of splenic parenchima by k light chain- and CD138-positive plasma cells and small lymphocytes was present; splenic artery vessel wall was infiltrated by plasma cells which caused dissection of the layers (Figure 1). Post-operative period was uneventful and the pt was discharged on day +15 from splenectomy. He subsequently received two additional courses of VAD therapy and eventually died of disease progression 5 months after diagnosis.

PU089

STEVENS-JOHNSON SYNDROME OCCURRING DURING FIRST-LINE THERAPY WITH LENALIDOMIDE AND DEXAMETASONE FOR MULTIPLE MYELOMA: REPORT OF A CASE

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Introduction. Dermatologic adverse events can occur during lenalidomide administration, albeit rarely. We report on a patient (pt) who developed Stevens-Johnson syndrome (SJS) while on Lenalidomide. Case report: A 56 yr-old female pt was transferred from another hospital with a diagnosis of multiple myeloma (MM) IgG/κ stage IIIA in Jan. 2009. Following vertebral arthrodesis, she was enrolled in a protocol (RV-MM-PI-2009) of Lenalidomide 25 milligrams /day, day 1-21 + dexamethasone 40 milligrams days 1, 7, 14. Therapy was started on Feb. 18th; concomitant medications were: low molecular weight heparin (LMWH), allopurinol, trimetoprim-sulfametoxazole, acyclovir, morphine. On Feb. 28th, mild skin erythema was noted; allopurinol and LMWH were discontinued and therapy with oral anti-hystamine resulted in transient rash attenuation. Ten days later (= day +21 from the start of Lenalidomide) she was hospitalized because of abrupt, marked worsening of skin lesions associated with itching and burning of the skin. Severe cutaneous edema and erythema, most prominent to the extremities, conjunctival erosions and low-grade fever were present; she received one course of Immunoglobulin and supportive therapy with antihystamines and intravenous fluids and electrolytes. Skin care included use of anti-septic (0.05% sodium hypoclorite) and sterile paraffin ointment; vaseline sterile gauze and sterile cotton gauze were used as final dressing. The lesions worsened - with confluent bullae developing at the forearms- during the first week, to slowly subside over the following 3 weeks with prominent skin desquamation and she was discharged. SJS was diagnosed based on clinical findings (the pt refused skin biopsy) and Lenalidomide was considered to be the offending drug.

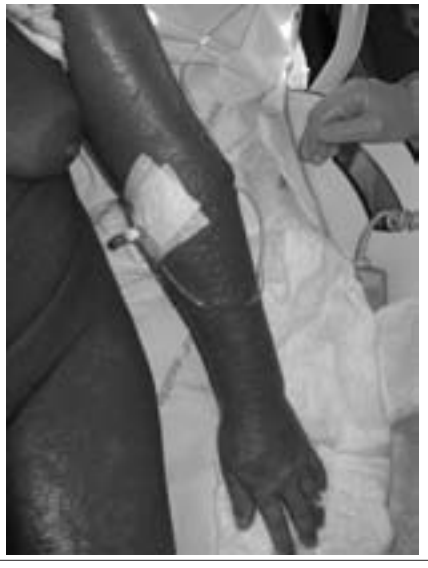

Figure.

Discussion. Cutaneous rash is reported to occur in approximately 15% of pts receiving lenalidomide. Celgene Corporation reports 12 cases of SJS, 3 cases of erythema multiforme and 1 case of toxic epidermal necrolysis among approximately 57,000 pts who received lenalidomide from Dec. 2005, through June 2008. Drugs, including allopurinol and dexamethasone, are well recognized inciting factors and the risk of developing SJS is reported to be highest during the first 2 months of treatment. Physicians prescribing Lenalidomide should monitor their pts for possible dermatologic adverse effects which may require prompt drug discontinuation.

PU090

IMMUNOPHENOTYPIC CHARACTERISTICS OF PERIPHERAL BLOOD MOBILIZED CD34+ HEMATOPOIETIC PROGENITOR CELLS AFTER DIFFERENT INDUCTION THERAPIES IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

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Introduction. The emergence of novel agents such as the immunomodulatory drugs are increasingly used in induction therapy as well as in maintenance treatment after stem cell transplantation for MM patients. However, little information is available regarding the impact of these drugs on CD34+ subset produced during mobilization and early hematopoietic engraftment. Objective: The aim of this study was to analyse retrospectively the subset of CD34+ cells from apheresis collections and early engraftment after transplantation in MM patients receiving different combination of drugs, including novel agents, as induction therapies. *Materials and methods.* A total of 26 newly diagnosed MM patients were included. Clinical characteristics were as follows: 13 M/13 F, median age 51 years (range 30-72), stage II/III according ISS, 2 out of 26 were in dialysis for end stage renal disease. Different induction-based therapies were used: lenalidomide (6 pts), bortezomib (12 pts) and VAD like (8 pts). Flow cytometry analysis was performed on cryopreserved apheresis samples, using the following three colours antibodies combinations (FITC/PE/PerCP): CD34/CD33/CD45, CD34/CD38/CD45, CD34/CD90/CD45, CD34/CD110/CD45, CD34/CD117/CD45, CD34/CD133/CD45, CD34/VEGF-R2/CD45. *Results.* Higher proportion of CD34+/CD133+ and CD34+/CD117+ cells (immature phenotype) was observed in pts treated with lenalidomide versus other induction therapies (52.7±28.4% vs 23.5±27.0%, p=0.05 for CD133+; 19.0±16.4% vs 5.4±5,4%, p=0.01 for CD117+, respectively). Similar expression of the remaining MoAbs was observed in the two groups of pts. A low proportion (range 0–0.8%) of CD34+/CD110+/CD45dim pattern, consistent with megakaryocytic committed CD34+ progenitors, was found in all apheresis analyzed. Conversely, high expression (range 76,8-98,3%) of CD34+/VEGF-R2+/CD45dim cells, indicating the presence of endothelial progenitor cells, was observed in all cases. In all transplanted patients, median time to neutrophil engraftment (ANC>500 microL) and platelet engraftment (plt>20000 microL) was 11 days (range 9-14) and 13 days (range 9-30), respectively. A correlation between CD34/CD90+ cell expression and faster platelet engraftment was found (r=-0.54, p=0.01). *Conclusion.* Analysis of CD34+ cell subsets may be utilized for a better understanding of hematopoietic stem biology in MM and may be a useful tool in predicting short term engraftment, especially in case of low number of CD34+ cell infusion.

PU091

PROPOSAL FOR THE MANAGEMENT OF THE MGUS

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The term monoclonal gammopathy of undetermined significance (MGUS) indicates the presence of a monoclonal protein (M-protein) without features of multiple mieloma (MM), Waldenström's macroglobulinemia, primary amyloidosis or other related disorders. The MGUS represent a large portion of the monoclonal gammopathies (MC). Even if MGUS don't require any therapy, the risk of progression to a malignant lymphoproliferative disorder is 1% per year, so It is recommended a prolonged follow up . At the present there are no formal guidelines regarding follow-up for patients with MGUS. We met the primary care

physicians to share the criteria of classification and to agree on management of the MGUS. Our aim is to provide suggestions that may help to discriminate, among the patients with MGUS, who could be cared by the primary care physician and who should be referred to the specialist (Figure 1). We hope that our work could be useful translated into other situations. MGUS is characterized by a serum monoclonal protein <30g/L, plasma cells in the bone marrow <10% and absence of end-organ damage: anemia (hemoglobin < 10 g/dL), renal failure (creatinine > 2 mg/dL), hypercalcemia (serum calcium> 12 mg/dL) , bone lesions (lytic lesions or osteoporosis with compression fractures). Serum protein electrophoresis is the most common method to detect and quantify a MC. The immunofixation, automatically performed, allows to confirm and to type the MC. Once a MC is detected we need: full blood count, serum creatinine, serum calcium, 24 h urine total protein (quantifiable, it represents a sure sign of nephrophaty it can reveal a nephrosic syndrome, due to myeloma or amyloidosis; unlike 24 h urine total protein, Bence Jones proteinuria is unquantifiable and often associated with a MGUS). Clinical care should be kept to the presence of constitutional symptoms, bone pain, lymphadenopathy and splenomegaly. In this phase β-2 microglobulin, serum quantitative imunoglobulins, urine protein electrophoresis, Bence Jones proteinuria are not necessary. In the absence of end-organ damage, and with a MC < 30 g/L, the bone marrow biopsy allows to discriminate the MGUS from the asymptomatic myeloma. Anyway, since the latter doesn't require any therapy, this discrimination is not essential. Once a MGUS is diagnosed the primary care physician will attend patients aged > 65 years with MC < 30 g/L and patients aged ≥ 65 years with MC <15 g/L ,without end-organ damage. Six-month follow-up testing is suggested (after 4 months, if the M.C. is > 15 g/L at the time of the diagnosis). It includes full blood count, serum creatinine, serum calcium, serum protein elecrophoresis and 24 h urine total protein; On the contrary an hematological evaluation is recommended for patients with: 1. MC and end-organ damage (not attribuable to any others causes); 2. MC >30 g/L; 3. aged ≥65 years and MC > 15g/L in the absence of severe co-morbidities 4. MC > 15 g/L with prompt increase (>2 g/L per year).

Figure 1.

PU092

DOUBLE AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) AS FIRST LINE THERAPY FOR MULTIPLE MYELOMA (MM): EXTENDED FOLLOW UP OF A PHASE II TRIAL

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In the last decade, the introduction of novel agents has changed the therapeutic options for MM patients. However, single ASCT is still considered the standard therapy for young patients (<65ys). Several studies showed that patients treated with double ASCT have a significant improvement in EFS, although data on timing and OS are not conclusive. We report results and long term follow up of a phase II trial performed to evaluate efficacy and feasibility of a tandem ASCT strategy. The study plan was the following: 1) three monthly VAD-like cycles as debulking therapy; 2) mobilization and peripheral blood stem cells collection after high dose cyclophosphamide (7 g/sqm) and G-CSF; 3) first ASCT after conditioning regimen with melphalan (60 mg/sqm) and busulphan (16 mg/kg); 4) second ASCT after conditioning regimen with melphalan 200 mg/sqm for patients not achieving CR after first ASCT or relapsed patients; 5) maintenance therapy with interferon alfa (IFN) used at conventional dose (3 MU x 3/week) in responding patients after single or double ASCT. Response was defined according to Bladè criteria. From January 1996 to December 2000, 39 consecutive patients (25 males, 14 females) with symptomatic MM were enrolled. Main characteristics were the following: median age 54 years (range 34-64), stage II/III 9/30 according to DS classification and I/II/III 18/10/11 according to ISS. Thirty-four patients completed first ASCT achieving 10 CR, 23 PR, and 1 NC. Fifteen patients underwent second ASCT, obtaining 5 CR, 8 PR and 2 NC. Of the responding patients, 15 received IFN maintenance therapy with acceptable and manageable toxicity. With a median follow up of 104 months (range 7-144) 11 patients remained alive and 7 free of progression. PFS and OS were 36 and 48 months, respectively. Better PFS and OS were observed in patients receiving IFN as maintenance therapy (p 0.0017 and 0.0063). Our experience suggests that IFN as maintenance therapy in a double ASCT program may prolong long-term PFS and OS. Further studies are warranted to compare efficacy, toxicity and costs of novel agents in the same setting of MM patients.

PU093

EFFICACY OF BORTEZOMIB AS SINGLE AGENT IN RECURRENT EXTRAMEDULLARY PLASMACYTOMA: A CASE REPORT

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Extramedullary plasmacytoma (EMP) is a discrete, solitary mass of neoplastic monoclonal plasma cells in soft-tissue. Represents approximately 3% of all plasma cell neoplasms, progresses to multiple myeloma in 11-30% of patients at 10 years and in 15% of the cases develops during the course of the disease. The standard treatment for EMP is surgery, radiotherapy, chemotherapy. The conventional antimyeloma agents, such as thalidomide, may not be effective especially as single agent. Among novel antymyeloma agents bortezomib seems to be very active on EMP also so single agent. We reported a case of recurrent extramedullary relapse of multiple myeloma (MM) after autologous HSCT treated with successful in first and in second relapse using bortezomib as single agent. A 63year old woman was diagnosed with IgG/lambda III A MM in March 2005.She was treated with 4 cycles VAD and autologous HSCT after Mel 200 achieving VGPR. She was given also montly acid zoledronic. In April 2007 she presented a sternal mass painful measurig 3.5x3 cm at ETG scan with bone destruction and intense tracer uptake on FDG-PET. Biopsy of lesion showed a diffuse infiltrate of plasma cells with a lot of atypical forms. Bone marrow plasma cells infiltration was not exceeding 6% of nucleated cell. Serum M protein concentration was the same that after HSCT time. She received bortezomib at the standard dose for 3 cycles. After 2 cycles the sternal plasmacytoma resolved completely, ETG scan and FDG-PET was negative. The M protein size was stable. In November 2008 the patient showed again rapidly progressive sternal mass very painful. At ETG scan it was 10x9 cm with sternal fracture .Serum M protein was not increasing , bone marrow plasma cells was low. Quickly was started standard dose bortezomib for 2 cycles . At the end of the first cycle the soft-tissue mass showed complete resolution and ETG scan was negative. The patient subsequently given local adjuvant radiotherapy (40 Gy) .At this time the serum M protein was disappeared. Bortezomib is a proteasome inhibitor that produces significant response in patients with relapsed/refractory M.M. There is little information on its effect in EMP. This case demonstre efficacy of bortezomib as single agent in recurrent manifestation of EMP. The stability of serum M protein suggests a specific and exclusive action of bortezomib in extramedullary site confirming it as very promising agent also in EMP.

PU094

A CASE OF EXTRAMEDULLARY PLASMACYTOMA (EP) 18 F-FDG PET/CT AND 99M TC-MIBI NEGATIVE

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Background. 18F-FDG-PET has demonstrated high sensitivity (85%) and specificity (92%) (Bredella AJR 05) in detecting myelomatous (MM) lesions. 99mTc-MIBI (MIBI) was positive in 91% of patients with MM lesions (57% with focal pattern) (Fonti JNM 08). We report a case of EP of the nasal cavities and paranasal sinuses with 18F-FDG PET/CT (PET/CT) and MIBI negative. Case history A 77 years old man suffering from recurrent epistaxis since December 2008 underwent a CT of the head (three-dimensional reconstruction), biopsy of the nasal cavities and paranasal sinuses, bone marrow (BM) biopsy, blood test, whole body X-ray (WBXR), magnetic resonance imaging (MRI) of the spine and pelvis, PET/CT. CT of the head showed solid tissue in the nasal cavities and paranasal sinuses with damage of the surrounding bone. Biopsy showed clonal plasma cells (CD 138 +++ K and IgG ++ ; L, CD 20, CD 45 RO, Cheratin, CK 5/6, CK 20, Cromogranin A: NEG) and diffuse necrosis. BM biopsy, WBXR, MRI, blood and urine tests were normal. Intravenous (i.v.) administration of 18F-FDG (420 MBq) after an 8 hour fast was performed. Blood glucose level was 108 mg/dL before tracer administration. Head-neck and whole-body PET/CT scans were acquired respectively at 40 and 60 min after i.v. administration of 18F-FDG. An emission scan was performed from the skull to the pelvis and transaxial, sagittal and coronal images were obtained. Four days after PET/CT the patient underwent MIBI scan after i.v. injection of 555 MBq of 99mTc-MIBI and by acquiring planar anterior and posterior whole-body views. PET/CT and MIBI showed no pathologic areas in the nasal cavities and paranasal sinuses. Discussion False negatives are about 30% when PET/CT is performed to asses the extent and severity of MM lesions (Zamagni Haematol 07). We suppose that necrotic tissue, cellular ipometabolism, deficit of glucose transmembran transporter and ipoperfusion present in the pathologic areas played a role to determine a false negative. Another cause could be the overexpression of P glycoprotein (Pgp) in this patient. This protein increase the energy dependent efflux of 99m Tc-MIBI and its washout can be associated with multidrug resistant myeloma (Fonti JNM 08). False negative cases by18 F-FDG PET/CT and 99m Tc-MIBI may be due to several co-factors. Conclusions PET/CT may be useful in assessing the extent and severity of MM lesions often overlooked by WBXR and/or MRI. It is important, however, to bear in mind the possibility of false negatives in MM lesions.

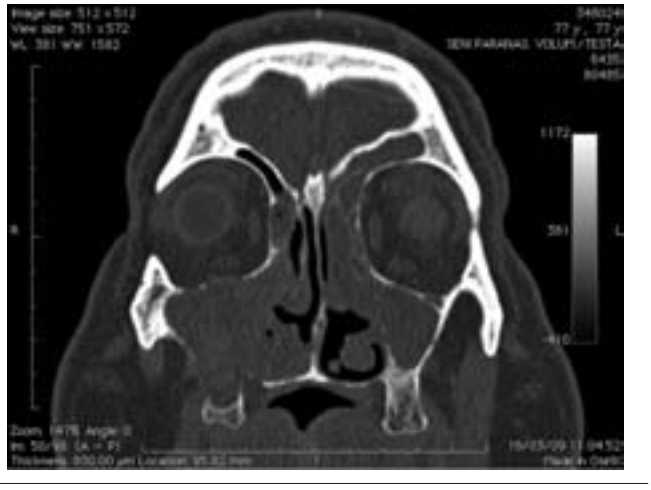

Figure 1. CT solid tissue in the nasal cavities and paranasal sinuses with damage of the surrounding bone.

PU095

A CASE OF LOCALIZED AMYLOIDOSIS OF THE TONGUE

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Amyloidosis is a disease characterized by the presence of extracellular deposits of proteinacious material causing organ failure. Amyloid involvement of the tongue is almost always secondary to systemic disease; the mean survival of patients with a systemic form of amyloidosis is between 5 to 15 months, whereas those with the localized form have an excellent prognosis. We observed a 66 years old female patient with hoarseness and dysarthria due to a tongue mass revealed by fiberoptic laryngoscopy. A laryngeal microsurgery was diagnostic for amyloid nodule, so the patient was send to the Hematology Department to exclude a systemic form of amyloidosis. At clinical exam, the patient resulted disarthric and asthenic, with a mild tongue basis hypertrophy and hepatomegaly, related constipation and loss of weight in the last 1 year period. The blood cell count showed Hb 12.7 g/dL, WBC $5.2x10^3$/L, PLTS 318.000/L with normal values of liver enzymes and serum creatinine; serum protein electrophoresis didn't show a monoclonal component, data also confirmed by serum and urine immunofixation; serum k and free light chain analysis was into physiological range (k 17.2 mg/L, 15.1 mg/L, ratio 1.14); the echocardiography showed a normal IVS thickness of 10 mm with good left ventricular performance and no hypertrophy; no plasma cells and no amyloid deposits were revealed by bone marrow sample and abdominal fat biopsy, respectively. Moreover, serum NT-proBNP and troponine I were, respectively, 39.2 ng/L and 0.02 ng/mL, both into range of normality; no proteinuria was revealed. The whole screening tests for systemic amiloidosis excluded this disease and confirmed the diagnosis of localized amyloidosis of the tongue. The patient actually is in good clinical conditions and has a follow-up period of 32 months from the diagnosis; clinical and biochemical assays are performed every 3 months together with periodic controls by ORL resulting in stable disease. The prognosis of localized forms of amyloidosis is better than systemic ones although recurrences are common and long-term follow-up is recommended. A thorough evaluation, including fat or rectal biopsy, is essential in every patient to identify any systemic involvement; the absence of systemic amyloidosis offers a much more favourable prognosis and may be treated with simple surgical endoscopic laser excision.

PU096

CHOD REGIMEN IN EXTRAMEDULLARY MULTIPLE MYELOMA PRESENTING AS RELAPSE AFTER PBSCT (PERIPHERAL BLOOD STEM CELL TRANSPLANTATION) OR SALVAGE THERAPIES

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Extramedullary relapse in multiple myeloma (MM) is a rare event characterized by highlymalignant histology, resistance to treatment and poor outcome. To evaluate the effectiveness and safety of CHOP-like chemotherapy in relapsed MM patients (pts) presenting with extramedullary disease, from June 2005 to January 2009, 9 pts with relapsed MM, aged less than 75 years and eligible for anthracycline-based chemotherapy, were treated with CHOD chemotherapy (cyclophosphamide 750 mg/sqm day 1, doxorubicin 50 mg/sqm day 1, vincristin 1.4 mg/sqm day 1, dexamethasone 40 mg days 1-4). Lower doses of doxorubicine (30 mg/sqm) and of dexamethasone (20 mg per days) have been administered to pts aged over 70 years old (4 pts). Median age at relapse was 65 years (48-75), 6/9 pts were female, 7/9 have systemic disease with a measurable monoclonal component, bone marrow plasmocytosis more than 30% and lytic bone lesions. All pts had already received 2-4 lines of therapy. Extramedullary disease, confirmed by fine-needle aspiration or surgical biopsies, consisted of skin lesions (4 pts), skull bone masses (2 pts), cerebral mass (1 pt), testicular mass (1 pt), abdominal nodal mass (1 pt), oral cavity mass (1 pt) and pancreatic mass (1 pt). LDH levels were high in all pts (median level 561 U/L, range: 316-923, with normal range 0-250 U/L). So far, 3 pts have progressed while on treatment.

Of the remaining 6 pts, 3 have completed 6 courses of CHOD, 2 pts have completed 5 courses of therapy and 1 is still on therapy after 4 courses. All these pts had an impressive reduction of the extramedullary disease. However, despite this reduction, 5/6 pts died because of progressive systemic disease, while the pt still responding have no systemic disease. The treatment was well tolerated and all pts experienced mild and moderate toxicities compatible with lymphoma-like chemotherapy, including only 1 case of grade 4 leukopenia, in absence of infective complications. Despite CHOD regimen had no activity versus systemic disease it was very effective on the extramedullary disease (6/9 mass reduction). Therefore, further studies are needed to verify whether or not CHOD regimen can be a useful therapeutic tool that can be associated to local radiotherapy in those pts who present only an extramedullary relapse of MM, in order to ameliorate the quality of response.

PU097

CLINICAL CHARACTERISTICS OF BICLONAL GAMMOPATHIES. REVIEW OF 79 CASES

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Introduction. Monoclonal Gammopathy of Undetermined Significance (MGUS) is a group of B-cell disorders wich result in the production of a specific and unique monoclonal immunoglobulin (M-component). It's reported in 3 to 5 percent of population, with the prevalence increasing with advancing age. Biclonal Gammopathy of Undetermined Significance (BGUS) is characterized by the simultaneous appearance of two narrow peaks, suggesting the existence of two monoclonal antibodies. The incidence is about 1% of all monoclonal gammopathies. The pathogenesis of BGUS is unknown, but several possibly related environmental factors have been identified. The type of monoclonal component (MC), level of uninvolved immunoglobulins (UI), Bence Jones proteinuria, light chains isotype, erytrosedimentation rate (ESR), percentage of bone marrow plasma cells are prognostic factors mentioned in the literature for the monoclonal gammopathies in defining the risk of malignant transformation. This study was performed in order to evaluate the baseline characteristics of a BGUS population and to determine the role of the same prognostic factors for the biclonal gammopathies's progression to myeloma. *Patients and methods*. We performed a retrospective study of 79 consecutive patients observed between 1999 and 2009. Males were 42 (53 %) and females were 37 (47 %). Patients were divided in three groups of age: younger than 50 years (5%, median 45y, range 37-47y), between 50-70 years (31%, median 66y, range 52-69 y) and older than 70 years (43 %, median 78y, range 71-91y). Clinical and laboratory features was selected for primitive BGUS so lymphoproliferative disorders, infections and solid tumors were excluded. Patients' characteristics are shown in Table 1. The median follow-up was twenty-five months (range 3-280). One patient (1.2 %) progressed to myeloma (a 71-year-old male whit a IgMκ-IgAλ biclonal gammopathy and a follow up of 280 months). *Conclusions*: Although the clinical features of biclonal gammopathies are similar to those of monoclonal gammopathy, this subject is of importance because of the lack of clinical data in the literature. Periodic follow-up of patients with BGUS allows earlier diagnosis of malignant plasma cell dyscrasias and prevent them for severe complications. Further a long term studies are ongoing to examine the natural history of BGUS using a prospective approach and a larger patients' cohort to identify variables associated with progression, the rate of progression, progression free survival and overall survival.

Table 1. Patients' characteristics (n=79)

Variable		N. (%)	Variable			N. (%)
Gender	M / F	42 (53) 37 (47)	**Type of light chains**	kappa/lambda		41 (52)
Age (y)	<50	5 (6)		kappa/kappa		27 (34)
	50-70	31 (40)		lambda/lambda		11 (14)
	>70	43 (54)	**Bence Jones**	negative	positive	Not evaluated	64 (81) 8 (10) 5 (6)
Isotype	IgG/IgA	27 (34)	**[UI] (mg/dL)**	normal	reduced	Not evaluated	30 (38) 22 (28) 27 (34)
	IgG/IgM	21 (27)	**PC (%)**	5	>5	Not evaluated	17 (22) 13 (16) 49 (62)
	IgG/IgG	15 (19)	**Albumine (g/dL)**	3.5	<3.5	Not evaluated	1 (1) 67 (85) 11 (14)
	IgM/IgA	7 (9)	**Beta2microG(mg/L)**	2.5	>2.5	Not evaluated	32 (41) 9(11) 38 (48)
	IgM/IgM	7 (9)	**ESR (mm/h)**	15	> 15	Not evaluated	25 (32) 34(43) 20 (25)
	IgA/IgA	1 (1)	UI = uninvolved immunoglobulins; PC = plasmacells in bone marrow; ESR = erytrosedimentation rate				
	IgMλ free	1 (1)					

PU098

OUTCOMES IN RELAPSED/REFRACTORY MULTIPLE MYELOMA TREATED WITH BORTEZOMIB-BASED REGIMENS. A SINGLE CENTER EXPERIENCE

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In the past decades, MP (melphalan/prednisone) and VAD (vincristine/adriamycin/dexamethasone) protocols have been the standard of care for multiple myeloma (MM) patients. More recently, new therapeutic approaches including proteasome inhibitor (bortezomib) and immunomodulatory agents (thalidomide, lenalidomide) in combination with corticosteroids and chemotherapeutic agents have been demonstrated to improve response rate, TTP and survival in these patients. Bortezomib (velcade) in monotherapy or in combination with other drugs is efficacious and safe for newly diagnosed MM patients as well as for advanced patient population with relapsed and/or refractory disease. In this retrospective study, we present data on outcome and toxicity of bortezomib-based regimens given to 35 patients with relapsed and/or refractory MM from a single center. The patients (17 female/18 male) had a median age of 68 years (range, 46-84). MM characteristics can be summarized as follows: isotype (71.5% IgG, 22.8% IgA, 5.7% light-chain); ISS (46% stage I, 40% stage II, 14% stage III); 40% in first (R1), 28.5% in second (R2), 14.4% in third or subsequent (R3+) relapse, and 17.1% refractory patients. All patients received prior therapies among the conventional combinations such as MP, VAD, VAD followed by cyclophosphamide mobilization and then by tandem high-dose melphalan with autoPBSCT, or combinations of multiple chemotherapeutic agents including ciclophosphamide. From January 2007 to January 2009, bortezomib-based regimens were administered for relapsed (82.8%) or refractory (17.2%) disease. Median interval between MM diagnosis and bortezomib initiation was 35 months (range, 4-99). In particular, 17 patients were treated with VD regimen (bortezomib 1.3 mg/mq d 1, 4, 8, and 11; dexamethasone 320 mg/21 d for up to 8 induction cycles, followed by consolidation cycles with bortezomib d 1, 8, 15, 22; dexamethasone 320 mg/35 d), and 18 patients with VTD (bortezomib 1.3 mg/mq d 1, 4, 8, and 11; thalidomide 100mg/d; dexamethasone 320 mg/21 d for up to 8 induction cycles, followed by consolidation cycles). The median number of delivered induction cycles was 6 (range, 4-8) plus 3 consolidation cycles in both VD and VTD groups. Bortezomib was discontinued, withheld or dose-modified in 34% of patients, mostly for peripheral neuropathy. Further, 20 patients (57%) received intravenous infusions of zoledronate at 4-week intervals. All patients on thalidomide were given low molecular weight heparin (n=10) or acetylsalicylic acid (n=8) prophylaxis. Responses were assessed according to IMWG criteria; the overall response rate was 79 % (14% CR, 14% VGPR, 44% PR, 17% SD, 11% PD). RR was 77%, 75% and 61% in R1, R2 and R3, respectively. Toxicity, scored by NCI criteria, included: peripheral neuropathy (grade 3/4 22.8%, grade 1/2 11%), gastrointestinal toxicity (constipation 14.2% and diarrhoea 5.7%), reversible thrombocytopenia (8.5%), neutropenia (5.7%), herpes varicella-zoster infections (5.7%), cutaneous rash (8.5%); a deep-vein thrombosis was observed in a patient while on acetylsalicylic acid prophylaxis. In conclusion, our experience confirms efficacy and safety of bortezomib-based regimens in relapsed and refractory MM, even in heavily pre-treated patients with predictable and manageable adverse events.

PU099

ATRIAL FIBRILLATION AND DIFFUSE MYALGIA IN A MYELOMA PATIENT DURING THE FIRST CYCLE OF BORTEZOMIB THERAPY

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Bortezomib therapy is known to be associated with thrombocytopenia and neurological side effects but cardiotoxicity has been rarely reported. We report the case of a 74-year-old woman with micromolecular myeloma with κ monoclonal light chains. The patient presented coxalgia. She had been previously treated with zoledronate, because of osteolytic lesions to ilio-pubic branch and acetabulum. Furthermore she suffered from hypothyroidism, hypertension, anti-HCV positivity and thrombophilic state (MHTFR [C677T] homozygosis, FV [H1299R] heterozygosis, MTRR [A66G] heterozygosis, GPIIIa [C1565T] homozygo-

sis). Physical examination was normal. Laboratory work-up showed only abnormal serum β2-microglobulin (9.2 mg/L). Creatinine, urea, uric acid, electrolytes and other serum clinical biochemistry values were within normal ranges. The blood cell count with differential was normal. Bone marrow aspiration showed 12% atypical plasmacells. Cytogenetic analysis (FISH) was negative. There was no sign of cardiac disease, ECG was normal and the left ventricular ejection fraction was 83%. Therapy with bortezomib (1.3 mg/m^2 on days 1,4,8,11) and dexamethasone (20 mg on days 1,2,4,5,8,9,11,12) was started. On day 5 of the first cycle, the patient experienced diffuse myalgia and at the same time LDH reached 7915 U/L (n.r. : 240-480 U/L) and AST 837 U/L (n.r. : 0-41 U/L) with normal ALT, cardiac troponins and serum electrolytes were normal, CK: 160 U/L (n.r. : 10-80 U/L), CK-MM: 99,9%; CK-MB: 0.02%. ECG and cardiac troponins remained normal at the subsequent controls (3,6, and 12 hours later). On day 6, the patient had an episode of atrial fibrillation. Cardiac troponins remained negative. LDH: 1240 U/L with LDH-3: 33.6% (n.r. : 19.8%-25,7%); LDH-4: 23.84% (n.r. : 7-10.4%); CK was almost unmodified; Fibrinogen: 722 mg/dL. Verapamil 120 mg, twice a day was added to therapy. Two days later, all biochemical values regressed to normal ranges and the atrial fibrillation had resolved. Bortezomib-dexamethasone therapy was stopped and it was restarted 21 days later, with a different schedule (bortezomib was reduced to 1 mg/m^2 on days 1,8,15, and 22, with dexamethasone 20 mg on days 1,2,8,9,15,16,22,23) every 5 weeks. To date, the patient has completed the second cycle and no further cardiac, muscular or laboratory alteration has been observed. We wonder if the atrial fibrillation and the diffuse myalgia of our patient were caused by bortezomib. Maybe the bortezomib action on the ubiquitin-proteasome system, or its NF-kappaB inhibition could be the responsible. Indeed, it is known that proteasome activity reduction is associated with increased muscle cells apoptosis, while NF-kappaB plays an essential role in myocardial cytoprotection. Other rare cases of cardiac complications are reported in the English literature. Even in those cases the patients were aged > 60. While in the other cases the cardiotoxicity appeared after several cycles of bortezomib therapy, in our patient the atrial fibrillation was recorded at the second infusion of the first cycle. Furthermore, we observed the absence of cardiac and/or muscular toxicity after reducing bortezomib dose. Therefore, in the elderly, a bortezomib dose reduction could be hypothesized in order to avoid cardiotoxicity.

PU100

LYMPHONODAL AMYLOIDOSIS: CASE REPORT

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We report on a case of AL amylodosis with IgM paraproteinemia manifestating as systemic deep-sited lymphadenopathy. An asymptomatic 59 years old lady seek medical attention because of a swelled right submandibular salivary gland (SSG) and a small IgM-k MC (1 gr/dL) but no BJ proteinuria. CT scan showed multiple deep-sited (mediastinum and abdomen) enlarged lymph nodes (aroud 2 cm) with a clear enhancement effects as seen in malignant lymphoma. Hystopatology of the SSG showed heavy B-lymphocytic infiltration (CD19+/CD5+) along with a few small areas of amyloid deposition. The same B-cell clone was found in bone marrow (BM) with an interstitial infiltration pattern (20%). Amyloid deposition could not be detected in BM nor in the abdominal fat and there were not laboratory or strumental signs suggestive of amyloid involvement in any other tissues. Diagnosis of small lymphocytic-lymphoma associated with localised amyloidosis of SCG was made ad R-FluCy therapy started. After 5 courses, MC and BM infiltrate were both non more detectable but the size and number of lymphopathies were unmodified. After one year, a new developed subclavicular lymphomegaly was biopsied and showed a massive amyloid deposition and no features diagnostic of lymphoma. After four R-CVP cycles the patient was considered resistant. We tried to achieve a response with 3 cycles of R-CHOP-(Myocet), but non change in the size of lymph nodes was observed and chemotherapy was discontinued. After eight months the size and number of lymph nodes started to gradually increase. Moreover, both lacrimal glands progressively enlarged. A core biopsy of an inguinal limph node was performed and showed the presence of massive amyloid deposition. Because of discomfort in the neck and inguinal area, therapy with sulindac (200 mg tid) was started and produced pain relief and about 25% regression of lacrimal glands tumor and inguinal lymphomegalies. After four years from diagnosis the patient is asymptomatic, without signs of lymphoprolipherative diseas and/or suggestive of amyloid deposition in other tissue but lymph nodes. AL amyloidosis with IgM paraproteinemia manifesting as systemic lymphadenopathy is a rare disorder that can be overlooked if a lymph node biopsy is not performed. According to the literature, we confirm that this clinical entity pursues a rather indolent but relentless course and appropriately tailored therapy for the underlying clonal disorder is necessary.

PU101

EFFICACY OF BORTEZOMIB COMBINED WITH DEXAMETASONE AND PEGYLATED LIPOSOMAL DOXORUBICIN FOR SOFT-TISSUE RELAPSE OF MYELOMA.

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The most common neurologic complication of Multiple Myeloma are spinal cord compression due to epidural plasmacytomas or vertebral fractures and peripheral polyneuropathy due to the presence of antibodies directed against myelin structures or due to amyloid deposits The development of soft-tissue plasmacytomas has been reported in 15-20% of the patients at the time of diagnosis and in an additional 15% during the course of the disease. Bortezomib has been reported to affect myeloma cell growth by NF-kB blockade, downregulation of adhesion molecules, inhibition of angiogenesis and by inhibiting DNA repair, all of which results in a proapoptotic effect on myeloma. This drug produces significant responses in about one-third of patients with relapsed/refractory disease. Pegylated liposomal doxorubicin (PegLD) was used because it was anticipated that many patients would have had prior anthracycline-based therapy, and there was evidence that PegLD may have less cardiac toxicity. Also, the prolonged half-life (t1/2) of doxorubicin in the liposomal preparation allowed maximal overlap between the 2 agents with a convenient dosing schedule. Finally, PegLD was the only anthracycline for which there was *in vivo* data showing enhanced antitumor efficacy in combination with bortezomib. Clinical Case: a 72-year-old man was diagnosed with IgG-k stage IIIA Multiple Myeloma in April 2002. He was treated with VAD (vincristine, doxorubicin and dexametasone) chemotherapy followed by high-dose therapy intensification with melphalan 200 mg/m^2 with autologous peripheral blood stem cell rescue achieving a complete remission. He relapsed in August 2007 with increasing serum M-protein and paraparesis which progressively worsened to paraplegia. MRI of the spine showed an extradural mass at the level of D4-D6 causing cord compression and multiple bony erosions from soft tissue masses. Figure 1 - FSE T1-weighted images at D6 level before the treatment assess the presence of pathological soft tissue spreading along the paraspinal fat, and along the spinal canal, with consequent narrowing.

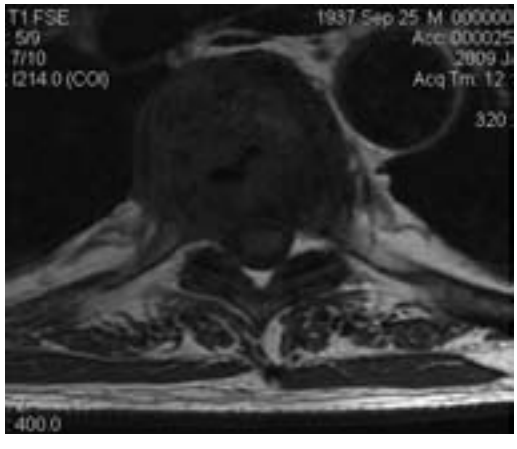

Figure 1.

A biopsy of the mass showed a diffuse infiltrate of plasma cells, with occasional atypical ones having enlarged nuclei, and multinucleated forms. Immunohistochemistry showed k light chain restriction and an

immunoglobulin G immunophenotype. Bortezomib was given at 1.3 mg/m2 (days 1, 4, 8, 11), dexamethasone at 40 mg (days 1-4) and pegylated liposomal doxorubicin (Caelix) at 30 mg/m^2 (day 4). After four cycle he achieved a second complete remission and the mass completely disappeared and had no recurrence of plasmacytoma or progression to multiple myeloma during follow up of 1 years. Figure 2 After treatment show markable reduction in the paraspinal space, and disappearance of the endocanalicular tissue. These data showed that Bortezomib, PegLD and Dexametasone is well tolerated and is active in clinical situation of extramedullary disease.

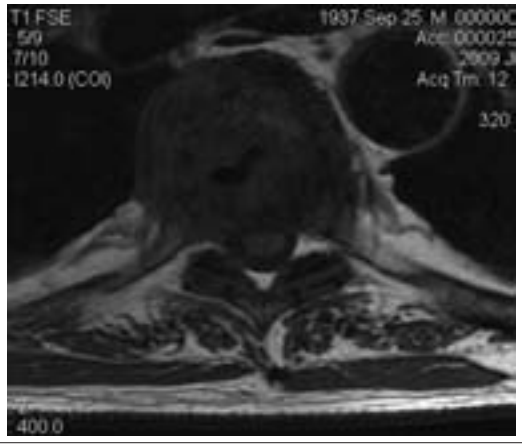

Figure 2.

PU102

RECURRENT DEEP VEIN THROMBOSIS AFTER INSERTION OF TWO VASCULAR ACCESSES IN A 13-YEARS THALASSEMIC GIRL WAITING FOR HAPLOIDENTICAL MARROW TRANSPLANT

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The increased risk of thrombosis in patients affected by β-thalassemia is well estabilished;[1] in particular, Eldor and colleagues[2] demonstrated that thalassemia carry on a chronic state of hypercoagulability, and also that platelet activation and enhanced thrombin generation exists since from childhood. Splenectomy is a biologic risk factor for thromboembolic events among thalassemic patients;[3] the most important Italian multicenter study by Borgna-Pignatti and colleagues[4] assesses the relationship between some known risk factors and thrombosis. We report here the case of a 13 years old girl, affected by β-thalassemia major, who came from Buenos Aires to our institution for a bone marrow transplant. The girl developed two consecutive episodes of vein thrombosis, after the insertion of a central, first, and peripheral vascular access, subsequently. Before these events, the patient underwent splenectomy. During the post-surgery period patient started low-weight molecular heparin (LWMH) treatment to prevent thrombosis. One month after splenectomy, a central venous access was positioned in the right subclavian vein to proceed through the pre-transplant preparation. Few days after, first episode of thrombosis of the right brachial-cephalic district appeared, coupled with the infection of the skin tunnel site of the device, nevertheless LWMH treatment; for this reason the catheter was removed. At the same time, we studied the molecular profile related to clotting factors. The patient showed a double heterozigosys mutation for the methylen-tetra-hydro-folate reductase enzyme, variant A1298C, and the prothrombin gene, variant G20210A; all the factors from second to twelfth, the lupus-like anticoagulant test and the APCR test were normal. Because of her poor peripheral vascular access and the scheduled pre-transplant treatment, we decided to insert with ultrasound-guided technique a brief-medium term peripheral catheter in the right cephalic vein. Two weeks after, in which patient was receiving only antithrombotic and antibiotic therapy, a second episode of thrombosis appeared, involving the right cephalic vein, with a totally obstruction of the blood flow; so the second catheter was promptly removed. To date, the patient is in good clinical condition, without any sign of thrombosis, confirmed by CT-scan of the upper vein district, waiting to start her transplant program. At the same time, we submitted her brother to a four day peripheral stem cells collection apheresis, after have been stimulated him with granulocyte colony stimulating factor, using a dialysis catheter located into femoral right vein; nevertheless previous studies on his blood have been negative for any thrombophilic state, several attemps have been required for the positioning of the vascular access, due to the rapid appearance of micro-clots over the catheter guide-wire. We suggest more investigations on this field, to better understand the complexity of these events in the thalassemic patients who need to be splenectomised before BMT, and also evaluate relative donors with specific blood tests to determine unexpected thrombophilic carrier conditions.

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PU103

ANEMIA IN MALE PATIENTS WITH CUSHING'S SYNDROME BEFORE AND AFTER CURE

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Glucocorticoids are known to exert a stimulatory action on white blood cells but little has been stated on modifications of red blood cells in hypercortisolemic states, i.e. Cushing's syndrome. We were able to follow blood cells parameters during the active disease phase and after surgery in a large series of patients with Cushing's syndrome. *Methods*. 84 patients with Cushing's syndrome (67 women, 17 men, age 38.9±9.98 yr) were evaluated prior to surgery and for up to 257 months (mean 47.7±2.6 months) after pituitary/adrenal surgery. *Results*. Leukocytosis (>9000/mm^3) was detected in 46% of patients with Cushing's syndrome; leukocyte counts fell promptly after remission of disease (9800±350 vs 7200±130/mm^3 in cured patients, $p<0.05$; 9280±81 vs 8050±210/mm^3 in surgical failures, N.S.) with a consistent drop in neutrophils (68.9 vs 54.5%, $p<0.05$) and slight increase in eosinophils (1.1 vs 2.4%, $p<0.05$) compared with presurgical values. Red blood cell counts were evenly distributed across the normal range in women with Cushing's syndrome (Hb 13.7±0.17 g/dL; RBC 4.5±0.65x10^6/mm^3) whereas, unexpectedly, male patients presented with low-normal hemoglobin (14.4±0.22 g/dL) and RBC (4.5±0.13x10^6/mm^3) and four patients were anemic (Hb <13 g/dL). Surgery per se was followed by a decrease in Hb levels by 1.5-2 g/dL, regardless of surgical outcome. Women cured of Cushing's syndrome rapidly restored and stabilized their Hb levels around 13.2±0.19 g/dl whereas a longer time (up to 3 years) was necessary in cured men to achieve normal RBC counts. Indeed, mean Hb levels in the middle quartiles of the normal range (15.2±0.31 g/dL) were observed on average 36 months after surgery. The recovery of RBC appeared independent of replacement therapy. In men, low RBC counts were correlated to testosterone levels (r=0.349, $p<0.05$) and possibly determined by the frequent, concomitant GH deficiency. *Conclusions*. This study highlights another unfavourable feature of men with Cushing's syndrome and shows, with a clinical approach, the important role of androgens and GH status in the regulation of erythropoiesis.

PU104

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA AND TREATMENT WITH ECULIZUMAB: EXPERIENCE OF SINGLE CENTRE

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Paroxysmal nocturnal haemoglobinuria (PNH) is a rare, chronic and life menacing blood disorder in which affected red blood cells are constantly damaged. PNH is characterized by the classic clinical triad of corpuscular hemolytic anemia, thrombophilia and cytopenia. Complement-mediated lysis of red blood cells is intravascular and it contributes significantly to the morbidity and mortality of patients. PNH is a genetic disorder caused by an acquired mutation of the PIG (phosphatidylinositol glycan) - A gene of the pluripotent hematopoetic stem cell involving a deficiency of GPI (glycosylphosphatidylinositol) - anchors and GPI-anchored proteins on the surface of affected blood cells and results in the deficient expression of CD55 and CD59 leading to excessive destruction of red cells and leukocytes. Chronic hemolysis, failures of the fibrinolytic system, increased leukocyte-derived tissue factor levels in plasma, procoagulant microparticles generated through complement-mediated damage of platelets and venous endothelium are related to the acquired hypercoagulable state. PNH is rare disease; 15.9 person for million of inhabitants are affect by its, about 8000-10000 people in North America and Europe. Allogeneic bone marrow transplantation is the only curative option in case of severe complications during the course of the diseases and it permanently abolishes the coagulation defect. A new targeted treatment strategy is the inhibition of the terminal complement cascade with a humanised mAb eculizumab (EC - marketed as Soliris®) that binds and prevents activation of complement C5 and the subsequent formation of the cytolytic membrane attack complex of complement. EC reduce complement mediated intravascular hemolysis, the need for transfusions, the risk for thromboembolic complications and improve the quality of life in patients with PNH. Although chronic treatment with eculizumab increases the risk of infections by Neisseria meningitides, the drug is usually safe and well tolerated. EC is expensive and treatment must continue indefinitely because C5 inhibition does not affect the process that underlies PNH. In our Unit we have managed 2 young "Molisani" patients, 1 male and 1 female affect by EPN and treated chronically, about for two years, with eculizumab every fortnightly. In Italy there are about 200 patients with EPN and in all probability 50 of these are in treatment with eculizumab; in our region Molise, that has a population of about 320,000 inhabitants, are only two known cases reported below-average. Our reporting is done to highlight that even if the EPN is a rare disease, it can be managed with the innovative drug eculizumab also in a peripheral Unit of Hematology . The benefit that the drug eculizumab significantly determines pays expenses that are incurred by the purchase, both in terms of hemolysis reduction, improvement of anemia, and in reducing transfusion, improvement in quality of life and reduction of hospitalization for the patient. The drug, prepared in our Hospital accordance with manufacturer's instructions, is manageable and its side effects are small. In our experience the disorder most frequently reported side, even if small claims, are headache, diarrhea, nausea and arthralgia. Both patients have undergone antimeningococcical vaccine and neither of the infections occurred. Both patients before the advent of eculizumab had high need frequency of blood transfusions and after EC treatment they have obtained the stabilization of hemoglobin to 9.5 g/dl (male patient) and 10.5 g /dl (women patient) with normalization of LDH. In conclusion the intravascular haemolysis of PNH is a consequence of deficiency of the complement inhibitory proteins decompose accelerating factor and membrane inhibitor of reactive lysis. PNH is an outstanding example of how an increased understanding of pathophysiology may directly improve the diagnosis, care, and treatment of disease. The EC is the first secure and efficient treatment for PNH, improving anemia, transfusion need, thrombotic risk and quality of life. Despite the EC is expensive and must be used indefinitely its positive effects are numerous.

PU105

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II IS CAUSED BY MUTATIONS IN THE SEC23B GENE

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CDA II, the most frequent type of congenital dyserythropoietic anemia family, is an autosomal recessive disease characterized by ineffective erythropoiesis, peripheral hemolysis, erythroblasts' morphological abnormalities and hypoglycosylation of some RBC membrane proteins. Recent studies indicated that CDA II is caused by a defect disturbing Golgi processing in erythroblasts. A linkage analysis located a candidate region spanning 5 cM on chromosome 20 termed CDAN2 locus in the majority of CDA II patients but the aberrant gene has not been so far elucidated. Recently we investigated the cytoplasmic proteome of human red blood cells (RBCs) using a combinatorial peptide ligand library as a capturing agent to amplify the signal of low- and very-low abundance proteins: 1578 proteins, most of whom unexpected, were identified allowing a deep exploration of the RBC pathways (*Roux-Dalvai, 2008*). In this study, we used a proteomic-genomic approach to identify the candidate gene for CDA II by matching data of cytoplasmic proteome of human RBC with the chromosomic localization of CDAN2 locus. On the basis of the functional properties of the 17 proteins detected in RBC proteome and codified by genes mapping on the region identified by linkage analysis, we selected SEC23B as a candidate gene for CDA II. The 20 exons and intronic flanking regions of SEC23B gene were analysed by direct sequencing in 13 CDA II patients from 10 families; 12 different mutations were detected among the 25 mutated alleles identified: six of them were missense, 2 frameshift, 1 splicing and 3 stop codon. All the missense mutations affected highly conserved aminoacids, and were not found in 100 normal alleles examined. Two of them (c.40C>T and c.325G>A) were detected in various unrelated patients. Patients' data are summarised on the table (*,° = members of the same family). SEC23B is a member of the SEC23/SEC24 family, a component of COPII coat protein complex which is involved in protein trafficking through membrane vescicles. Even if the exact function of human SEC23B is not completely clarified, abnormalities in this gene are likely to disturb ER-to Golgi trafficking affecting different glycosylation pathways and ultimately accounting for the cellular phenotype observed in CDA II.

Table 1.

Case	Sex	Origin	Hb (g/dL)	Retics (10^9/L)	Bd3 Deglycos.	Mutation	Exon	Effect
1	F	N Italy	9.7	160	yes	c.40 C>T c.428 A>CG	2 5	Arg 14 Trp Frameshift
2	F	C Italy	11.4	44	yes	c. 1821 delT ?	16	Frameshift ?
3	F	Bolivia	9.9	61	yes	c.568 C>T c.1808 C>T	5 16	Arg 190 STOP Ser 603 Leu
4	M	Rumania	9.8	102	yes	c.40 C>T c.1660 C>T	2 14	Arg 14 Trp Arg 554 STOP
5	M	S Italy	10.4	-	yes	c.325 G>A c.325 G>A	4 4	Glu 109 Lys Glu 109 Lys
6	F	C Italy	8.3	103	yes	c.40 C>T Ivs6 +1g>a	2 Ivs6	Arg 14 Trp Splicing
7	M	C Italy	9.7	100	yes	c.1489C>T c. 2101 C>T	13 18	Arg 497 Cys Arg 701 Cys
8 °	F	N Italy	9.2	121	yes	c.325 G>A c.325 G>A	4 4	Glu 109 Lys Glu 109 Lys
9 °	M	N Italy	11.3	115	yes	c.325 G>A c.325 G>A	4 4	Glu 109 Lys Glu 109 Lys
10 °	F	N Italy	11.7	63	yes	c.325 G>A c.325 G>A	4 4	Glu 109 Lys Glu 109 Lys
11	F	N Italy	11.6	104	yes	c.40 C>T c.1043 A>C	2 9	Arg 14 Trp Asp 348 Ala
12*	M	S Italy	11.9	90	yes	c.40 C>T c.649 C>T	2 6	Arg 14 Trp Arg 217 STOP
13 *	F	S Italy	7.8	61	yes	c.40 C>T c.649 C>T	2 6	Arg 14 Trp Arg 217 STOP
Ref. value			12.2-16.7	24-84				

PU106

PHOSPHOGLYCERATE KINASE DEFICIENCY: CHARACTERIZATION OF MUNCHEN, MATSUE AND KYOTO VARIANTS

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Phosphoglycerate kinase (PGK) is glycolytic enzyme that catalyzes the reversible phosphotransfer reaction from 1,3-bisphosphoglycerate (1,3-BPG) to ADP to form 3-phosphoglycerate (3-PG) and ATP. Humans have two PGK isozymes, PGK1 and PGK2, where PGK1 is ubiquitous and PGK2 is testis-specific. The PGK1 gene is located on the X-chromosome q-13.1. Mutations of the PGK1 gene result in an enzyme deficiency that is characterized by mild to severe hemolytic anemia, neurological dysfunctions and rhabdomyolysis. Patients rarely exhibit all three clinical features. To date, 20 different mutations with worldwide distribution have been described. To provide a molecular framework to the disease, we have undertaken an in-depth characterization of the PGK mutants. In this study we present the molecular charaterization of the L89P, D268N and A354P PGK mutant enzymes obtained from E. coli as recombinant proteins. The corresponding mutations c.266T>C, c.802G>A and c.1060G>C have been identified in patients with PGK deficiency. Patients bearing L89P and A354P mutant forms were affected by severe hemolytic anemia and progressive mental retardation, whereas patient with D268N did not displayed any clinical manifestations. All recombinant enzymes were purified to homogeneity. The D268N enzyme (Munchen variant) exhibited kinetic and heat stability properties similar to those of the wild-type (wt) enzyme, in agreement with the fact that the patient was not affected by the disease. The reduced erythrocyte activity found in the patient still remains intriguing. L89P and A354P mutants (Matsue and Kyoto variants, respectively) turned out to be heavily affected in their heat stability (T50, both approximately 10 °C lower than that of the wt enzyme; t1/2 at 37°C, 9 and 30 min, respectively, vs >2 h of wt). The A354P was also characterized by a Km value vs 3-PG 15 times higher than that of the wt enzyme, accounting for a catalytic efficiency 18-fold lower. These data indicate that the deficiency in PGK activity displayed by both patients is primarily due to a low level in enzyme concentration as a consequence of an intrinsic protein instability, rather than to a crucial decrease in activity. The Matsue and Kyoto variants, more easily unfolded, may became a good target for proteases, thus reducing their lifetime into red cells. Depletion of glycolytic ATP, caused by the reduced concentration of the defective enzymes, may be the reason for both neurological and hematological disorders.

PU107

NOT PUBLISHED

PU108

MITOGEN-STIMULATED DIRECT ANTIGLOBULIN TEST IN PATIENTS WITH HEREDITARY SPHEROCYTOSIS

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Hereditary spherocytosis (HS) is a common inherited haemolytic anemia due to defects in the red cell membrane proteins (band 3, spectrin, ankyrin, or protein 4.2) that cause loss of membrane surface area, reduced deformability, trapping and destruction in the spleen. Naturally occurring autoantibodies (NAbs) against band 3 are thought to be involved in the removal of senescent and damaged erythrocytes. Mitogen-stimulated-DAT (MS-DAT) is a new functional and quantitative method for the detection of anti-RBC antibodies in mitogen-stimulated whole blood cultures; the test was shown able to reveal cytokine modulation of anti-RBC antibody production in AIHA, and found positive in a fraction of B-CLL patients without clinically overt AIHA, suggesting that *in vitro* mitogen stimulation could disclose a latent anti-RBC autoimmunity in B-CLL. The aim of this study was to evaluate MS-DAT in 23 consecutive patients (12 male and 11 female) with HS: Diagnosis was made on the basis of clinical history, physical examination and the results of laboratory tests: complete blood count, blood smear examination, reticulocyte count, bilirubin concentration, red blood cell osmotic fragility tests. All patients underwent SDS-PAGE analysis of the red cell membrane proteins. MS-DAT was performed by stimulating whole blood with PHA, PMA and PWM and antibodies were detected by competitive solid phase ELISA. We found that 12 out of 23 patients displayed positive MS-DAT (427+/-113 versus 89+/-13 ng/mL RBC-bound IgG, mean+/-SE; cut off for positive values=150 IgG ng/mL. The clinical and hematological parameters were comparable in MS-DAT positive and negative cases, as well as the results of osmotic fragility tests and the type of membrane protein defect. These findings suggest that in half patients with HS mitogen-stimulation is able to induce the production of NAbs which might be responsible for immune-mediated destruction of erythrocytes, independently of the membrane structural alteration.

PU109

ACUTE SPLENIC SEQUESTRATION CRISIS (ASSC) IN SICKLE CELL SYNDROMES

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ASSC is a severe complication of Sickle Cell Syndromes (SCS). It is caused by intrasplenic trapping of red blood cells (RBC) causing a precipitous fall in haemoglobin (Hb) level and potential hypovolemic shock. ASSC may be defined by a decrease of at least 2 g/dl from the steady-state Hb concentration, evidence of increased erythropoiesis such as an elevated reticulocyte count and an acutely enlarging spleen with pain. We describe 2 cases of HbS/β Thalassemia patients observed at our Centre. Case1: a 28-year-old woman was admitted to Emergency Room for weakness, fever and abdominal pain in left hypochondrium. She referred tonsillitis in the previous weeks. Laboratory tests showed: Hb 5,7 g/dl, LDH 2472 U/l, hypertransaminasemia, positivity for IgM EBV; normal Chest X-Ray. The abdomen US showed an enlarged splenic volume (25 cm) and multiple hypoechogene areas. The patient was transfused with packed RBC and underwent vaccinations for splenectomy. Afterwards we decided to wait for splenectomy because of improving clinical and ecographic condition during the following weeks. Four months later the Hb levels returned to patient's usual value (9,5 g/dl), the splenic volume was reduced (20 cm) and no hypoechogen area was detected. Case 2: a 54-year-old man was admitted to Hospital for fever and acute abdominal pain. In the previous days he had flu-like syndrome. The abdomen US showed an increased splenic volume (17.2 cm) with an hypoechogen area extended to the superior 2/3 of the spleen, that was confirmed by CT as a haemorrhagic area. During the following days the Hb levels decreased to 8.9 g/dL. He underwent vaccinations in view of splenectomy. Nevertheless, as the clinical condition improved quickly in the next 2 weeks (increased Hb levels, reduction of splenic volume), the patient was not transfused and surgery was not performed. The pathogenesis of ASSC is not well known; it may derive from an acute obstruction of the venous flow in the spleen with consequent sequestration of RBC. ASSC is more frequent in childhood (common in SS genotype patients) but it may occur also in HbS/β Thalassemia adult patients. The treatment consists in early diagnosis, packed RBC transfusion and clinical support. A delay in diagnosis may result in hypovolemic shock and patients death. Splenectomy may be performed if transfusion therapy failed. ASSC has to be suspected in SCS in presence of acute abdominal pain. A prompt diagnosis and care prevent severe complication and death.

PU110

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)-ASSOCIATED PURE RED CELL APLASIA (PRCA) AND RITUXIMAB

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PRCA is a rare and often life threatening complication of CLL. Corticosteroids, recombinant-erythropoietin (r-Epo) and packed red cell trans-

fusions are frequently given. We report 6 patients (mean age 66 yr; range 40–78 yr) affected by acquired PRCA complicating CLL, recently seen at our Institutions. Two patients developed PRCA after rituximab, fludarabine and cyclophosphamide (R-Flu-Cy) therapy (first and fourth cycle, respectively), and treated with corticosteroids and r-Epo. Of them, one patient responded to therapy, one other died after 3 months without response. The remaining 4 patients were treated with rituximab. Five out of 6 patients were given packed red cell transfusions. Steroids and r-Epo were also administered as first-line therapy and only 1 patient showed a response. After a mean time of 57 days (range 23-62 days) from PRCA diagnosis, 4 non responding patients received rituximab at a dosage of 375 mg⁄m2⁄week for 4 consecutive weeks. First injection side effects of rituximab were minimal. All patients showed an increase in hemoglobin levels in response to rituximab, in 1 patient just after the first dose, in another patient after the second and in 2 other patients after the third dose. Three patients (75%) were considered in complete remission (CR) and one patient (25%) in partial remission 4 weeks after the last rituximab infusion, despite a CR was obtained later (16 weeks following the beginning of the therapy). At the last follow-up (mean 18.5 months, range 2-60 months), all patients were alive and in continue CR. Despite very limited in number, these results suggest that rituximab is very effective in the treatment of PRCA complicating CLL. Why in some cases rituximab have not a protective role for the onset of a PRCA in CLL (2 cases developing the disease after R-Flu-Cy) need to be better elucidated.

PU111

DIFFERENTIAL DIAGNOSIS BETWEEN HAEMATOLOGICAL MALIGNANCIES AND VISCERAL LEISHMANIASIS. A CASE REPORT

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A 51-year-old woman was admitted in our department due to flu-like symptomes, weakness, severe fever (39-40°C), night sweats and progressive pancytopenia. From familiar history, father deceased for non Hodgkin lymphoma. In the previous month, dental extraction and urinary infection resolved with levofloxacin. Physical examination revealed a slight paleness, spleen enlargement no lymphonodes, weight loss and fever. Blood analysis revealed hematocrit 29%, hemoglobin 9,7 g/dl, leucocytes 1350/mm^3 (granulocytes 260/mm^3), platelets 102000/mm^3, erythrocyte sedimentation rate of 78 mm/h, C-reactive protein 4,8 mg/dl, β2 microglobulin 4.52 mg/L and policlonal increase serum gamma-globulin with total proteins 9.5 g/dL and a small monoclonal IgG-band. Viral and rheumatic/autoimmune disease screening was negative. Chest radiograph was normal. CT scan of chest and abdomen showed only splenomegaly(spleen 16 cm). Bone marrow biopsy revealed no haematological malignancies. The bone marrow smear revealed a good deal of extracellular Leishmania amastigotes and PCR for Leishmania on bone marrow was also positive. Leishmania serum fluorescent antibodies were positive (1/1280). Western blot leishmania was positive (p 16 +). The patient was administered liposomal Amphotericin-B 3 mg/kg/day for 5 days and another 3 mg/kg eight and fifteen days later. After a month the patient is doing well, the spleen is no more palpable. Complete normalization of clinical and serum parameters occur nine month later. Visceral leishmaniasis is a severe disease associated with infection of the reticuloendothelial system by Leishmania species. The infection is acquired through female sandfly bites. The clinical picture depends on the virulence and tropism of the parasite and genetic background, immunity, nutritional status and age of the host. Visceral leishmaniasis is an important differential diagnosis among the clinical syndromes have as a feature fever, pancytopenia, and splenomegaly. An accurate travel and clinical anamnesi is therefore of paramount importance. The present patient comes from Turin/Piemont, an endemic area for canine leishmaniasis. Though visceral leishmaniasis was considered in differential diagnosis, other causes of severe fever, progressive pancytopenia, weight loss and weakness were investigated. Without treatment, the case fatality rate is high. A complete recovery is usually achieved with adequate therapy (liposomal amphotericin B, miltefosine).

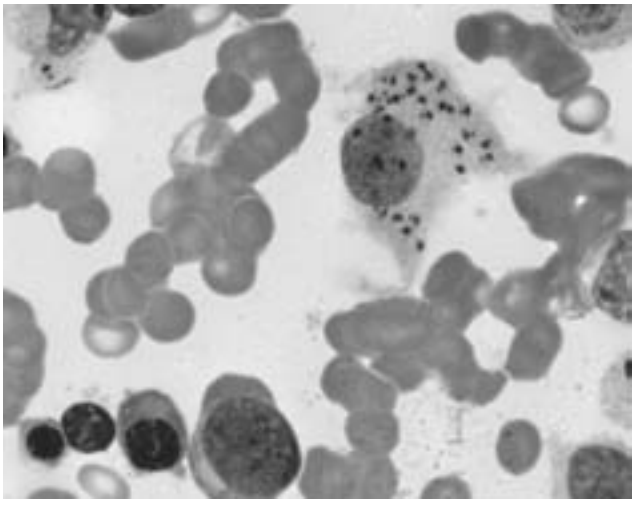

Figure.

PU112

ATYPICAL THERAPEUTIC RESPONSE TO PLASMA EXCHANGE IN A CASE OF MIXED CRIOGLOBULINEMIA HCV ASSOCIATED

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The Mixed Crioglobulinemia (MC) is a pathology characterized by the presence of serum Immunoglobulins (Ig) which are able to precipitate at a cold temperature (<37°C). Traditionally, it is classified in three types: I, II, III. Numerous studies have shown, in approximately 80% of the cases, a correlation between HCV infection and CM appearance. The therapy of the CM is performed by immunosuppressive treatments (Interferon in HCV positive patients) and plasma exchange (PE). Rituximab (Ab anti-CD20) could represent a safe and effective alternative to standard immunosoppression. AIMS. In the present study, we report a case of a patient affected by MC HCV positive with discontinuous and partial therapeutic response to the PE treatment. The PE was carried out with the following cellular separators: AS.TEC and COM.TEC FRESENIUS removing from 1 to 1,5 L of plasma/session. It was executed 3 PE/weeks for 2 weeks. A 54-year-old man was observed by our Day Hospital in the 2005 year for a suspected MC HCV correlated. The patient showed purpura, weakness, arthralgias accompanied by fever and symptoms of incipient neuropathy and nephropathy. The cryoglobulins were isolated at 4°C for 72 hours as reported. Circulating Immunecomplex (CIC), Igs and C3 serum concentrations confirmed the MC diagnosis. The first treatment of the MC was focused on eradication of HCV by combined Interferon-Ribavirin drugs. In order to stoop and quickly resolve the clinical syndrome, the PE procedures was performed as described in the methods. The patient, after an initial response to the PE treatment, began not responder in the successful PE sessions and an immunosuppressive therapy with Cortisone and Interferon was performed. After approximately a year, the patient began again the PE therapy returning to respond to the treatment. PE has been continued every periodically 2 months in the course of the successive years in order to prevent clinical neuropathy and nephropathy. The treatment with PE is still in course. The Plasma Exchange is currently the main treatment of the clinical syndrome correlated to MC HCV associated. The data of our work confirm that in the case of MC HCV + the PE therapy, even if in discontinuous way, is much efficacy in order to improve the clinical symptoms by removal the CIC and reducing the overload works of the reticulum-endothelial system.

PU113

USE OF RED BLOOD CELL TRANSFUSIONS IN ONCOHAEMATOLOGICAL PATIENT IN PALLIATIVE CARE: THE NIGUARDA CA' GRANDA HOSPITAL HOME CARE SERVICE EXPERIENCE

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The prevalence of anemia among the population in palliative care (PC) has never been described, probably because of the high costs and of the invasiveness of treatments. Anaemia can have harmful effects on physical, emotional and cognitive functions; if these are managed, the quality of life, besides dyspnoea and asthenia, will benefit. The study describes the conditions of transfusion on the oncohaematological patient afferent the HCS and assesses the costs supporting. From January 2005 to December 2007 39 patients were transfused at HCS: 21 women and 18 men (median age: 75 years). 20% of the 39 patients presented a chronic oncohematologic disorder, associated to heavy co-morbidity; 80% presented an acute oncoheamatologic disorder, with a life expectancy of 90 days. Among these, 61% were in palliative chemotherapy (oral or intravenous); 26% presented a cardiac failure, about 19% received erythropoietin, but unsuccessfully. 35% were transfused at home. The blood sample for blood count and matching was taken at home, haemoglobin and symptoms were evaluated and transfusion in hospital or at home was then organized. All the 39 patients were transfused and were all symptomatic: dyspnea and asthenia were the most frequent symptoms. The pre-transfusion Hb median was lower than 7 gr/dl, in hospital 2 units of red blood cells (RBC) were transfused in each session. The median survival has been of 7.5 months (1-30 months; 2 patients alive). The median transfusion rate was 6.8/patients (1-41) in hospital; 2.7/patients (0-40) at home. Totally, we performed 240 transfusions in hospital and 103 at home. The costs for each unit transfused in hospital were 158 per unit of RBC without buf fy coat and 50 for the staff, in out-patient transfusion, costs rised to 128 for the staf f (loaded on the competent ASL). The patient transfused in hospital with 41 units of RBC costs 8528 to the Health Ser vice, which equals the cost of thirteen-day unit hospitalization or of about 33 days in a hospice-like structure. While the study is retrospective and observational, in HCS transfusion support reliefs asthenia and improve well being feeling, with sustainable costs. Further studies will better define transfusion support guidelines in PC: haemoglobin value, life expectancy, symptoms, and costs.

PU114

IMPACT ON QUALITY OF LIFE OF ONCO-HAEMATOLOGICAL NO LONGER CURABLE PATIENTS OF AN ONCOLOGICAL HOSPITALIZATION AT HOME

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In June 2002 it was set up, within the oncological department of Molise and of directed derivation from U.O.C. of Medical Oncology of Cardarelli Hospital of Campobasso, the oncological ospedalization at home service, in order to support the onco-haematological patients no longer susceptible of curative treatments and their families, through the take over of the sick and the global solution of the problems of palliativistic medical order (therapy of the pain, emotrasfusions, nutrition, medication of the pressure injuries, metabolic resuscitation, treatment of clinical signs), generally psychological help and support to the mourning and social order. Since that date there have been assisted 619 patients, of which 82 haematological, through nearly daily nursing accesses and specialistic medical visits 2 or 3 times a week. The number of the patients suffering of haematological malignant diseases (acute and chronic leukaemias, mielodisplastic syndrome, myelomas and lymphomas) increased over time, so it was necessary to the ever more frequent, as well as palliative treatment in the strict sense, support transfusion, avoiding painful movements of the sick at the hospital. It is necessary to clarify that the choice of the U.O. about the transfusion therapy has always been to provide such support - in particular to oncohaematology patients - also in contrast with the guidelines of palliative medicine in general; this choice is motivated by the conviction that the maintenance of discrete values of haemoglobin and platelet encourages better control of symptoms. Have been carried out N. 238 transfusions of concentrated red blood cells and platelet pool and, in 45 patients, was positioned at home a central venous access (GROSHONG PICC). Patient care were also provided in full by the drugs needed, with direct service delivery of Hospital Pharmacy. It is been fully achieved the goal of humane treatment, as demonstrated by the questionnaires completed by the joint approval of the sick and their families, who daily demonstrate the indispensability of this form of care and satisfaction of being able to attend in person, along with us as part of the family home until the end, their loved ones. Objective is also the advantage of this initiative, since the average cost per day of hospitalization in O.D.O ranged in these 7 years-from 55 to 67 euros, with an obvious saving of about 450 for day compared to the hospital that would be inevitable for onco-hematological patients, at least in the critical stages of terminal illness.

PU115

COEXISTING OF POLYCITHEMYA VERA (PV) JAK2 V617F-POSITIVE AND MONOCLONAL GAMMOPATHY (MG)

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PV is a clonal disease which originates from abnormal hematopoietic progenitor cells at the pluripotent stem cell level. The somatic mutation JAK2 V617F in exon 12 of the jak2 gene, with the constitutive activation of the gene, has been identified in about 81% (range, 65–97%) of patients with PV; in addition JAK2 V617F is not associated with Multiple Myeloma as recently showed. Concomitant cases of MG with PV have been described but MG has been related to the precedent therapy for PV and JAK2 V617F was not performed. Here we report 2 cases of concomitant JAK2 V617F- PV and MG in which the MG has been diagnosed before or at the same time of PV. The first is about a 70-year old female who was referred to our institution because of elevated blood cell count and MG. Blood test showed an increased number of white blood cells (WBC) and thrombocytosis of $12x10^9$/liters and $0.7x10^{12}$/liters, respectively, and hemoglobin of 178 grams/liters. Immunoelectophoresis showed 0.8 grams/liters of IgG/kappa monoclonal protein. BJ proteinuria was negative and renal function was normal. BM aspirates was consistent with PV and showed plasmacells (PC)< 10% and eythroid iperplasia. Karyotype analysis revealed 46 XX. JAK2 V617F was identified in whole blood peripheral leucocytes with RT-PCR. PV was diagnosed according to WHO 2008. The second one is about a 67-year old male referred to our institution for MG. Blood test showed increased WBC, platelets and hemoglobin ($11x10^9$ liters, $0.6x10^{12}$/liters, 180 grams/liters respectively). He had 2 grams/liters of IgG/λ monoclonal protein. BJ proteinuria was negative and renal function was normal. BM aspirates was consistent with PV and showed PC < 10%. Two osteolitic lesions in the skull were showed on X-ray. JAK2 V617F analyzed with RT-PCR from whole blood peripheral leucocytes was positive. Both patients were treated for PV with low dose aspirin and with periodic phlebotomy to reduce hematocrit to 47%. Laboratory blood test check for MG was performed every six months; a X-ray total body every year.

Our reports suggest that the coexistence of two different diseases from the same bone marrow milieu probably takes origin from different cell clones. In literature coexistence of PV and MG has been described but it has been associated to a precedent therapy for PV. Here the two diseases were diagnosed at the same time and the presence of JAK2 V617F mutation provides a molecular basis for the identification of PV-clone.

PU116

ABSENCE OF THE V617F JAK2 MUTATION IN THE LYMPHOID COMPARTMENT IN A PATIENT WITH ESSENTIAL THROMBOCYTHEMIA AND B-CHRONIC LYMPHOCYTIC LEUKEMIA AND IN TWO RELATIVES WITH LYMPHOPROLIFERATIVE DISORDERS.

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The Ph- myeloproliferative disorders (CMPDs), are through to originate at the level of the pluripotent hematopoietic stem cell, likely involving both myeloid and lymphoid lineages. Nevertheless the coincidence of chronic myeloproliferative and lymphoproliferative diseases in the same patient is a rare phenomenon. Recently a recurrent mutation in Janus kinase (JAK2) gene in the hematopoietic cells of patients with Ph-MPDs has been reported, and 50% of patients with essential thrombocythemia (ET) has this mutation. Conflicting data have been reported on the presence of the JAK2 mutation in B or T cells and that led us to search for JAK2V617F mutation in lymphocytes in a patient having ET and B-Chronic Lymphocytic Leukemia and in two relatives with lymphoproliferative disorders (B-LLC and NHL). Peripheral blood granulocytes were separated by differential centrifugation. T cells were isolated by depletion of non-T cells. Furthermore, a combination of direct magnetic labelling steps and the combination of a depletion and positive selection approach allow to isolate B cell subsets that are not defined by a single cell surface antigen. Patients were genotyped for the JAK2V617F mutation by an allele-specific (ASO) polymerase chain reaction (PCR). In our patient with ET and B-CLL we identified homozygous JAK2 mutation in the granulocytic compartment. Both relatives were heterozygous for JAK2 mutation, whereas no mutation signal could be detected in B and T-lymphocyte populations of all three patients. Our results seem then to confirm some previously observations that CLL cases are constantly negative for JAK2V617F mutation in B and T-lymphocyte populations. In our patient analysis of JAK2V617F mutation seem to testify that lymphatic leukemic clone derives from a V617F negative stem cell and that myeloid and lymphoid lineages are totally distinct. Chance coincidence of both disorders is possible, and different mutagenic events would induce independently the lymphoid and myeloid malignant proliferation. Patient assumed hydroxyurea, and the mutagenic role of hydroxyurea remains controversial, but no increased frequency of lymphoproliferative syndrome has been reported following hydroxyurea treatment. However, evidence of JAK2 mutation in myeloid lineage in two relatives with lymphoproliferative disorders could also be a intriguing starting point to discuss relationship between lymphoproliferative disorders and CMPDs.

PU117

TREATMENT OF REFRACTORY POLYCYTHEMIA VERA BY ERYTHROCYTAPHERESIS

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The Polycythemia Vera (PV) is a familial chronic myeoproliferative disorder derived by clonal expansion of transformed stem cells, characterized by the JAK2V617F mutation. Small molecule inhibitors of JAK-STAT signalling offer potential for molecularly targeted therapy. However, therapeutic management of the PV is actually founded on phlebotomy and cytotoxic therapy. Therapeutic erythrocytapheresis is also performed in selected PV cases. In the present work we report the case of a patient affected by PV, in treatment from 2005 with Pipobroman and presenting serious collateral effects, up to interrupt the pharmacological treatment. Erythrocytapheresis (EA) was performed using cellular separator Fresenius COM.TEC, trough the program of red cells depletion. It was executed 1session/weeks for 3 weeks. A 69-year-old man was hospitalized by our Hemathology Division in the 2004 where a PV was diagnosed. After some months from the diagnosis, he developed an acute coronary syndrome which followed a block of right and left branch. At first, the patient was treated with Interferon 3 MU/3 times to week; this treatment, in consequence of the considerable collateral effects, was suspended after some months. In the January 2005, Pipobroman treatment was begun 1 mg/kg/day; this dose was reduced to 0.5 mg/kg/day after partial disease remission. The treatment was suspended in the February 2007 for the progressive decrease of the PLT (<50.000/mm^3) accompanied from a series of further collateral effects and a raising of the Hct 62%(nv: 35/55%) (not responder). The patient was then candidate to phlebotomy therapy in order to reduce the danger of a thrombosis, but this therapy was contra-indicated because of his cardiovascular problems. For these reasons, the patient was treated by erythrocytapheresis as rescue therapy in order to avoid further reduction of the PLT and haemodinamic disequilibrium. At the end of the treatments the patient not presented some collateral effect and reached a Hct value of 45% and PLT>50.000/mm^3. At present, the patient is in clinical phase of remission and submitted to hematological follow-up. The data of our work show that the EA used as a rescue therapy is a therapeutic tool of high safety and efficacy for the PV, resolving in short time the disease symptom, avoiding the PLT decrease and allowing the treatment also in an elderly subject with cardiovascular troubles.

PU118

MYELOPROLIFERATIVE DISEASE COESISTING WITH CRONIC LYMPHOCYTIC LEUKEMIA IS A RARE EVENT: THE REPORT OF THREE CASE

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Introduction. The coexistence of B-cell chronic lymphocytic leukemia (B-CLL) and solid tumor is sometimes observed.The concomitant diagnosis of B-CLL and chronic myeloproliferative disorders is on the contrary an unusual event.We report the cases of three patients, simultaneously affected by B-CLL and myeloproliferative disorders who referred to our institution between December 2002 and December 2008. *Case 1.* A 85-year-old men was diagnosed with concomitant CLL and chronic myelomonocytic leukemia (CMML). At the time of diagnosis his leukocyte count was 100.000/mmc with relevant monocytosis Important splenomegaly was detected at physical examination. Interphase FISH analysis detected a 13q14.3 deletion in lymphocytes nuclei but the abnormality was not observed in monocytes nuclei. The PCR analysis of IgH gene rearrangement showed monoclonal IgH configuration. The patient was originally treated with hydroxyurea (1.5 g/day) without hematological improvement and he died three months later because of progression of both leukemias.*Case 2*. A 51-year-old men presented at diagnosis with abnormal leukocyte count, morphological lymphocyte abnormality and a large number of circulating immature myeloid cell (no blast). Immunophenotyping performed on peripheral blood showed expansion of CD5$^+$/CD23$^+$ monoclonal B-cells. The conventional cytogenetic analysis performed on bone marrow demonstrated translocation t(9;22) with rearrangement p210 (b3a2) and an abnormal karyotype with deletion 17p13 (96%), 13p14 (20%), 11q23 (30%). PCR analysis of IgH gene revealed rearrangement. The patient was treated with tyrosine kinasis inhibitor imatinib and after six months he obtained a complete cytogenetic remission of the t(9;22) clone, with the persistence of the remaining cytogenetic abnormalities. After 3 years from diagnosis the patient is alive with CML remission and persistent asymptomatic CLL which did not required therapy. *Case 3*. A 66-year-old woman was admitted to our hospital due to splenomegaly and increased lymphocyte count (75.000/m^3) associated with relevant anemia (7 g/dl). The bone marrow biopsy showed a monoclonal B-cell infiltrate diagnostic for CLL associated with massive myelofibrosis with abnormal megacariocytes; JAK2 gene mutation was detected. The patient was treated with chlorambucil and she obtained a significant leukocyte count reduction, however no improvement in splenomegaly was observed and the patient is now waiting for experimental anti JAK2 therapy.

PU119

ANALYSIS OF CLINICAL COMPLICATIONS IN ESSENTIAL THROMBOCYTEMIA: A RETROSPECTIVE STUDY OF 74 PATIENTS

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Essential thrombocytemia (ET) is a chronic myeloproliferative disorder, characterized by an indolent clinical course. ET does not generally shorten life expectancy. As the propensity of this disorder to develop thrombohemorrhagic complications, myelofibrosis or secondary malig-

nancies, a clinical supervision of individuals with ET is important to prevent or treat complications. In this study, we retrospectively investigated long-term development of hematological and non-haematological second malignancies and thrombohemorragic complications in 74 patients (male 26/ female 48) with ET. Our working group followed 74 patients with diagnosis of ET from 1987 to 2008, with median follow-up of 72 months (range 1-144 months); median age was 70 years (range 23-90). We considered risk factors, as reported by others author, the preesistent presence of arterial hypertension, diabetes, smoking, and hypercholesterolemia. Of the 74 patients observed, we found the one cardiovascular risk factor in 24 pts (32%), two risk factors in 7 pts (9%) and three risk factors in 1 pts (1, 3%). At the diagnosis we found 7 cases (9%) of primary malignancies: 3 breast (4%), 1 prostate (1.3%), 1 skin cancer (1.3%), 1 bladder (1.3%) and 1 endometrial (1.3%). After a median follow-up of 36 months from the start of treatment (range 0-144 months), 20 patients (27%) developed a clinical complication. In 11 of 20 patients (55%) a second non-hematological malignancy was documented (2 colon, 1 prostatic, 1 pancreas, 3 endometrial, 2 bladder, 1 breast and 1 large B-cell NHL). Nine patients (45%) developed vascular complications including: ischemic stroke (n=1, 11.1%), acute myocardial infarction (n=2, 22, 2%), deep vein thrombosis of the of the lower extremities (n=5, 55, 5%). No clinical progression to myelofibrosis or leukaemia occurred. Of the 62 pts (83, 7%) who were treated with chemotherapy, 45 (72, 5%) received only hydroxyurea (HU), 3 pts (0, 48%) only anagrelide (ANA), 2 pts (0, 32%) only interferon (IF), 6 pts (0, 96%) HU followed by ANA, 6 pts (0, 96%) other combination chemotherapy.

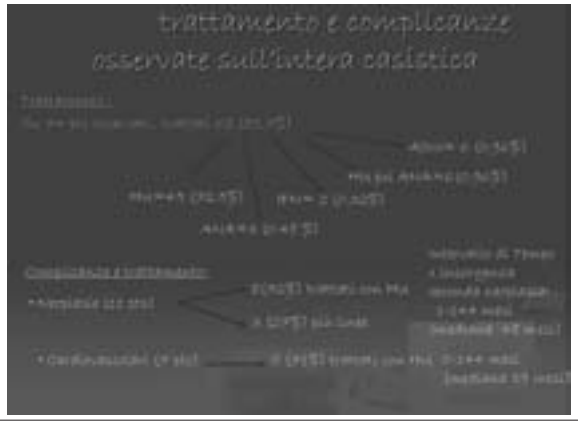

Figure.

According to the type of complications, 8/11 pts (72%) with second malignancies were treated with only HU, 3/11 pts (27%) with more than one citotoxic agent; of the 9 patients with vascular complications 5/9 pts (62%) received only HU, 3/9 more than 1 line of therapy and 1 no treatment. The findings from this study provide that ET is a real chronic and indolent disorder which affected patients having a long survival exceeding 144 months. The main risk is clinical complication, while myelofibosis and leukaemia are rare and late complications. In our group all patients who developed second malignancy or cardiovascular complication were in treatment with HU; no patients in clinical observation developed any complication.

PU120

AN INITIAL DIAGNOSTIC HYPOTHESIS NOT VALIDATED BY CLINICAL COURSE:LMMC'S BLASTIC EVOLUTION VERSUS SEPSIS

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A correct interpretation of laboratoristic data in haematological patients together with an aimed diagnostic phase are basical for reaching a precocious diagnosis to avoid serious results. We describe a case of a 66 years old woman affected by LMMC in chronic phase, coming from another hospital in coma with kidney failure, and methabolic acidosis. The patient has got fever, diarrhoea and dehydratation, with elevated values of K+ (7,2 milliequivalents/liter), creatinine (2,6 milligrams/liter), white blood cells (152000/microlitro; neutrophils 83%), anemia and lower platelets count. Antibiotic therapy and hydratation with bicarbonates were started; an abdomen TC scan had warned a very large spleen with necrosis diffuse areas and abscessualization. The initial hypothesis of blastic evolution of LMMC was ruled out and the patient underwent, even if with very high risk, a surgical operation. At laparatomy purulent peritonitis of pelvis was evident. The patient died in post-surgical phase. In haematological patients a possible quickly expanding disease must be differentiated by a sepsis, because prognosis is up to different therapy approach.

PU121

UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS ARE MORE IMMUNOCOMPETENT THAN ADIPOSE TISSUE-DERIVED ONES IN A FBS-FREE MEDIUM: AN INTEREST APPROCH TO GRAFT VERSUS HOST DISEASE

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Background. The immunosuppressive capacity of mesenchymal stromal cells (MSCs),a population of multipotent stem cells, have generated clinical interest in the field of hematopoietic stem cell transplantation in order to prevent-control graft versus host disease (GvHD). Aims In our laboratory we defined growth factors cocktails (GFC) for the expansion of MSCs isolated from adipose tissue (AT) and umbilical cord (UC) to bypass the limitations of fetal bovine serum (FBS) and bone marrow derived MSCs without influencing differentiation capacity and pluripotency.

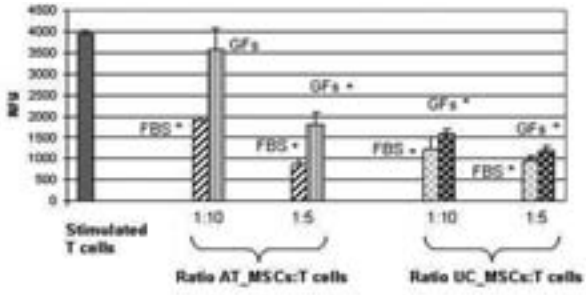

Figure 1. Immunosuppressive effect of AT and UC MSCs expanded in the two medium supplements, FBS 10% and GFs cocktails (EGF-PDGFbb for UC MSCs, EGF-PDGFbb-bFGF for AT MSCs each at 10 ng/ml). MSCs were plated in 96 well plates in diminishing concentration (20.000, 10.000, 1.000, 100, 0 cells/well) and co-cultured with lymphocytes (100.000/well) stimulated with PHA.
* p<0.001 when data are compared with T cell proliferation without MSCs.

Culture expansion conditions of MSCs may alter their fundamental biological properties. Thus, we investigated if our GFC containing epidermal growth factor and platelet-derived growth factor-bb (EGF-PDGF-bb) for UC MSCs and EGF-PDGFbb-basic fibroblast growth factor (bFGF) for AT MSCs, could influence T cell proliferation. Methods AT and UC MSCs (n=4) were expanded in DMEM supplemented with:1) FBS 10%;2) human platelet poor plasma (hPPP 3%) with growth factors (GFs) at 10 ng/ml.At the end of passage 3 MSCs were plated in 96 and 24 well plates in diminishing concentration. After 24 hours their proliferation was blocked with Mitomycin C. Human allogeneic T cells were added and stimulated with phytohemagglutinin (PHA) for 7 days. T cell proliferation was measured with BrdU Proliferation Assay. Results UC MSCs immunosuppressive effect was confirmed both in FBS and in GFs combination and was significant when MSCs:T cell ratio was 1:10 (Figure 1). AT MSCs immunomodulation was revealing at 1:5 ratio in the presence of GFC and it was less marked than in FBS,probably because GFs, inducing cell maturation,reduce immunomodulatory potential and an increased AT MSCs:T cell ratio is required.UC MSCs, having relatively primitive nature, seem to be less influenced by cytokines than mature MSCs. We did not observe a significant immunosuppression when AT/UC MSCs and lymphocytes were separated by transwell, in contrast with other published data that registered inhibition and asserted that soluble factors are involved in the effect on T cell proliferation. This suggests that the suppressive factor(s) are not constitutively secreted by MSCs and probably a pre-activation mediated by cell contact is required. *Conclusions*. UC MSCs expanded with GFs are more suitable for the treat-

ment of GvHD and immune-mediated diseases than UC MSCs in FBS.In addition, they exert an immunomodulation more remarkable than that of AT MSCs exposed to GFs.

PU122

PERIPHERAL BLOOD STEM CELLS MOBILIZATION WITH PEGFILGRASTIM OR LENOGRASTIM FOLLOWING CHEMOTERAPY IN LYMPHOMA AND MYELOMA PATIENTS: A RANDOMIZED STUDY

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High-dose chemotherapy with autologous peripheral blood progenitor cells rescue has been established in patients with myelomas and lymphomas during first-line treatment or for advanced/relapsed disease. Growth factors are routinely used, in combination to chemotherapy, to mobilize $CD34^+$ peripheral stem cells from bone marrow. *Aims.* We performed a randomized study to evaluate the $CD34^+$ cells mobilizing efficacy of pegfilgrastim versus lenograstim and the possible reduction of the poor mobilizer phenomenon in patients with lymphoprolipherative disorders. Methods. From March 2005 to November 2008, 96 patients (55 non Hodgkin Lymphoma, 16 Hodgkin Lymphoma, 25 Multiple Myeloma) were randomized to receive 6 mg pegfilgrastim on day plus 5 or daily doses of lenograstim (10 micrograms/Kilogram/day) from day 5 after mobilization chemotherapy. A total of 114 harvesting procedures was performed. No significant differences concerning clinical characteristics are tested between the two cohorts of patients. *Results.* A median of 2 apheresis (range 1-3) was performed. No difference was observed regarding the day of CD34 peak and maximal CD34 count in the peripheral blood. The median number of $CD34^+$cells collected was higher in the lenograstim [$6{,}81x10^6$/kg (range 1-32,84)] than pegfilgrastim cohort [$5x10^6$ (range 1,06-24,88); p=0.01]. A total of 86/92 patients (94%) harvested >=$2x10^6$/Kg $CD34^+$cells: forty-two (48%) and 44 (51%) patients in the lenograstim and pegfilgrastim group, respectively (p=ns). Poor mobilizers (<=$2x10^6$/Kg $CD34^+$cells) were 25 (22%): 9 (36%) in the pegfilgrastim and 16 (64%) in lenograstim group, (p=0.1). Fifty-nine patients underwent ASCT. Patients mobilized by pegfilgrastim received a lower number of $CD34^+$ cells/Kg [$3{,}9x10^6$ (range 1.04-7.3)] in comparison to lenograstim group [$5{,}98x10^6$ (range 0.88-21.3); p=0.03]. Despite the lower number of $CD34^+$ infused cells, the haematological engraftment and the day +90 immunological reconstitution were comparable to filgrastim. Thirty-nine patients (68%) experienced a febrile neutropenia (18 patients (46%) in the pegfilgrastim and 21 (54%) in the lenograstim group, not significant p). *Conclusion.* In summary, a single dose of pegfilgrastim associated to chemotherapy was effective as conventional daily lenograstim to mobilize a sufficient number of CD34+ cells to achieve a stable engraftment and immunological reconstitution after ASCT. Pegfilgrastim seems to be associated with a trend in reduction of poor mobilizers. These data are encouraging and probably optimal dosing of pegfilgrastim may improve the mobilizing capacity results.

PU123

LANGHERANS-CELL-HISTIOCYTOSIS IN AN OLD PATIENT

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An 81-year old man with progressive asthenia, weight loss, cramps and peripheral neuropathy was admitted to our Istitution. Hemogram revealed normocytic anemia (Hb: 7,7 gr/dL) and thrombocytopenia (PLT: 56.000/mm^3) and laboratory tests showed severe hypoalbuminemia with coagulation defects. A bone marrow aspirate and biopsy was performed after the exclusion of common causes of anemia. Morphologic analysis of bone marrow aspirate indicated an increased cellular density constituted by 35% of blasts with signs of differentiation through monocyto-macrophage lineage associated with histiocytic hemophagocytosis. Myeloid and erythroid series were hypoplastic whereas increased dysplastic megakaryocytopoieis was present. The bone marrow biopsy was strongly suggestive for the diagnosis of Langherans Cell Histiocytosis because a proliferation of $CD1a^+$, $CD68^+$, $S\text{-}100^{low}$, CD3neg, TdT^-, $CD34^-$ and $c\text{-}Kit^-$ cell population, frequently nested in granulomatous-like formation, was documented. Total skeleton x-ray did not show bone involvement. EMG was consistent with a bilateral sensitive-motory assonal neurophaty of medium grade. After an initial treatment of continous oral prednisone (40 mg/m^2 daily) and weekly doses of vinblastine (6 mg/m^2) no changes of the hemocytometric parameters were observed althoguh peripheral neuropathy signficantly improved.

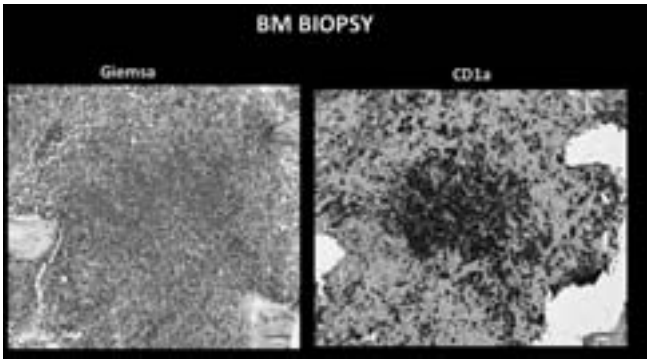

Figure.

PU124

OUTPATIENT BONE MARROW HARVEST WITH EPIDURAL ANESTHESIA

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Bone marrow harvest (BMH) has historically been performed on inpatient basis with a minimum of overnight inpatient stays. However, in the past years several authors have demonstrated the feasibility of BMH in an outpatients setting using general anesthesia. On the basis of other surgery experiences, from 2004 our Division in collaboration with the Day Surgery Unit started a program of outpatient BMH using epidural anesthesia. This choice was related to reduced complications of this kind of procedure compared with other as spinal anesthesia (headache, hemodynamic and lower limb involvement). A retrospective analysis showed that 21 patients underwent BMH with epidural anesthesia and four of them were healthy donors. All patients were medically assessed in the two weeks prior to BMH and they were AB0 Rhesus typed and cross-matched for 2 units of packed red cells. Healthy normal donors underwent autologous predeposit of two units of whole blood after stimulation with herytropoietin and iron oral administration. The procedures were carried out in the early morning. All patients underwent epidural anesthesia with high concentration of bupivacaine or levobupivacaine 0.5% (12-14 mL) in the lumbar tract L3-L4. Two operators aspirated bone marrow from both posterior iliac crests simultaneously. Multiple aspirations of 1-2 mL of bone marrow were performed using between 5-10 skin puncture sites. The harvest continued to achieve a minimum target of $2x10^8$ mononucleated cells/Kg body weight of recipient without exceeding 20 mL bone marrow/Kg body weight of patient or donor. To minimize the risks related to BMH induced hypovolemia, patients and donors were transfused with two cross-matched packed red cells or two autologous whole blood predeposit units, respectively, and received intravenous fluids. At the end of the BMH patients and donors were recovered in the Day Surgery Unit, medically reassessed, given adequate oral analgesics, if requested, and discharged after 6.00 p.m. No episodes of vomiting, headache or hypotension occurred; only one donor needed one overnight admission because of anemia BMH induced. No perioperative problems were reported and no patients were readmitted due to complications of BMH. In conclusion, in our experience, BMH using epidural anesthesia in an outpatient setting seems to be feasibility and safe, with patient and donor satisfaction and probably with a reduced cost and minor bed pressure for the clinical transplant unit.

PU125

ROLE OF HEME OXYGENASE 1 DURING *IN VITRO* OSTEOBLASTIC DIFFERENTIATION FROM HUMAN MESENCHYMAL STEM CELLS CULTURED IN HIGH GLUCOSE MEDIUM

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Bone-mineral density and other biochemical markers of bone turnover are very much affected in people with diabetes. The role of the HO system in diabetes and other pathologies is a burgeoning area of research. Human bone marrow–derived mesenchymal stem cells (MSCs) are multipotent cells that have the potential to proliferate and differentiate into a variety of cell types characteristic of bone, skeletal and cardiac muscle, adipose tissue. The progenitor cells in the presence of -glycerophosphate, dexamethasone, and ascorbic acid differentiate into osteoblastic lineages. We hypothesized that the induction of heme oxygenase (HO-1) and increased HO activity, during differentiation of Mesenchymal stem cells (MSCs) in osteoblasts, following high glucose exposure, would ameliorate the osteogenic process. Glucose concentrations used during osteoblastic differentiation corresponds to healthy individuals (5.5 mM) and to level frequently recorded in patients with hyperglycemia (30 mM). In the present study, we show a negative effects of high glucose on the osteoblastic differentiation revealed by osteoblastic markes expression such as osteocalcin, osteonectin , osteoprotegerin and by increase of ROS formation. Furthermore, mRNA adiponectin expression was found to be strongly increased during high glucose osteoblastic differentiation suggesting an antinflamatory or antioxidant properties. Using cobalt protoporphyrin IX (CoPP), an inducer of HO activity, we report here that induction of HO-1 during high glucose differentiation is associated with reduction of ROS formation and is able to ABROGATE THE NEGATIVE EFFECT OF GLUCOSE ON osteoblastic markers. In addition, HO-1 induction reduces adiponectin expression. In conclusion the overexpression of HO-1 could be useful as a protective agent against high glucose toxicity during osteoblastic differentiation and could provide a possible therapeutic strategy in metabolic diseases such as diabetes.

PU126

IMPLEMENTATION OF AN INTERNAL QUALITY CONTROL FOR MONITORING HPC-CB PRODUCTION PROCESS

Bova I., Marcuccio D., Pontari A., Pucci G., Dattola A., Garreffa C., Spiniello E., Princi D., Monteleone R., Gallo G.A., Scaramozzino P., Surace R., Iacopino P.

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Calabria Cord Blood Bank (CCBB) started its activity for umbilical cord blood (UCB) collection in January 2006. In September 2007 obtained the certificate of quality ISO 9001:2000 related to collection, manipulation and cryopreservation of CB units, reconfirmed in September 2008. CCBB has its office at the Regional Bone Marrow Transplant and Cell Therapy Center "Alberto Neri", Azienda Ospedaliera "Bianchi-Melacrino-Morelli" in Reggio Calabria (RC). CCBB bases his work on standard operative procedures in order to assure the ability to satisfy quality requirements. The security of the production process of Haematopoietic Progenitor Cells-Cord Blood (HPC-CB) units is part of the Quality's Policy, in order to guarantee clinic efficacy for the patients. Aim of our work is to define an internal quality control (QC) of cryopreserved HPC-CB units as useful tool to monitoring the production process. Materials and Methods: From June 2008 to December 2008, n° 72 UCB units have been evaluated using frozen cell aliquots as reference samples of units and destined to internal QC. We performed viability and count of total nucleated cells (TNC) and $CD34^+$ cells. In addition, hematopoietic progenitor cells cultures on Methylcellulose and sterility control were performed. The viability is determined using flow cytometric method (7AAD) and vital coloration by Trypan Blue. The determination of $CD34^+$ cells is by cytometric flow with BD FACS Calibur and monoclonal antibodies Becton Dickinson. Cell Cultures are incubated at 37° C in 5% CO2 and 95% humidity (Stem Cell Technologies Methocult H4434 e H4534). The incubation time is 14 days. The above mentioned determinations are compared with the same parameters performed on samples before cryopreservation. *Results*: Our results in internal control of frozen cell aliquots as reference samples showed high recoveries for TNC (88%) and for viability CD34+ cells (82%). Recovery of total colony-forming units (CFU) was 76%. Microbiological controls were negative. *Conclusion*: It is very important to know quality and security of cryopreserved UCB units. Quantification of viability cell recovery post thawing out represents a basic side. Performing these simple tests, it is possible to have always under control our production process of HPC-CB units.

PU127

INFLUENCE OF TYPE OF DELIVERY ON THE HEMATOPOIETIC POTENTIAL OF UMBILICAL CORD BLOOD UNITS

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Umbilical cord blood (UCB) is a source of hematopoietic progenitor cells and is used as an alternative to bone marrow or peripheral blood for treatment of several onco-hematological diseases in patients without sibling donors. It is difficult to predict the number of hematopoietic progenitor cells in each UCB before cell processing. The purpose of the present study was to investigate the correlation between many maternal/neonatal factors and hematopoietic potential of UCB units. These factors included: type of delivery, gestational age, mother's age, Apgar score after 5 minutes, neonatal weight, UCB volume, nucleated cell count/ml (NCC), $CD34^+$ cells/microlitres, number of colony-forming units (CFU) Granulocyte-Macrophage: CFU-GM; CFU Granulocyte-Erythroid-Macrophage- Megakaryocyte: CFU-GEMM; Burst-Forming Unit-Erythroid: BFU-E. We studied 221 UCB units voluntarily collected at birth centers in Calabria and arrived at Calabria Cord Blood Bank for unrelated transplantation from January at April 2009. A total of 101 UCB units were obtained from normal vaginal deliveries while the placenta is still within the uterus. The mean volume of UCB collected was 98,9 (SD +/- 56). The mean neonatal weight was 3.300 gr. (SD+/- 600). The mean gestational age was 39 (SD+/- 1,04). NCCs/ml (mean 7,7 x 10(6)+/- SD 3,4) was significantly correlated with vaginal delivery (r= 0,37 $p<$ 0,001) and with $CD34^+$cells/microlitres (r=0.32, $p<0.001$). Therefore, a significant correlation was found between the $CD34^+$ cell count/microlitres with the numbers of CFU at day 14 (r=0.59, $p<0.001$) and then, the vaginal delivery, was significantly correlated with Lymphomonocytes count (r=-0,19, $p<0.004$) of UCB unit. Our results showed that the type of delivery influences the quality of UCB unit and this is comparable with data reported from other cord blood banks.

42° Congress of the Italian Society of Hematology

Milan, Italy, October 18-21, 2009

MAIN PROGRAM

ALLOGENEIC CELLULAR GENE THERAPY IN HEMOGLOBINOPATHIES

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Thalassemia is a growing public health problem in many countries worldwide. Despite improvements in conservative treatment these patients continue to have progressive disease and treatment –related complications which cause severe morbidity and shortened life expectancy. Allogeneic cellular correction of the genetic defect through the hemopoietic stem cell transplantation (HSCT) is the only treatment able to definitively cure thalassemia. Our past experience showed that risk Classes based approach to transplantation in thalassemia led to a high disease-free survival rate. This presentation focuses on the current status of stem cell transplantation for thalassemia and in the sickle-cell anemia in a large multi-racial population. These results are of relevant interest for the treatment of these genetic hemoglobinopathies in the increasing multi-racial population of immigrants in Europe.

Since 2004 in Roma we have treated 18 Class 1, 33 Class 2 and 44 Class 3 patients aged less then 17 years with HSCT from HLA identical related donors. The patients were ethnically very heterogeneous, and the vast majority of them were not regularly transfused/chelatedand, and therefore were highly sensitized due to red blood cell transfusions without leucodepletion filters. Consequently they could have a high risk of graft failure. Therefore we have made some modification in our treatment protocols to avoid this complication. The probability of overall survival, thalassemia-free survival, rejection and transplant-related mortality were 94%, 89%, 6% and 6% in Class 1 patients, 97%, 91%, 6% and 3% in Class 2 patients and 86%, 79%, 10%, and 13% in Class 3 patients respectively. With current treatment protocols adult thalassemia patients also have encouraging results. Graft failure or rejection is one of the major obstacles to successful transplantation for thalassemia. Our current treatment protocol for second transplantation showed a higher engraftment rate and disease-free survival, 94% and 79% respectively. The major limitation of stem cell transplantation is the lack of an HLA-identical sibling donor for the majority of affected patients. In fact, approximately 30% to 40% of thalassemic patients could have a matched sibling donor. Therefore there is need to develop alternative stem cell donations. Currently high resolution HLA- typing has enabled physicians to perform transplant from unrelated volunteer donors for thalassemia with results comparable with those obtained employing an HLA-identical sibling. Patients who do not have matched family or unrelated donor could benefit from haploidentical family members, predominantly the mother in our experience. Accumulated experience has shown that stable mixed chimerism is a common event after transplant in thalassemia which could provide a rational for the use of less intensive conditioning regimens and future autologous cellular gene therapy.

The protocols used for the preparation to the transplant in thalassemia are very effective also in the other severe hemoglobinopathy, the sickle cell anemia. Today, when a thalassemic or a SCA patient has a HLA identical family member, the cellular gene therapy through the transplantation of the allogeneic hemopoietic cell should be performed. Tomorrow, hopefully, the autologous genetically corrected stem cell will break down the wall of the immunological incompatibility.

MOLECULAR BASIS OF TARGET THERAPY FOR DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease, with patients exhibiting a wide range of outcomes that are clinically predicted by the International Prognostic Index (IPI). Although advances in treatment and identification of clinical indicators have led to improved prognosis and have allowed some tailoring of therapy, a significant fraction of DLBCL patients still fail treatment and die of their disease.

Clinical models predicting DLBCL outcome do not take into account the high degree of molecular heterogeneity and the pathogenetic mechanisms of DLBCL. Knowledge of the main molecular pathways sustaining DLBCL growth are a prerequisite for a rational approach toward target therapy of DLBCL. Two main approaches have concurred to the clarification of DLBCL biology. Conventional and high throughput studies of structural genetic lesions, predominantly gene rearrangements and mutations, have identified genes that are recurrently affected in DLBCL patients. Transcriptional profiling and associated functional analyses have further increased our understanding of DLBCL, and have recognized pathological subtypes of the lymphoma with shared features and reliance upon targetable survival pathways. This knowledge has been instrumental in defining novel biological prognosticators and in devising strategies for target therapy of DLBCL. In addition to the molecular features of DLBCL tumor cells, some genetic characteristics of the host, such as the pharmacogenetic background of DLBCL patients, may be important for molecularly tailored therapies.

Therapeutic strategies targeting molecular pathways and genetic lesions of DLBCL tumor cells

Studies of the molecular pathogenesis of DLBCL have revealed a number of potential targets for rational therapeutic strategies. Molecular targets that have been exploited for rational therapeutic strategies of DLBCL in pre-clinical models or in early phase clinical trials include (Table 1): i) protein kinase Cβ; ii) the BCL6 proto-oncogene; iii) histone deacetylase; iv) the B-cell receptor (BCR) cascade; v) the NF-κB system.

Protein kinase Cβ as a therapeutic target for DLBCL

Gene expression profiling and immunohistochemistry studies have shown that protein kinase Cβ (PKCb) expression associates with poor prognosis and reduced survival in DLBCL. PKCb is a serine/threonine kinase phosphorylating the scaffolding protein CARD11 (also known as CARMA1). PKCb brings into close proximity two kinases, known as transforming growth factor-activated kinase 1 (TAK1) and Ik-inhibitor kinase (IkK). This PKCb-mediated molecular proximity renders TAK1 able to phosphorylate IkK. In turn, phosphorylation of IkK represents the initial step of the cascade activating the pro-survival NFkB pathway. PKCb is also an essential component of the VEGF signaling pathway. This fact is of note since tumor angiogenesis mediated by VEGF has been proposed to herald poor prognosis in DLBCL. The expression of PKCb in DLBCL with unfavorable prognosis and the role of the kinase in critical signaling pathways, including NFκB and VEGF-mediated tumor angiogenesis, suggested that PKCb might be a rational therapeutic target in DLBCL and prompted analysis of molecules representing possible inhibitors of the PKCb target. Enzastaurin HCl is an adenosine triphosphate-competitive, selective inhibitor of PKCβ that induces apoptosis and inhibits the proliferation of cell lines representative of different tumors, including DLBCL, glioblastoma, and colon carcinoma. Notably, in animal models, enzastaurin HCl is also able to antagonize

proliferation of tumor xenografts at low micromolar doses.

After determining doses and safety profile in a phase I study, a phase II multicenter trial of oral enzastaurin has been conducted in patients with relapsed/refractory DLBCL. Twelve of 55 patients with relapsed DLBCL (22%; 95% CI, 13% to 46%) experienced freedom from progression (FFP) for > two cycles, and eight patients remained free from progression for > four cycles (15%; 95% CI, 6% to 27%). Four patients (7%; 95% CI, 2% to 18%) continued to experience FFP 20+ to 50+ months after study entry. Overall, treatment with enzastaurin was well tolerated and associated with prolonged FFP in a small subset of patients. These pilot data prompted the development of additional multicenter phase III trials of standard induction therapies (rituximab-CHOP) with or without enzastaurin as initial therapy in patients with high intermediate/high risk DLBCL.

BCL6 as a therapeutic target for DLBCL

Chromosomal alterations affecting band 3q27 and several alternative partner chromosomes are a frequent recurrent abnormality in DLBCL. The cloning of the 3q27 chromosomal breakpoints revealed the *BCL6* gene, a transcriptional repressor containing zinc fingers, a protein sequence motif able to mediate the protein binding to specific DNA sites. *BCL6* expression is topographically restricted to the germinal center (GC), where *BCL6* is expressed by both centroblasts and centrocytes, whereas expression of BCL6 is absent in pre-GC B cells (naïve B cells) and post-GC B cells (memory B cells and plasma cells) (Figure 1). The observation that BCL6 is expressed within the GC, but not before entrance into or after exit from the GC is consistent with the fact that BCL6 is needed for GC development and sustainement, while its downregulation is necessary for further differentiation of B cells. *BCL6* determines the ability of GC B cells to tolerate their extremely high proliferation rate while undergoing DNA remodeling in the GC. In fact, BCL6 suppresses apoptotic and cell cycle arrest responses by directly suppressing TP53 transcription and by suppressing the activation of the cell cycle arrest gene p21.

BCL6 rearrangements are detectable in 35% of DLBCL. In all of *BCL6* rearrangements, the entire coding sequence of *BCL6* is juxtaposed downstream to heterologous sequences which may originate from different chromosomal sites in different patients. The common functional consequence of *BCL6* translocations is the juxtaposition of heterologous promoters to the *BCL6* coding domain, a mechanism called promoter substitution. The substitution of the *BCL6* promoter by heterologous regulatory sequences causes deregulated *BCL6* expression in lymphomas carrying *BCL6* rearrangements. Thus, *BCL6* rearrangements may prevent downregulation of *BCL-6* and, in turn, block the differentiation of GC B cells toward the stage of plasma cells. In particular, BCL6 translocations abrogate the NF-κB mediated induction of the IRF4 transcription factor that, in normal B cells, represses BCL6 expression. Another way whereby BCL6 contributes to lymphomagenesis is functional inactivation of TP53. BCL6 functions normally to suppress TP53-mediated apoptosis of GC B cells in response to DNA damage during the GC reaction. Constitutive expression of BCL6 decreases the TP53-mediated apoptotic response to DNA damage, promoting persistence of malignant clones.

The fact that BCL6 is lymphomagenic and is frequently activated in *de novo* DLBCL represent optimal prerequisites for its exploitation as a therapeutic target. Recently, a peptomimetic inhibitor of BCL6 has been shown to display potent anti-lymphoma activity both in vitro and in animal models. This peptide inhibitor, known as RI-BPI (for retroinverso BCL6 peptide inhibitor), can inhibit BCL6 biologic functions, including its transcriptional repressor activity, and selectively kills DLBCL cells associated with the BCR gene expression profile. In animal models, RI-BPI was nontoxic and nonimmunogenic even after prolonged administration, and could powerfully suppress the growth of DLBCL xenografts in a dose-dependent manner. Although RI-BPI could kill primary DLBCL cells, it had no effect on normal lymphoid tissues or other tumors, confirming the specificity of action of this compound. Rational design of a peptidomimetic inhibitor of BCL6 demonstrates that this oncoprotein is an excellent therapeutic target for anti-lymphoma therapy, and provides the basis for future clinical studies in DLBCL patients.

Targeting HDAC in DLBCL. Acetylation plays a major role in down regulating BCL6, with histone deacetylase (HDAC) being required to lift this repression. Pharmacologic inhibition of HDAC in lymphomas expressing BCL6 may lead to tonic acetylation and inhibition of this pathway. This deacetylation pathway interferes with the TP53 pathway, providing further rationale for inhibiting deacetylation in DLBCL. On these basis, several HDAC inhibitors are under investigation in DLBCL. Because HDAC inhibitors are not specific for BCL6, inhibiting HDAC in DLBCL can have a number of effects on the cell, some of which may be mediated through BCL6 modulation, and some through chromatin remodeling that is a well known effect of HADC inhibitors in general. Tonic BCR signaling as a therapeutic target for DLBCL. A subset of DLBCL have a transcriptional profile characterized by increased expression of multiple components of the BCR signaling cascade including the SYK tyrosine kinase. Recent studies highlight the role of BCR-mediated survival signals in normal B cells and B-cell lymphomas. Engagement of the BCR recruits and activates SYK and downstream pathways. Although BCR signaling is triggered by antigen binding, emerging data highlight the role of *tonic* BCR survival signals in the absence of receptor engagement.

Table 1. Molecular targets for rational therapeutic strategies of DLBCL.

Target	*Function*	*Drug(s)*	*Reference*
PKCβ	serine/threonine kinase phosphorylating the scaffolding protein CARD11	Enzastaurin	7
BCL6	Transcriptional repressor	RI-BPI	2
SYK	BCR signaling	Fostamatimib	3
NFκB pathway	regulates cell survival, cell proliferation, and cell adhesion Bortezomib, Lenalidomide, other NF-kB	inhibitors	5
HDAC	Deacetylation of histones and of BCL6	HDAC inhibitors	6

SYK plays a critical role in tonic BCR signaling, transmitting downstream events and amplifying the original signal. The activity of SYK is tightly regulated by BCR-associated phosphorylation and protein tyrosine phosphatase-mediated inhibition. These reasons prompted assessment of the role of SYK-dependent tonic BCR survival signals in primary DLBCL and DLBCL cell lines as well as evaluation of the in vitro efficacy of a competitive SYK inhibitor, R406. R406 induced apoptosis in the majority of DLBCL cell lines and primary tumors and specifically inhibited tonic BCR signaling.

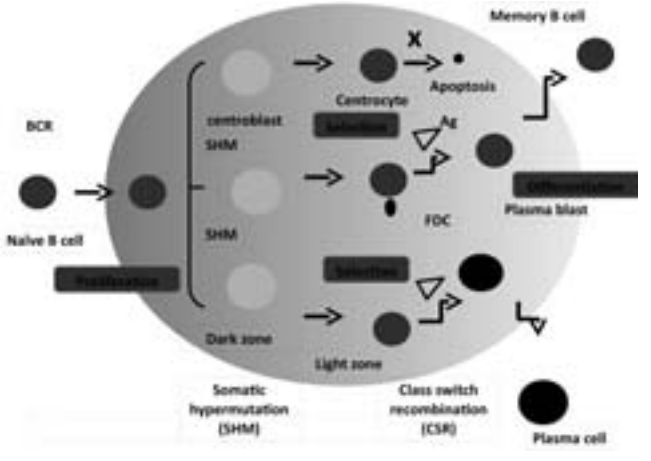

Figure 1. The germinal centre reaction. Naïve B cells differentiate into centroblasts and undergo clonal expansion in the dark zone of the germinal centre. During this process, somatic hypermutation (SHM) targets the IGV regions of the B cell receptor (BCR); some of these mutations may change the aminoacid sequence and increase, or decrease, the BCR affinity for antigen (Ag). Centroblasts subsequently differentiate into centrocytes, which reside in the germinal centre light zone. With help from T cells (not shown) and follicular dendritic cells (FDC), the mutated BCR is selected for antigen binding. Newly generated centrocytes whose mutations decreased BCR affinity for antigen undergo apoptosis and are removed. A subset of centrocytes undergoes class switch recombination. Antigen selected centrocytes eventually differentiate into memory B cells or plasma cells. Expression of BCL6 is restricted to GC centroblasts and centrocytes.

The *in vitro* studies indicated that SYK-dependent tonic BCR signaling was a targetable survival pathway in certain B-cell lymphomas, prompting further clinical investigation. A phase I/II trial of an oral version of the SYK inhibitor, R788/fostamatimib disodium (FOS D), was recently completed. The oral SYK inhibitor was evaluated in relapsed/refractory DLBCL, follicular lymphoma and additional B-cell malignancies. In this clinical trial, there was clear evidence of activity in DLBCL.

NF-κB as a potential therapeutic target for DLBCL. Sustained activity of NF-κB signaling leads to aberrant expression of NF-kB target genes, including loci involved in cell survival, cell proliferation, cell adhesion, and inflammation (Figure 2). In DLBCL, activation of the NF-kB system in tumor cells may be due to molecular lesions intrinsic to the tumor clone and affecting genes belonging to or regulating the NF-kB system. Five subunits combine into hetero- and homodimers to create the NF-kB transcription factor family (p50, p52, c-Rel, p65/RelA, and RelB). Such dimers are inactive in the cytoplasm in most normal cells, due to the interaction of NF-kB dimers with IkB inhibitors. Activation of NF-κB signaling may follow two general pathways. In the classical (or canonical) pathway IKKb phosphorylates the inhibitory subunits IκBa, IκBb, or IkBe, leading to their degradation in the proteosome. As a result, the NF-kB heterodimers p50/p65 and c-rel/p65 accumulate in the nucleus. In the alternative (or non-canonical) pathway, IKKa homodimers phosphorylate p100/NFKB2, resulting in proteasomal removal of an inhibitory C-terminal domain and generating the NF-κB p52 subunit. As a consequence, p52/RelB heterodimers preferentially accumulate in the nucleus. These two pathways, however, show much interplay and overlap: many signals activate both NF-kB pathways, many of the same cytoplasmic effector proteins are used in both pathways, and many target genes are activated by both pathways.

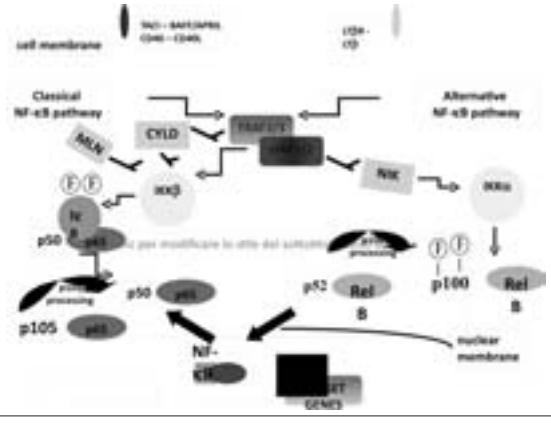

Figure 2. The NF-κB pathway and its involvement in DLBCL. Five subunits combine into hetero- and homodimers to create the NF-κB transcription factor family (p50, p52, c-Rel, p65/RelA, and RelB). Such dimers are inactive in the cytoplasm in most normal cells, due to the interaction of NF-κB dimers with IkB inhibitors. Activation of NF-κB signaling may follow two general pathways. In the classical (or canonical) pathway, IKKb phosphorylates the inhibitory IkB molecule, leading to degradation (*data not shown*) in the proteosome. As a result, the NF-κB heterodimers p50/p65 and c-rel/p65 accumulate in the nucleus. In the alternative (or non-canonical) pathway, IKKa phosphorylates p100/NFKB2, resulting in proteasomal removal of an inhibitory C-terminal domain and generating the NF-kB p52 subunit. As a consequence, the p52/RelB heterodimers preferentially accumulate in the nucleus. Following translocation to the nucleus, p50/p65, c-rel/p65 and p52/RelB activate transcription of target genes. In DLBCL, the NF-κB pathway is altered by several structural alterations, whose common effect is to activate the NF-κB cascade.

Gene expression studies have shown that the most aggressive biological type of DLBCL, i.e. activated B cell-like (ABC) DLBCL, is associated with constitutive activation of the NF-kB transcription complex. In >50% of ABC-DLBCL, NF-kB activation is due to somatic mutations in negative and/or positive regulators of NF-kB. Negative regulators of NF-kB mutated in DLBCL include the *TNFAIP3* gene, also known as A20. Positive regulators of NF-κB mutated in DLBCL include the *CARD11, TRAF2, TRAF5, MAP3K7 (TAK1),* and *TNFRSF11A* (*RANK*) genes. Of these, the A20 gene, which negatively regulates NF-κB, is most commonly affected, with 30% of patients displaying biallelic inactivation by mutations and/or deletions. When reintroduced into cell lines carrying biallelic inactivation of the gene, A20 induces apoptosis and cell growth arrest, indicating its pathogenetic role in DLBCL. Activation of NF-κB by one or more of the aforementioned genetic lesions may provide suitable targets for rational therapy by drugs interfering with NF-κB. However, available candidate inhibitors have either not been potent enough for clinical development or have multiple other likely mechanisms of action. For example, the proteasomal inhibitor, bortezomib, blocks the proteasomal degradation of the cytosolic inhibitor of κB, IkKB, but also affects many other cellular proteins and pathways. IMIDs may interfere with NF-kB among several other effects of this class of drugs. Studies of lenalidomide combined with CHOP are in progress in the context of DLBCL.

The host pharmacogenetic profile as a molecular tool to personalize therapy in DLBCL.

Beside the biology of tumor cells, also the genetic background of the host may be relevant for cancer prognostication and for tailored therapy. In particular, pharmacogenetic studies have documented that host's single nucleotide polymorphisms (SNPs) affecting genes involved in drug metabolism, detoxification, and transport are responsible, at least in part, for the inter-individual variability in efficacy and toxicity of a given pharmacologic treatment. Until recently, the impact of pharmacogenetics as a predictor of outcome and toxicity in the context of DLBCL had been poorly investigated.

R-CHOP pharmacogenetics in DLBCL

Rituximab-CHOP (R-CHOP) is the standard treatment for DLBCL. To address the potential role of the host pharmacogenetic profile in DLBCL treatment and management of toxicity, pharmacogenotyping was performed on a consecutive series of 106 newly diagnosed DLBCL treated with R-CHOP21 at our institution. This study documented that: i) host SNPs affecting alkylating agent detoxification (GSTA1--4621C>T) and doxorubicin pharmacodynamics (CYBA-4185C>T) are independent predictors of event free survival (EFS) in DLBCL treated with R-CHOP21; and ii) NCF4--368A>G, a SNP belonging to NAD(P)H-oxidase p40phox and regulating reactive oxygen species (ROS) generation, recurs as an independent protective host factor against both hematologic and non-hematologic toxicities of R-CHOP21. The independent predictive value of GSTA1--4621C>T, CYBA-4185C>T and NCF4--368A>G was validated in a prospectively collected dataset provided with a large number of tumor and host-related covariates, including IPI, organ function, comorbidities, and treatment feasibility.

The association of GSTA1--4621C>T and CYBA-4185C>T with R-CHOP21 efficacy and DLBCL outcome is biologically plausible and consistent with previous observations in settings other than lymphoma. GSTA1 encodes an alpha1 class glutathione S-transferase that catalyses the conjugation of cyclophosphamide and its active metabolites with glutathione in order to increase water solubility and facilitate excretion. The GSTA1--4621 T minor allele associates with reduced levels of GSTA1 enzyme in healthy individuals, and predicts for reduced detoxification of alkylating agents, thus increasing tumor cell exposure to the drug. DLBCL patients carrying the GSTA1--4621 CT/TT genotypes displayed a better EFS compared to DLBCL patients who carried the GSTA1--4621 CC genotype. Interestingly, in addition to DLBCL, the prognostic relevance of GSTA1--4621C>T in cancer patients treated with cyclophosphamide containing regimens is also documented in breast cancer. Conceivably, improved outcome in both DLBCL and breast cancer may be related to increased levels of cyclophosphamide derivatives mediating increased tumor cell killing.

The CYBA gene encodes the p22phox subunit of the NAD(P)H oxidase complex. Individuals carrying the CYBA-4185 T minor allele have a substantial reduction in ROS generation by NAD(P)H oxidase. Since ROS generation is one of the antitumor mechanism of doxorubicin, the CYBA-4185 T minor allele is expected to reduce the tumor cytotoxicity of doxorubicin based regimens. According to this model, DLBCL patients treated with R-CHOP21 and carrying the CYBA-4185 TT genotype displayed a poorer EFS compared to DLBCL patients who carried the CYBA-4185 CT/CC genotypes.

The genetic background of the host may add information useful for tailoring therapy when utilized in combination with IPI. In fact, in the study by Rossi et al. both GSTA1--4621C>T and CYBA-4185C>T identified a subgroup of DLBCL patients that, despite presenting with favorable IPI 0-2, eventually failed R-CHOP21. In addition, DLBCL patients presenting with both unfavorable IPI 3-5 and GSTA1--4621 CC genotype failed R-CHOP21 in virtually all cases.

In addition to outcome, the pharmacogenetic background of the host might be relevant for predicting R-CHOP toxicity. In fact, carriers of the NCF4--368 G minor allele experience less frequently hematologic, infective, and cardiac toxicity, conceivably because of reduced generation of ROS that are well known mediators of cardiac toxicity by doxorubicin and of neutrophil death upon exposure to chemotherapy. The protective effect of NCF4--368A>G appears to be independent of potentially confounding clinical variables, such as comorbidities, organ function, performance status and dose intensity.

Overall, SNPs of genes involved in the pharmacogenetics of R-CHOP may provide valuable markers for predicting failure of therapy and toxicity in DLBCL patients. To date, most of the new molecular markers identified for DLBCL have been derived from the biological characterization of the tumor clone. Based on the example of colon cancer and childhood leukemias, the results of pharmacogenetic studies in DLBCL highlight the need to improve the characterization of the host genetic background for devising strategies of molecularly tailored therapy.

Perspectives

From a clinical standpoint, understanding DLBCL biology may serve two major purposes: i) provide biologically-based prognosticators that might be both highly informative and independent of conventional predictors; ii) identify genes and molecular pathways suitable for target therapy approaches. Biological prognosticators of DLBCL are now well established, and their wide application in the clinical practice merely awaits simple and affordable technical approaches for their assessment. Target therapy of DLBCL is at an earlier stage, but significant advances have been made during the very last few years. Most studies have been focused on de novo DLBCL, although molecular targets are being currently identified also for transformed DLBCL, in particular Richter syndrome. Although target therapy is frequently looked upon as an *all problem-solving* strategy, it may not be so in such a molecularly heterogeneous disease as DLBCL has revealed to be. The genetic asset of DLBCL is far more complex than that, say, of chronic myeloid leukemia, and a prerequisite for the exploitation of target therapies in the single patient will be a detailed biological analysis of the lymphoma sample. Also, given the results achieved so far with immunochemotherapy in DLBCL, target therapy is expected to be used in combination strategies with conventional approaches, rather than be an immediate substitute for these well established modalities.

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STANDARD AND NOVEL CHEMOIMMUNOTHERAPY APPROACHES AS FIRST LINE TREATMENT TO IMPROVE THE OUTCOME IN AGGRESSIVE LYMPHOMA AT HIGH RISK

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Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma accounting for 30% of all newly diagnosed cases and more than 80% of aggressive lymphomas. The CHOP (Cyclophosphamide, Doxorubicine, Vincristine and Prednisone) regimen has been the mainstay of therapy for several decades but a marked improvement in the outcome was showed with the development of the anti-CD20 monoclonal antibody Rituximab. A first randomized study conducted by Groupe d' etude des Lymphomes de l'Adulte (GELA) reported a significant advantage of adding Rituximab to CHOP for the treatment of elderly patients with newly diagnosed DLBCL.[1] The US Intergroup and the RICOVER 60 trials evaluated R-CHOP in elderly patients, while MINT trial investigated its use in young patients with a favorable prognostic profile.[2,3] Although the combination of Rituximab with CHOP as standard regimen has led to improved outcomes, there is a group of patients that have a lower chance to be cured with standard R-CHOP. Early identification of poor-risk patients may allow for alternative treatment strategies.

Prognostic factors

Clinical and molecular models were devised to better discriminate patients with poor prognosis at presentation. The International Prognostic Index (IPI) has become the primary clinical tool to predict outcome for patients with aggressive NHL.4 Based on the number of negative prognostic features (age>60, stage III-IV, LDH level, PS >1 and more than one extranodal site), four distinct risk groups were identified, with a 5-years OS ranging from 26% to 73%. Recently, on the basis that risk assessment is a moving target, a Revised International Prognostic Index (R-IPI) was retrospectively applied to patients with DLBCL treated with R-CHOP distinguishing three separate prognostic groups with different 4-year OS rates: very good risk 94%, good risk 79% and poor risk 55%.[5]

During the last decade positron emission tomografy (18F-FDG PET) has been showed to be a powerful tool for monitoring response to therapy in most lymphomas and has been introduced into all steps of treatment.

Many studies showed that PET at the end of treatment is highly predictive of PFS and OS in aggressive lymphomas with or without residual masses detected with CT scan. PET scan is able to distinguish between viable lymphoma and necrosis or fibrosis in residual masses. The combination of International Workshop Criteria (IWC) and PET was evaluated in a retrospective analysis of 54 patients with NHL and on the basis of this study the International Harmonization Project has provided new recommendations for response criteria for aggressive malignant lymphomas, incorporating PET into the definition of response at the end of treatment.[6]

Some studies have shown that early PET scan after 2-3 cycles of chemoimmunotherapy may be predictive of the final response. Haoiun *et al.*[7] prospectively studied 90 patients with aggressive NHL treated with or without Rituximab and found a higher PFS and OS rates in patients with early PET negative compared to early PET positive (PFS: 82% *vs.* 43%; OS: 90% *vs.* 60%). However contradictory results were reported by others including our group that in 82 patients did not show differences in outcome based on early PET results (2-yr PFS: early-PET neg 84% vs early-PET pos 74%). The most crucial problem with interim PET analysis in DLBC is its low positive predictive value. Indeed, in another series, positive early PET after two courses of R-CHOP was not predictive, whereas end of treatment PET strongly correlated with the outcome.8 Some trials are currently investigating whether patients with early or mid-treatment PET positive results would benefit from early escalation to more intensive regimens or even high dose therapy with ASCT. However so far, outside clinical trials, a modification of treatment, based on early PET results, is not supported by clinical evidence. Determination of the best timing and guidelines for interpretation of interim PET are warranted. PET scan in follow up setting is still a debated issue. One of the main concern over using PET for surveillance is actually the high rate of false positive results (especially in patients with aggressive NHL), which may lead to an unnecessarily salvage therapy.

Table 1. Most recent studies in poor-prognosis DLBCL treated with (Rituximab) dose-dense chemotherapy with or without R-HDC and ASCT.

AUTHOR	TREATMENT	INCLUSION	FFS/OS	CR%	TD%
Brusamolino Haematologica 2006	RCHOP14	<71yr, stage II-IV	2yr FFS 72% OS 68%	74	2
Coso BMT 2006	RISC	<61yr, aa-IPI 2-3, stage II-IV	5yr FFS 63% OS 65%	72	3
Glass Blood 2006	MegaCHOEP	<61yr, aa-IPI 1-2-3, stage III-IV	5yr FFS 62% OS 67%	70	4.5
Intragumtornchai Leuk Lymphoma 2006	CHOP	<66yr, aaIPI2-3, stage III-IV	5yr FFS 16% OS 24%	36	8
	CHOP-ESHAP-HDT		5yr FFS 34% OS 43%	44	17
	RCHOP-ESHAP		5yr FFS 61% OS 61%	67	11
Rueda Hematol Oncol 2007	RCHOP14	<71yr, stage II-IV	30m PFS 72% OS 86%	73	1
Stewart Blood 2006	CHOP+DICEP+BEAM	<65yr, aa-IPI 2-3	4yr EFS 72% OS 79%	n.a.	1.8
Tarella Leukemia 2007	RHDS-maps	<66yr, aa-IPI 2-3, stage II-IV	4yr FFS 73% OS 76%	80	5
Arranz Eur J Haematol 2008	MegaCHOP (+/- IFE) + BEAM	18-65yr, low IPI with beta2microglobulin or Intermediate/high risk	5yr PFS 56% OS 64%	n.a.	3.5
Haioun Ann Oncol 2009	ACE + HDT+ASCT +/- R	18-60yr, aa-IPI 2-3	4yr EFS 71-80% OS 48-53%	72	4
	ACVBP + HDT+ASCT +/- R				
Vitolo Haematologica 2009	RMegaCEOP-RMAD-BEAM	<61yr, aa-IPI 2-3, stage III-IV	4yr FFS 73% OS 80%	82	5

FFS: Failure-Free Survival; OS: Overall Survival; EFS: Event-Free Survival; CR: complete remission; TD: toxic death; aa-IPI: age-adjusted International Prognostic Index; n.a. not applicable

Further studies are warranted to investigate the cost effectiveness and the benefit of using PET during the follow up phase.

Recently, the WHO classification underlines the neediness to determine the proliferation index with MIB1. Cases with MIB1 > 80-90% should be further investigated by an experienced pathologist with immunoistochemistry and cytogenetics (FISH) or molecular biology in order to detect c-myc translocation and/or other findings and to rule out typical Burkitt lymphomas or the new entities recognized by WHO classifications such as *unclassified aggressive lymphomas*, double hit lymphomas with features that are intermediate between classical Burkitt and DBCL. These patients have a dismal prognosis if treated with standard R-CHOP chemotherapy. Although the proper therapy is not well established, these cases should be recognized and referred to centers with more experience into the treatment of aggressive lymphomas for the proper treatment.

Treatment

The addition of Rituximab to CHOP21 or dose-dense CHOP14 significantly improves the overall and event-free survival compared with CHOP alone either in elderly or in young low-risk patients with DLBCL. However, patients with poor prognosis had still a chance of cure of no more than 45-55% and they should be considered for investigational approaches in the context of clinical trials designed to ensure that potentially curative therapy is not compromised.

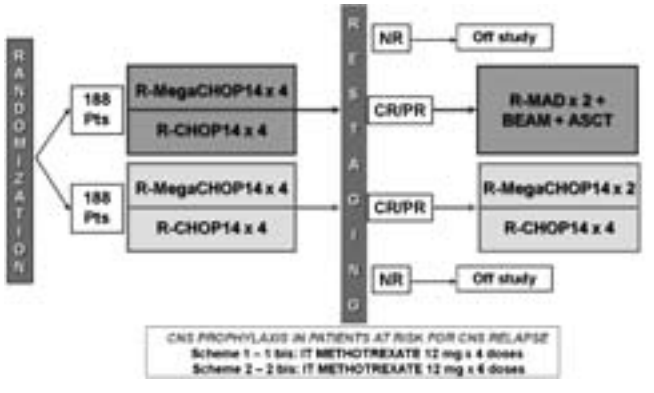

Figure 1. Phase III randomized, multicenter study in poor-prognosis (aaIPI2-3) DLBCL young patients. Dose-dense chemotherapy + Rituximab +/- intensified and HDC with ASCT.

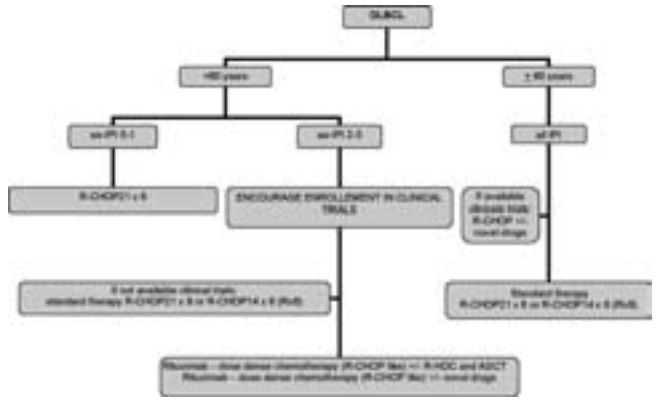

Figure 2. Management of DLBCL: first line treatment.

Young patients

Several phase II non-randomized studies incorporating Rituximab into dose-dense or dose-intense schemes, R-CHOP14-like, but without ASCT showed that such approaches are feasible and effective in high-risk young DLBCL patients. Brusamolino *et al*.[9] showed the feasibility of R-CHOP14 with pegfigrastim support in 50 patients with aggressive lymphoma at diagnosis with a relative dose intensiy of 95% and a low incidence of febrile neutropenia. The estimation of the outcome of young patients with poor prognosis treated with dose-dense or dose-intense schemes, including Rituximab, namely R-CHOP14, but without ASCT is difficult; the reported two or five-year PFS for patients with aa-IPI intermediate-high or high risk score did not exceeded 45-61% indicating that there is a place for new approaches in these patients that are unlikely to be cured by standard R-CHOP.[9-11]

High Dose Chemotherapy (HDC) and autologous stem cell transplantation (ASCT) is actually recommended in young (<65 years) patients with diffuse large B-cell lymphoma (DLBCL) without a complete response (CR) after first line chemotherapy or in patients with chemosensitive DLBCL at relapse. According to Italian Society of Haematology guidelines and others, this approach, as first line treatment should be considered in young patients with an intermediate/high or high-risk according to the age-adjusted International Prognostic Index (aa-IPI) only in approved clinical trials.[12] In the pre-Rituximab era, conflicting results were generated in randomized studies, with similar survival rates in patients receiving either first-line HDC and ASCT, or standard chemotherapy without Rituximab.[13] From June 2002 to December 2005 the Gruppo Italiano Multiregionale Linfomi e Leucemie conducted a phase II trial (registered at *http://www.clinicaltrials.gov, NCT00556127*) aimed to explore the combination of Rituximab with dose-dense chemotherapy and HDC with ASCT in 94 untreated young DLBCL patients with a poor prognosis (age-adjusted IPI score 2-3). Data were promising: complete remission was obtained in 82% of patients with a 4-years failure free survival and overall survival of 73% and 80% respectively.[14]

On this basis, the cohort treated with Rituximab and HDC with ASCT was compared retrospectively to a group of DLBCL with the same clinical characteristics treated with a similar approach of HDC, but without Rituximab. The significant benefit of adding Rituximab was shown in terms of 4-years FFS (73% R-HDC vs 44% HDC) and OS (80% R-HDC vs 54% HDC) respectively. Recently, similar results were presented in a case controlled study comparing a dose dense Rituximab-ACVBP followed by ASCT vs ACVBP and ASCT: PFS was significantly higher in R-ACVBP group.15 A summary of the most recent studies in poor-prognosis DLBCL treated with (R) dose-dense chemotherapy with or without R-HDC and ASCT is shown in Table 1.

These studies suggest that HDC supplemented with anti-CD20 antibodies and ASCT approach may be effective in young DLBCL patients with a poor prognosis. However, the issue if Rituximab-HDC may be more effective compared with Rituximab-dose-dense chemotherapy in these patients will be addressed only by randomized phase III trials that are currently ongoing into the major cooperative groups. A randomized phase II trial of the German group is now comparing dose dense R-

CHOEP14 with dose escalated R-CHOEP+ASCT. The Italian Lymphoma Intergroup is currently conducting a randomized trial (IIL-DLCL04 registered at *http://www.clinicaltrials.gov, NCT00499018*) comparing full course of Rituximab-dose dense chemotherapy (RCHOP14 or R-MegaCHOP14) alone with a brief Rituximab dose dense chemotherapy followed by Rituximab-HDC with ASCT in young patients with DLBCL at intermediate-high and high risk. (Figure 1).

Sistemic chemoimmunotherapy, also with HDC and ASCT support, does not exclude the possibility of CNS progression in patients with involvement of specific extranodal sites such as the testes, paranasal sinuses, hard palate, orbit, paravertebral masses and bone marrow or in those presenting with a high-intermediate/high IPI score, particularly reflecting the presence of a high level of LDH and involvement of more than one extranodal site. In this subset is always strongly recommended a proper CNS prophilaxis with intrathecal Metotrexate administration.[12]

Elderly patients

Two large randomized studies clearly showed that the addition of Rituximab to CHOP given every 21 days or 14 days significantly improved the outcome of elderly patients (>60 years) compared to CHOP or CHOEP (with the addition of etoposide) but without Rituximab. The former study was reported in 2002 by the GELA group and updated in 2005 in 202 patients.[1] Patients were randomized to receive 8 courses of CHOP with or without Rituximab every 21 days. The addition of Rituximab to CHOP21 significantly increased the CR rate (76% vs 63%) and reduced the risk of treatment failure and death (risk ratios 0.58 and 0.64). The results were updated in 2005 by Feugier *et al*.[16] The superiority OS rate of RCHOP was confirmed in both low and high risk aaIPI group. However the 5-yr OS rate in high risk patients did not exceed 50% even in patients treated with RCHOP (48% *vs.* 39%) . The latter study was conducted by German High Grade Non Hodgkin's Lymphoma Study Group (DSHNHL) (Ricover 60) that randomized 1222 patients to receive six or eight courses of R-CHOP14 with or without Rituximab and radiotherapy to sites of initial bulky disease.[2] Six cycles of R-CHOP14 with eight doses of Rituximab, significantly improved EFS, PFS, and OS over six cycles of CHOP-14 treatment (3-yr OS 78.1% *vs.* 67.7%). Moreover no advantages were reported with the addition of two further courses of chemotherapy suggesting that response-adapted addition of chemotherapy beyond six cycles, though widely practiced, is not justified. The comparison between these two studies is difficult because of different inclusion criteria, different use of radiotherapy, supportive therapy and so on. A formal demonstration that R-CHOP14 is superior or not to R-CHOP-21 is lacking. Randomized trials between R-CHOP14 and R-CHOP 21 are ongoing by GELA and British National Lymphoma Investigation Therefore, both R-CHOP21 and R-CHOP14 should be regarded as standard of care for elderly patients with DLBCL. The choice between them should be based mainly on the expertise of the center, performance status of the patients, co-morbidities favoring the choice of less aggressive treatment (R-CHOP21) in more advanced aged patients. Hopefully, the results of the ongoing randomized trials will possibly clarify this issue. Aimed at improving the efficacy of R-CHOP14 in elderly patients, the DSHNHL explored a "Dense" R-CHOP14 with an increased dose of Rituximab. Actually, the optimal dose of Rituximab is not yet well known. In fact Rituximab serum levels build up slowly after infusion and it may be that also dose dense immunotherapy may improve the efficacy of the treatment. *Dense-R-CHOP14* explored in elderly patients a supplemented dose intense Rituximab during the first 2 cycles of R-CHOP14, maintaining a single dose in the subsequent cycles for a total of 12 doses of Rituximab delivered in six courses of chemotherapy. One hundred patients were recruited, the results historically compared with those achieved in Ricover 60 study, showed a markedly increase in Rituximab serum level and suggested a higher efficacy in poor-risk patients (1-yr EFS 74% vs 65%) but also showed an increased incidence of infection, mainly interstitial pneumonia. This strategy is currently under investigation in a controlled randomized study.

Novel approaches

Several novel agents are undergoing evaluation in DLBCL, as both single agent in the relapsed setting or in combination with standard chemotherapy R-CHOP. These agents have varying degrees of single agent activity and some of their mechanisms are incompletely understood. These new approaches include immunomodulating agents (IMiDs) such as Lenalidomide, SGN 40, m-TOR inihibitors as Temsirolimus and Everolimus, proteasome inhibitors as Bortezomid, histone deacetylase inhibitors as Vorinostat and anti-angiogenetic agents (anti-VEGF) as bevacizumab.[17]

IMIDs inhibit angiogenesis and tumor necrosis factor (TNF)-alpha, stimulate immune responses, alter cytokines and inhibit interleukin-12, affect stromal cells, induce apoptosis and inhibit pro-survival factors (Akt). Lenalidomide was tested as single-agent in relapsed or refractory aggressive NHL: overall response rate (ORR) was 34% for all patients and 24% for DLBCL patients.[18] On this basis, the Italian Lymphoma Intergroup is running a prospective multicenter dose finding phase II pilot trial to evaluate efficacy and safety of treatment with lenalidomide plus R-CHOP21 (LR-CHOP21) for elderly patients with untreated diffuse large B-cell lymphoma (registered at *http://www.clinicaltrials.gov, NCT00907348*).

Bevacizumab was used in aggressive lymphoma as single-agent with poor results; unlike single-agent, the use in combination with standard therapy R-CHOP was promising. In a pilot trial on 13 untreated DLBCL, the association of Bevacizumab, Rituximab and CHOP (RA-CHOP) demonstrated: 85% overall response rate and a 12-month progression-free survival of 77%.[19,20] An international phase III double-blinding study (RA-CHOP versus R-CHOP) is actually ongoing (registered at *http://www.clinicaltrials.gov, NCT00486759*).

Conclusions

R-CHOP21 and/or R-CHOP14 are the standard of care for DLBCL (Figure 2 for details and indications). However the identification of poor-prognosis patients is a priority because these patients have no more than 50% chance of cure with R-CHOP. The enrollment of these patients into clinical trials aimed to test different therapeutic strategies is warranted to improve their outcome.

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NEW SALVAGE TREATMENT OPTIONS FOR RELAPSING-REFRACTORY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA PREVIOUSLY TREATED WITH CHEMO-IMMUNOTHERAPY

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Diffuse large B cell-lymphoma (DLBCL) patients who relapse or fail to achieve a CR after first-line therapy have a poor outcome. Since less than 10% of these patients obtain a long-term disease-free survival with salvage chemotherapy alone and it is well established that in a chemosensitive patient salvage chemotherapy should, whenever possible, be followed by consolidation with high dose chemotherapy (HDT) and an autologous stem cell transplant (ASCT).[1] All patients are now treated with front-line Rituximab and chemotherapy and data registry analyses have confirmed the major impact on survival of this combination for both young and elderly patients with DLBCL.[2,3] In the post-Rituximab era fewer relapses (10-20%) are seen among patients with low IPI risk (0-2) compared to the values (30-50%) recorded in the high risk IPI (>2) group.

Salvage regimens

Advances in salvage therapy are needed mainly to overcome resistance to chemotherapy, enabling more patients to achieve a complete remission (CR) in order to proceed and optimize the transplantation procedures. Various chemotherapy regimens have been employed for DLBCL in the salvage setting. The effectiveness of these regimens has been evaluated mainly in non-randomized phase II studies and their outcome is generally expressed in terms of response rate and possibility of collecting an adequate number of stem cells for an ASCT. Consequently, in the absence of randomized trials no clear superiority of a given regimen over another has been demonstrated (Table 1).

The combination of Rituximab with salvage chemotherapy regimens has significantly improved the CR rate in patients with relapsed/refractory disease. This assumption has been demonstrated in a series of 36 patients where the CR rate was significantly higher (53% vs 27% p=0.01) in the group treated with Rituximab and ICE (R-ICE) compared with an historical controls of patients treated with ICE alone.[4] Other phase II studies with various regimens have reported similar results. The more robust demonstration of the potential benefits of the addition of Rituximab to platinum-based salvage regimens has been reported by the HOVON group in a prospective randomized trial in 239 patients with relapsed/refractory DLBCL who received a salvage regimen consisting of DHAP-VIM-DHAP with or without Rituximab followed by ASCT. A marked difference in terms of CR (75% vs 54%) and EFS at 24 months (50% *vs.* 24%) was observed in favor of the Rituximab-containing salvage regimen.[5] However, the prior Rituximab use in each group of patients was not well described. While the benefits of Rituximab combined to salvage chemotherapy is clearly established, the optimal chemotherapy regimen still needs to be determined. Recently, the preliminary data of the CORAL international randomized trial, which compares R-DHAP to R-ICE followed by ASCT in 396 relapsed/refractory DLBCL after first line therapy, have been reported. The overall response rate was 63% with 38% of CR. There was no difference in terms of response rate between R-ICE (63.5%) and R-DHAP (62.8%), in mobilization-adjusted response rate (52% vs 54%) and in 3-year EFS (26% vs 35 p=0.6). Fewer serious adverse events were reported in the R-ICE regimen compared to R-DHAP.[6]

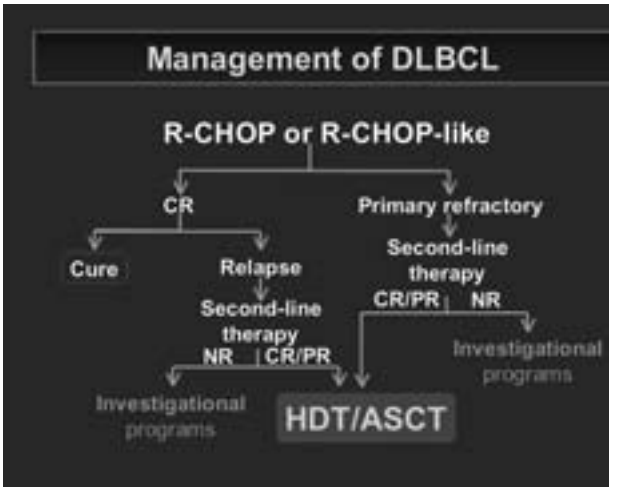

Figure 1. Treatment algorithm of DLBCL.

Table 1. Salvage Rituximab combined chemotherapy regimens for DLBCL

Regimen	N°pts	ORR/CR	ASCT performed	ASCT survival
R-ICE	36	78% / 53%	70%	67%
R-ESHAP	163	73% / 45%	65%	57%
R-ASHAP	20	75% / 45%	40%	62%
R-DHAP/VIM/DHAP +/-R	225	75% / 54%	63% vs 46%	50% vs 24%
R-ICE vs R-DHAP	396	63% / 38%	52%	49%
R-DHAOX	33	70% / 27%	40%	n.r
R-DHAP	57	70% / 20%	50%	n.r

Table 2 Outcome after RIC allogeneic transplant in relapsed DLBCL

Study	Diagnosis	N° pts	Regimen	Survival
Nagler et al	DLBCL	8	Flu,Bu, ATG	13% OS, 63% TRM
Fulkner et al	DLBCL	8	BEAM, Alemtuz	17%OS, 88% relapse
Spitzer et al	DLBCL	20	Cycl,ATG	25% EFS
Escalon et al	Aggres.NHL	20	Flu,Cyclo,Ritux	31% OS
Dean et al	NHL	28	Flu,Cyclo	49% OS 25% TRM
Robinson et al	Aggres.NHL	62	Various	47% OS 37% TRM
Corradini et al	Aggres. NHL	61	Cyclo,Thiotepa, Flu	63% OS 15% TRM

Prognostic factors at relapse

The International Prognostic Index (IPI) is a validated scoring system to predict survival rate in newly diagnosed DLBCL patients. Even in the setting of relapsed/refractory disease the IPI is predictive of OS and PFS. In the analysis of the PARMA trial, Blay et al demonstrated that an IPI score >1 at relapse correlated with a poor survival 7. In the same PARMA trial also the time to relapse, defined as less than 12 months from diagnosis, was considered a significantly poor prognostic factor 8. In the recent analysis of the CORAL trial these data were again confirmed and the EFS was significantly affected by early relapse (20% *vs.*45%, $p<0.0001$) and by a high IPI risk at relapse (18% *vs.* 40%, $p<0.0001$). Of note, the 3-year EFS was significantly affected by prior treatment with Rituximab (21% *vs.* 47%, $p<0.0001$). In the Cox model, all three parameters - early relapse, IPI at relapse and prior treatment with Rituximab - were significantly associated to response, OS, EFS and PFS, but not to the treatment arm 6. So that now the prior exposure to Rituximab should be added as a significant poor prognostic factors; however, considering that all patients should be treated with monoclonal antibodies at onset, we are now experiencing patients at relapse more refractory to any available salvage treatment.

Response evaluation and role of PET scan prior to transplant

Fluorine-18-deoxyglucose positron emission tomography (PET), when performed 6-8 weeks after the last cycle of first line chemotherapy, is able to distinguish between fibrosis and active tumor predicting the survival outcome in patients with DLBCL. Similar findings are evident in patients with DLBCL who relapsed after primary treatment. The results of a recent phase II study have indicated that a PET-positive result prior to ASCT was associated with a poor duration of response and a higher risk of relapse, particularly if the post-ASCT PET scan was still positive.[9,10] Conversely, a PET negative result prior to ASCT would predict a better duration of response. Thus, the quality of response prior to transplant is highly predictive of the outcome and PET scan should be incorporated in the definition of response.

Role of Rituximab pre- and post-transplant

In a study in which Rituximab was administered during stem cell mobilization and for consolidation of response at days 1 and 8 after transplant in relapsed/refractory DLBCL, the 2-year DFS was 67% compared with 43% in historical controls who underwent an ASCT without Rituximab (p=0.004) 11. Rituximab can also consolidate the response to transplant when given once weekly at weeks 4 to 8 after transplantation and repeated at months 6 over a 4 week period. In 21 patients with relapsed DLBCL the EFS and OS rate was 81% and 85%, respectively, at a median follow-up of 30 months 12. Since most patients with DLBCL received Rituximab as part of their first line therapy, the role of adjuvant immunotherapy after salvage therapy and ASCT should be defined more precisely in a prospective study. In the CORAL trial, all responding patients were randomized after ASCT to either Rituximab maintenance for 1 year or observation. The trial is ongoing and a longer follow-up is needed before the final analysis.

Improving the conditioning regimen

The development of radiolabelled immunotheraphies, such as 90Y-ibritumomab tiuxetan (Zevalin), has increased the targeting ability to deliver therapeutic doses of radiation to the tumor sites. Recently, in various studies 90Y-ibritumomab tiuxetan has shown a promising role as a component of the conditioning therapy to augment standard chemotherapy regimens (immunotherapy-enhanced conditioning), but also as single conditioning agent extending the option to a wide range of patients. These studies have also demonstrated that the use of 90Y-ibritumomab tiuxetan in combination with HDT has no negative effect on engraftment and has a toxicity profile similar to conventional regimens.[12,13] Future phase II or III studies are now needed to confirm the role of 90Y-ibritumomab tiuxetan (Zevalin) in combination with HDT (Z-BEAM) or alone prior to ASCT in different subgroups of relapsed/refractory DLBCL patients with poor prognosis risk.

Allogeneic SCT in relapsed DLBCL

The use of allogeneic SCT for relapsed/refractory DLBCL is still limited considering the reasonable good outcome and low toxicity of HDT and ASCT. Most patients who are referred for stem cell allografting have generally refractory disease, or disease that has failed a prior ASCT, or are precluded from stem cell autografting because of difficulties with stem cell collection or bone marrow infiltration. Comparative studies have shown a lower relapse rate and a longer DFS after allogeneic SCT than after ASCT.[14–16] However, the high transplant-related mortality (TRM) often offsets any potential survival benefit. The response to DLI lends credence to the existence of a graft-versus lymphoma (GVL) effect. In an effort to reduce the TRM of allogeneic SCT several groups have reported some preliminary encouraging results with reduced–intensity conditioning (RIC) regimens (Table 2). A recent study has reported the outcome of 48 relapsed/refractory DLBCL treated with a RIC transplant 17. The great majority of patients (69%) had a prior ASCT and 17% resulted chemorefractory at the time of transplant. All patients engrafted and only 13% experienced an extensive chronic graft-versus-host disease (GVHD). The TRM at four years was 32% with a relapse risk of 33%, giving a 4-years PFS and OS of 55% and 54%, respectively. Prior chemorefractory patients had a very poor outcome. On the basis of these data, a RIC transplantation should be considered in future prospective trials for patients with poor prognosis risk who have a chemotherapy sensitive relapse. However, if an allogeneic transplant is considered for young patients with high risk features and good performance status it should be performed in the context of a clinical study.

Salvage treatment strategies without transplant

Because of age or comorbidities not all patients are eligible for a transplant. We can assume that about 40-60% of elderly patients with DLBCL treated with R-CHOP will be refractory or experience a relapse disease during their clinical course. Effective and less toxic chemotherapy approaches are still therefore needed. New chemotherapy drugs or biological agents both alone or in combination are also worth considering for this specific and broad group of patients. More recently, gemcitabine and oxaliplatin have been shown activity in relapsed or refractory DLBCL. In a single arm study involving 46 patients with relapsed DLBCL who received Rituximab, Gemcitabine and Oxaliplatin in combination (R-GEMOX), 38 patients (83%) responded and 23 (50%) were in CR by the end of treatment. At a median follow-up of 27 months the 2-year OS was 66% with a median duration of response of 18 months 18.

New biological drugs with different mechanisms of action should be encouraged in this unfavorable and frail group of patients. Among these, Lenalidomide has been evaluated as single agent in a series of 73 relapsed/refractory DLBCL patients with an overall response rate of 29%. Toxicity was mild and mainly hematological.[19] In another series of 49 heavily pretreated relapse/refractory DLBCL patients treated with Lenalidomide 25 mg daily an objective response was observed in 35% of cases, including 12% of CR. The most common grade 3 adverse events were neutropenia and thrombocytopenia.[20]

Enzastaurin is a selective inhibitor of the PKC and protein kinase 3 (AKT) pathways known to promote tumor angiogenesis, as well as tumor-cell survival and proliferation.[21] A phase II trial with oral Enzastaurin plus GEMOX is ongoing. Oral Enzastaurin is also being tested in DLBCL patients as maintenance after the end of first-line chemotherapy. New monoclonal antibodies - new generation anti-CD20, anti-CD22 or anti-CD22 conjugated with calicheamycin (CMC-544) - are currently being investigated to assess their activity in DLBCL. In the near future, the combination of these new biological drugs together with conventional chemotherapy should be considered for the first line management of elderly DLBCL patients in an attempt to reduce the high incidence of relapses.

Conclusions

Autologous stem cell transplantation represents today the standard care for patients with relapsed/refractory DLBCL. Most salvage regimens are giving the same degree of response and there is no difference between the two most widely used salvage treatment modalities (R-ICE and R-DHAP). The addition of Rituximab to salvage regimens enhances the response rate. Patients who relapse after a Rituximab-based combination as first-line treatment have a poor prognosis. The quality of response, evaluated by PET scan before transplant, is highly predictive of the outcome. Patients with early relapse and/or with residual disease on functional imaging prior to ASCT should be considered as poor prognosis relapse/refractory and enrolled in prospective trials

aimed at evaluating intensified conditioning regimen or allogeneic SCT. The clinical use of targeted drugs for relapsed/refractory DLBCL patients is evolving and the possibility and efficacy of incorporating these new agents into the current treatment strategies needs to be defined. Finally, the identification of unknown biologic markers through newly developed technologies could improve our understanding of the mechanisms underlying the disease and foster the development of innovative and targeted therapeutic strategies.

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INCIDENCE AND SUSCEPTIBILITY TO THERAPY-RELATED MYELODYSPLASTIC SYNDROME/ACUTE MYELOID LEUKEMIA (T-MDS/AML)

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Therapy-related-MDS/AML is a well recognized disease arising after treatment with chemotherapy, radiation therapy, immunosuppressive agents, such as azathioprine, or after documented exposure to environmental carcinogens, such as benzene. It is clearly defined and separated from other secondary leukemias following a previous haematological disease, such as a myelodysplastic syndrome or a chronic myeloproliferative disorder, where AML may represent the natural evolution of the disease (strictly defined as *secondary leukemias*). In 2001, the World Health Organization (WHO) recognized therapy-related MDS/AML as a distinct entity, and this has been confirmed by the 2008 WHO classification where *therapy-related myeloid neoplasms* are included among *Acute myeloid leukemias and related neoplasms* (*Vardiman et al. Blood 2009*). Although MDS and AML occasionally occur in cancer survivors treated with surgery only, suggesting a possible predisposition to malignant diseases, the inducing role of chemotherapy is proven by the occurrence of therapy-related leukemia in patients with non-malignant disorders who received cytotoxic treatment.

The first classifications of t-MDS/AML distinguished two well-defined groups depending on whether the patient had received alkylating agents (melphalan, cyclophosphamide, nitrogen mustard, etc.), radiotherapy, or drugs binding to the enzyme DNA-topoisomerase II (epipodophyllotoxins and anthracyclins) as treatment for the primary malignancy. More recently, since most cancer patients receive combination therapies, this division is less actual (*Vardimar et al. Blood 2009*).

Incidence of t-MDS/AML

Recent papers, reporting on longer follow-up of high number of patients treated with new cytotoxic drugs and combinations with radiotherapy and growth factors, have more precisely defined the incidence of secondary malignancies among long-term cancer survivors (Table 1 shows incidence in t-MDS/AML, according to recent reports on over 100 patients). The most frequent primary malignancies associated to t-MDS/AML are lymphoproliferative diseases, ovary, breast and testicular cancer in adults, acute lymphoblastic leukemias and central nervous system tumors in children (*Leone et al, Hematologica 2007*).

Up to 10% of patients with a lymphoproliferative disorder treated with conventional chemotherapy and especially high-dose therapy and autologous stem-cell transplantation (SCT) may develop t-MDS/AML within 10 years following primary therapy. In patients with Hodgkin lymphoma (HL), the risk of t-MDS/AML ranges between 1% and 10%, depending the type of therapy administered, the study population size, and the follow-up duration. A higher long-term leukemia risk has been observed in patients treated with the MOPP regimen, when compared to ABVD. Delwail et al. reported a significantly higher 15-year leukemia risk for the MOPP regimen (3.4%) versus ABVD (1.3%) followed by high-dose irradiation, in 761 patients treated between 1972 and 1998, (*Br J Hematol, 2002*). For the MOPP subgroup, the risk of leukaemia increased to 13.9% for extended irradiation. The lower incidence of t-AML for ABVD, has been confirmed by the long-term follow-up of 120 patients with non-bulky stage I-IIA Hodgkin lymphoma, treated with 4 cycles of ABVD and limited radiotherapy, where no cases of secondary leukemias have been observed (*Brusamolino et al, Cl Can Res 2006*). A significantly

Table 1 Incidence of t-MDS/AML according to primary cancer (Publications from year 2000).

Reference (year)	No. of pts	Therapy	Cases of t- AML	Cumulat. Risk (%)	Time to AML	Median Follow-up	Cases of solid tumors
Hodgkin lymphoma	373	ABVD + Radiotp	4	1.3	15 yrs		
Delwail *et al.* (2002)	462	MOPP + Radiotp	9	3.4			
Josting *et al.* (2003)	677	RT	4	0.6	12.5 mths	4.5 yrs	127 (lung: 30,
	1775	COPP+ABVD	15	0.8	(0-128)		GI: 26
	304	ABVD	1	0.3			Breast: 13)
	550	BEACOPP baseline	2	0.4			RR 2% at 6 yrs
	460	BEACOPP escal.	8	1.7			
Brusamolino et al (2006)	118	ABVD (4-6 cy) + Radiotp	0	0	–	10 yrs	6 (5/6 in irradiated field)
Non-Hodgkin lymphoma							
Andre' *et al.* (2004)	2837	ACVBP	11		40 mths		64 (exc-risk lung)
Mudie *et al.* (2006)	1219	CHOP	17	1.5		15 yrs	106 (exc-risk lung)
	752	Chlorambucil					
Sacchi *et al.*(2008)	154	Alkylating (Alk)	12	2.1	25 mths	5 yrs	27 (lung: 8, GI:7)
	204	Alk + Anthracyclin					
	205	Alk+Anthr+Fludara					
Leleu *et al.*(2009)	193	Fludara/2-CDA	3	1.6	60 mths	5 yrs	
	136	Non- NA	0	0			
Gyan *et al.*(2009)	80	CHOP	1			10 yrs (estimated)	0
	86	HDT (TBI-Cy)	6	8			5
Acute lymphoblastic leukemia							
Pui *et al.*(2005)	827	Protocols XI-XIV	22	5.1	64 mths	10 yrs	18 (CNS: 14)
Breast/Testicular Cancer	7110	Epirubicin regimens	28	0.55			
Praga *et al.*(2005)	1427	CMF	1	0.07		8 yrs	
	903	Hormone therapy	1	0.11			
Howard *et al.* (2008)	10007	Surgery only	10	1.6		Over 1 yr	
	13682	Radiotherapy	39	2.7			
	5283	Chemotherapy	8	3.9			
	842	Chemo+Radiotp	2	4.5			

increased t-MDS/AML risk has been also associated with the escalated-BEACOPP regimen (*Josting et al, JCO 2003*). In 5411 patients treated between 1981 and 1998 with 4 different schedules there were 36 t-AML and 10 t-MDS, with an overall incidence of 0.85%, rising to 1.7% for escalated BEACOPP versus 0.4% for the baseline BEACOPP regimen.

In patients with indolent non Hodgkin's lymphoma (NHL), an increased risk of secondary malignancies including t-MDS/AML has been reported, in particular when fludarabine-containing regimens or SCT are used. At a median follow-up of 62 months, the cumulative incidence of secondary cancer at 12 years was 10.5 in 563 italian patients with indolent non-Hodgkin's lymphoma treated with different regimens, including SCT. Significant risk factors were older age at the time of diagnosis, male sex, and fludarabine-containing therapy (*Sacchi, Hematologica 2008*). Similarly, an increased incidence of secondary malignancies has been reported for nucleoside analogs (NA, fludarabine and 2-chlorodeoxyadenosine) in Waldenström macroglobulinemia (WM). At a median follow-up of 5 years, of 193 WM patients treated with NA, 12 patients (6.2%) developed disease transformation (n = 9; 4.7%) or t-MDS/AML (n = 3; 1.6%), compared with one patient (0.4%) who did not receive NA and none of the untreated patients. (*Leleu et al JCO 2009*).

A British study reported 123 second malignancies in 2456 NHL patients (mostly follicular and diffuse large cell lymphoma), treated between 1973 and 2000 (*Mudie et al, J Clin Oncol 2006*). The main secondary cancer sites were lung, colon-rectum, and breast, but 17 acute leukemias were also reported, with a relative risk of 10.5. A significantly increased risk of leukemia was associated to chemotherapy, combined or not with radiotherapy, while no cases of leukemia were observed in patients treated with radiotherapy alone. The relative risk of leukemia was 14.2 (95%CI, 6.8 to 26.2) for CHOP and 19.2 (95% CI, 9.6 to 34.3) for chlorambucil-treated patients. The relative risk was similar in aggressive and indolent NHL, and diminished with increasing age at the time of first treatment. At a median follow-up of 74 months, the GELA group reported 81 secondary malignancies in 2837 patients with aggressive NHL treated between 1984 and 1998 with the ACVBD (adriamycin, bleomycin, vindesine, cyclophasphamide and prednisone) regimen, with a a statistically increased risk of t-MDS/AML (Andre et al, Blood 2004).

High-dose therapy and autologous stem cell transplantation (SCT) are associated to the highest t-MDS/AML risk in NHL. In 2739 patients (955 HD and 1784 NHL) treated between 1989 and 2005, 56 t-MDS/AML were reported in a multicenter case-control study, corresponding to 3.7% 7-year risk (3.3% for HD and 3.9% for NHL) (Metayer, Blood 2003). Similarly, at a 9-year follow-up of 172 patients with untreated follicular lymphoma (FL) randomized to receive SCT vs chemotherapy, there was a significantly increased risk of secondary malignancies associated to SCT (6 t-MDS/AML and 6 second solid tumor vs 1 t-AML, p=0.01). Although there is a PFS advantage, the increased rate of secondary malignancies may discourage the use of purged ASCT in combination with TBI as first-line treatment for FL (*Gyan et al, Blood 2009*). The incidence of t-MDS/AML after SCT is related to the type of conditioning regimen, mostly associated to TBI, but also the type of previous chemotherapy, its effects on harvested hematopoietic stem cells and the use of growth factors. The development of t-MDS/AML after SCT has been shown to be associated with and preceeded by markedly altered telomere dynamics in hematopoietic cells, which may reflect increased clonal proliferation and/or altered telomere regulation in premalignant cells. Genetic instability associated with shortened telomeres may contribute to leukemic transformation in t-MDS/AML (*Chakraborty et al., J Clin Oncol 2009*).

Secondary carcinogenesis remains a major late complication in patients with acute lymphoblastic leukemia (ALL), particularly in children. A retrospective study recently analyzed the cumulative incidence

of secondary neoplasms after childhood ALL over 30 years (Hijiva et al, JAMA 2007). Secondary neoplasms developed as the first event in 123 of 2169 patients and included 46 myeloid malignancies, 3 lymphomas, 22 brain tumors and 16 meningiomas. The cumulative incidence of secondary neoplasm was 4.17% (SE, 0.46%) at 15 years and increased substantially after 20 years, reaching 10.85% (SE, 1.27%) at 30 years. The risk of t-AML is higher in ALL children who receive a high cumulative dose and prolonged epipodophyllotoxin therapy in weekly or bi-weekly schedules, with short-term use of G-CSF and central nervous system irradiation as additive risk factors. It has been shown to range from 2.7% in patients who did not receive G-CSF or radiotherapy to 11% and 12.3% in patients treated with G-CSF or radiotherapy, respectively (*Relling et al, Blood 2003*).

When looking at solid tumors, an increased risk of t-MDS/AML has been reported in breast cancer patients treated with chemo-radiotherapy. Praga et al. analyzed 9796 breast cancer patients treated in 19 randomized trials. The cumulative 8-year risk of t-AML was 0.55%, but there was a wide variability between patients treated with standard or high cumulative doses of epirubicin (0.37% *vs.* 4.97%, respectively) (*Praga et al, J. Clin Oncol 2005*). In a large French case-control study, the risk of t-MDS/AML in women treated for breast cancer was higher with mitoxantrone-based chemotherapy, than with anthracycline-based chemotherapy and was further increased by the addition of G-CSF (*Le Deley et al, J Clin Oncol 2007*). These data were confirmed also by other studies, stressing the need for monitoring the long-term effects of G-CSF support. In multiple sclerosis, acute promyelocytic leukemia has been reported following mitoxantrone therapy. The specific promyelocytic leukemia-inducing activity of mitoxantrone lies on the induction of preferential DNA damage sites by mitoxantrone in PML and RARA. Breakpoints in 5 mitoxantrone-treated patients fell within an 8-bp region corresponding to the *hotspot* reported in mitoxantrone-induced APL in breast cancer (*Hasan et al, Blood 2008*).

Susceptibility to t-MDS/AML

Since only a small fraction of patients exposed to cytotoxic therapy develop t-MDS/AML, specific predisposing features and the pathogenesis of leukemia remain elusive. It is unlikely that the development of t-MDS/t-AML is a stochastic event, occurring by chance. A heritable predisposition, including DNA-repair defects or altered drug metabolism due to enzymatic polymorphisms, has been hypothesized. This has been particularly relevant for t-MDS/AML with abnormalities of chromosomes 5 and/or 7, where susceptibility factors are similar, and different from radiotherapy-induced AML. A specific susceptibility seems not only associated to the treatment, but also to the primary disease, like shown for APL in breast cancer and multiple sclerosis treated with mitoxantrone (*Hasan et al, Blood 2008*).

Drug or xenobiotic metabolizing enzymes (DME) play central roles in the metabolism, biotransformation, and detoxification of xenobiotics and foreign compounds. Phase I DME primarily consist of the cytochrome P450 (CYP) superfamily, while the Phase II drug metabolizing or conjugating enzymes consist of many superfamilies of enzymes, including sulfotransferases, glucuronosyltransferases, NAD(P)H:quinone oxidoreductase (NQO), epoxide hydrolases (EPH), glutathione S-transferases (GST) and N-acetyltransferases (NAT). Conjugation by Phase II DME increases hydrophilicity and enhances excretion of the compunds in the bile and/or the urine, and consequently affects detoxification and ultimately elimination of many drugs and xenobiotics. Under certain situations, conjugation with Phase II enzymes can result in activated metabolites and increased toxicity.

Epipodophyllotoxins, etoposide and teniposide, as well as cyclophosphamide, ifosphamide, vinblastine, and vindesine are substrates for metabolism by CYP3A, the most abundant component of the CYP system in the human liver. A variant in the 5' promoter region of the CYP3A4 gene (CYP3A4-V) impacts production of the epipodophyllotoxin catechol metabolite, which is the precursor of the potentially DNA-damaging quinine. Several authors, including our group, have shown that the prevalence of CYP3A4-V is reduced in t-AML suggesting that the CYP3A4-wild type genotype may increase production of potentially DNA-damaging reactive intermediates and increase the t-AML risk (*Felix et al, PNAS 1998; Voso et al, Ann Oncol 2007*).

Another enzyme important for benzene metabolism is NAD(P)H:quinone oxidoreductase (NQO1), which convert hydroquinone and related hydroxyl-metabolites to less toxic hydroxyl-metabolites. Defects in the NQO1 pathway also cause accelerated telomere shortening and a switch to clonal hematopoiesis due to oxidative stress. Hematologic impairment in benzene workers has been associated to single-nucleotide polymorphisms, the MPO –463GG and NQO1 465CT, in particular when combined. Accordingly, it has been shown that the frequency of NQO1 heterozygous was higher among leukemia patients than expected in the general population, and homozygotes mutants were 4% in patients with primary AML, and 11% in t-AML. This proportion further increased when looking at patients with chromosome 5 or 7 abnormalities, alone or combined, or when looking at patients treated with alkylating agents for their primary tumor (*Lan et al, Science 2004; Larson et al, Blood 1999, Voso et al, Ann Oncol 2007*).

Many cytostatic drugs such as adriamycin, BCNU, bleomycin, chlorambucil, cisplatin, etoposide, melphalan, mitomycin C, mitoxantrone, vincristine and cyclophosphamide are inactivated by GST. Several GST are polymorphically expressed, in particular the *GSTP1* gene present a variant allele, with a substitution of isoleucine to valine at amino acid codon 105 (Ile105Val), which occurs at a frequency of about 30% in Caucasian populations and is associated with decreased enzymatic activity. In central Europe, *GSTM1* is homozygously deleted in about 50% and GSTT1 in 20% of Caucasian individuals. *GSTM1* or *GSTT1* deletions have not been specifically associated with susceptibility to t-AML, while individuals with at least one *GSTP1* codon 105 Val allele were significantly over-represented in t-AML (*Allan et al, PNAS 2001*). In particular, the highest t-AML risk was present in patients with *GSTP1* codon 105Val allele with prior exposure to chemotherapy, particularly to known GSTP1 substrates and not among t-AML patients with prior exposure to radiotherapy alone.

Another mechanism of secondary leukemogenesis are DNA-repair defects. Double-strand breaks (DSBs) in DNA are the most important class of DNA damage because they lead either to cell death or loss of genetic material, resulting in chromosomal aberrations or to translocations. Too little repair leads to the acquisition and persistence of mutations, whereas elevated levels of repair can inhibit the apoptotic pathway and enable a cell with damaged DNA to attempt repair, potentially mis-repair, and survive. DSBs are predominantly repaired in mammalian cells by homologous recombination (HR) or nonhomologous end-joining.

One of the central proteins in HR repair pathway is RAD51 which binds to DNA and promotes ATP-dependent homologous pairing and strand transfer reactions. RAD51 interacts with BRCA1 and BRCA2 and is essential to the viability and genetic stability of the cell: knock-out models in mice are embryonically lethal, probably due to an accumulation of chromosomal breaks.

The *XRCC3* protein also functions in DSB repair pathway and directly interacts with and stabilizes RAD51. The *RAD51* gene present a G/C polymorphism at position –135 (RAD51-G135C), while XRCC3 has a polymorphism at codon 241. In 51 t-AML patients, the frequency of the RAD51-G135C polymorphism was significantly higher than in normal controls, matched for ethnicity and age, translating in a 2.66-fold increased t-AML risk (*Seedhouse et al, Clin Can Res 2004*). The risk of the development of AML was increased 4-folds when both variant RAD51-135C and XRCC3-241Met alleles were present, whereas the risk of t-AML development was increased 8-folds.

Also the nucleotide excision DNA-repair mechanism seems to be involved in t-AML. Reduced activity of the xeroderma pigmentosum group D (XPD) gene due to a genomic polymorphism, was associated with reduced AML survival and a 2-fold increased risk of developing t-AML after chemotherapy (*Allan et al, Blood 2004*).

DNA mismatch repair is important to correct replicative errors that escape DNA polymerase proofreading. In humans, the MSH2/MSH6 complex and the MSH2/MSH3 complex recognize and bind to single base mismatches and insertion/deletion loops in DNA. Subsequent recruitment of MLH1 and PMS2 facilitates degradation and resynthesis of the region in the newly synthesized daughter strand. Although defects in any of the proteins can impair the DNA mismatch repair system, the process is most greatly affected by abnormal MSH2 or MLH1 function. By examining 3 quasi-monomorphic mononucleotide markers the distribution of microsatellite instability was found to be low in *de novo* AML (0–30% of cases), while it was detected in 38% of t-AML cases (*Worrillow et al, Clin Can res 2003*). In this line also MLH1 polymorphisms were overrepresented in t-AML (Worrillow et al, J Med Gen 2008). Mutations of Caspase-5, FANCD2 and NF1 may also induce MSI in t-AML (*Offman et al, Mol can Res 2005)*.

Mutations of the p53 tumor suppressor genes, which directs the cel-

lular response to many DNA-damaging agents, are important for the pathogenesis of t-MDS/AML. In this line it has been shown that 2 common functional p53-pathway variants, leading to reduced p53 activity, interacted in increasing the t-AML risk, particularly in in patients with loss of chromosomes 5 and/or 7 (*Ellis et al, Blood 2008*). A high frequency of microsatellite instability and evidence of MSH2 loss in t-AML suggests that DNA mismatch repair dysfunction may be an initiating event in disease evolution. Subsequent accumulation of secondary genetic changes may promote the emergence of cells with reduced p53 activity, overexpression of homologous recombination genes (including RAD51), excess of DNA-repair, and increased chromosomal instability, leading to the typical abnormalities seen in t-MDS/AML.

This could be further enhanced by the presence of xenobiotic-metabolizing enzymes polymorphisms, since reactive species, escaping detoxification mechanisms or produced in excess, damage DNA which is inefficiently repaired due to defective DNA-repair. This leads to clonal expansion of the injured cells and the onset of MDS. Transformation to leukemia is then a continuum and t-MDS/AML can be considered a unique disease. This hypothesis is supported by the significantly higher number of t-AML patients carrying a combined detoxification/DNA repair defect.

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CYTOGENETICS AND MOLECULAR STUDIES FOR DEFINING THE PATHOGENESIS AND REFINING THE DIAGNOSIS OF THERAPY-RELATED ACUTE MYELOID LEUKEMIAS (T-AML)

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Cytogenetics and molecular analyses have highlighted that AML is a genetically pleiomorphic disease. At first, this suggestion was revealed by chromosomal studies which showed that AML comprises several biological and clinical entities marked by distinctive karyotype abnormalities.[1-3] Subsequently, it became apparent that most chromosomal defects, which are mandatory for the diagnostic work-up and for predicting the clinical outcome of every de novo AML patient, result in the rearrangement of genes responsible of the leukemogenic process4. Afterwards, it was observed that in therapy-related myelodysplastic syndromes (t-MDS)/t-AML chromosomal abnormalities have an additional role, being strictly related to the type of cytotoxic treatment administered for a previous cancer or to the immunosuppressive treatment administered after hematopietic stem cell (HSC) or solid organ transplantation. Former treatments (chemotherapy agents and radiotherapy, [RT]) interact with DNA and cause somatically acquired mutations directly responsible of t-MDS/t-AML, whereas immunosuppressive treatments probably select an already genetically unstable myeloid clone with a mutator phenotype by reducing anti-neoplastic immuno-surveillance.[4] Recently, eight different and alternative genetic pathways marked by specific chromosomal defects were proposed for the pathogenesis of t-MDS/t-AML.[5-7] In addition, it was revealed that in each of these pathways specific genes with a pivotal role in normal hematopoiesis are targeted by point mutations and/or internal tandem duplications. Thus, a cooperation between specific chromosomal defects and distinctive gene lesions was suggested.

Considering all these points, it is obvious that the study of t-MDS/t-AML is of particular interest since these entities not only represent the most serious complications of current cancer therapies, but also because they share many cytogenetic abnormalities and gene mutations with de novo AML, allowing to extrapolate between observations in the two subtypes of the disease. In addition and in contrast to de novo MDS/AML, patients submitted to chemo/radiotherapy for a previous cancer are accurately monitored in order to predict relapse and possible complications. So, in these patients a phase of t-MDS is never overlooked and the complete genetic and clinical evolution from exposure to cytotoxic/immunosuppressive treatment to the development of frank leukaemia can be easily studied.[4,7]

This review will analyse all these points and the molecular signals responsible for progression of t-MDS to t-AML.

Incidence of chromosomal defects

In t-MDS/t-AML the overall incidence of chromosomal abnormalities is 70-90%, versus 40-60% in de novo MDS and 50-60% in de novo AML (Table 1).[1-6] Based on chromosomal analysis MDS/AML either *de novo* or therapy-related can be subdivided in three groups. The first cytogenetic group includes patients with non-random unbalanced abnormalities (monosomies or deletions of chromosomes 5 and 7 and trisomy of number 8), the second patients with recurrent balanced rearrangements without any visible loss of chromosomal material, the third chromosomally normal patients. In de novo and therapy-related MDS unbalanced defects are revealed in 15-25% and 50-70% of patients respectively, whereas balanced translocations are very rare. In *de novo*AML unbalanced defects are less frequent than balanced defects, in t-AML the opposite occurs. Chromosomal instability, which accounts for the development of non-clonal defects and unrelated clones in 10% and 5% of patients respectively, is a common feature of *de novo* MDS and t-MDS/t-AML and rarely occurs in de novo AML. These differences suggest that de novo MDS and t-MDS/t-AML may have a similar pathogenesis which seems to be different from that of *de novo* AML.

Table 1. Incidence of chromosomal abnormalities in *de novo* and therapy-related MDS and AML: a comparison.[1-6]

Karyotype	*Frequency (%) De novo / t-MDS*	*Frequency (%) De novo t-AML*
Abnormal	40-70/80-95	50-60/85-90
Balanced rearrangements	10/4	25-30/15-20
Unbalanced rearrangements	30/12	15-20/40-50
5/7 defects	20/80	20/90
Specific abnormalities		
-5/del(5q)	10-20/40	10/50
-7/del(7q)	5-10/50	5/50
+8	10/10	10/10
del(20q)	5/7	2/10
-Y	10/-	–
17p rearrangements	7/10	1/20
11q23 translocations	5-6/3	5/20
Compl. karyot. (≥three defects)	10-20/90	2/90

Cooperation between chromosomal defects and gene lesions

The pathogenesis of t-MDS/t-AML has been linked to the following eight alternative genetic pathways which combine chromosomal abnormalities and gene lesions (Table 2).[7] Pathway I: includes patients who harbour a monosomy 7 (-7) or a deletion of the long arm of chromosome 7 (7q-), normal chromosomes 5 and no recurrent balanced chromosomal translocation. These patients, who have been previously exposed to alkylating agents (AA) alone or in combination with radiotherapy (RT), nucleoside analogs, immunosuppressive agents usually present as t-MDS, which develops 5-7 years after the end of these treatments and progressively evolves into t-AML. Monosomy 7/7q deletions are significantly associated to RUNX1 mutations. The Copenhagen team has reported a 38% incidence of RUNX1 mutations in patients belonging to this pathway. These mutations are distributed throughout the full length of the protein but those which are significantly associated with -7/7q- are inframe mutations of the N-terminal region (Ni mutations)8. In fact, Ni mutations are observed in young patients with a previous history of radiation and/or chemical exposure and are associated with t-MDS/smouldering t-AML presenting with peripheral blood cytopenia and hypo-cellular marrows. Ni mutations, due to missense and insertion type mutations replacing some amino acids, are located within three protein loops which mediate DNA-binding potential. So, Ni mutations are associated with partial or total of DNA-binding ability, but maintain the ability to bind CBFBeta. RUNX1 N-terminal region can also be affected by truncated mutations (Nt) which include nonsense and frameshift mutations. Nt mutations cause the partial deletion of the RHD domain with the consequent loss of the nuclear localization signal and the C-terminal region of the protein. Thus, Nt mutations cause a loss of DNA-binding ability and trans-activating potential. Other mutations, mainly frameshift mutations, target the C-terminal region and maintain intact RHD domain. Despite all these structural differences, RUNX1 mutants function through a simple haploinsufficiency mechanism. Ni mutants can also inhibit the trans-activation activity of the wild-type RUNX-1 in a dose-dependent manner. A reduced RUNX1 DNA binding and transcriptional activity may be also due to an interaction with EVI1 proteins, which cooperate with RUNX1 mutants in determining the progression of t-MDS to t-AML. An increased methylation pattern of the p15INK4B (p15) promoter, which occurs in about 68% of patients with t-MDS/t-AML, is another gene abnormality significantly associated with 7q deletions. The p15 gene, together with p14arf (p14) and p16INK4a (p16), maps to a 25kb fragment on chromosomal band 9p21 and along with p16 directly binds and negatively regulates CDK4- and CDK6-mediated phosphorylation and inactivation of the Rb protein. Thus, p15, being a negative regulator of the cell cycle, is considered a putative tumor suppressor gene (TSG). In t-MDS the methylation frequency and the methylation density of the p15 promoter, being higher in advanced t-MDS, are significantly correlated with disease stage and the methylation frequency of the p15 promoter, which is an independent prognostic factor, increases with the increase of marrow blast cell percentage. In t-AML the methylation frequency of the p15 promoter is lower in M5 subtype and independent of the type and the latency period of previous anti-cancer treatment, platelet counts and presence of p53 mutations.[7]

Table 2. The eight alternative genetic pathways in secondary MDS/AML.[7]

Inducing agent		Chromosomal defect	Additional chrom. defect	Principal molecular lesion	Other molecular lesion	Last genetic defect
Alkylating agents	DELETIONS	-7/7q-	None	*RUNX1* mutations	*RAS* mutations, *TP53* mutations	Frequent *CDKN2B* promoter methylation
		-5/5q-	-7/7q- 17p- complex karyot.	*TP53* mutations	*MLL* and *RUNX1* amplif./duplicat.	
Topoisomerase II inhibitors	GENE FUSIONS	11q23 rearr.	None	*MLL* rearr.	*RAS* mutations *BRAF* mutations	Frequent *CDKN2B* promoter methylation
		21q22/16q22 rearr.	-7/7q-	*RUNX1* or *CBFB* rear.	*KIT*	
		t(15;17)	None	*RARA*	*FLT3* ITD	
		11p15 defects	None	*NUP98*	??	
De novo leukemia		Normal karyot.	None	*FLT3* mutations *RAS* mutations *MLL* PTD	*RUNX1* mutations	Less common *CDKN2B* Promot.methyl.
		Other defects	None	??	??	

Recent evidence suggests that an over-expression of the isoform 4 of the G-CSF receptor, which is defective in signalling cellular differentiation, with a consequent constitutive activation of the STAT1/STAT5 signal-transduction pathway is another molecular lesion frequently observed in MDS patients who develop -7 after G-CSF stimulation. Interestingly, this G-CSFR mRNA isoform confers resistance to apoptosis by up-regulating the Akt signalling pathway and mimics the effects of the G-CSFR dominant mutation observed in children with severe congenital neutropenia.

Pathway II: includes patients previously treated with AA alone or in combination with RT who develop t-AML after t-MDS and harbour monosomy 5/5q deletion, no recurrent balanced chromosomal translocation and frequent complex karyotypes (≥3 chromosomal defects). Some patients within this pathway may by chance have -7/7q- as the only recurrent partner abnormality, but do not show any type of RUNX1 mutation. Many studies have analysed genes mapped within the deleted region of chromosome 5 in order to reveal any putative TSG. A study focused on EGR1 haplo-insufficiency and another emphasized haploinsufficiency and promoter methylation of the CTNNA1 gene, but none of these genes still has a definite role in t-MDS/t-AML pathogenesis.

Molecular studies have revealed a significant association between p53 mutations and -5/5q-. The p53 protein, activated by several types of DNA damage, causes a cell-cycle arrest at the G1 and G2 checkpoints in order to allow DNA repair. If this last event does not occur, the p53-dependent apoptotic pathway is activated. A p53 loss of function translates into genome instability with a high rate of mutations, chromosomal translocations, gene amplification, deregulation of centrosome replication. This association is further strengthened by the demonstration that in t-MDS/t-AML somatically acquired p53 mutations, which occur in 30% of patients and are due to single-base substitution at A:T pairs, are strictly associated to complex karyotypes showing centromeric breakages and highly rearranged chromosome derivatives. Interestingly, some t-MDS/t-AML patients may present a loss of p53 due to a deletion at band 17p13 and a loss of p53 heterozigosity at the DNA or mRNA level, suggesting a duplication of the homologous chromosome 17 carrying the mutated p53 allele. These gene lesions are more common in older patients and are associated with an extremely poor prognosis. Recent data suggest that in doxorubicine-resistant cell lines drug resistance is due to a decreased p53 induction, high levels of activated Raf/MEK/ERK signalling and decreased induction of apoptosis.

Pathway III: includes patients previously treated with topoisomerase II inhibitors (TI) (epipodophyllotoxin derivatives: etoposide and teniposide) or DNA intercalating agents (mitoxantrone, doxorubicin) with balanced chromosomal translocations involving band 11q23, which contains the MLL gene. In contrast to t-AML following treatment with AA, these secondary AML, especially classified as M4-M5, are not preceded by t-MDS and usually develop 1-3 years after the end of cytotoxic treatments. From a clinical point of view these patients present high leucocytes counts and, although overall survival is unsatisfactory, they often experience a good response to induction treatment. Balanced translocations of band 11q23 always produce a fusion gene formed by MLL and one of its numerous partners and an increased aberrant expression of the Homeobox (HOX) genes, especially of the *5'-HOXA (HOXA5-11)* gene cluster. *HOXA5*, *HOXA9* and *HOXA10*, prominent members of a gene expression signature found in leukemia stem cells (LSC), are immediately induced after MLL-AF9 expression, suggesting a hierarchical model of leukaemia initiation by this chimeric gene. This possibility is further strengthened by the observation that a multiprotein complex formed by MLL fusion oncoproteins regulates the chromatin structure near the 5'-HOXA cluster. Some patients within pathway III may present point mutations of the BRAF gene.

In addition, since the telomeric BCR region of the MLL gene, which contains all the breakpoints of t-MDS/t-AML translocations, corresponds to a site-specific apoptotic cleavage and co-localizes with a DNAase hypersensitivity site, this gene may be involved in pro-leukemogenic translocations induced by apoptotic effector nucleases. The mechanism controlling the sensitivity to these enzymes is supposed to be an earliest apoptotic fragmentation at sites of DNA attachment to the nuclear matrix. Three steps are required to create these rearrangements: a double strand break within the MLL and MLLT3 genes, a subsequent fusion of these breaks in a biologically active MLL-MLLT3 translocation, maintenance of division ability by the cells with the apoptosis-induced MLL-MLLT3 translocation.

Pathway IV: includes patients exposed to TI, especially antracyclines, with balanced translocations of band 21q22 and inversions/translocations of band 16q22, which contain the *RUNX1* and the *CBFBeta* genes respectively. As in pathway I, -7/7q- are the most common secondary abnormalities within this pathway, a datum which further stresses a link between these defects

and *RUNX1* mutations/chimeric gene fusions.

The rare t(8;16) translocation which develops after previous treatments with TI in combination with AA may also be included within this pathway. Recent data demonstrate that t-AML with this chromosomal abnormality is never preceded by t-MDS, is mostly diagnosed as M4-M5a and more frequently affects female patients. Genes profiling has revealed that the t(8;16) clusters close to t(11q23)/MLL, but also that the t(8;16) translocation is associated to a highly unique signature which includes the over-expression of the CHD3 gene. CHD3 proteins are ATP-dependent chromatin remodelers , contribute to the repression of developmentally regulated genes and might de-regulate the MYB pathway.

Pathway V: includes breast cancer patients treated with doxorubicine and/or mitoxantore who harbor a t(15;17) translocation which creates the PML-RAR_ fusion. Recently, this translocation has also been revealed in patients affected with multiple sclerosis (MS) previously treated with mitoxantrone. Interestingly, molecular studies have demonstrated that in breast cancer and MS patients chromosome 15 breakpoints fall within the same 8-bp *hot-spot* region of PML intron 6.

Pathway VI: includes rare patients with chromosomal abnormalities involving band 11p15, the chromosomal locus where the NUP98 gene resides. No association to any specific type of previous anticancer treatment has been reported.

Pathway VII: includes chromosomally normal patients who had not been exposed to any specific type of previous chemo/radiotherapy but have had an intense mutagenic exposure. In these patients multicolour FISH has been unable to unmask chromosomal defects which might have been overlooked by conventional cytogenetics. Molecular studies have revealed that these patients may harbour mutations of the NPM1, FLT3, RAS and less frequently *RUNX1* genes. About half of these patients may present a NPM1 mutation along with the internal tandem duplication (ITD) of the *FLT3* gene. Similarly to de novo AML, t-MDS patients with NPM1 mutation present an up-regulation of the HOX genes and their leukemic cells are CD34 negative.[9] An intriguing point is whether these NPM1 mutated t-AML patients are really secondary leukemias or whether they represent *de novo* AML incidentally arising in patients with a history of previous treatment.

Pathway VIII: includes patients with unique chromosomal abnormalities, especially balanced translocations. The incidence of these defects is about 14% and none of them is directly related to any specific type of previous therapy.

Table 3. Cytokines and cytokines receptors differently expressed in the signalling pathways of early and advanced t-MDS and t-AML.[10]

Signaling pathways	Low-risk MDS	High-risk MDS	AML
Bcl-2	↓	↑	↑
Apo-2/?	↑	↓	↓
TRAIL	↑	↓	↓
Fas/CD95	↑	↓	↓
P38	↑	↓	↓
TNFR1	↑	↓	↓
TNFR2	↓	↑	↑
FLIP$_S$	↑	↓	↓
FLIP$_L$	↓	↑	↑
FLIP$_S$/ FLIP$_L$ ratio	↓	↑	↑
NF-kB	↓	↑	↑
Akt	↓	↑	↑
ERK1/2	↑	↓	↑

↑ = increased levels; ↓= reduced levels; ∫= involved in the progression from low-risk to high-risk MDS and AML.

Cooperation between class I and class II mutations

It has been suggested that in *de novo* chromosomally normal and abnormal AML the hematopoietic stem cell (HSC) and not the hematopoietic progenitors are the true targets of leukemic transformation and that at least two types of mutations are required to cause the disease, namely class I and class II mutations.[3,7] The former mutations target genes involved in signal transduction (FLT3, RAS, KIT, ect), while the second target genes encoding transcription factors (AML1, RARA, EVI1, WT1 ect.). The analysis of the eight alternative genetic pathways listed above has revealed that this cooperation is also operative and more complex in t-AML with class I and class II mutations not only showing a significant association, but also a mutual exclusiveness. In fact, only one chromosomal translocation is always observed, *RUNX1* mutations are never revealed in combination with other chromosomal translocations, FLT3 mutations are never associated with RAS, BRAF, KIT and PTPN11 mutations and, more importantly, leukemic transformation is accompanied by point mutations of *RUNX1* and RAS genes.

In contrast, the demonstration of such a cooperation is still lacking in de novo and therapy-related MDS which relevant genetic lesion seems to be the loss of a TSG followed by a sub-microscopic deletion or a mutation of the other allele localized on the apparently normal homologue chromosome, an assumption confirmed only for the 17p deletion. In fact, up to now only putative TSG have been mapped within the common deleted regions of chromosomes 5 and 7. An alternative possibility could be genetic instability due to mutations of genes (especially NPM1) involved in DNA repair. Anyhow, a cooperation between class I and class II mutations cannot be completely excluded even in MDS since mutations in RAS signalling molecules, cell cycle regulators and transcription factors frequently occur in chromosomally normal/abnormal MDS patients upon AML progression.

Despite all this evidence, the assumptions that the leukemogenic event occurs at the level of normal HSCs and is caused by a cooperation between class I and class II mutations is challenged by some genetic defects. The latter seems to be itself sufficient to alter the self-renewal and differentiation/proliferation programs of the most primitive normal hematopoietic cells and recreate the functional and phenotypic hierarchy of AML and the environment required for acquiring additional genetic changes. These genetic defects are represented by the TLS-ERG, MOZ-TIF, MLL-ENL and MLL-AF9 fusion genes present in various AML sub-types. It has been revealed that in mouse experimental models the MLL-AF9 fusion gene is itself able to generate LSCs from committed progenitors through the activation of a leukemia self-renewal-associated signature and without a widespread reprogramming of gene expression.

Molecular signals for MDS progression to AML

The transcription factor RELA/NFKB1 and the PI3KC2A/AKT1 axis are in tight cooperation and play a key role in such a process, an assumption underlined by the fact that RELA/NFKB1 activation correlates with t-MDS stage, being higher in advanced disease (Table 3).[10] In addition, in early t-MDS RELA/NFKB1 levels are lower in $CD34^+$ than in CD34– cells, suggesting a high incidence of apoptosis in early hemopoietic precursors, whereas in advanced t-MDS and t-AML RELA/NFKB1 levels are higher in CD34– than in $CD34^+$ cells, suggesting the activation of anti-apoptotic signals. In high-risk t-MDS and t-AML the constitutive RELA/NFKB1 activation is due to the constitutive activation of the CHUK/IKBKB/IKBKG complex. The frequency of cells with RELA/NFKB1 nuclear translocation correlates with blast counts, irrespective of cytogenetics, with apoptosis suppression and disease progression. Bortezomib inhibits RELA/NFKB1 and causes enhanced mitochondria-mediated apoptosis in high- but not low-risk t-MDS.

The mechanisms of RELA/NFKB1 activation are still undefined. TNF-_ is supposed to be a potent stimulus for RELA/NFKB1 activation, but this assumption contrasts the high TNF-α and the low RELA/NFKB1 expression in early t-MDS. Thus, other molecules, especially adaptor proteins involved in *downstream* events, can interfere with the autocrine TNF-α stimulation and can cause RELA/NFKB1 constitutive activation. However, this effect can also be determined by variations in the expression of TNF-α receptors. A contribution of downstream effectors of the RAS-GTPase pathway to RELA/NFKB1 activation is also possible, since RAS activation significantly correlates with RELA/NFKB1 DNA-binding activity. In contrast, the contribution of constitutive FLT3 activation is still debated.

RELA/NFKB1 controls the transcription of many anti-apoptotic genes including BCL2, BCL2L1, the inhibitor of apoptosis protein and Fas-asso-

ciated death domain like IL-1Beta-converting enzyme)-inhibitory protein (CFLAR) which expression is higher in advanced t-MDS and t-AML than in early t-MDS.

Few data support the role of the PI3KC2A/AKT1 axis in determining the evolution from t-MDS to t-AML. This pathway, constitutively activated in t-AML, is needed for increased survival, proliferation and myeloid transformation. Recently, it was revealed that mononuclear cells from high-risk t-MDS and t-AML, in contrast to those from early t-MDS, presented the activation of the AKT1 kinase along with that of the mammalian target of rapamycin.

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OUTCOME AND THERAPEUTIC APPROACHES OF THERAPY RELATED ACUTE MYELOID LEUKEMIA

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Therapy-related neoplasm is the definition recently proposed by the World Health Organization to identify a spectrum of malignant disorders, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), which supervene as a late complication of preceding cytotoxic chemotherapy. The term therapy-related is descriptive and refers to the patient's history of previous treatment with cytotoxic drugs thus that a relationship of causality is implied but the underlying mechanism is far from being fully elucidated. These neoplasms are supposed to occur as a direct consequence of mutations induced by chemotherapy or by the emergence of a clone carrying a mutator phenotype. The interval between the initial diagnosis and the secondary disease is function of a number of factors such as: cumulative dose and dose intensity of cytotoxic drugs, exposure to specific agents. We will focus on therapy-related AML, in particular, on their clinical picture and available therapeutic strategies.

Factors which impact on clinical outcome

Therapy-related AML (t-AML) is generally a fatal disease whose course is progressive and resistant to chemotherapy conventionally used in AML arising *de novo*. The life threatening complications are consequence of the prolonged and profound hematopoietic failure and there is universal consensus that survival for patients affected with t-AML is shorter than for those with *de novo* AML. A number of factors can explain the poor outcome of t-AML: the persistence of primary cancer contributing to morbidity and mortality, independently from the hematopoietic failure caused by t-AML; tissue damage from prior treatment may compromise the ability of these patients to receive intensive chemotherapy or stem cell transplantation. In this view, depletion of normal hematopoietic stem cells can worsen cytopenias following induction chemotherapy, rendering it more profound and persistent. Damage of bone marrow stroma from previous therapy, especially combination of radiochemotherapy, is a further cause of hematopoetic deficiency. Patients with t-AML are often chronically immunosuppressed from prior disease or therapy and, due to dysfunctional phagocytosis, may be colonized with pathogenic flora. Patients may be refractory to transfusions following prior supportive regimen, and therefore, not ideal candidates for intensive myelosuppressive chemotherapy. Finally, the high frequency of unfavorable cytogenetic and expression of P-glycoprotein associated with t-AML can favor the rapid emergence of chemotherapy resistance.

Treatment of t-AML

Since patients with t-AML are very often excluded from frontline clinical trials, there is a paucity of data of prospective, randomized studies. In a Japanese study, 256 patients with therapy-related MDS (t-MDS) (41%) or t-AML (59%),were analyzed for outcome and prognostic factors. Median age was 61 years, the majority (72%) received chemotherapy, or standard 3+7 either low dose ARAc, including 7 patients with therapy-related acute promyelocytic leukemia (t-APL) who were treated with low dose ARAc and ATRA. Overall survival (OS) was 9.7 months, 85 (46%) patients achieved complete remission (CR) and median duration of response was 8.2 months. The authors also identified a number of factors significantly associated with unfavorable outcome and these were: abnormalities of chromosome 5, hypoproteinemia, a high level of C-reactive protein, thrombocytopenia, and persistence of the primary malignancy. In a review of 644 patients with t-AML treated with a variety of chemotherapeutic approaches at the M.D.Anderson Cancer Center, only 182 (28%) achieved a CR; such a figure is remarkably lower than 65-80% CR rate observed in patients with de novo AML. In general, even other small and individual experience, report CR rate < 50% with short duration of response. One exception, not isolated, is represented by the GIMEMA experience, focusing on comparison between 38 cases of t-AML and 114 matched de novo AML enrolled in sequential EORTC/GIMEMA programs. Although few cytogenetic data are presented, thus that a full risk assessment is difficult to establish, the authors emphasize the finding of a similar outcome for the two groups, in terms of CR rate, OS and disease free survival (DFS). Kern et al, reported a series of 93 patients with t-AML, treated on intensive chemotherapy, whose median OS was worse than that of patients with de novo AML enrolled in the same program. However, a more detailed analysis indicated a higher frequency of unfavorable karyotype in the group of t-AML leading to the author's conclusion that karyotype makes the difference between t-AML and de novo AML. In fact, among t-AML, median OS of patients with favorable karyotype was 26.7 months (not dissimilar from that of de novo AML) versus 5.6 of those with unfavorable pattern. In line with these results, an analysis of MRC AML10 protocol demonstrated that, for 119 patients with secondary AML (also including t-AML) treated on identical standard chemotherapy as *de novo* AML patients, response rate depended entirely on the cytogenetic group, very much as one would expect from any *de novo* AML. This observation raises the question as to whether t-AML should be regarded as any other leukemia with specific unfavorable cyogenetic and/or molecular marker, rather than drawing emphasis to the clinical history of previous drug exposure. Some authors claim that t-AML should probably not be considered as a separate entity, being synonymous with any AML that presents with unfavorable cytogenetics, whether or not they have a typical history. Based on this, and due to the reluctance among many physicians to enter patients with t-AML on clinical trials, recommendations for therapy have often been intuitive and derived from informations of select phase II studies. There have been no phase III studies of induction or post-remission therapy of t-AML and no evidence has been generated proving that any regimen is better than standard AML induction therapy consisting of an anthracycline and cytarabine (3+7), or similar. Although up to 50% of patients with t-AML might have an initial CR, no more than 10% are expected to be long-term survivors. Therefore, patients achieving CR should be offered allogeneic stem cell transplantation (ASCT), especially if they are younger adult and/or carriers of unfavorable cytogenetics. ASCT is the only potential curative approach and a number of small case series have reported survival rates of 20-30% for these patients, even though, chronic and cumulative toxicities from prior chemo-radiotherapy negatively impact on survival and hamper the applicability of ASCT on a larger scale; early transplant related mortality after ASCT is more common for t-AML than for primary AML. Recently, Kroger and colleagues on the behalf of the European Group for

Blood and Marrow Transplantation (EBMT) reported on the outcomes of 461 patients with therapy-related neoplasm (including 308 t-AML) who underwent ASCT between 1981 and 2006. Overall, one-third were cured and multivariate analyses predict a more favorable outcome for younger patients (<40 years), for those with normal cytogenetics, and those transplanted in first CR; this suggests that ASCT can be effective for patients who have chemotherapy-responsive t-AML. Non-myeloablative, reduced intensity ASCT is under investigation for those who are not eligible for myeloablative procedures. The EBMT registry has also reported on 65 patients with therapy-related neoplasm (including 58 t-AML) who underwent autologous SCT. Median age was 39 years (range 3-69), median duration of OS and DFS at 3 years was 35% and 32%, respectively. Relapse rate was lower for patients transplanted in first CR (48% vs. 89%, p=0.05) and age > 40 years was associated with a higher transplant-related mortality (47% *vs.* 7%, p=0.01). In contrast to the general poor prognosis of t-AML, patients who develop forms with balanced chromosomal translocations such as t(15;17) or core binding factors abnormalities (CBF) have been reported to share similar outcomes with those who have the same chromosomal rearrangements, associated with a diagnosis of *de novo* AML. In a report on 106 cases of t-APL identified between 1982-2001 in France, Spain, and Belgium, the clinicobiologic features and treatment outcome of t-APL patients were similar to those of de novo APL patients. In fact, 80% CR rate was observed for those treated on anthracycline-based regimen plus ATRA. Median OS was 58% at 8 years, and no differences were even noted when patients were segregated by primary treatment (chemotherapy, radiotherapy, or both) or prior exposure to specific drugs (alklyating agents, topoisomerase-II inhibitors, or both). Concordant results were reported in the GIMEMA experience, where 51 cases of t-APL were identified and compared to 641 matched cases of *de novo* APL. CR rate, 4-year event free survival (EFS) and 4-year OS rates were 97%, 93%, 65% and 68%, 85%, 78% in t-APL and *de novo* APL groups, respectively, reflecting probable genetic similarities. As far as t-AML with CBF abnormalities it concerns, earlier reports indicated an outcome which was super imposable to that of de novo CBF AML. Among patients analyzed at the International Workshop in Chicago in 2000, 33 of 39 (85%) patients with CBF t-AML, treated with intensive chemotherapy, achieved a CR. The patients had previously been exposed to topoisomerase-II inhibitors, but importantly, 21% of them had received only radiotherapy. Median OS, after receiving intensive AML therapy, was 29 months and 12 of 33 (36%) relapsed. However, long-term follow-up of this category of patients has provided arguments against the assumption that CBF t-AML and de novo CBF AML share the same favorable prognosis. In a recent review of 188 patients with CBF AML, Borthakur et al identified 17 cases (9%) of t-AML. In a matched analysis, by performance status, age and additional cytogenetic abnormalities, they found that t-AML had a significantly worse OS and EFS and that in multivariate analysis CBF status appeared to be of marginal significance. Similarly, Gustafson et al analyzed retrospectively 13 cases of CBF t-AML and 38 of de novo CBF AML, diagnosed between 1995 and 2008. With a median follow up of 13 months, 10 patients with CBF t-AML have died and the median OS for CBF t-AML versus de novo CBF AML is 19 months versus not reached (p=0.002). The authors concluded that, although CBF t-AML and *de novo* AML have in common several biologic features, the outcome of patients with CBF t-AML is generally worse. Finally, an analysis of the Medical Research Council trials of intensive chemotherapies confirmed that as compared with *de novo* AML, unfavorable karyotypes were more frequent in t-AML (27% v 11%), however, the diagnosis of t-AML resulted in an inferior outcome also within favorable cytogenetic risk groups.

Investigational therapy

The historic literature on the treatment of t-AML is strikingly deficient in prospective studies directly comparing results for this group of patients. On the other hand, it must be realized that, owing to lack of a full compatible donor or poor performance status, ASCT represents a feasible option for a small proportion of patients with t-AML. Therefore, despite attempts to seek for alternative sources of stem cells (haploidentical donor, umbilical cord blood) or to implement techniques such as reduced intensity conditioning, a therapeutic dilemma still remains as to the most adequate approach for those who are not candidates for any form of ASCT. It would seem appropriate to offer these patients investigational programs using new drugs. Accordingly, in the next section, the role of some novel agents in the treatment of t-AML, will be addressed.

Amonafide

Over-expression of P-glycoprotein has been related to resistance to classical Topo II inhibitors used in the treatment of AML and is common in patients with poor-prognosis, such as those with t-AML. Amonafide (amonafide l-malate, Xanafide®) is a unique Topo II catalytic inhibitor that, unlike the classical Topo II inhibitors, is ATP-independent and, more importantly, is not a substrate of P-glycoprotein; therefore it might represent the ideal candidate to bypass this frequent mechanism of chemo-resistance. In an initial phase 1 study of amonafide given as a single agent, 4/14 patients with secondary AML or relapsed/refractory AML achieved CR. A 46% CR rate was observed in a subsequent combination study of amonafide given with standard dose cytarabine to treat 88 patients with secondary AML (54% with t-AML, 47% with unfavorable cytogenetics). This has led to the activation on an international basis, of a "Phase III Randomized Study of Amonafide (AS1413) and Cytarabine Versus Daunorubicin and Cytarabine in Patients With Secondary Acute Myeloid Leukemia" which is currently recruiting participants.

Hypomethylating agents

Epigenetic alterations have been shown to play a role in neoplastic transformation and in prognosis of MDS and AML. They consist in DNA and chromatin modifications which are inherited by cell progeny, and which are able to alter gene expression without changing DNA sequence and without generating any additional genetic information; more importantly, epigenetic alterations are potentially reversible. DNA methylation is one of the mechanisms capable to induce epigenetic alterations. It takes place by the addition of a CH3 group at the carbon 5 position of cytosine situated in the sequence contest 5'CG3'. DNA methylation is regulated by DNA methyltransferases (DNMT) and it results in gene silencing by disrupting transcription factors binding at respective promoters and inhibiting regional recruitment of specific transcription repressors. Therefore, reverting DNA methylation might be of therapeutic relevance, and in fact, DNMT inhibitors (azacitidine, decitabine) have been approved by FDA as specific agents for treatment of MDS. Formal studies focusing specifically on t-AML are not available, however some observations suggest that hypomethylating agents might be successfully used in particular categories of these patients. In a recent survey on the use of azacitidine to treat patients with AML entered into an Italian named patient program, of 63 patients analyzed, 19 had secondary AML and 13 unfavorable karyotype. Although, in multivariate analysis secondary AML and unfavorable karyotype were associated with a poor outcome, responses were seen within these categories. In particular, in patients with abnormalities of chromosome 7 and complex karyotype. This is in line with several new analyses indicating that patients with MDS who have monosomy 7 abnormalities (either alone or within complex cytogenetics) may be particularly sensitive to treatment with DNMT inhibitors. At the American Society of Hematology meeting in 2007, Lübbert et al presented data on the use of continued low dose decitabine in 178 elderly patients with AML. Fifty-six of them had unfavorable karyotype (16 abnormalities of chromosome 7 or 5, 32 complex karyotype, 4 others); the overall response rate (CR plus PR) for these group was 26%. Finally, Voso et al, on the behalf of the GIMEMA group, have demonstrated the efficacy of combining azacitidine an valproic acid (± ATRA) to treat high risk MDS. Of 62 patients enrolled, 8 carried abnormalities of chromosome 7 and 11 a complex karyotype. The overall response rate (CR plus PR) after 8 cycles was about 31% and, importantly, multivariate analysis identified valproic acid serum concentration as an independent parameter associated with response. All together, these observations will lead to the use of this class of drugs even in t-AML with unfavorable cytogenetics, when ASCT cannot be performed. Based on this, protocols of azacitidine or decitabine to treat patients with de novo or secondary AML who are ineligible for intensive chemotherapy, have been activated.

Clofarabine

Clofarabine is a second-generation nucleoside analog, which was developed as an hybrid molecule to combine the most favorable pharmacokinetic properties of both fludarabine and cladribine. In the BIO-121 study of clofarabine as a single agent, to treat elderly patients unsuitable for intensive therapy, a CR rate of 47% and 31% was reported for the group of patients with unfavorable cytogenetics and secondary AML, respectively. Such figures were confirmed in the Classic II study, recruiting patients with similar characteristics as BIO-121. In fact, CR

rate was 50% and 43% for patients with secondary AML and unfavorable cytogenetics, respectively. Such promising results underline the potential benefit from using clofarabine in t-AML.

Conclusions

Planning appropriate programs of treatment for patients with t-AML requires considerations related to performance status, age, co-morbidities, status of primary malignancy and presence of unfavorable clonal abnormalities. Patients with available source of stem cells and with adequate performance status should be considered for ASCT, whereas encouraging the remaining patients to participate in prospective clinical trials should represent a major goal.

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CLASSIFICATION, PATHOPHYSIOLOGY AND EPIDEMIOLOGY OF THROMBOCYTOPENIAS

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Introduction

Thrombocytopenia may appear as the prevalent manifestation in various clinical conditions. Different pathogenetic mechanisms, including decreased platelet production, increased platelet destruction and/or consumption, platelet sequestration, and platelet dilution may be operating. When, as in the majority of cases, no apparent causes of thrombocytopenia can be detected by a complete physical and laboratory investigation, the "isolated" thrombocytopenia is called idiopathic, or immune if an immune mechanism is thought to play a central role. Currently, the terms idiopathic and immune are used interchangeably.

Classification and denomination of Immune Thrombocytopenias

Recently, an International Working Group (IWG)1 proposed to define cases of isolated thrombocytopenia as Primary Immune ThrombocytoPenia (Primary ITP). The "old" term "idiopathic" is avoided, preferring "immune", to emphasize the purported immune-mediated mechanism of the disease and choosing "primary" to indicate the absence of any obvious initiating and/or underlying cause. So, the diagnosis of primary ITP remains one of exclusion. The term "secondary ITP" has been proposed to broadly include all forms of immune-mediated thrombocytopenia due to an underlying disease or drug exposure. This classification seems clinically relevant, since in secondary forms the treatment is often direct toward the associated disease.

Definitions for "newly diagnosed ITP" (from diagnosis to 3 months thereafter); "persistent ITP" (4 to 12 months from diagnosis) and "chronic ITP" (more than 12 months from diagnosis) have been proposed, recognizing the different therapeutic goals in each of these phases (Figure 1). The term "severe ITP" should be reserved to cases of clinically relevant bleeding symptoms demanding active treatment. The term "refractory ITP" defines ITP not responsive to (or relapsed after) splenectomy and requiring treatment for severe ITP or high risk of bleeding.

Figure 1. Therapeutic goals in the different phases of ITP.

Pathophysiology of Immune Thrombocytopenias

Very recently, many comprehensive reviews have been published, focusing on the current understanding of the pathogenetic mechanisms 2-4 of ITP. Solid experimental evidence shows that, in addition to platelet destruction caused by autoantibody (by phagocytosis by the reticuloendothelial system) and, probably, by a direct T-cells-mediated cytotoxycity, a defective, immune- and non immune-mediated platelet production also contributes to the platelet count decrease.4,5 The emergence of antiplatelet auto-antibodies produced by B-lymphocytes (detected only in 60-70% of ITP patients with currently available tests) remains the central pathogenetic mechanism. T-lymphocytes play an important role, not only by stimulating B-cell antiplatelet antibodies production, but also through a direct antiplatelet cytotoxic action. "In vitro" studies have shown that antiplatelet antibodies are direct also against the megakaryocytes, which share with platelets many surface antigens. Electron microscopy studies confirmed the presence of apoptotic features in megakaryocytes from ITP patients. Moreover, the homeostatic mechanism of thrombopoietin (TPO) - the growth factor regulating the production of platelets - seems to fail its scope in most cases.6 Indeed, in ITP, TPO often does not sufficiently increase to provide maximal platelet production stimulation. This is now clearly shown by the efficacy of second generation thrombopoietin receptor agonists, like romiplostim and eltrombopag, which increase the platelet count to a safe level in 70-80% of patients with ITP unresponsive to one or more lines of treatment.

A very promising and interesting research perspective is actually looking at the genetic susceptibility for ITP. So far, no association between ITP and the major histocompatibility-complex-susceptibility-gene HLAB8DR3 has been found. Moreover, ITP is infrequently found in family members. Despite these negative findings, prospective projects on the genetic susceptibility of ITP are in progress within the ITP-United Kingdom adult registry (www.ukitpregistry.com) and Pediatric and Adult Registry on Chronic ITP-study (www.itpbasel.ch).

In secondary ITP, many different pathogenetic mechanisms may contribute to the development of antiplatelet antibodies and to thrombocytopenia.8 In ITP associated with infection (self-limited ITP in children; HCV, Helicobacter Pylori, Cytomegalovirus, Varicella zoster and HIV associated ITP) the antibodies primarily directed against the infectious agent may cross-react with some platelet glycoproteins (molecular mimicry). In ITP associated with HCV and HIV, the genesis of thrombocytopenia is probably multifactorial, with megakaryocyte infection by the virus contributing to impaired platelet production. Reduced TPO production is also present in these forms. In ITP associated with autoimmune diseases (like systemic lupus erythematosus, Evans syndrome, antiphospholipid antibodies syndrome) a central tolerance defect probably rep-

resent the main pathogenetic mechanism, with an involvement of additional cell types.

Table 1. Incidence of ITP in general population (cases per 105 subjects/year).

	Male (95% CI)	Female (95% CI)	Total (95% CI)
PLT < 100 x 10^9/L Ref. a	2.06 (1.62-2.50)	3.28 (2.74-3.82)	2.68 (2.33-3.03)
PLT < 50 x 10^9/L Ref. a	1.78 (1.37-2.19)	2.71 (2.22-3.20)	2.25 (1.92-2.57)
PLT < 50 x 10^9/L Ref. b	-	-	1.6
PLT < 150 x 10^9/L Ref. c	3.4 (3.1–3.7)	4.4 (4.1–4.7)	3.95 (3.7–4.1)

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Table 2. Prevalence of adult ITP in general population (cases per 105 subjects).

	Male	Female	Total
Ref. d	6.1 (5-7.03)	11.3 (10-13)	9.5 (8.5-10)
Ref. e	17 (16.2-17.7)	24.5 (23.6-25.4)	20 (19.8-20.2)
Ref. f	-	-	176* (161-193)

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Epidemiology of Primary ITP

In some recently published epidemiological studies in adult ITP, incidence has been estimated between 1.6 and 3.95x105 subjects/year, with some relationship with the selected cut-off ranging from < 150 to < 50x10^9/L (Table 1). The incidence tends to increase with age, doubling in subjects over 60 years. Moreover females have been shown to have a higher incidence than males in patients younger than 60 years. Prevalence estimates were obtained analysing administrative data from Health Care databases in USA, with conflicting results and estimates ranging from 9.5 to 20/105 subjects (Table 2) in two different studies, perhaps reflecting a non - standardized and heterogeneous methodology in disease classification and/or different samples population. Among all cases of ITP, 20% are secondary forms, with coexisting, underlying conditions.8 Secondary ITP may develop in 1/40000 children after measles-mumps-rubella vaccination, 5/100 children after varicella-zoster virus infection, 5-30/100 HIV infected patients, 0.2/100 HCV infected patients. Between lymphoproliferative diseases, ITP can complicate 1-5/100 cases of chronic lymphocytic leukemia,9-10 0.2-1/100 Hodgkin disease, 0.7/100 non Hodgkin lymphoma, and 1/100 large granular T cell leukemia/lymphoma. Moreover, a high ITP incidence can be found in patients affected by common variable acquired immunodeficiency (10%) or by autoimmune lymphoproliferative syndrome (20%). The prevalence of Helicobacter Pylori (HP) infection in ITP patients is comparable with that of the general population, however eradication of HP may be effective in a significant proportion of ITP patients.[11]

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IMMUNE THROMBOCYTOPENIA AND ITS MANAGEMENT

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In 1735, P.G. Werlhof first described a new disorder under the name of *Morbus Maculosus Hemorrhagicu*s, subsequently identified as idiopathic or immune thrombocytopenic purpura. More recently, Rodeghiero *et al*. on behalf of an international panel of experts, suggested to uniform its definition as Immune ThrombocytoPenia (ITP), to emphasize the immune-mediated mechanism of the disease and a platelet count <100x10^9/L was established as the threshold for diagnosis. ITP is a relatively common disorder, affecting around 5-10 adults per 100.000 in the western world.

Diagnosis is one of exclusion and the main elements to consider in an otherwise healthy individual who presents with isolated thrombocytopenia are: patient and familial history, physical examination, peripheral blood smear examination, exclusion of other underlying disorder that may cause secondary immune thrombocytopenia (Table 1). Generally bone marrow examination in not routinely performed, but it is mandatory in case of patients over 60 years of age and before splenectomy. Antiplatelet antibodies may be of some utility in the ITP diagnosis, however antibodies have also been detected in 10% to 20% of patients with non-immune thrombocytopenias; moreover a standardization of the method is lacking.

ITP should be termed *primary* to indicate the absence of any obvious initiating and/or underlying cause. The term *secondary* should be reserved to all forms of immune-mediated thrombocytopenias except primary ITP. Secondary forms include the thrombocytopenias due to underlying disease or to drug exposure. The presence of antinuclear antibodies (ANA) and/or antiphospholipid antibodies (aPL) on their own, in the absence of distinctive clinical manifestations suggestive of SLE and/or antiphospholipid syndrome, does not qualify these cases as secondary ITP. The distinction between primary and secondary immune thrombocytopenia is clinically relevant, due to their different natural histories and distinct treatments.

Moreover, the definition "newly-diagnosed ITP" has been proposed for all cases at diagnosis instead of "acute", in absence of reliable predictive clinical or laboratory parameters of disease duration; whereas the

term *persistent ITP* was considered to define the period of disease lasting between 3 and 12 months from diagnosis. The term "chronic" was reserved for patients with ITP lasting for more than 12 months.

The disease should be judged *severe* exclusively when bleeding is clinically relevant. The international panel of experts defined some criteria of response to treatment, in order to uniform and improve communication among investigators, to enhance comparability among clinical trials, to facilitate meta-analyses and development of therapeutic guidelines and to provide a standardized for regulatory agencies. A "Complete response" (CR) was defined as any platelet count 100x10^9/L; "Response" (R) identified any platelet count ≥30<100x10^9/L and at least doubling of the baseline count, and *No response* (NR) any platelet count <30x10^9/L or less than doubling of the baseline count.

Table 1. Incidence of ITP in general population (cases per 105 subjects/year).

ITP DIAGNOSTIC CRITERIA

HISTORY
- Familial
- Concomitant/previous medications
- Infection
- Risk factors for HIV and/or HCV
- Symptoms related to other autoimmune disorders

PHYSICAL EXAMINATION
- Liver and/or spleen and/or lymphonodes enlargement
- Signs related to infective or autoimmune disease
- Bleeding characteristics

ISOLATED THROMBOCYTOPENIA: EVALUATION OF PERIPHERAL BLOOD SMEAR

ABSENCE OF:
- Pseudothrombocytopenia
- Intravascular Disseminated Coagulopathy
- Drug induced thrombocytopenia
- Collagenopathy
- MDS
- *Helicobacter Pylori*
- Congenital or hereditary thrombocytopenia (MYH9, Bernard-Soulier, ecc.)

Indications for initial treatment

Literature data show a direct relationship between platelet number and bleeding. However, since about 20% of ITP patients are asymptomatic at diagnosis regardless their platelet count, it is important to establish criteria for timing the initial treatment. Caution is recommended in view of the possible adverse effects of treatment and the unpredictable and frequently transient outcome.

There is no evidence based on randomized trials to guide management decisions on this issue, although several guidelines and reviews on this topic suggest to consider 30x10^9/L as platelet threshold to start treatment. More recent inclusion criteria adopted by clinical trials suggested a platelet count below 20x10^9/L as threshold to start treatment in asymptomatic patients, because fewer than 10% of adults rapidly show spontaneous remission; moreover treatment is mandatory for patients with platelet count over 20-30x10^9/L and active bleeding, because the main goals for initial management of severe ITP are to avoid major bleeding attaining a hemostatic platelet count (>30x10^9/L) and preserve patient activity.

Oral prednisone represents the initial generally accepted therapeutic strategy of choice for ITP patients who require treatment. Prednisone mechanisms of action in ITP are probably related to its capability to impair the clearance of antibody-coated platelets, increase platelet production by interfering with the platelet destruction of the macrophages within the bone marrow, and stimulate megakaryocyte progenitors.

There is a large variability in treatment regimens regarding the type of steroids (prednisone or dexamethasone), the dose used, the duration of full-dose treatment (two to six weeks) and the mode of tapering. About two-thirds of patients achieve a complete or partial response with prednisone 1 to 2 mg/kg, usually within seven to ten days. Treatment is regarded to have failed when platelet count doesn't rise significantly and/or bleeding symptoms don't improve within three weeks. Most patients relapse when the dose is reduced, whereas 20 to 40% have a durable remission. Lower doses of corticosteroids (0.25 to 0.50 mg/kg/day) have been shown to have similar efficacies to conventional doses (1 mg/kg/day) in adults. Recently, high-dose dexamethasone (40 mg/day four days consecutively every two weeks for 1-4 courses) was given as first-line treatment. Cheng *et al*. initially achieved a platelet count >50x10^9/L in 106/125 (85%) of treated patients with only one course; the platelet count increased by at least 20x10^9/L by the third day of treatment. Among the 106 responding patients, 53 (42% of the 125 treated patients) had a sustained response (for at least 6 months); the other 53 (50%) relapsed within six months, mostly (94%) within the first three months. A platelet count less than 90x10^9/L on day 10 was associated with a high risk of relapse.This finding could suggest that the short course of treatment with dexamethasone warrants a good and quick response, but it is unable to induce a complete and reliable control of disease in the half of ITP patients.

Mazzucconi *et al*. treated 90 patients with 4 cycles (cycle repeated every 14 days) of high-dose dexamethasone, achieving an overall response (platelet > 50x10^9/L) in 85.6% of the cases. Relapse-free survival (RFS) at 15 months was 81%; long-term responses, lasting for a median time of 8 months (range 4-24 months), were observed in 67 of 90 (74.4%) patients. In both studies, therapy was well tolerated.

Anti-D immunoglobulin is active only in Rh-positive patients and in pre-splenectomy setting. Anti-D binds to the erythrocyte D-antigen and the immune-complex is cleared by the Fc receptors in the reticuloendothelial system, impairing removal of antibody-coated platelets. The response rate to intravenous anti-D at the dose of 50 μg/kg was 70%; the platelet increase occurred after 72 hours and lasted more than three weeks in 50% of the responders. At doses of 75 μg/kg anti-D increases more rapidly and for a longer period of time the platelet count when compared to the standard dose of 50 μg/kg. The repeated administration of anti-D globulin allows to avoid splenectomy in approximately 40% of ITP adults. However, more recently a randomized controlled trial failed to demonstrate the potential of anti-D to avoid or defer the need for splenectomy in newly diagnosed adults with ITP and a platelet count <30x10^9/L. Anti-D immunoglobulin should be used with care, because can rarely cause intravascular haemolysis and disseminated intravascular coagulation.

Hospitalisation and emergency treatment should be considered for patients with extremely low platelet counts (<5-10x10^9/L) and/or significant bleeding. The treatment generally consists of intravenous immunoglobulin (IVIG) (1g/kg/day for 1-2 days) in addition to steroid. IVIG is effective to quickly increase the platelet count at safe level and stop the bleeding, in approximately 80% of patients. Regardless their high cost and transient effect, IVIG may be usefully administered in preparing patients for surgery or delivery.

Platelet transfusion may be necessary in case of severe haemorrhage, although the survival time of transfused platelet is short.

Second-line treatment

Spleen is the primary site of antibody production and clearance of antibody-coated platelets and splenectomy is generally considered to be the second-line treatment in the management of adults with ITP, in whom first-line treatment and in particular prednisone have failed to achieve a stable safe platelet count. Splenectomy offers a 60-70% chance of cure for patients with chronic ITP, without requiring additional therapy.

Kojouri et al. reviewed 47 case series including 2.623 patients, in which the median complete remission (platelet>150x10^9/L) rate was 66% (range, 37% to 100%). Of 707 evaluable patients with at least five years of follow-up, 456 (64%) maintained their response status. No clinical or laboratory preoperative characteristic has been reported to be able to consistently predict the response to splenectomy. Patients refractory after splenectomy should be evaluated for an accessory spleen, which is the case in 11%. Relapse mostly occur during the first two years after splenectomy.

Laparoscopic splenectomy represents a good alternative to conventional open splenectomy. This procedure may warrants reduced blood loss, more rapid recovery time and a lower mortality rate compared with open splenectomy (0.2% and 1.0%, respectively). However splenectomy in not without risks, perioperative mortality is 0.3 to 0.9%, and the long-term risk of sepsis and thrombosis has been described to be up to 6.3%.

Although splenectomized patients have a relatively small risk for overwhelming infections and there are no data on the efficacy of vaccination, immunizations against encapsulated bacteria (Streptococcus pneumoniae, Haemophilus influenzae B and Neisseria meningitides) are advised at least two weeks before surgery.

Refractory ITP management

Patients were defined refractory when low platelet count (≤20 x10^9/L) persists after splenectomy, if attempted, and/or continue active treatment is required to maintain a "safe" platelet count. Adopting these criteria, fewer than 10% of patients who are initially diagnosed with ITP would eventually be included. On the contrary, other Authors reported an higher rate of chronic refractory patients, up to 25-30% of cases.

The objective of treatment for these patients is to achieve stable "safe" platelet count (>20-30x10^9/L), minimizing the adverse side effect of medication.

Cyclophosphamide, azathioprine, vinca alkaloids, danazol, cyclosporine, mycophenolate mofetil, dapsone, campath-1H, are reported to be efficacious in maintaining a safe platelet count, globally in about 20-40% of cases, with no negligible side effect and toxicity, derived from prolonged treatment. However, results are often transient and all studies were case reports or included small cohorts of patients.

Combination chemotherapy and intensive chemotherapy with peripheral blood stem cell support have been employed with good results in some cases, but with the occurrence of some deaths due to major bleeding, probably related to treatment toxicity.

Recently, two different treatment strategies have been employed with two different aims: 1) to interfere with the B-lymphocytes compartment, thus reducing the capability of the immune system to produce antibodies against autologous platelets; 2) to increase platelets turnover, by the enhancement of the platelets medullary production.

Concerning the first strategies, Rituximab, a monoclonal anti-CD20 antibody that induces transient depletion of B cells, represents an effective and relatively safe therapeutic option for refractory patients. In a large systematic review of the published literature, Arnolds suggests that Rituximab induces an initial response in approximately 60% (range 25-75%) of cases, with a 15-20% rate of long-term complete response and no significant difference between splenectomized and non-splenectomized patients. The median response duration was 10.5 months (range, 2-48 months).

Medeot et al. evaluated the long-term activity and toxicity profile of Rituximab in 26 adult patients with refractory or relapsed ITP; median time from diagnosis to Rituximab was 34.5 months. CR (platelet>100x10^9/L) and PR (platelet 50-100x10^9/L) were obtained in 14/26 (54%) and 4/26 (15%), respectively. Median time of observation was 56.5 months (range 39-77). Nine of the 18 responding patients relapsed after a median of 21 months (range 8-66); 9/26 patients (35%) maintained the response, with a median follow-up of 57 months (range 39-69), and 11/26 (42%) did not need any further therapy. A higher relapse rate occurred among PR patients (6/14 CR vs. 3/4 PR), in accordance to what previously reported by Cooper *et al*. Estimated 5 yr relapse free survival (RFS) and therapy free survival (TFS) were 61% and 72%, respectively. Younger age and shorter interval from diagnosis to rituximab appeared indicators of better outcome.

On the basis of these observations, it could be hypothesized a role for Rituximab in an earlier phase of the disease, when its administration might warrant better short- and long-term results, as a splenectomy-sparing agent. With this purpose, in a phase II multicenter French trial Rituximab was used as an alternative to splenectomy, leading to a durable response (>1 yr) in 40% of patients. At 2 years, 24/60 (40%) patients had platelet counts 30x10^9/L or more off treatment. Among the 36 nonresponders, 25 underwent splenectomy, with 23 undergoing surgery during the year following Rituximab infusions. Splenectomy led to good responses for 15/25 (60%) after a median follow-up of 18 months (range 2-36 months).

Taking together, these data suggest that Rituximab could be considered as a good pre-splenectomy therapeutic option, at least for those patients who are unfit or unwilling to undergo surgery.

Recently, lower doses of Rituximab were employed in 28 adults with refractory ITP, and seemed to show similar activity to standard dose. Overall (platelet count >50x10^9/L) and complete (platelet count >100x10^9/L) responses were achieved in 21/28 (75%) and 12/28 (43%) patients, respectively. However, the median time to response and time to CR were 31 and 44 days respectively, much longer when compared to standard dose. After a median follow-up of 11 months (range 3-18) 7/21 (33%) patients relapsed. Further data with longer follow-up are needed, to confirm these finding.

Zaja *et al*. conducted a prospective randomized study comparing Rituximab and Dexamethasone vs Dexamethasone alone in previously untreated adult ITP patients, with the aim to assess the role of Rituximab as first line therapy.

One hundred and one patients were randomly assigned to receive dexamethasone 40 mg/day for 4 consecutively days with (arm B) or without (arm A) rituximab 375 mg/m^2 weekly for 4 weeks. Patients refractory to dexamethasone alone could receive salvage therapy with dexamethasone plus rituximab. Sustained response (platelet count ≥ 50 x 109/L at month 6 after treatment initiation), was higher among patients assigned to dexamethasone plus rituximab (n= 49) than among those assigned to dexamethasone alone (n=52) (63% *vs.* 36 %, p=0.004). Dexamethasone plus Rituximab was an active salvage therapy in 15/27 (56%) patients refractory to dexamethasone alone. Twelve patients of arm A, 27 of arm B and 19 of salvage therapy group with sustained response, were followed up beyond month 6, for a median period of observation of 18 months (range10-34 months). The relapse rate (platelet < 50x10^9/L) in these three groups was 25% (3/12), 11% (3/27) and 10.5% (2/19) respectively. The safety profile was good without significant difference between the two arms.

On the other hand, another recently introduced but relevant therapeutic option is represented by thrombopoietic growth factors Romiplostim and Eltrombopag. These agents increase platelet production. Up to now, several literature data have been published regarding their efficacy and safety, also when used for a long period of time, both in splenectomized and in non-splenectomized refractory ITP patients. A role for these molecules in the improvement of the quality of life has also been suggested. Stasi will described in detail this topic in the next section of this chapter.

Gasbarrini *et al*. firstly described in 1998 the relationship between *Helicobacter Pylori* (HP) infection and ITP, when observed a significant increase in platelet count in 8 of 11 ITP patients in whom the bacterium was eradicated. More recently, Stasi and Arnold reviewed the data coming from several publications on this topic and published along the last ten years. The prevalence of HP infection is particularly higher in some regions such as Japan and Italy with respect to United States or other European regions.

Globally, the response (platelet ≥30x10^9/L) to *HP* eradication therapy is roughly 50%. Shorter ITP duration seems to be associated with better chances to response, whereas there are conflicting data concerning the predictive value of age and baseline platelet count. The response rate tends to be higher in countries with a high background prevalence of HP infection. The odds to achieve a platelet count response following eradication therapy has been demonstrated to be higher among HP positive in comparison to HP negative patients.

Pregnancy

ITP occurs in 0.1-1/1000 pregnancies, accounting for approximately 3% of women who are thrombocytopenic at delivery. The differential diagnosis in this condition should consider pregnancy-induced ipertension, hemolysis, elevated liver enzymes and low platelet count (HELLP), disseminated intravascular coagulation, microangiopathic processes and gestational thrombocytopenia. The latter, also referred to as incidental or benign thrombocytopenia of pregnancy, is found in 5% to 8% of healthy women with an uneventful pregnancy and accounts for at least 75% of all cases of thrombocytopenia at term. In the absence of symptoms, maternal platelet count should be monitored at least monthly through the first 2 trimesters, biweekly in the third, weekly as term approaches and more often, if indicated.

Steroids and IVIG are the two drugs usually employed to maintain the maternal platelet count over 20x10^9/L. Splenectomy should be avoided and, if necessary, delayed until the second trimester when necessary.

The mode of delivery is based on obstetric considerations. A platelet count > 50x10^9/L is generally considered to be sufficient safe to perform epidural anesthesia. About 4% of ITP neonates born with platelet count < 20x10^9/L and the severity of neonatal thrombocytopenia is often most marked 1 to 3 days after birth, with a risk of ICH estimated to be less than 1%.

There are no antenatal maternal parameters that predict reliably the neonatal platelet count. Only prior neonatal outcome represents a useful predictor of neonatal platelet count in subsequent pregnancies.

Conclusion

ITP is a relatively benign disease, but in some cases its management may represent a challenge for hematologist. Around 5% to 10% of patients have chronic refractory disease with considerable morbidity

and mortality, approaching 10-15% in some series. Often bleeding and infection (treatment related) contributed equally to the death. The recent introduction of new promising therapeutic molecules may significantly modify and improve the ITP management in the next future.

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THE ROLE OF NOVEL THROMBOPOIETIC AGENTS FOR THE TREATMENT OF IMMUNE THROMBOCYTOPENIA

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Primary immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by isolated thrombocytopenia, defined as a platelet count below 100x10^9/L, and the exclusion of other causes of thrombocytopenia.[1] Several issues regarding the optimal treatment of adult patients with chronic ITP remain unresolved. Splenectomy is traditionally considered to be the second-line treatment in adults with ITP in whom achieving a safe platelet count with initial prednisone therapy has failed. For those who are refractory or relapse after splenectomy, there is a long list of available approaches.[2] With the possible exception of rituximab,[3] all have modest response rates, and in the case of immunosuppressive agents, an increased risk of infection. Evolving concepts about the mechanisms of the thrombocytopenia in ITP have led to the investigation of thrombopoietic growth factors for the treatment of patients with this disease.

Rationale for the use of thrombopoietic agents IN ITP

Current treatments for ITP aim at suppressing the production of platelet autoantibodies and/or inhibiting macrophage-mediated destruction of opsonized platelets. However, in several patients the main mechanism of thrombocytopenia may be an impaired platelet production rather that an increased platelet destruction.[2] Studies using indium-111 (111In)-labeled autologous platelets showed considerable heterogeneity in platelet turnover in chronic ITP. Although the platelet lifespan is often markedly decreased, in some patients the lifespan is only mildly reduced; furthermore, platelet turnover (a measure of platelet production) is frequently subnormal. Overall, approximately 40% of patients with ITP had a reduced platelet turnover. In keeping with this finding, autoantibodies against platelet glycoproteins have been shown to interfere with the maturation of megakaryocytes, resulting in reduced platelet production. Furthermore, most ITP megakaryocytes show ultrastructural features of apoptosis or para-apoptosis, and these morphologic changes could be induced in cultured megakaryocytes with ITP plasma. In vitro studies have also shown that antibodies that target the GpIb-IX-V complex may induce thrombocytopenia both by inhibiting megakaryopoiesis, and by inhibiting proplatelet formation. Accordingly, growth factor stimulation of megakaryopoiesis might be expected to increase the platelet count in patients with ITP.

Thrombopoietin receptor agonists. First-generation molecules

Two recombinant thrombopoietins have been used in clinical trials. Recombinant human thrombopoietin (rhTPO), which has a circulatory half-life of 20 to 40 hours, is a glycosylated molecule produced in Chinese hamster ovary (CHO) cells consisting of the full-length, native human amino acid sequence. A number of clinical trials were carried out with a nonglycosylated, truncated form of TPO produced in Escherichia coli composed of the first 163 amino acids of the native molecule and chemically coupled to polyethylene glycol (PEG). The recombinant protein, called *megakaryocyte growth and differentiation factor* (MGDF), had important differences compared to native TPO that probably explain its immunogenic potential. When administered subcutaneously to platelet donors, some of the donors produced antibodies against MGDF that cross-reacted with endogenous TPO, thereby causing severe thrombocytopenia.[4] This adverse event led to the discontinuation of clinical research with both MGDF and the full-length form of thrombopoietin. Nevertheless, early reports of the use of pegylated recombinant human MGDF suggested that megacaryocyte stimulation may actually be effective in ameliorating the thrombocytopenia associated with ITP,5 which further lent support to the use of second-generation thrombopoietic agents in this disorder.

Second-generation molecules

The theoretical advantage of the second generation thrombopoietic agents is that they bear no structural similarity with native TPO, and should not trigger auto-immune anti-TPO antibodies like PEG-MDGF. These compounds, also referred to as TPO receptor agonists or TPO mimetics, bind and activate the TPO receptor. The molecules for which phase III clinical trials in patients with ITP have been completed are romiplostim, a TPO peptide mimetic, and eltrombopag, a TPO non-peptide receptor agonist (Table 1). Several other second-generation thrombopoietic growth factors are in early stages of development, but the discussion here will focus on the two main agents.

Romiplostim

Romiplostim (formerly AMG 531) is a recombinant protein known as a "peptibody". It is made of 2 disulphide-bonded immunoglobulin IgG1 heavy-chain and κ light-chain constant regions (Fc fragments) each of which is covalently bound at residue 228 of the heavy chain with 2 identical peptide sequences linked via polyglycine. The carrier Fc component of the molecule binds to the FcRn salvage receptor and undergoes endothelial recirculation, resulting in a substantially longer half-life than the peptide alone. The peptide component binds to the extracellular domains of the human TPO receptor (TPO-R) resulting in its activation. The efficacy of romiplostim in the treatment of ITP was demonstrated in large multinational placebo-controlled phase III trial conducted over a 24-week period in splenectomized and nonsplenectomized patients. Patients were randomized (2:1) to receive romiplostim or placebo once weekly by subcutaneous (SC) injection over the study period.6 The initial romiplostim dose was 1 μg/kg and subsequent doses were adjusted based on platelet response to achieve target counts of 50x10^9 to 200 x 10^9/l. The maximum permitted dose was 15 μg/kg. A total of 125 patients were enrolled in the studies. These patients had severe and refractory ITP, with baseline platelet counts ranging from 2 to 31 (median 16)x10^9/l. Almost two-thirds had received at least three previous ITP treatments and almost one-third were receiving concomitant ITP therapy. The median duration of ITP was approximately 8 years in splenectomized and approximately 2 years in nonsplenectomized patients. Romiplostim achieved a durable platelet response in 16 of 42 (38%) splenectomized and 25 of 41 (61%) nonsplenectomized patients. Corresponding figures for the placebo group were 0 of 21 (0%) and 1 of 21 (5%), respectively Overall, durable or transient (≥4 weeks with counts ≥50x10^9/l without use of rescue medication in the previous 8 weeks) platelet responses were achieved in 88% (36/41) of nonsplenectomized and 79% (33/42) of splenectomized patients treated with

romiplostim, compared with only 14% (three of 21) of nonsplenectomized and 0% (0) splenectomized placebo recipients (p<0.0001). Romiplostim treated patients were able to maintain a platelet count of 50x10^9/l or more for a mean of 15.2 weeks (nonsplenectomized patients) or 12.3 weeks (splenectomized patients), compared with only 1.3 or 0.2 weeks for placebo recipients.

Table 1. Pharmacological characteristics of AMG 531 and eltrombopag. From Stasi et al.[5]

	*ROMIPLOSTIM**	*ELTROMBOPAG***
Chemical structure	Peptibody	Hydrazone organic compound
Molecular weight	29,542 Da	564.6 Da
Mechanism of binding to TPO-R	Similar to endogenous TPO	Different from endogenous TPO
Formulation	Vials for Injection	Capsules
Route of administration	Subcutaneous†	Oral
Frequency of administration	Once weekly	Once daily
C_0 (pg/mL)	2,810 ±1,170 at 0.3 μg/kg IV 12,900±1,800 at 1.0 μg/kg IV 21,1000±32,000 at 10 μg/kg IV	–
C_{max}	–	7.3 μg/mL at 75 mg/day
AUC	964±1,310 pg · h/mL at 0.3 μg/kg IV 26,700±19,100 pg · h/mL at 1.0 μg/kg IV 153,0000±260,000 pg · h/mL at 10 μg/kg IV	79.0 μg · hour/mL at 75 mg/day
CL (mL · kg-1 · h-1)	754±435 at 0.3 μg/kg IV 63.0±55.7 at 1.0 μg/kg IV 6.69 ± 1.03 at 10 μg/kg IV	NR
Vc (mL/kg)	122±51 at 0.3 μg/kg IV 78.8±10.7 at 1.0 μg/kg IV 48.2±7.4 at 10 μg/kg IV	NR
$t_{\_}$ (h)	1.50±2.83 at 0.3 μg/kg IV 2.41±1.56 at 1.0 μg/kg IV 13.8±3.9 at 10 μg/kg IV	>12
t_{max} (median)	13 days at 0.3 μg/kg IV 12 days at 1.0 μg/kg IV 15 days at 10 μg/kg IV	15 days at 75 mg/day

**In the phase I study, eligible subjects were randomized in a ratio of 2:1 to receive a single injection of AMG 531 at escalating doses or placebo. **In the phase I study, subjects received eltrombopag or placebo as oral capsules once daily for 10 days at doses of 5, 10, 20, 30, 50, or 75 mg. TPO-R: thrombopoietin receptor; TPO: thrombopoietin; IV: intravenous; C0: maximum serum concentration at time 0 after IV bolus administration; C_{max}: maximum plasma concentration; AUC: area under serum concentration-time curve; CL: systemic clearance; Vc, central volume of distribution; $t_{\_}$: half-life; t_{max}: time when peak platelet count was observed; NR: not reported. †Romiplostim was given intravenously to normal volunteers.*

The majority of the romiplostim-treated patients (87%) were able to discontinue concomitant treatments or substantially reduce dosage (by >25%) compared with only 38% of placebo recipients. Moreover, fewer romiplostim-treated patients required rescue medications compared with placebo recipients (26.2 versus 57.1% of splenectomized and 17.1 versus 61.9% of nonsplenectomized patients). Although adverse events were reported in most patients treated with romiplostim or placebo, most events were mild to moderate and appeared to be related to the underlying disease. Among those treated with romiplostim, there were few serious treatment-related adverse events (increased bone marrow reticulin and arterial embolism were each observed in 1 case) or discontinuations because of adverse events (3 cases). Bleeding events of at least grade 3 severity were more common with placebo than with romiplostim (12% versus 7%). There was no evidence of an increased risk of thromboembolic events during romiplostim treatment: such events were equally uncommon in patients receiving romiplostim or placebo (2.5%). No antibodies against romiplostim or TPO were detected. Patients on any of the previous romiplostim phase 1 to phase 3 studies have been enrolled in an open-label study of long-term administration of the drug. Data from 142 patients treated for periods of up to 3 years have been reported.7 Altogether, 87% of patients (n=124) achieved a platelet response (>50x10^9/L and double the baseline value in the absence of rescue medication in the previous 8 weeks) and, on an average, this response occurred for 67% of the weeks on study in patients who responded. Long-term romiplostim treatment was generally well tolerated and treatment-related serious events occurred in 13 patients (9%). Thromboembolic events occurred in seven (5%) patients, six of whom had pre-existing risk factors such as cardiovascular disease and/or a history of thrombosis. Bone marrow samples were taken from 16 patients: eight patients were found to have presence of increased bone marrow reticulin. Reticulin deposition is often present in the bone marrow of healthy individuals and patients with ITP, and increased reticulin has been observed in patients treated with various TPO mimetics.[6] The clinical significance of these findings is unknown, but close monitoring has shown no evidence of progression to collagen fibrosis or clonal myeloproliferative disorder after romiplostim treatment. One patient transiently developed neutralizing antibodies to romiplostim (absent on retesting >4 months after discontinuation of treatment), but these did not cross-react with endogenous TPO or affect the platelet response.

Eltrombopag

Eltrombopag olamine (formerly SB497115) is a small, orally available, hydrazone organic compound developed by GlaxoSmithKline. In preclinical studies eltrombopag has been shown to stimulate human megakaryocyte differentiation and proliferation in a dose dependent manner and to activate the TPO receptor in human and chimpanzees platelets, but is not active on the rat, mouse, ferret, or cynomolgus monkey TPO receptor. Its activity is therefore species-specific. In vitro experiments suggest that eltrombopag interacts with TPO-R at a distance from the binding site for endogenous TPO and appears to initiate signal transduction by a mechanism different from TPO. The results of a 6-month, randomized, double-blind, placebo-controlled, phase III study of eltrombopag (RAISE [RAndomized placebocontrolled ITP Study with Eltrombopag]) have been presented recently.8 RAISE included adult patients with chronic ITP who had platelet counts of <30 x 10^9/l and who had been previously treated for ITP. A total of 197 patients were randomized to individualized treatment with eltrombopag (n = 135; initial dosage 50 mg/day, then adjusted according to individual response) or placebo (n = 62). The primary endpoint for RAISE was the odds of responding (i.e. achieving a platelet count of 50–400x10^9/L) during the treatment period. Patients who received eltrombopag were 8 times more likely to achieve platelet counts 50x10^9/L to 400x10^9/L during the 6-month treatment period compared with patients on placebo (OR [95% CI] = 8.2 [4.32, 15.38]; p<0.001). Baseline median platelet counts were 16x10^9/L in both groups and never exceeded 30 x 10^9/l in the placebo group. Patients responded to eltrombopag regardless of splenectomy status, use of baseline ITP medications, or baseline platelet counts. Significantly fewer patients treated with eltrombopag had any bleeding (WHO Grades 1-4; p<0.001) or clinically significant bleeding (WHO Grades 2-4; p<0.001) throughout the trial compared with patients treated with placebo. More patients in the eltrombopag group (59%) stopped or dose-reduced their concomitant ITP medications than in the placebo group (32%; p=0.016). Patients in the eltrombopag group (19%) required less rescue therapy compared with the placebo group (40%; p=0.001) during the treatment phase of the study. Eltrombopag also had a positive impact on QOL. Quality of life was evaluated using the following instruments: SF-36v2, a 6-item subset of FACT-thrombocytopenia (FACT-Th), and the fatigue subscale of FACIT-Fatigue. After 6 months, patients receiving eltrombopag had significantly greater improvements from baseline than placebo in physical and emotional role, vitality and overall mental health. Furthermore, eltrombopag recipients had greater improvements from baseline in FACT-Th scores than placebo, indicating statistically and clinically significant benefits in concerns for bleeding and bruising symptoms and physical and social activities. Overall, patients treated with eltrombopag perceived an improved ability to participate in activities of daily living and a reduction in fatigue symptoms.

Eltrombopag was generally well tolerated. The frequency of grade 3–4 adverse events during treatment (eltrombopag, two [3%]; placebo, one [3%]) and adverse events leading to study discontinuation (eltrombopag, three [4%]; placebo, two [5%]), were similar in both groups. Other adverse events that were reported with eltrombopag, but not with placebo, were nausea (8%) and vomiting (5%). The interim results of an ongoing long-term study (EXTEND [Eltrombopag eXTENded Dosing]) are available in abstract form.[9] EXTEND enrolled patients with chronic ITP who had previously completed an eltrombopag trial. Patients received individualized dosages (initial dosage 50 mg/day, adjusted to 25-75 mg/day depending on platelet count). A total of 207 patients had evalu-

able data available; median duration of therapy was 91.5 days. In EXTEND, platelet counts of >50x10^9/L were seen in 79% of eltrombopag patients at least once during the study, and similar results were observed among patients regardless of whether they received concomitant ITP medication at baseline or whether they had undergone a splenectomy.

Potential risks of thrombopoietic agents

Rebound thrombocytopenia, i.e. thrombocytopenia below a patient's baseline level, has been observed in 10% of patients recruited for the trials with romiplostim and eltrombopag when thrombopoietic agents were stopped. This phenomen is thought to be due to the higher platelet counts during treatment that may have absorbed and thereby suppressed plasma levels of endogenous TPO. Thrombosis may be a potential risk, but clinical trials have not yet observed a difference in either arterial or venous thrombotic events between treatment and placebo groups. Reversible increase of bone marrow reticulin has been observed in a few patients in the clinical trials with romiplostim.[10] This also requires patient monitoring and a prospective bone marrow study in a larger number of patients to provide a clearer view of the frequency, reversibility, and clinical consequences of bone marrow changes associated with these new agents in ITP patients. Because TPO promotes the viability of hematopoietic progenitor cells of all lineages and TPO receptors are present in hematologic malignancies, there is the potential risk that thrombopoietic agents may accelerate growth of malignant cells.

Conclusion

The results of clinical trials investigating romiplostim and eltrombopag unequivocally demonstrate the efficacy of these agents in elevating the platelet count and reducing bleeding events of patients with chronic ITP. Since these drugs are supposed to be a chronic treatment for ITP but long-term safety data are still lacking, we believe that their initial use should be confined to patients refractory to splenectomy. However, if newer data will support the lack of toxicity over longer periods, they will probably shift to second line as a splenectomy sparing therapy. Apart from their efficacy both prior to and after splenectomy, these new drugs are appealing for a number of reasons, including the fact that they are not blood products, thereby avoiding the potential risk of infectious diseases, and that unlike most of the current conventional therapies they are not immunosuppressive. They have significantly expanded our armamentarium of available drugs for ITP, revolutionizing the therapeutic approach to patients with a severe form of the disease.

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GENETICS AND CYTOGENETICS

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Myelodysplastic Syndromes (MDS) are heterogeneous hematological malignancies. As cytopenia and bone marrow dysplastic features may be found in a number of non-malignant conditions, including vitamin B12 deficiency, heavy smoking, copper deficiency, viral infections, diagnosis is sometime difficult and cytogenetics are required. Chromosomal aberrations identify malignant clones and most importantly, cytogenetic-pathological associations identify clinical MDS subsets . In the clinical-hematological continuum between MDS and AML, typical AML translocations, such as inv(3); t(8,21); t(15;17) and NUP98 changes, are virtually always associated with high-risk MDS. Cytogenetic MDS subgroups have inspired well-validated prognostic stratifications, such as the IPSS score and its recent modification, the WPSS score.

Although MDS are acquired disorders, congenital syndromes, such as Fanconi Anemia, Dyscheratosis Congenita, the Shwachman Syndrome, Neurofibromatosis and the Noonan Syndrome, are preludes to the development of MDS and acute leukaemia, suggesting that the genes causing these syndromes are involved in major pathogenetic mechanisms, particularly in cases of MDS with hypoplastic bone marrow.

A clear overlap emerged between MDS and myeloproliferative disorders (MPD) without BCR-ABL1 or JAK2/MPL mutations. Genetic investigations have made essential contributions to the classification of these disorders and to successful therapies (see below).

MDS/MPD

Jan Cools wrote a brilliant synthesis of MDS/MPD genetics as the *story of tyrosine kinases*. The recent WHO Classification fully incorporated PDGFRB, PDGFRA, FGFR1, KIT, as master genes for diagnosis and treatment. Here we focus on PDGFRB as a tyrosine kinase which may be involved in clinical-hematological phenotypes including typical chronic myelomonocytic leukaemia, MDS and atypical MPD, all of which have eosinophilia as a common morphological denominator . The PDGFRB gene, which behaves as a promiscuous partner in reciprocal translocations, always maintain its 5' end containing the tyrosine kinase domain which is activated by diverse genomic partners. All PDGFRB rearrangements (at least 24 partners) maintain a high sensitivity to imatinib. Now the frontline therapy, imatinib provides complete, long-term clinical and molecular remission.

MDS with hypocellular /aplastic bone marrow

Bone marrow is hypoplastic and increased fibrosis may be observed.

Studies on MDS with hypoplastic bone marrow helped trace a genetic bridge between congenital bone marrow failures and acquired myelodysplastic syndromes. In Fanconi Anemia chromosomal instability predisposes to clonal selection and evolution to MDS and acute myeloid leukaemia. In heterozygous subjects MDS may be the first clinical evidence of the congenital disorder. In the Shwachman syndrome bone marrow failure is rather peculiar as cytopenia is not always associated with bone marrow dysplasia and emergence of clonal chromosomal aberrations, such as 20q- or isochromosome(7q) is not predictive of evolution to MDS. Mutations of genes encoding for specific telomerase complex subunits, such as TERT and TERC showed they played a major role in MDS . Mutations may be either congenital or acquired in familial and sporadic cases of hypoplastic MDS supporting the hypothesis that in congenital diseases a mutator effect results in MDS. Another example is *AML1/RUNX1* germline mutations which underlie a rare familial thrombocytopenia which may evolve to MDS and AML. In addition N-terminal-mutations are found in acquired hypoplastic MDS. A congenital mutator effect has been also hypothesized in MDS in siblings with rare familial monosomy 7. Notably, acquired monosomy 7, a common motif in MDS arising from various congenital bone marrow failure syndromes, is a warning not to administer G-CSF treatment because in cases of congenital neutropenia G-CSF stimulates growth of monosomy 7–bearing cells and evolution to MDS/AML. 5-azacytidine has elicited a good response in the subgroup of MDS associated with monosomy 7.

The 5q deletion in MDS

The 5q- syndrome, as described by Van den Berghe et al in 1974, is the prototype of clinical/cytogenetic associations in MDS. Deciphering

the molecular significance of 5q- has been a big challenge since it is an interstitial deletion with loss of a very large genomic region containing a huge number of genes. Furthermore the deletion may vary in size because of individual variations in the proximal and terminal chromosome breakpoints. A starting point in the identification of critical gene(s) in 5q- was detecting the minimal common deleted region which is at 5q31 in MDS and at 5q33 in AML. Using RNA interfering Golub's group recently showed that RPS14 is involved in the 5q deletion. Its mono-allelic loss plays an important pathogenetic role since haploinsufficiency in a mouse model induced bone marrow dysplasia, increased MCV and a high platelet count as typically found in the 5q- syndrome. However other deleted genes such as catenin alpha , SPARC, EGR1, IRF1, NPM1, DIAPH1, also intervene in the pathogenesis of the 5q- syndrome, suggesting it is an acquired contiguous deletion disease and that variations in phenotype may be related to haploinsufficient genes one copy of which is included in the lost material. Gene expression profiling showed a specific 5q- syndrome signature in CD34+ cells. Interestingly, when an isolated 5q- is found in AML, the genetic characteristics of the 5q- clone are the same as those in the typical 5q- syndrome. Consequently, factors other than simple 5q- may be responsible for disease aggressiveness. Indeed, when the 5q-syndrome is associated with additional cytogenetic aberrations, it is definitively more aggressive, manifesting as AML or type II MDS which rapidly progresses to AML. The majority of MDS induced by chemo- and radio-therapy and by environmental toxic exposure fall into this group.

Why lenalidomide therapy is successful in the 5q- syndrome is not completely understood although immunological mechanisms and stem cell competition in the bone marrow seem to play a role. The appearance of new abnormal unrelated cytogenetic clones under lenalidomide raises crucial biological and clinical questions.

TET2: the earliest pathogenetic event so far

TET2 mutations were discovered in 40% of MDS. Indeed TET2 undergoes LOH,UPD and mutations in other haematological malignancies, such as chronic myeloproliferative disorders, acute myeloid leukaemia, chronic myelomonocytic leukaemia and systemic mastocytosis. There is no evidence of TET2 inactivation by mean of methylation. TET2 belongs to a family of three members (TET1, TET2, and TET3) with undefined functions. The three protein TET family has zinc-binding CXXC motifs which are typically found in DNA- and in histone-modifying complexes. TET1 is known as a leukemic gene as it rearranges with MLL in acute myeloid leukemia. TET2 contains 11 exons and two transcripts are known, namely a short and a long isoform. Both low- and high- risk MDS may show deletion and/or mutations of TET2 at 4q24. In the hematopoietic system TET2 lesions involve a totipotent stem cell which does not acquire malignant properties unless additional genetic events superimpose. Experimental evidence demonstrated TET2 mutations precede the JAK2 in MPD. We also have evidence of germline TET2 deletion without hematopoietic malignancies (*manuscript in preparation*). Moreover TET2 deletion/mutation may occur simultaneously and independently of 5q- in 5q- syndrome (*unpublished*).

New knowledge from new technologies

According to cytogenetics MDS were defined as deletion diseases when compared with AML, which is a typical translocation disease. Today, whole genome analysis with high resolution technologies is opening new horizons in understanding the genetic basis of MDS.. High resolution technologies such as single nucleotide polymorphisms (SNP) arrays will be an important complement to standard cytogenetics as they not only confirmed loss of heterozygosity in MDS but also discovered a high incidence (around 20%) of segmental uniparental disomy with an impact on the overall survival. Heinrichs et al found a specific 7q UPD region in high risk MDS and hypothesized it mirrored the bad prognosis 7q MDS subset that had been identified by cytogenetics. UPD at chromosome 11q helped discover acquired c-cbl gene mutations encoding for a protein that interfered with tyrosine kinase signalling and identify them as the molecular lesion underlying MDS with an 11q- chromosome. New insights are emerging from microRNA investigations in MDS. At least one recurrent chromosomal translocation, i.e., a t(2;11)(p21;q23) involves miR-125b-1. Moreover, miR155 over-expression induced the MPN/MDS phenotype in mice.

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WHO CLASSIFICATION OF THE MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are hematopoietic stem-cell disorders characterized by ineffective hematopoiesis producing marrow failure and a propensity to progress into acute myeloid leukemia[1] MDS display impressive clinical heterogeneity running the gamut from an indolent disease with a near normal life expectancy to an aggressive malignancy overlapping acute myeloid leukemia (AML). Following the original 1982 FAB classification scheme,[2] various additional systems to improve prognostic predictive power in MDS have been proposed. In 2001, the original FAB guidelines were revised and integrated into the World Health Organization (WHO) Classification System.[1] The WHO Classification System has been recently revised and the current updated version (termed 4th edition) was published in the fall of 2008 (1). In parallel to the FAB and WHO classification systems, a prognostic scoring system [International Prognostic Scoring System (IPSS)] based on bone marrow blast percentage, cytogenetic subgroups of outcome, and number of cytopenias has been in clinical use since 1997. More recently, the integration of transfusion dependency within the WHO system has produced the so called WHO classification based prognostic scoring system (WPSS) which has effectively brought together within a unified diagnostic approach, MDS classification and prognostic scoring system.[4]

The WHO classification as an integrated diagnostic approach

The conceptual basis for the WHO Classification System is that the diagnostic categorization of hematopoietic neoplasms should be based not only on morphologic findings, but also that clinical, genetic, immunophenotypic, and biologic information should be used to define separate disease entities.[1] However because a single biologic or genetic marker that reliably identifies all or most of the cases of MDS has not yet been discovered, bone marrow morphology remains the most important tool for classifying the majority of patients with MDS. Although concordance of the diagnosis of MDS is generally reported to be ~ 80%, this only applies to cooperative clinical trials. Outside of this setting, concordance among different observers is often considerably less. The factors which are largely responsible for inconsistencies in diagnosis and classification include the variability in the quality of the specimens, inaccurate enumeration of blasts, and the erroneous inclusions in the MDS category of patients with non-clonal dysplastic hematopoiesis. Therefore, the importance of a critical evaluation of morphology in the light of other laboratory results and clinical information

cannot be overemphasized. Because morphology alone is often insufficient to reach a firm diagnosis, it should be supplemented whenever possible by other tests such as immunophenotypic analysis, cytogenetics, and molecular genetics techniques.[1,5] The best approach is decided case by case on the basis of a preliminary morphological observation and clinical input at the time when the diagnostic material is initially "triaged". Morphological examination requires peripheral blood smear, bone marrow aspirate and bone marrow trephine biopsy. Biopsies should be of an adequate size (at least 1.5 cm, excluding cortical bone) and bone marrow aspirates should be both particulate and cellular enough to perform a 500 cells differential count.[1] The blood and marrow aspirate smears should be examined for dysplasia (defined as >10% of the cells for each given lineage), the percentage of blasts, and the iron stained marrow aspirate for ring sideroblasts. Cells counted as *blasts* include myeloblasts, monoblasts, and megakaryoblasts. Micromegakaryocytes are not blasts, and erythroid precursors are also not counted as blasts, except in rare cases of *pure* erythroid leukemia, in which abnormal proerythroblasts account for the majority of cells.[1]

Flow cytometry diagnosis of MDS is still a *work in progress*. Substitution of the percent of blasts determined by flow cytometry (e.g. by counting CD34 positive cells) for a visual blast count is discouraged because not all blasts express CD34. Furthermore, dilution of the marrow sample by peripheral blood, as well as further cellular loss during processing of the sample may cause discrepancy between the visual estimate and the blast value obtained by flow cytometry.[1] Besides the blast count, multiparameter flow cytometry can also provide evidence of abnormal maturation of the myeloid lineages. There is no single abnormality that is specific, but abnormal light scatter properties, abnormal antigen density, loss of antigens and/or anomalous expression of antigens have all been reported in MDS and may even correlate with the grade of disease. However, the specificity of aberrant antigen expression for MDS, as compared to diseases in which secondary dysplasia may be seen, has not been extensively studied, and aberrant antigen expression has been documented in non-hematologic disorders, such as autoimmune disease, that morphologically can mimic MDS.[1] The WHO approach recommends that cases with inconclusive morphologic and cytogenetic findings and three or more aberrant features by flow cytometry should be re-evaluated over several months for definitive morphologic or cytogenetic evidence of MDS.[1] The value of bone marrow biopsy in this group of disorders is generally well established.[5] It increases the diagnostic accuracy and helps in refining the prognostic scoring system.[6] Besides a *quality check* of the adequacy of the marrow aspirate, the biopsy provides information on cellularity and stroma (e.g. presence of fibrosis, microvascular density - angiogenesis) and can be used to perform a number of studies including immunohistochemistry, in-situ hybridization, or molecular procedures that can provide additional diagnostic information.[5] Additionally, bone marrow biopsy may help in confirming a suspected diagnosis of MDS by excluding reactive conditions in which dysmyelopoietic changes may be at times prominent (e.g. HIV infection, autoimmune diseases). An underappreciated role of the biopsy is that it may provide evidence for another disease that can mimic MDS clinically, such as hairy cell leukemia, lymphoma, or a metastatic malignancy.[5] Among the alterations detected by bone marrow biopsy, a prognostically important finding is the presence of aggregates or clusters of blasts, a typical finding in aggressive subtypes of MDS.[1,5] These can also be identified by immunohistochemistry with CD34.[1,5,7,8] The blast enumeration by immunohistologic analysis is especially helpful in cases of MDS with fibrosis (MDS-F)[7,8] and cases of MDS associated with hypocellular marrows (hypoplastic MDS).[9,10] In both these variants, the presence of reticulin fibrosis (in the former) or fatty changes (in the latter) can make accurate disease characterization very difficult or impossible using bone marrow aspirates. Moreover, the often low cellular yield of the bone marrow aspirate in these cases may also be insufficient to obtain adequate cytogenetic information. Specific diagnostic examples include the separation of MDS-F from primary myelofibrosis or other types of myeloproliferative neoplasm (MPN) with secondary myelofibrosis, as well as from several subtypes of acute myeloid leukemia characterized by bone marrow fibrosis such as, e.g., acute panmyelosis with myelofibrosis, acute megakaryoblastic leukemia,[11] or therapy-related AML, and the identification of hypoplastic MDS and its separation from aplastic anemia.[9,10] While an aggressive clinical course is expected in cases of MDS-F,[7,8] it is still unclear whether hypoplastic MDS differs from the more common cases of MDS with an hypercellular or normocellular bone marrow.[12] Cellularity per se does not seem to affect response to immunosuppressive therapy significantly and does not seem to be the major factor affecting improvements in response to lenalidomide, stem cell transplantation, or hematopoietic growth factors.[12] A description of these two morphologic variants of MDS is, for the first time, included in the current 4th WHO edition (*MDS Overview* section).

Subtyping MDS. Refractory cytopenia with unilineage dysplasia

This designation which is now present in the 4th WHO edition, encompasses those MDS that present a refractory cytopenia (of at least 6 months duration, in the absence of a confirmatory cytogenetic abnormality) associated with dysplasia in >10% of cells in a single cell line. Subtypes include refractory anemia (RA) and the rare cases of isolated refractory neutropenia (RN) and refractory thrombocytopenia (RT). Refractory bi-cytopenia may be included in this category if accompanied by unilineage dysplasia, but refractory pancytopenia that is associated with unilineage dysplasia should be considered as MDS, unclassifiable. The clinical presentation, in most cases, is related to anemia (these cases are properly designated as RA). In RA, anemia may be normocytic and normochromic, but is often macrocytic. In the blood, blasts are absent or represent <1% of circulating leukocytes, and they account for fewer than 5% of the nucleated marrow cells. The marrow is usually hypercellular due to erythroid hyperplasia, and dyserythropoiesis is present, but ring sideroblasts account for fewer than 15% of the erythroid cells. In RA, fewer than 10% of the cells in the granulocytic or megakaryocytic lineages show dysplasia. Monocytes are $<1x10^9$/L in the blood, and there is no monocytosis (<5% monocytes; normal range 0–4%) in the bone marrow. No Auer rods are present. The diagnosis is one of exclusion—other causes of anemia with dyserythropoiesis, such as megaloblastic and congenital dyserythropoietic anemia must be carefully excluded. In general, RA can be considered as a *low-grade* MDS. Median survival times with unilineage dysplasia are reported to be 6 to 7 years, and only 10% progress to overt acute leukemia. RN and RT are very rare and likely account for less than 1% to 2% of all cases of MDS. Other causes of neutropenia and thrombocytopenia need to be excluded, and extreme caution should be used in making such diagnoses.

Refractory anemia with ring sideroblasts (RARS)

RARS is an MDS characterized by unexplained anemia, morphologic dysplasia in the erythroid lineage, and ring sideroblasts comprising 15% or more of the erythroid precursors. There is no significant (<10%) dysplasia in granulocytic or megakaryocytic lineages. No circulating blasts are seen. The RBCs often exhibit a *dimorphic* pattern of normochromic and hypochromic cells, but macrocytosis is frequently observed as well. The criteria are similar to those described for RA with unilineage dysplasia, except that in the bone marrow, ring sideroblasts account for >15% of the erythroid precursors. Like RA, RARS is a "low-grade" process. In most series, RARS is reported to have the best prognosis and lowest rate of conversion to AML of all of the subtypes of MDS. Median survival times of 7 to 9 years or longer are commonly reported, and the conversion rate to acute leukemia is <5%. Whether some patients diagnosed with RARS, who have no evidence of a cytogenetic abnormality, may have ring sideroblasts only due to mitochondrial DNA abnormalities without clonal abnormalities in nuclear DNA is not clear. If the platelet count is greater than $450x10^9$/L, and the megakaryocytes have features of those described in the MPNs, an analysis for a JAK2 V617F mutation and assignment to the provisional entity of refractory anemia with ring sideroblasts and thrombocytosis (RARS-T, see MDS/MPN below) should be considered.

Refractory cytopenia with multilineage dysplasia (RCMD)

This subcategory was an addition introduced in the classification of MDS by the WHO 2001 that was not recognized in the FAB system. Its identification has improved the prognostic utility of the morphologic classification of MDS. RCMD is characterized by one or more cytopenias in the peripheral blood and dysplastic changes in >10% of cells in two or more of the myeloid lineages: erythroid, granulocytic, and/or megakaryocytic series. In RCMD, there are <1% blasts in the blood and <5% blasts in the bone marrow, and Auer rods are not found. If 15% or more of the erythroid precursors are ring sideroblasts, the designation of RCMD with ring sideroblasts (RCMD-RS) can be made; although in the setting of multilineage dysplasia, there is no clear prognostic impact if ring sideroblasts are found or not. Cases that meet the criteria for RCMD,

but have persistently 1% blasts in the blood, should be considered as MDS, unclassifiable (see below), while those with multilineage dysplasia and <5% blasts in the bone marrow but 2% to 4% blasts in the blood should be classified as RAEB. Patients with RCMD have a worse outcome (reported median survival times are 17–33 months) than patients with RA.

Refractory anemia with excess of blasts (RAEB)

RAEB is used to describe MDS with 5% to 19% blasts in the bone marrow or blood. However, if there are <5% blasts in the bone marrow, the finding of 2% to 4% blasts in the peripheral blood is sufficient for the diagnosis. Two subcategories are recognized. RAEB-1 is defined as having 5% to 9% blasts in the bone marrow or 2% to 4% in the blood. If blasts are 10% or more in the marrow, or 5% or more in the blood, the designation should be RAEB-2. Several investigators have shown that patients with 10% or more blasts in the marrow and 5% or more in the blood (RAEB-2) have a worse survival and higher rate of transformation to AML than those with 5% to 9% blasts in bone marrow (RAEB-1). RAEB is a serious disorder, regardless of whether it transforms to overt acute leukemia. Although 30% to 40% of patients with RAEB develop AML, more will die from the complications of neutropenia, thrombocytopenia, or anemia. Median survival times for RAEB-1 are 18 months versus 10 months for those with RAEB-2.

MDS associated with an isolated del(5q) chromosomal abnormality

This subtype of MDS is characterized by anemia, with or without other cytopenias, and interstitial deletion of the long arm of chromosome 5q as the sole cytogenetic abnormality. Myeloblasts comprise<5% of nucleated bone marrow cells and<1% of peripheral blood leukocytes; Auer rods are not seen. An interstitial deletion of the long arm of chromosome 5 is a common abnormality in MDS, and may be seen in isolation or as part of a more complex karyotype. A unique syndrome occurs in which del(5q) is the sole chromosomal abnormality, associated with a prevalence in females with a refractory anemia, normal or high platelet count, megakaryocytes with nonlobated or mono/hypolobated nuclei, fewer than 5% blasts, and a relatively good prognosis. Patients with isolated del(5q) have a high response rate to the drug lenalidomide.

Myelodysplastic syndrome, unclassifiable (MDS, U)

This subtype encompasses those cases that do not fit easily into the other categories of MDS. Three possible situations which qualify a patient for this category include (1) patients with RCUD or RCMD with 1% blasts in the blood found on two occasions, (2) MDS with morphologic unilineage dysplasia associated with pancytopenia, and (3) patients with persistent cytopenias lacking diagnostic morphologic features of MDS or of any specific subgroups of MDS (i.e., <10% dysplastic cells in any lineage) but with cytogenetic abnormalities considered as presumptive evidence of MDS.

MDS related disorders: Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN)

The MDS/MPN are rare conditions which demonstrate hybrid characteristics with myelodysplasia plus atypical findings more suggestive of MPN, such as leukocytosis, thrombocytosis, and/or significant organomegaly.[1,13] They do not fit well into any given category and can only be accurately categorized by using a systematic approach similar to that previously illustrated for the MDS (13). The best characterized members of the MDS/MPN group include chronic myelomonocytic leukemia[13,14] and, in pediatric patients, juvenile myelomonocytic leukemia.[1] Other less well characterized subtypes (because of their rarity) include atypical myeloid leukemia (aCML),[15] a BCR-ABL and JAK2 negative[15] leukemic disorder most often seen in older individuals which is characterized by a poor prognosis, as well as several other poorly understood syndromes. One such syndrome is termed refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T).[16] The hallmark of this condition is the presence of erythroid cells similar to those seen in patients with refractory anemia with ring sideroblasts (RARS), which are found in association with large megakaryocytes, more similar to those seen in cases of Ph' chromosome negative MPN (e.g., in essential thrombocythemia).[13] The demonstration in RARS-T of a high frequency of positivity for JAK2 V617F mutation, possibly acquired as a secondary genetic event, gives further support to a possible relationship with a classical myeloproliferative neoplasm.[13,16] Less frequently MPL W515K/L mutation has also been described.[17] Patients with RARS-T, at least in one series,[18] were shown to have a significantly longer survival than those with MDS of RA or RARS type, but shorter than that of patients with essential thrombocythemia, a myeloproliferative neoplasm with which RARS-T may be easily confused, particularly if an iron staining of bone marrow aspirate is not performed. An additional example of an MDS/MPN–like condition includes rare cases of MDS with isolated 5q- abnormality and myeloproliferative characteristics (19). This hybrid condition must be differentiated from the classical 5q- syndrome, a well defined subtype of MDS. Further examples of a MDS/MPN-like overlapping syndrome are rare cases of chronic myeloid neoplasms associated with isolated isochromosome 17q.[20] This syndrome is characterized by neutrophilia with dysplasia (hyposegmentation of neutrophil nuclei), variable monocytosis, and a hypercellular marrow with pleomorphic megakaryocytes, variable fibrosis, and a high rate of transformation to AML.

Conclusion

In conclusion, while eagerly awaiting a *genetically based* classification scheme free of morphologic subjectivity, the WHO 2008 classification offers a valuable tool in the diagnosis and classification of MDS. The overall goal remains the same: keeping the classification flexible and usable worldwide while, at the same time, making it open to the inclusion of new information.

Table.

Refractory cytopenia with unilineage dysplasia (RA, RN, RT)	Mono-/bi-cytopenia[1] No or <1% blasts[2,3]	Dysplasia (≥10%) unilineage <5% blasts <15% ring sideroblasts
Refractory anemia with ring sideroblasts	Anemia No blasts	Erythroid dysplasia only <5% blasts ≥15% ring sideroblasts
Refractory cytopenia with multilineage dysplasia	Cytopenia(s) No or <1% blasts[2,3]	Dysplasia in ≥10% of the cells of two or more myeloid lineages <5% blasts ±15% ring sideroblasts

[1] Pancytopenia= MDS,u
[2] If 1% blasts in PB (<5% blasts in BM)= MDS,u
[3] If 2-4% blasts in PB (< 5% blasts in BM)= RAEB-1

	PB	**BM**
Refractory anemia with excess blasts -1	Cytopenias <5% blasts* No Auer rods	Uni- or multi-lineage dysplasia 5–9% blasts* No Auer rods
Refractory anemia with excess blasts -2	Cytopenias 5–19% blasts Auer rods +/-	Uni- or multi-lineage dysplasia 10–19% blasts Auer rods +/-
MDS with isolated del(5q)	Anemia, usually normal or mildly increased platelets No or <1% blasts No Auer rods	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts No Auer rods

* Also RAEB-1 if 2-4% PB blasts in cases of RCUD or RCMD with <5% blasts in BM

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RISK-ADAPTED TREATMENT OF MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are usually characterized by clonal proliferation of hematopoietic cells, which partly retain their capacity to differentiate and maturate, but do so in an inefficient manner. Their clinical heterogeneity is best illustrated by the observation that these disorders range from indolent conditions with a near-normal life expectancy to forms approaching acute myeloid leukemia (AML). A risk-adapted treatment strategy is mandatory for conditions showing a so highly variable clinical course, and definition of the individual risk has been based so far on the use of prognostic scoring systems. We have developed a prognostic model that accounts for the WHO categories, cytogenetics and transfusion dependency. This WHO classification-based prognostic scoring system (WPSS) is able to classify patients into five risk groups showing different survivals and probabilities of leukemic evolution. WPSS predicts survival and leukemia progression at any time during follow-up, and may therefore be used for implementing risk-adapted treatment strategies. The approach to a patient with myelodysplastic syndrome should always begin with a period of observation, with sequential peripheral blood counts - and sometimes bone marrow examinations - to assess the rate of progression, if any. Several therapeutic tools have been proposed in the last decades but only few survived the evidence-based criteria of efficacy. Patients with very low WPSS risk do not need any treatment and can be just followed regularly. Transfusion dependent patients with low WPSS risk can be treated with recombinant human erythropoietin. Responsive patients are mainly those with early disease, inadequate endogenous erythropoietin productions and low need for blood transfusion. Transfusion-dependent patients with myelodysplastic syndrome associated with deletion (5q) may respond to lenalidomide with cytogenetic remission and abolishment of transfusion requirement. A recent survival study comparing azacitidine versus conventional care showed that treatment with azacitidine increases overall survival in patients with higher-risk MDS, and the European Medicines Agency (EMEA) has recently approved azacitidine for the treatment of adult patients with high-risk myelodysplastic syndrome who are not eligible for allogeneic hematopoietic stem cell transplantation. The only treatment that can cure a patient with myelodysplastic syndrome is still allogeneic stem cell transplantation. It can be estimated that approximately one third of patients receiving an allogeneic transplantation are cured with this treatment, but only a minority of all MDS patients are eligible and have a donor.

PATHOGENESIS OF APLASTIC ANEMIAS

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Aplastic anemias (AA) are rare and heterogeneous diseases in which the number of normally functioning circulating blood cells is reduced because of a deficient production by bone marrow. AA may involve all cell lineages (Global AA) or some of them (Selective AA). Selective AA can over time transform to Global AA. Both Global and Selective AA can be sub-classified in Ereditary or Acquired AA. Table 1 reports a comprehensive classification of AA. Acquired AA (AAA) are the less rare (2 new cases/million inhabitants/ per year in Western countries i.e. about 120 new cases/year in Italy; incidence in three-folds higher in East Asian Countries) and the most clinically relevant forms in adulthood. AAA (Table1) can be secondary to Radiations, Chemicals and Drugs, Viral infections, Immune diseases, Pregnancy. In most cases however AAA is Primary and Idiopathic (IAAA) i.e. no evident cause is demonstrable.

In-spite of the little knowledge of initial triggering mechanisms, more is known regarding the steps closer to the destruction of the hematopoietic stem cells (HSC) in the marrow of IAAA.

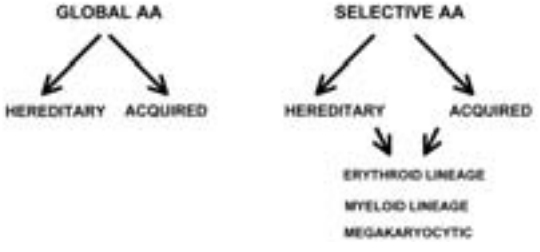
These "*closer* "mechanisms involve the stroma, the haematopietic stem cells, and the immune system. It is likely that in a proportion of patients more than a single pathway is operating.

Though for many years the marrow stroma of IAAA has been considered relatively innocent, recent studies focused on selected cell species demonstrated that mesechimal stem cells from aplastic marrow, differently from normal counterpart, fail to inhibit T cells' proliferation and their release of myelosuppressive cytokine IFN-γ.[1]

HSCs of IAAA are dramatically reduced in quantity (< 1% than those of normal subjects)[2,3] and this may be due, at least in part, to some intrinsic mechanism that renders these cell particularly prone to death. The best characterized of these mechanism so far is the excess telomere shortening. Integrity of telomere is due to a multi-protein complex named telomerase that maintain the length of the telomere impeding excess shortening that leads, via activation of p53, block of cell cycle in G1,up-regulation of cell cycle inhibitor p21, to proliferation arrest and apoptosis and chromosomal instability.[4] Abnormal telomere shortening in HSC has been observed in 30-50% of IAAA subjects[5] suggesting both an excess proliferation of the surviving cell pool and a defect of genes involved in telomerase and telomere maintenance. About 4% of apparently IAAA, have heterozygous mutations of TERC (4) the gene encoding for the RNA template of the telomerase, which is mutated in the autosomal dominant form of hereditary marrow failure disease Dyskeratosis Congenita (DC). Often mutations are similar to those of true DC patients but apparent IAAA subjects neither have the classical stigmata

nor obvious family history, even if show low telomerase activity and very short telomeres. Relatives also, in spite of normal or near normal count, were found to have more subtle abnormalities such as mild anemia, microcytosis, reduced progenitors. Whereas no apparently IAAA patients were found to carry mutation of NOLA 1,[6] a protein stabilizing the telomerase complex, another 4% of apparently IAAA[4] have mutations of TERT, the gene encoding the telomerase reverse transcriptase (the enzymatic component of telomerase) which at the homozygous state characterizes the autosomal recessive form of DC. Similarly to TERC, healthy relatives of TERT mutated IAAA subjects have short telomeres and mild haematological defects whereas clonal diseases (MDS/AML) are reported in family histories. Occasional IAAA patients carrying mutations of *TERF1* and *TERF 2*, genes encoding for shelterin component (the complex protecting the end of the telomere) TRF 1 and TRF 2 were found and so did subjects with heterozygous mutations of Schwachman Bodian Diamond gene that, at the compound heterozygous state, define the hereditary marrow failure Schwachman Bodian Diamond Syndrome. Also SBD mutated subjects were found to have very short telomeres.[4]

TABLE 1
CLASSIFICATION OF APLASTIC ANEMIAS (AA)



GLOBAL AA

ACQUIRED

IDIOPATHIC APLASTIC ANEMIA
- Secondary to
- Radiation
- Drugs and chemical agents
- Virus
- EBV, Hepatitis non A, non B, non C, non E, non G
- HIV
- Immunological diseases
- Eosinophilic Fascitis
- Hypoimmunoglobulinemia
- SLE
- Thymoma
- Pregnancy

Associated to Myelodysplasia and or Paroxysmal Nocturnal Hemoglobinuria.

HEREDITARY

- FANCONI ANEMIA (Aut. Rec, X-linked)
- SHWACHMAN-DIAMOND Syndrome (Aut. Rec).
- PEARSON Syndrome (Mitochondrial DNA deletion i.e.maternal transmission)
- CONGENITAL DYSCHERATOSIS (X-linked and sporadic, Aut.Rec. and Dom)
- AMEGACARYOCYTIC THROMBOCYTOPENIA (Aut. Rec)
- RETICULAR DYSGENESIS (Aut. Rec).
- HAIR CARTILAGE HYPOPLASIA (Aut. Rec).
- FAMILIAL APLASIAS
- DOWN, DUBOWITZ, SECKEL Syndrome

SELECTIVE AA

HEREDITARY

ERYTHROID LINEAGE
- · Blackfan Diamond Anemia (Aut Rec, Dom, Sporadic)
- · Congenital Diserythropoietic Anemia (Aut, Rec Dom).
- · Pearson Syndrome (Mitochondrial DNA deletion. Maternal transmission)

MYELOID LINEAGE
- * Severe Congenital Neutropenia (Aut. Rec.Dom. Sporadic)
- * Cyclic Neutropenia (Aut. Dom. Sporadic)
- * Shwachman-Bodian- Diamond Syndrome.(Aut. Rec)
- * Pearson. Syndrome. (Mitochondrial DNA Deletion. Maternal Trasmissiion)

Dysgenesia Reticolare (Aut.Rec).
Mielocatexis (Aut. Dom)
Familial benign or ethnical neutropenia
Neutropenias associated to methabolic diseases
Neutopenias associated to immunodeficiency

MEGAKARYOCYTICLINEAGE
Microthrombocytopenias
- Wiskott-Aldrich (WAS) Syndrome (X linked)
- X linked Thrombocytopenia

Normothrombocytopenias
- Amegakarocytic Thrombocytopenia with and without congenital somatic abnormalities (Aut.Rec)
- Thrombocytopenia absent radii (Aut. Rec).
- Autosomal dominant Thrombocytopenia
- Familial Platelet Disorder with predisposition to AML (Aut. Dom).
- Thrombocytopenias associated to Metabolic diseases (Organicoacidosis)

Macrothrombocytopenias
- -Bernanrd soullier syindrome. (Aut. Dom).
- Cardio - Facio palatina Syndrome. (Aut. Dom)
- Pseudo von Willebrand disease. (Aut. Dom)
- Benign Mediterranean Macrothromboyitopenia (Aut. Dom).
- Thrombocytopenia with Dyserithropoietic Anemia. (X-linked)
- Thrombocytopenia with thalassemia. (X-linked)
- Paris-Trousseau Thrombocytopenia type (Aut. Dom).
- °Thrombocytopenis due to mutations of MYH9 gene (Aut.Dom).
- Gray Platelet Syndrome. (Aut. Dom)
- Montreal Syndrome (Aut. Dom)
- Macrothromboyitopenia with platelet expression of Glicoforina. (Aut. Dom)

° inclodes the following types wich are considered different expression of the same disease due to MYH9 gene mutations: Sebastian Syndrome, Epstein Syndrome, May-Hegglin Abnormality

ACQUIRED

ERYITHROID LINEAGE
- Idiopathic Pure Red Cell Aplasia (immunomediated)
- Pure Red Cell Aplasia associated to autoimmune disease
- Transient Erithroblastopenia of Childhood
- Post-infections Pure Red Cell Aplasia
- Anemia due to drugs and toxics
- Anemia associated to nutrition deficiency
- Anemia associated to lympho-myelo prolipherative disorders

MYELOYD LINEAGE
- Idiopathic Neutropenia
- Neutropenia due to drugs nd toxicsPost-infection neutropenia
- Neutropenia associaed to autoimmune diseases
- Neutropenia associatated to nutrition deficiencies
- Neutropenia associated to lympho-myelo prolipherative disorders

MEGAKARYOCYTIC LINEAGE
- Idiopathic Thrombocytopenia
- Thrombocitopenia due to drugs and toxics
- Post-infection thrombocytopenia
- Thrombocytopenia associated to autoimmune diseases
- Thrombocytopenia associated to nutrition deficiencies
- Thrombocitopenia associated to lympho-myelo prolipherative disorders

Regarding the role of telomere shortening in the pathogenesis of IAAA there is a discrepancy between the 30-50% of patients having short telomere and the less than 10% found with telomerase and shelterin mutations. This may be due to involvement of additional genes (other telomere binding proteins, DNA repair proteins) to be investigated or to the fact that telomere shortening is largely dependent on HSC replications. At the current state a telomerase mutation *per se* can not be con-

sidered causative of IAAA but a just a genetic predisposition factor. Another mechanism leading to the massive reduction of the number of HSC in IAAA marrow, is their immuno-mediated destruction. Indeed the autoimmunity has been deeply investigated over many years and is so far the major pathogenic mechanism of IAAA, as inferred also by the high rate of response -up to 80% -[7] observed after combined immunosuppression. Various abnormalities of the immune system have been demonstrated in IAAA. Patients have more frequently than normal controls, over-representation of polymorphism of myelosuppressive cytokines like IFN gamma[8] and up-regulation of Th1 transcription Factor (T- bet) that binds to IFNγ promoter.[9] In addition to the stroma, also PB and BM lymphocytes were shown to over- produce myelosuppressive cytokines IFNγ and TNFα[10,11] that non specifically inhibit haematopoiesis by blocking mitosis and increasing apoptosis of HSC. Oligoclonal $CD4^+$and $CD8^+$ T cell populations were shown sometimes to fluctuate according to response to immunusuppression.[12] $CD4^+CD25^+FOXP3^+$ T-regulatory cells, believed to control the development of autoimmune diseases by suppressing autoreactive T-cells, are reduced in IAAA[13] thus suggesting a similarity of this type of marrow failure with other autoimmune diseases. Aspecifically myelosuppressive cytokines IFNγ and TNFα are known to act also by up-regulating FAS expression on HSCs as confirmed in mice model where abnormal FAS or FASL expression contributed to marrow hypoplasia.[14] Heterozygous mutations of Perforin gene, responsible of the severe disease Familial Haemophagocytosis 2 (FHL2) were seen in IAAA along with low level of the protein in T cells and decreased natural killer cell cytotoxicity.[15] SAP, a small modulator molecule inhibiting IFN gamma production that when mutated causes a fatal X-linked lymphoproliferative disorder (XLPD) occurring in response to Herpes viral infection, classically EBV, is also under-expressed in AAA.[16] These findings points to two additional genetic predisposition factors to development of AAA and strengthen the role of aberrant proliferation and activation of cytotoxic T-cells associated to aplastic anemia as an effective arm for HSC depletion. In addition to antigen- non specific mechanisms, also antigen- specific autoimmune pathways are advocated in IAAA. HLA class II-restricted cytolysis of autologous HSCs has been shown by T cell clones of some IAAA.[15] HLA-DR2 is over expressed and is predictive of response to Cyclosporine.[17] Also auto antibodies were demonstrated in a number of IAAA of patients.[18] In 40 % of subjects antibodies anti kinectin, a protein expressed on HSCc, liver, ovary testis and brain cells were detected but no anti kinectin T cell were found. Antibody anti Diazepam-binding related protein- 1 an enzyme oxidating unsaturated fatty acid, largely diffuse in the tissues, was occasionally seen in AA subjects and found capable of stimulating T clones in rare patients.[16] However, in spite of these evidence the role of auto antibodies in the pathogenesis of IAAA is still to be elucidated.

According to a widely accepted vision, IAAA is considered in most cases as deriving from an autoimmune attack triggered by various stimuli like infections mainly viral, drugs, chemicals, genetic events that may activate auto-reactive T cells. Genetic mechanisms may potentiate the effect of autoimmunity. This may either occur indirectly by weakening the HSC via shortening the telomere, or directly reinforcing autoimmune attack by polymorphisms of high production of myelosuppressive cytokines, by perforin mutations, by HLA-DR2 molecules and auto antibodies production.

Whatever the precise sequence of events, the extracellular common final effector of the HSCs destruction is the release from activated T cells of myelosuppressive cytokines IFNγ and TNFα that kill HSC via different pathways such as increase of apoptosis and block of mitosis. The role of these cytokines, particularly TNFα has been outlined by a recent study[19] showing that also patients responding to IS still retain an excess of this cytokine in the marrow which might account for the relapse risk after immunosuppression leading to the hypothesis to strengthen this therapy with specific agents targeted against this key molecule.

In percentage varying from 30-50% the outcome of AA is complicated by the rise of PNH and MDS clones. Detailed explanation are outside the scope of this abstract but it is worthy mentioning that according to the most substantiated model, the PNH clone (cells lacking GPI-A molecules on their surface) is spared by the immune attack to the marrow and, maintaining normal proliferative capacities, takes advantage, on the weak and unpopulated marrow of AA subjects,[16] thus constituting part of the hematopoiesis.

In the case of MDS a genetic mutations, possibly triggered by immune attack, occurs at the level of HCSc and confer them a proliferative advantage, more easy to realize if the marrow is empty, thus leading to the development of MSD. This is well substantiated in MDS cases in which the clone bears trisomy 8. In these patients, who respond to immunosuppression, there is an oligo clonal expansion of T-cells capable of recognizing highly expressed antigens on trisomy 8 cells (*WT1* antigen). These cell however are not killed since they resist to apoptosis because of up- regulation of anti-apoptosis genes as c-myc, survivin and *CDK1*, which confers them proliferative advantage.[16] MDS with monosomy 7 have worse prognosis due to easier evolution to Refractory Cytopenias or to frank acute leukemia. Monosomy 7 is reckoned to arise after use of G-SCF. In fact monosomy 7 cells were shown to take proliferative advantage when exposed to high concentration of G-CSF that selects cells carrying a short isoform of the G-CSF receptor capable of inducing proliferation but not differentiation response.

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CURRENT CHALLENGES AND FUTURE GOALS IN IMMUNOSUPPRESSION FOR APLASTIC ANEMIA

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Idiopathic Aplastic Anemia (AA) is the typical acquired bone marrow failure syndrome (BMFS), characterized by low peripheral blood counts in the context of an empty or fatty bone marrow. The clinical management of AA depends on disease severity (as assessed by blood counts) and patient characteristics; however, immunosuppression (IS) is a key strategy for all AA patients. This is due to the pathophysiology of AA, which in most cases is immune-mediated; in fact, a number of experimental evidences support the pivotal role of the immune system in the damage of hematopoiesis, even if the actual target of such immune-attack – namely, the molecular antigen(s) – is still obscure. However, a minority of patients may harbor disease mechanisms other than immune-mediated; inherited mutations of genes involved in the human telomerase complex have been identified, bridging acquired AA to constitutional forms of AA with late onset. Historically, the benefit of IS in AA patients was initially perceived by Mathe' in the '70s, from the observation that some AA patients experiencing graft failure after stem cell transplantation (SCT) may subsequently recover with hematopoiesis of recipient origin. The success of a transplant procedure lies on both the disease-sparing effect of the conditioning regimen (which is essentially immunosuppressive for AA patients) and on the sustained engraftment of donor hematopoietic stem cells (HSC); thus, such autologous recovery suggested that IS itself may be sufficient to counteract disease mechanisms. From the '80s IS has been tested as sole treatment for AA patients ineligible to SCT; preliminary results were more robust than a simple proof of principle, leading in a few years to the establishment of IS as a worthy treatment option for many AA patients. IS treatment (IST) may include several different drugs; anti-thymocyte globulin (ATG) has been the key agent since the beginning of the development of IST for AA. In comparison to the coeval best supportive care, ATG alone demonstrated a substantial response rate and a superiority in overall survival. Larger studies confirmed such good results, with response rate in the range of 30-70%; this broad range was at least partially due to heterogeneous definitions of clinical response. In fact, responses may range from simple hematological improvement to partial response (PR, transfusion independence with still reduced blood counts) and to complete response (CR, transfusion independence with normal blood counts). Recent reports try to follow common criteria, as those proposed by Camitta, to allow comparison of results among studies; thus, CR + PR were in the range of 30-50%. However, it came out that many responses were not stable, and subsequent worsening of blood counts up to clinical relapse may occur. With the aim to increase and to prolong over time the response rate several studies investigating combined IS were set; different agents were associated to ATG, including steroids, androgens and cyclosporine A (CyA). Only this latter has been proven effective in increasing the response rate, as initially demonstrated by the German Aplastic Anemia Group; in addition, with a very long follow up, CyA was able to reduce the relapse rate, leading to superior failure free survival.[1] Since the end of the '80s ATG + CyA has been considered the standard IST for AA patients, with an expected 50% probability of response and 60% overall survival at 1 year. The following decade of IST experience resulted in a further 10-20% increase in overall survival;[2] this improvement essentially lied on better supportive care, which reduced fatal events in patients waiting for clinical response, and allowed prolonged survival even in non-responders. In the '90s, the availability of standardized IST for AA patients led to the issuing of open questions, which need to be answered to further improve clinical results.

Standard IST consists of ATG and CyA; however, it is less homogeneous than thought. In fact, different ATGs exist, and in some cases have been used without distinction. Most available data coming from large randomized clinical trials refer to polyclonal ATGs obtained from horse (h); of note, the US most utilized hATG (ATGAM®, Upjohn) 2 is different from that used in Europe (Lymphoglobuline®, Genzyme),1 which on the other end is not available anymore. Rabbit (r) ATGs are available too (Thymoglobuline®, Genzyme; ATG-Fresenius); Thymoglobuline has been utilized in AA patients, and retrospective data as well as prospective series demonstrated a substantial efficacy. In most cases, rATG has been used as second-line IST (after initial hATG) to prevent side effects due to possible sensitization to horse proteins, resulting in response rate up to 68% (in relapsed patients).[3] However, large prospective studies and comparisons with hATG (even to assess dose-equivalence) are lacking; the NIH is currently running a randomized study comparing hATG vs rATG in new AA patients, while the EBMT has an ongoing pilot study to assess the best Thymoglobuline dose, the only available ATG preparation in Europe. Regardless the specific preparation, treatment related morbidity may be significant after ATG therapy; in fact, allergic reactions against heterologous proteins are relatively frequent and may even lead to anaphylaxis. In addition, serum-sickness disease is a well known risk, requiring adequate pharmacological prevention. However, in most if not all patients appropriate procedures (steroids/antihistamines premedication, slowing down of the administration as long as 24 hours) result in a safe completion of the scheduled treatment. In any case, ATG therapy remains an intensive treatment requiring specialized medical care; in addition, ATG treatment is burdensome for patients, because prolonged hospitalization and central line are needed, regardless the initial medical conditions. This leads to the question which AA patients need to be intensively treated. Few studies have addressed this question; while it is well established that early IST results in a better survival in severe AA (SAA) patients (regardless their clinical presentation), data on moderate AA (MAA) patients are less robust in terms of survival. However, given the proven efficacy of IST in this setting, it seems appropriate to treat all symptomatic patients and individuals with long life-expectancy, while the treatment may be deferred in asymptomatic patients with comorbidities (or older patients), which may be considered for a try with CyA alone. On the other side, very sick patients might be thought not eligible for IST; it has to be remarked that IS is a necessary treatment for these patients, and no absolute contraindication exists (even fever, infection or hemorrhages). Of course, if a rapid medical improvement may be expected, a short delay does not impact treatment outcome (in contrast to deferring the IST of weeks or months). Similar considerations apply to elderly patients: IST in subjects >70 years shows similar efficacy as in the younger ones (even if long-term survival is of course less impressive). Safety concerns about the increased risk of infections and bleeding in this population has raised the idea to lower ATG doses to increase its tolerability; unfortunately, the tested dose resulted ineffective and treatment decision is usually based on individual performance status (even if ATG is usually not utilized in patients >80 years).

The majority of AA patients achieve some clinical benefit from IST; with current regimens, the response rate is about 60-70%, equally distributed between CR and PR.[1,2,4] Hematological improvement is sometimes seen after 3 months and usually within 6 months, even if later responses are possible. The quality of response is heterogeneous, and most patients cannot be considered cured. In fact, many of them require long-term maintenance IS treatment by CyA to sustain the response: even in recent studies, CyA-dependency has been in the range of 25-50% of patients.[1,2,4] Nevertheless, relapses after an initial response to IST are frequent: in about 30-50% of cases the disease comes back within months or years from IST discontinuation.[1,2,4] In the most recent experiences, the extension of CyA therapy beyond 6 months resulted in a reduction of the relapse rate, but a shared policy on CyA tapering (when and how) has not been achieved. Late relapses remain possible, maybe also due to a suboptimal therapeutic range of CyA in long-term responders (due to tolerability or scarce compliance). Treatment-failure free survival at 5 years may be estimated in the range of 30-50%;[1,2,4] however, overall survival remains significantly higher (55-85%),[1,2,4] because of available salvage treatments. In fact, patients relapsing after a successful IST may be rescued by further IST courses (or even by SCT); the expected response rate in these relapsed patients is 50-70%,[3] proving the principle that standard IST may be insufficient in some patients. In these relapsing patients, even a third course of ATG may be considered, while this strategy is usually useless in refractory patients; however, given the increased risk of systemic reactions, all these patients seem candidate for alternative experimental IST.

Treatment-failure remains a major problem after IST, but possible causes are still elusive. Several attempts have been done to identify possible factors predicting response to IST; different studies have shown the presence of HLA-DR15 and of a paroxysmal nocturnal hemoglobinuria (PNH) granulocyte population confer higher chances of response (as surrogate biomarkers of immune-mediated pathophysiology). Even some distinct AA subentities (e.g., hepatitis-associated, pregnancy-associated, drug-induced) may respond to IST. Very recently, baseline absolute reticulocyte and lymphocyte counts (>25x10^9/L and >1x10^9/L, respec-

tively) have been found associated with a higher response rate and a better long-term survival,[5] while data on baseline absolute neutrophil count remain controversial (especially in adults). To understand why some patients do not benefit from IST, a distinction between primary and secondary failures is needed, because in the latter the previous response to IST supports the underlying immune pathophysiology. In primary failures, seen in one third of patients, an easy explanation is that some AA are not immune-mediated; of course, misdiagnosis has to be considered too (hypo/aplastic myelodysplastic syndromes [MDS] may be erroneously classified as AA). In a limited number of cases, inherited defects of the telomerase complex genes may contribute to disease development interfering with the self-renewal capability of HSCs. For these patients, IS cannot be considered an etiologic treatment; in contrast, androgens have been proven effective in vitro to increase telomerase function, and may be useful in vivo. Second, the efficacy of IST relies on the possibility that residual HSCs repopulate the bone marrow, once the injuring event has been removed. Thus, regardless the pharmakodynamically activity of IST, treatment failures may occur because HSC may have been exhausted, or due to an impaired function resulting from a sublethal damage of residual HSCs. The third possible explanation for IS failure is that the treatment utilized was not able to control the ongoing immune-attack, as confirmed by possible response to further IST in patients experiencing secondary failure. Last, a more rare reason for long-term treatment failure is the so-called disease clonal evolution (better defined as transition to malignant clonal diseases). In fact, emerging long-term complications of IS-treated AA patients are the progression to MDS and/or to acute myeloid leukemia (AML), and the emergence of a PNH clone. These events have to be considered substantially distinct: in fact, while PNH is consistent with the underlying immune-process (leading to selection and expansion of the PNH HSCs, spared from the immune-attack), MDS and AML represent the development of a malignant clone resulting from oncogenic mutation(s) in the residual, likely stressed, HSCs. Few (>1%) PNH granulocytes may be detected in 10-35% of AA at baseline; such population may expand during disease course, independently from any IST. In addition, a PNH population may emerge after IST in about 10% of AA patients; given the embedded pathophysiology of AA and PNH, it is questionable whether PNH may be considered a possible complication of IST or of AA itself. In contrast, in some AA patients the clonal evolution represents the transition to a malignant hematological disorder, namely MDS or AML; this event is estimated in the range of 10%. To note, karyotyping should be interpreted cautiously in this context of oligoclonal hematopoiesis, because some abnormal karyotypes might also simply be considered a marker of clonality reflecting the fixation of a neutral mutation (e.g., trisomy 8), rather than the intrinsic malignant propensity of such clone (e.g., monosomy 7). The causal relationship between development of MDS or AML and IST (or AA itself) is still elusive; in a Japanese retrospective study in children, the cumulative dose of G-CSF has been associated with a higher risk of secondary MDS/AML (especially with monosomy 7). G-CSF has been used as supportive therapy in the context of IST to speed neutrophil recovery, resulting in higher neutrophil counts in responders and early identification of non-responders in a GITMO study. However, it did not translate in a reduction of infectious events, nor in any improvement of survival at 3 years. The safety concerns about the routine use of G-CSF have been partially confirmed by an EBMT retrospective survey, while two subsequent randomized studies in adult population did not.[4] The issue likely will be cleared by the forthcoming data from a large EBMT study which has randomized more than 200 AA patients to IST ± G-CSF, and has completed its enrollment in 2008. At the moment, it seems reasonable to not recommend a broad and prolonged use of G-CSF to artificially sustain neutrophil count, while a short term administration in case of infectious complications may be reasonable. Finally, the development of secondary solid malignancies is not so rare after IST for AA (about 10%). Given the recent data demonstrating inherited genetic lesions even in some (apparently) acquired AA, it remains questionable whether such solid tumors are linked to the IST, to the underlying disease, or whether they are just random events.

A number of challenges are still open in IST for AA: i. to increase the initial response rate; ii. to improve the quality of initial response (increasing CRs, and reducing CyA-dependency); iii. to reduce the relapse rate; iv. to reduce the risk of long-term complications; v. to treat patients failing standard IST. Different strategies have been employed to improve IST in AA patients, and a list of candidate agents have been hypothesized to potentiate IS; in most studies, ATG and CyA remained the key agents, while a third IS drug (possibly with a different mechanism of action) has been added. A purine synthesis inhibitor, mycophenolate mofetil, associated with ATG and CyA, did not result in increased response in a NIH study (62% at 6 months), with a relapse rate unchanged at 37% (despite continuing the maintenance therapy).6 A mammalian target of rapamycin (mTOR) inhibitor (rapamycin or sirolimus) has been investigated in a randomized trial at the same institution; surprisingly, it has not resulted in any improvement of hematological response in comparison to the control arm (ATG + CyA), and a myelotoxic effect has been hypothesized (possibly explaining the low response rate in the experimental arm).[7] A different approach employed IS agents alternative to ATG and CyA; high-dose cyclophosphamide (CTX) has been proved effective in an initial study, with a response rate of about 70% (which were delayed up to 12 months from treatment).[8] However, a subsequent randomized study (ATG + CyA as control arm) has been stopped due to fatal infectious complication in the experimental arm (CTX + CyA), likely related to the prolonged neutropenia resulting from CTX toxicity.[9] In this latter study, even the initial observation that CTX may reduce the risk of MDS/AML development has not been confirmed. In the recent years, many new IS medications pertaining to the class of biological agents have been hypothesized for the treatment of AA and related bone marrow disorders, such as pure red cell aplasia (PRCA) or pure white cell aplasia (PWCA). Some pilot studies have tested the anti-CD25 monoclonal antibody daclizumab, which inhibits IL-2 dependent lymphocytes activation pathway, with some efficacy in MAA and PRCA. Some inhibitors of the cytokines involved in the suppression of hematopoiesis have been tested in MDS, especially the TNF-α inhibitors etanercept and infliximab; their potential use in AA patients may be suitable, but so far experiences have been anecdotic. Newer biological IS agents recently approved for other immune-mediated diseases (e.g., alefacept, efalizumab, etc.) or still under development (e.g., anti-IFN agents) may be of interest even for AA in the future years. Anti-lymphocyte monoclonal antibodies have also been hypothesized as an alternative to ATG; while the rationale for using rituximab (an anti-B cell antibody) is quite weak, the anti-CD52 monoclonal antibody seems a good candidate for a T cell-mediated disease such as AA. Given its lymphocyte depleting effect, alemtuzumab is a very powerful IS agent aiming to give a more profound (and possibly prolonged) IS. Some small series have documented its efficacy,10 and two randomized trials are currently ongoing at NIH to assess its efficacy as first and second line treatment. Recently, an EBMT survey including a prospective phase II pilot study (SIE 2009 abs. #572) has shown a global response rate of 53% in AA (which was as high as 84% in the prospective trial, which also included newly diagnosed patients). Of note, alemtuzumab was given subcutaneously, with no safety concerns (the infectious complications were below the expected rate), excellent tolerability and no treatment-related hospitalization (except in case of pre-treatment unstable medical conditions). The same regimen has been utilized for PRCA and PWCA, with response rate higher than 80%, demonstrating an excellent feasibility and tolerability in comparison to ATG-based IST (even in case of retreatment due to relapse), and possibly comparable efficacy.

In conclusion, IST represents a valid etiologic treatment for most patients suffering from AA and other related BMFS, even if it is not considered as curative as SCT. The choice between a conservative treatment (IST) and a more aggressive approach (SCT) lies on the risk-to-benefit assessment of IST and even more of SCT, which grossly depends on patient age and type of available donor. Current data support ATGs (h-ATG or r-ATG) associated with CyA as the current standard IST for SAA patients, even if some newer IS agents seem worthy of future investigations. Further challenges of IS strategies should aim to improve treatment-related toxicity and patient tolerability, as well as to improve initial and sustained hematological response and to reduce long-term complications. The achievement of such goals will shape the future scenario of the clinical management of AA patients.

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CHRONIC MYELOID LEUKEMIA: IMATINIB THERAPY

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Chronic myeloid leukemia is a unique disease, characterized by the presence of an aberrant chromosome (Philadelphia chromosome) derived from the reciprocal translocation between the long arms of chromosomes 9 and 22, the t(9;22)(q34;q11) translocation. This transposition juxtaposes the Breakpoint Cluster Region (BCR) adjacent to the Ableson gene and generates the chimeric gene BCR-ABL, which encodes a constitutively activated tyrosine kinase. Recognition that BCR-ABL expression was causally linked to the pathogenesis of CML and that the aberrant tyrosine kinase activity was vital for the malignant clone led to the development of the first molecularly targeted therapy for CML.

Imatinib (Glivec®, Gleevec®, Novartis Pharmaceuticals, East Hanover, NJ) is a 2-phenylaminopyridimine compound, rationally designed to selectively inhibit the Bcr-Abl tyrosine kinase. The preclinical program, initiated in the early 1990s, moved slowly because of the concern that the molecule would not be selective enough to be clinically useful, and would have targeted also other protein tyrosine kinases essential for normal cell survival. Further, it was possible that targeting of a single molecular defect would not be an effective anticancer strategy. However, study of cell lines and primary material collected from patients suggested that it did indeed selectively inhibit proliferation of CML cells. Phase 1/2 clinical studies started in 1998, involving patients resistant or intolerant to IFN-alpha. The new agent formulated for oral administration, then termed STI571, is eliminated predominantly by hepatic metabolism; it has a plasma half-disappearance time of 18 hours, which provides the pharmacokinetic rationale for once daily administration.

In phase 2 clinical trials, imatinib was tested in patients with CML in late chronic phase (LCP), who had previously failed IFN-alpha therapy; 95% of these patients achieved a complete hematologic response and 60% a major cytogenetic response after a median follow-up of 18 months. At that timepoint, estimated rates of freedom from progression to accelerated and blastic phase (PFS) and overall survival (OS) were 89% and 95%, respectively. At last update (6-year follow-up), PFS and OS were 61% and 76%, respectively. These results confirmed those observed in the phase 1 studies and led to FDA approval of imatinib in May 2001.

Thanks to the introduction of imatinib into clinical practice, CML is perhaps the most dramatic example of how therapies with a specific molecular target can change the natural course of a malignancy. In this review, we discuss the evolution of imatinib therapy for patients with newly diagnosed CML, starting with imatinib standard and high dose, then discussing current investigational trials of imatinib in combinations or in rotations with other agents, and finally providing a glimpse at the possibility of imatinib dose reduction/discontinuation in the long-term.

Evaluating response to imatinib

The response to treatment in CML proceeds in an orderly manner, first with restoration of spleen size to normal and normalization of the blood count, then with reversion of the bone marrow to Ph negativity, and eventually with reduction in the number of BCRABL transcripts in the blood and marrow to very low or undetectable levels. These criteria are also used sequentially to monitor the response in an individual patient (Figure 1).

The majority of patients (more than 95%) achieve a complete hematologic response (normal blood counts and spleen size) by 3 months after start of treatment. Because of the well-documented association between cytogenetic response and improved survival, conventional cytogenetic analysis is the best indication of outcome. Approximately 65% will have achieved a complete cytogenetic response (CCgR) (absence of Philadelphia-positive metaphases in bone marrow samples) after 1 year of therapy. Once a patient has achieved a CCgR, the most sensitive method for monitoring the residual disease is to measure BCR-ABL transcript numbers. The current methodology involves real-time quantitative polymerase chain reaction (RQ-PCR) whereby the BCR-ABL transcript numbers are related to transcript numbers of a control gene (ABL) that ideally is expressed in the leukemia cells at approximately the same level as in normal cells. The result is expressed as the ratio of BCR-ABL control gene transcript numbers on a log10 ABL and then converted to the International Scale. A Major Molecular Response (MMolR) is defined as Bcr:Abl transcriptIS < 0.1%. Patients should be monitored by bone marrow cytogenetics at diagnosis and at 3-month intervals until achieving CCgR. Thereafter, marrow cytogenetics may be performed at 6 -12 months intervals, depending on the availability and on the frequency of molecular rests. Molecular studies can reasonably be performed at 3-month intervals until a MMolR is achieved, and then every 6 months, indefinitely.

Imatinib in the treatment of early chronic phase CML: standard dose

The issue of the optimal dose of IM is not yet settled. In Phase 1 studies the maximum tolerated dose was not identified while a dose of 300 mg and above resulted to be able to induce significant therapeutic benefits (long-lasting complete hematologic responses); a starting dose of 400 mg daily (which induces a blood concentration of IM consistently higher than that required to inhibit 50% of BCR-ABL TK activity in vitro) was therefore recommended. Table 1 summarizes the results of the most important clinical trials on imatinib in chronic phase CML.

The IRIS study

In June 2000, the International Randomized Study of Interferon and STI571 (IRIS) study was initiated for newly diagnosed, early chronic phase CML patients. Overall, 1106 patients were randomized to imatinib 400mg daily or Interferon-α subcutaneously (5 MIU/m^2 of body surface area) in combination with Ara-C (20 mg/m^2 of body surface area). There were no significant differences in prognostic features on the two arms. With a median follow-up of 19 months, patients randomized to imatinib had significantly better results in all evaluations, including rates of complete hematologic response (97% vs 56%, $p<0.001$), CCgR (74% vs 8%, $p<0.001$), discontinuation of assigned therapy due to intolerance (3% vs 31%), and progression to accelerated-blast phase (3% vs 8%, $p<0.001$).

After a 6-year follow-up, 364 out of 553 (66%) of the patients randomized to imatinib were still on treatment, while 239 out of 359 (67%) patients who discontinued Interferon-alpha plus Ara-C crossing over to imatinib remained on second-line imatinib. The cumulative best CCgR was 82% but 17% have subsequently lost the CCgR. The overall survival was 88% (95% when only CML-related deaths were considered). An estimated 93% of imatinib-treated patients remain free from disease progression to the accelerated phase or blast crisis. Event rates (defined as death from any cause, progression to accelerated or blast-phase, loss of a complete hematologic response or major cytogenetic response, or a rising white blood cell count to more than 20x10^9 cells/L) continue to decline. To date, only 15 patients (3%) who obtained a CCgR have progressed to advanced phase.

Due to the substantial superiority of imatinib, most (65%) of the patients randomized to Interferon-α plus Ara-C crossed over to the imatinib arm (while only 3% of patients on the imatinib arm crossed over to the Interferon-α plus Ara-C arm). Consequently, a formal comparison of the longer term results of treatment between the two treatment arms was not possible and the IRIS study is now a long-term follow-up studyof patients who received imatinib as initial therapy.

Intention-to treat analyses

The IRIS data have a number of limitations. First, the censoring rate was nearly 20%. Second, only 60% of the original cohort continued participation and remained evaluable, with a substantial number of patients discontinuing the study protocol after the commercial availability of imatinib in 2001. Third, the definition utilized for EFS conflicts with the current management guidelines provided by both the National Comprehensive Cancer Network (NCCN) and the European LeukemiaNet. Finally, the IRIS data excludes those patients from their EFS calculations who discontinued imatinib either due to poor tolerance or those failing to achieve a MCgR but not having lost their CHR.

The Hammersmith group recently reported an intention-to treat analysis on the outcome of 204 CML patients treated with imatinib 400 mg daily in early chronic phase. Median follow-up was 38 months. Patient demographics were similar to the IRIS trial, with a higher percentage of high Sokal risk (28.9% vs 18.5%). Despite this difference, the majority of outcomes appear quite similar, including cumulative best 5-year estimates for CHR (98.5% vs. 96%), CCgR (82.7% vs. 81%), and MMolR at 1 year (50.1 vs. 53%). Utilizing the previously described definition of EFS in the IRIS trial, these rates (81.3% vs. 83%) as well as OS (83.2% vs. 89%) were also similar in the two trials. However, when the entire population study was included, the probability of remaining in a CCgR while still receiving imatinib at 5 years was reduced to 63% in the de Lavallade cohort.

At the 14th Congress of the European Hematology Association, the GIMEMA CML WP reported on treatment outcome (intention-to-treat) in 559 consecutive Ph+ CML chronic phase patients treated frontline with imatinib 400 (76%) or 800 (24%) mg daily. The patients were enrolled in 3 simultaneously running trials of the GIMEMA CML WP: CML/022 (NCT00510926), phase III, IM 400 vs 800 mg in high Sokal risk (with the contribution of the Nordic, Turkish and Israel CML Study Groups); CML/021 (NCT00514488), phase II, IM 800 mg in intermediate Sokal risk; CML/023, observational, IM 400 mg. The definitions of failure and events were according to the ELN criteria (failures: no CHR at 6 months, no CgR at 6 months, no PCgR at 1 year, no CCgR at 18 months, loss CHR, loss CCgR, progression to accelerated/blastic phase and death; events: failures, off-treatment for toxicity, refusal and lost to follow-up).

Beyond standard-dose: Imatinib high dose

Driven by the identification of resistant disease as well as interest in understanding whether earlier molecular responses would improve outcomes, a number of recent trials have attempted to improve on frontline imatinib therapy. The MD Anderson group treated 114 CML patients in first chronic phase with imatinib 800 mg daily; with a median follow-up of 15 months, the overall CCgR rate was 90% and the overall survival was 98% (median follow-up, 15 months; range, 3-27 months). The overall incidence of MMolR of the 112 evaluable patients was 63%.

The Australasian Leukemia and Lymphoma Group recently reported the results of their Therapeutic Intensification in De Novo Leukemia ("TIDEL") trial. Overall, 103 newly diagnosed CML patients were treated with imatinib 600 mg/day, with dose escalation to 800 mg/day for suboptimal response and combination of intermittent standard-dose Ara-C if the response criteria were still not met after a further 3 months. When compared to the IRIS results, significantly more patients achieved a CCgR at 12 months (88% vs. 69%; $p<0.001$) as well as at 24 months (90% *vs.* 80%; $p=0.002$). MMR rates were similar at 12 months; by 24 months, the MMR rates were 55% and 72% for the IRIS and TIDEL trials, respectively. Dosage adjustments were indicated in nearly 20% of patients by 9 months and by 80% of patients by 12 months, yet only half

Table 1. Results of Clinical Trials using imatinib (IM) Frontline in Chronic Phase-Chronic Myeloid Leukemia.

Study	*no of patients*	*drug and dose*	*6 months*		*12 months*		*last update*			
			CCgR	*MMolR*	*CCgR*	*MMolR*	*CCgR*	*MMolR*	*OS*	*EFS/PFS*
IRIS O'Brien et al., NEJM 2003 Hochhaus A et al., Leukemia 2009	1106	IM 400 mg	51%	NA	69%	50%	82%	88%	88%	97%/93% (7 yrs)
De Lavallade et al., JCO 2008	204	IM 400 mg	35%	5%	57%	7%	82.7%	50.1%	83.2%	62.7%/82.7% (5 yrs)
TOPS Cortes J, ASH 2008;112:335	476	IM 400 mg IM 800 mg	45% 57%	20% 39%	66% 70%	46% 54%	NA	NA	NA	NA
Kantarjian H et al., Blood 2004	114	IM 800 mg	82%	NA	90%	63%	NA	NA	96%	NA/100% (2 yrs)
TIDEL Hughes T et al., Blood 2008	103	IM 600 mg (dose escalation in suboptimal response)	NA	NA	88%	47%	90%	73%	NA	NA
CML022 Baccarani et al. Blood 2009	216 (high sokal)	IM 400 IM 800 mg	50% 52%	25% 31%	58% 64%	33% 40%	NA	NA	84% 91%	66%/86% 62%/88% (1 yr)
CML021 Castagnetti et al., Blood 2009	78 (intermediate sokal)	IM 800 mg	81%	38%	88%	56%	91%	73%	97.5%	85%/NR (2 yrs)
CML011 Baccarani et al., Blood 2004	76 IFNα	IM 400+Peg	60%	58%	70%	47%	87%	83%	96% (5 yrs)	NA/95%
SPIRIT Rousselot et al., EHA 2009;94:441	636	IM 400 mg IM 600 mg IM 400 mg +Ara-C IM 400 mg+PegIFNα	48% 67% 55% 56%	21% 33% 27% 39%	57% 65% 66% 71%	40% 52% 51% 61%	NA	NA	NA	NA
CML study IV Hehlmann et al., EHA 2009;94:193	710	IM 400 mg IM 600 mg IM 400mg +Ara-C IM 400mg+PegIFNα	NA	16%	52%	32%	94%	75%	95% ***	NA/89% (4 yrs)

NA: not available. CCgR: compete cytogenetic response. MMolR: major molecular response. OS: overall survival. EFS: event-free survival. PFS: progression-free survival.

were able to undergo the modification, and only 37% of patients whose doses were adjusted obtained a CCgR by 24 months.

An additional risk adaptive investigation was recently reported by the GIMEMA CML WP in newly diagnosed CP-CML patients with high Sokal risk scores The patients were randomized to receive either 400 mg/day or 800 mg/day of imatinib. No significant difference was noted in the rates of CCgR at 3 months (19% vs 25%), 6 months (49% vs 52%), or 12 months (58% vs 64%). Moreover, the rates of treatment failures were also equivalent at these time points. A second risk-adaptive clinical study promoted by the GIMEMA CML WP investigated the efficacy of high-dose imatinib (800 mg/day) in 78 newly diagnosed CML patients at intermediate Sokal risk. At 12 months, the cumulative incidence of CCgR was 88% and the cumulative incidence of MMolR of CCgR patients was 56%. Fifty-five of the patients received a mean daily dose more than 600 mg daily and more than 50% of the patients taking the full dose after 1 year of therapy.

The phase III TOPS trial randomized patients with newly diagnosed CP-CML to either 400 mg/day or 800 mg/day of imatinib with stratification according to the Sokal risk score and results were recently updated. Although CCgR and MMR rates occurred faster in the 800 mg/day cohort, the MMR percentages were equivalent by 12 months, including patients with a high Sokal risk score.

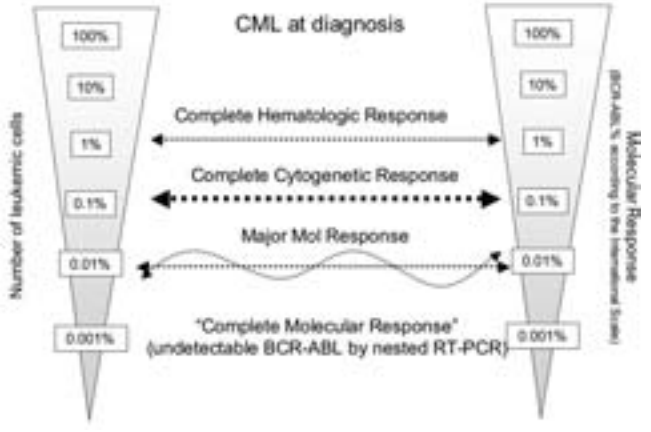

Figure 1. Degrees of response to tyrosine kinase inhibitors and approximate relationship between response, number of leukemic cells, and level of BCR-ABL transcripts.

Alternative Therapeutic Options for Upfront Therapy

Imatinib combination therapies

In the attempt to improve responses obtained with imatinib alone, a growing interest turned to revisit the use of other agents in combination therapy with imatinib. A particular focus was reserved to Interferon-alpha, which has proved to induce durable remissions in a small fraction of patients, with undetectable Bcr-Abl levels enduring even after Interferon-alpha discontinuation in some cases.

In 2004, the GIMEMA CML WP reported the short-term results of a multicentric, phase 2 study of the combination therapy of Imatinib 400 mg/day and pegylated Interferon-alpha in the treatment of 76 early chronic phase patients. After one year, 70% of the patients achieved a CCgR and 47% a MMolR. However, 45 of 76 (59%) patients had discontinued Interferon-alpha due to adverse events. After a 5 year follow-up, the overall CCgR rate was 87%, and the MMolR rate raised to 95% in complete cytogenetic responders. While compliance to IM was excellent, just a few patients continued the combined treatment beyond 2 years, confirming that the compliance of the patients was poor.

The French group has presented the preliminary results of a multicenter phase III investigation (SPIRIT), where patients were randomized to receive either imatinib (400 mg/day or 600 mg/day), imatinib (400 mg/day) plus Ara-C, or imatinib (400 mg/day) plus interferon-alpha. The study showed that the rate of MMolR at 6 months was significantly higher for IM+interferon as compared with IM 400mg (39% vs 21%, p=0.01), but also confirmed that the feasibility was not optimal, since the discontinuation rate of patients in the imatinib+interferon arm was 46% in the first year.

The German group designed an ongoing 5-arm study (CML Study IV) which randomized early chronic phase patients to 1) imatinib 400 mg; 2) imatinib+interferon; 3) imatinib+low dose araC; 4) imatinib after IFN failure (for low and intermediate-risk patients); 5) imatinib 800mg (for high-risk patients). Overall, 1242 patients have been randomized. The interim analysis covers 710 patients randomized by the end of 2005 to allow a follow-up of at least 3 years, for a median observation time of 45 months. 5-year overall survival of all patients was 92%, while the rate of CCgR was 94% and of MMolR was 88% with no significant survival differences recognizable.

Imatinib rotation therapies

It is acknowledged that the use of a tyrosine kinase inhibitor (TKI) alone and indefinitely might lead, sooner or later, to the emergence of resistance. Based on this rational, in 2008 the GIMEMA CML WP proposed to investigate the use of more than one TKI in combination or in rotation, according to the principles of cancer polychemotherapy. This study is an open-label, multicentric, exploratory, Phase II study of nilotinib, administered orally twice daily, for a total daily dose of 800mg, and of imatinib, administrated orally once daily, for a total daily dose of 400mg. The two drugs are administered following a rotating schedule (3 months nilotinib, 3 months imatinib, and again), for 24 months (study core), and then extended for another 36 months, if it is in the interest of the patient (study extension). At the time of writing, 120 patients have been enrolled.

Looking at the future: Imatinib dose reduction and discontinuation

Although imatinib has changed the natural course of chronic myeloid leukemia, all patients must continue the treatment indefinitely. The continuative nature of the treatment might become a substantial issue, because of its psychological implications, especially in young patients, and also because of the progressively increasing economic burden. Moreover, even mild side effects may severely compromise quality of life, when continuative.

The GIMEMA CML WP presented preliminary results of a multicentric study of "Intermittent Imatinib treatment" (INTERIM). The study aims to investigate whether the CCgR achieved with standard imatinib could be maintained with the same dose of imatinib given intermittently, in a cohort of elderly (>65 years) patients, in CCgR for at least 2 years. Imatinib was given according an intermittent schedule: 1 week on/1 week off for the 1st month; 2 weeks on/2 weeks off for 2 months; 1 month on/1 month off from the 4th month thereafter. At the time of writing, 114 patients were enrolled. Notably, 17 patients refused to enter the study, due to the concern that lower doses would not ensure to maintain the response.

The French group has recently presented the preliminary results of a French Multicenter Study "Stop Imatinib" (STIM) Study, with the aim to evaluate the persistence of complete molecular remission (CMR) after stopping imatinib, and to determine the factors that could influence the persistence of CMR. Patients treated with imatinib for at least 3 years and with BCR-ABL/ABL levels below a detection threshold ("CMR") for at least 2 years were eligible. The study included 70 patients, 34 of whom treated in late chronic phase. After 18 months, a molecular relapse was observed in 36 patients (15 in late chronic phase), for a relapse-free survival of 46%.

Conclusions

The concepts behind the development of IM were truly revolutionary and the therapeutic development in CML represents a success story for molecular medicine. The outlook for a patient who is treated with imatinib standard dose in early chronic phase is remarkable; IM high dose and newer combinatorial therapies have registered additional gains in molecular responses. However, it is currently unclear whether these early kinetic improvements will translate into reduced treatment failures, nor it is known, yet, whether it will be possible to definitely discontinue imatinib therapy, without losing the response.

Although imatinib has exceeded most individuals expectations, there is more room for improvement. Some patients do not achieve an optimal response, others achieve a good response but eventually relapse, and some patients are unable to tolerate therapy. In addition, many patients

carry residual disease measurable by PCR despite achieving a complete cytogenetic response. Disease eradication and further improvements in prognosis in CML remain high on the agenda.

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TREATMENT OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) RESISTANT TO IMATINIB

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Imatinib (IMA) 400 mg daily is the current standard treatment for patients with Philadelphia-positive CML in chronic phase (CML-CP). Data from the International Randomized Study of Interferon and STI571 (IRIS), 6 years after the last patient was enrolled, showed that 82% of patients treated with IMA achieve a complete cytogenetic response (CCgR = 0% Ph+ metaphases) and that the overall survival (OS) rate and the progression – free survival rate (PFS) are 86% and 93%, respectively.[1] Moreover, the IRIS study showed that long-term OS is correlated with response, as 97% of patients who achieve CCgR and near 100% of patients with CCgR and major molecular response (MMR = 0.1 - 0.001 BCR/ABL x 100IS) at 12 months are likely to be alive 5 years after starting IMA.[1] Other non-randomized studies gave independent confirmation of the IRIS study results,[2] but, despite this advance, there is still a group of patients who do not benefit from IMA because of resistance or intolerance. Indeed, some patients do not reach or loose hematologic response (HR), cytogenetic response (CgR), or molecular response (MR). The time-based landmarks for evaluation of response to IMA were critically reviewed from an expert panel on behalf of the European LeukemiaNet (ELN). They classified the response to IMA (standard dose, 400 mg daily) into three categories, namely optimal, suboptimal, and failure[3,4] (Table 1).

According to ELN guidelines, primary (or intrinsic) resistance was defined as failure to achieve complete hematologic response (CHR) by 3 months, partial cytogenetic response (PCgR = Ph+ cells > 35%) by 12 months and CCgR by 18 months and secondary (or acquired) resistance as loss of response in patients who initially responded to treatment.[3]

The proportion of patients who can be considered resistant to IMA is not well established. Using ELN criteria this proportion may vary from ~ 40% in the IRIS study to 26% in the study by de Lavallade *et al*.[2]

Importantly, in addition to patients defined as having primary or secondary resistance, ELN guidelines identify a subset of patients who respond suboptimally.[3,4] This latter group is represented by those patients who may still benefit from IMA but are unlikely to have a favourable outcome. They are the patients who achieve less than CHR, PCgR, CCgR and MMR at the 3rd, 6th, 12th and 18th month, respectively.[3,4] Anyway, despite the outstanding results obtained so far, there is still room to improve the outcome of CML patients and for the patients with failure or suboptimal response alternative therapies should be considered promptly. Second-line therapeutic approaches may be different and they include: IMA dose escalation, the use of second generation tyrosine kinase inhibitors (TKIs), namely Dasatinib and Nilotinib, or allogenic stem cell transplantation (allo-SCT).[5,6] The therapeutic strategy should be based on the knowledge of the underlying mechanism of resistance and in this field many advances were made in order to clarify the causes of resistance to IMA. Resistance to IMA can develop from a number of mechanisms that can be defined as Bcr-Abl dependent, such as the genomic amplification or, most commonly, the onset of point mutations in the Abl kinase-domain; or as Bcr-Abl independent, including clonal evolution or the constitutive activation of downstream signalling molecules (e.g. SRC-family kinases) which could result in the activation of the oncogenic pathways regardless of Bcr-Abl inhibition.[7,8] Among the Bcr-Abl independent mechanisms, those strictly related to IMA-metabolism which may influence the drug plasma levels [e.g. pertubations of cytochrome P450 system (CYP3A4 and CYP3A); or the activity of several drug transporter influx or efflux proteins (hOCT-1; PgP)] have to be mentioned, too[8] (Figure 1). Not all these mechanisms of resistance have the same clinical relevance. Mutations at the Bcr-Abl kinase domain are the clinically dominant and the best-studied mechanism of resistance, with more than 70 mutations having already validated in vitro and profiled for resistance not only to IMA but also to Nilotinib and Dasatinib.[7] The most relevant consequence of IC50 determination of Abl mutations is that it is possible to modify the clinical strategy, by choosing whether to increase the dose of IMA, or replace it with a second generation TKI or, in the case of T315I mutation, by enrolling the patient into a stem cell transplant program.

Table 1. Operational definition of failure and suboptimal response for previously untreated patients with ECP CML who are treated with 400 mg IMA daily (slightly modify from ref 4).

Time	*Failure*	*Suboptimal response*	*Warnings*
Diagnosis	–	–	High risk, del9q+, ACAs in Ph+ cells
3 mo after diagnosis	No HR (stable disease or disease progression)	Less than CHR	–
6 mo after diagnosis	Less than CHR, no CgR (Ph+ > 95%)	Less than PCgR (Ph+ >35%)	–
2 mo after diagnosis	Less than PCgR (Ph+ > 35%)	Less than CCgR	Less than CCgR
18 mo after diagnosis	Less than CCgR	Less than MMolR	–
Anytime	Loss of CHR*, loss of CCgR, mutation	ACA in Ph+ cells, loss of MMolR, mutation	Any rise in transcript level; other chromosome abnormalities in Ph- cells

Imatinib dose escalation may be an appropriate initial option for patients with CML-CP who are experiencing suboptimal response o resistance.[5] The rationale for this therapeutic approach is that: i) several studies showed that some Bcr-Abl mutations have less sensitivity to IMA, but not complete resistance, ii) Bcr-Abl amplification induces relative resistance which could be overcome by dose escalation and iii)

low through plasma levels of IMA have been shown to be correlated with treatment failure, suboptimal response or low compliance. Referring to this last point, the assessment of drug-plasma levels is assuming more and more importance and have become mandatory not only for treatment optimization but also to early recognize patient resistant to IMA or failing an optimal response, for a better selection of alternative therapies.[5]

Before 2009, only limited clinical data published to support the use of higher dose of IMA in resistant patients were available. Recently, Kantarjian et al reported that dose escalation of IMA in patients with newly diagnosed CML-CP in the IRIS study was effective in patients who had a disease not responding optimally to IMA 400 mg daily. Approximately, 20% of patients had a dose increased from 400 mg to 600 or 800 mg daily,[5] and among the patients who received dose escalation according to ELN recommendations, 67% achieved or regained hematologic response within 12 months of dose escalation, and 38% achieved or regained a CyR. Three years after the dose increase, the PFS was 89% and the OS rate was 84%.[5]

However, some Bcr-Abl mutations or other concomitant mechanisms may render the disease completely resistant or significantly resistant to IMA (e.g. T315I mutation; or mutations for which the IC50 of IMA is increased significantly). For these patients, a change to a second generation tyrosine kinase inhibitor (e.g. Dasatinid, Nilotinib) may be more beneficial than escalating the dose of IMA.[6]

Dasatinib is a potent, orally bioavailable, dual Bcr-Abl/Src kinase inhibitor, and is the first TKIs approved in the U.S. and Europe for IMA resistance and intolerant patients across all phase of CML. Despite targeting Bcr-Abl, Dasatinib is structurally unrelated to IMA and binds multiple conformation of the Abl kinase domains. In vitro, Dasatinib demonstrated 325-fold greater activity against native Bcr-Abl compared with IMA and has shown efficacy against IMA resistant Bcr-Abl mutations with the exception of T315I. In the START-C trial, which evaluated 288 IMA-resistant and 99 IMA-intolerant patients with CML-CP, responses were achieved irrespective of the presence and location of Bcr-Abl mutations (with the exception of T315I, where no activity was observed).[9] Of clinical importance is the fact that Dasatinib have undiminished activity across subgroups, including patients with P-loop mutations, and the 15 months progression free survival (PFS) rate was 88%. Furthermore, the results from START-R trial comparing Dasatinib (70 mg b.i.d) with high dose IMA (800 mg/day) in CML-CP that was resistant to IMA (400 - 600 mg/day) provide clinical evidence to support the rationale that a broader targeted and more potent agent, such as Dasatinib, is more appropriate for the treatment of IMA-resistant patients than high-dose IMA. CCgR were achieved in 40% receiving Dasatinib and in 16% (p=0.004) receiving IMA. Furthermore, treatment with Dasatinib resulted in significantly longer PFS compared to high-dose IMA (p<0.0001).[10]

Nilotinib is an analogue of IMA approved for the treatment of patients with CP or accelerated phase (AP) CML, resistant or intolerant to IMA or to other prior therapy. According to the *in vitro* profile, Nilotinib is effective against all IMA resistant Abl Kinase mutations except T315I, although a significantly lower activity (10 to 35 fold) has been noted against-P-loop mutations compared with wild type. This lower in vitro activity against P-loop mutations was correlated with a poor clinical response to Nilotinib in terms of probability to achieve a CCgR. Briefly, CHR, MCyR and CCgR were observed in approximately 90%, 50% and 30% of IMA-resistant CML-CP patients treated with Nilotinib.[6]

Other TKIs are developing (i.e. SKI-606, INNO-406 and MK-0457) and will provide further opportunities for treating patients with CML, either alone or in combination. The use of these new agents is still experimental. They are employed in phase I-II clinical trials and the results are coming up. However, it should not be forgiven that patients resistant to IMA with T315I mutation, or with clonal evolution, or non-responsive within 6–12 months of second generation TKIs can or must be addressed to allo-SCT. Allo-SCT remains the only curative therapy for CML and nowadays it can be offered also to patients older than 50 years, due to the extended use of reduced conditioning or non-myeloablative procedures and by the availability of alternative stem cell sources, including cord blood. One important question is: can we prevent the resistance to Imatinib?. In CML we have the advantage of having excellent surrogate markers (CCgR and MMR) for overall and event-free survival. Thus, improving the rate of patients who achieve early optimal response would be desirable and it is the main objective of new strategies such as the front-line use of higher dose of IMA, Nilotinib or Dasatinib, or sequential rotation of two or more TKIs. However, for those patients who obtain an early response, it is still unclear whether these therapeutic strategies may really improve their long-term outcome or whether these responses may be transient and cosmetic.

The excellent results achieved to date with IMA should not be translated into complaince with the outcome. This is not the time to slow down the efforts to better understand the disease and improve the therapy.

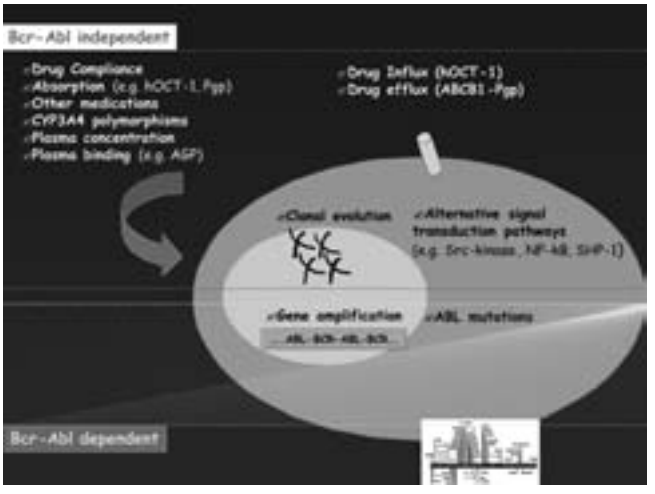

Figure 1. Bcr-Abl independent and Bcr-Abl dependent mechanisms of IMA resistance.

Acknowledgments: Baccarani M. Coordinator of GIMEMA CML Working Party and Martinelli G., Rosti G, Soverini S., Testoni N., Amabile M., Fogli M., Palandri F., Castagnetti F., Saglio G, Pane F., Alimena G. Bocchia M., Abruzzese E., Carella A.M., Specchia G. on behalf of GIMEMA CML Working Party.

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MINIMAL RESIDUAL DISEASE CONTROL

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In the last decade imatinib has become the frontline treatment of choice for all chronic myeloid leukaemia (CML) patients in early chronic-phase basing on the impressive response rates and the good tolerability of the drug. Similarly to what has already been demonstrated for Interferon therapy, the degree of tumour load reduction during treatment represents an important prognostic factor for CML patients treated with imatinib. (1) Although hematological and cytogenetic parameters allow to monitor response in the first part of the treatment, given the high rates of complete cytogenetic remission (CCyR) achieved during imatinib therapy, molecular monitoring of BCR-ABL transcript levels by Real-Time Quantitative PCR (RQ PCR) has become the method of choice to assess the amount of residual disease below the cytogenetic threshold. The molecular study associated to the IRIS trial offered the first demonstration of the ability of molecular monitoring to evaluate response to tyrosine kinase inhibitor (TKI) therapy and to further stratify the CCyR patients in different prognostic subgroups (2). Therefore it is mandatory nowadays to have precise and adequate procedures for the evaluation of clinical, cytogenetic and molecular responses to optimize the CML treatment with TKIs.

Hematologic and cytogenetic responses

Hematologic remission (HR) can be defined when platelet count is <4509/L and WBC <109/L; the differential count must not present immature granulocytes and basophils must be less than 5%; the spleen must not be palpable. Haematological resistance is rare in imatinib-treated early chronic phase CML patients (2-3% of all cases). If HR is not reached in 3 months since start of therapy this is considered suboptimal, whereas overt failure occurs when complete HR is not reached in 6 months (3). It is well established that hematological response does not represent a sufficient therapeutic goal in CML, as patients in hematological remission who still maintain 100 % of the methaphases Ph-positive invariably progress to a blastic phase.[1] Therefore, HR is considered preliminary to cytogenetic response, whose degree, conversely, has been shown to represent a strong prognostic indicator in patients treated with IFN-α[1] as well as in patients treated with imatinib.[2,4]

The cytogenetic response is established on the basis of the proportion of residual Ph-positive metaphases and is defined as complete (0 % of Ph-positive metaphases), partial (1-35 %), minor (36-66 %), minimal (67-95 %) and none (>95%), whereas a major response represents the sum of the complete and of the partial cytogenetic responses. Only complete and partial cytogenetic remissions have been shown to be associated with an increased survival, whereas the impact on prognosis of minor or minimal cytogenetic responses remains negligible.[2,4]

In imatinib-treated patients the dynamics of the cytogenetic response is very important. Patients without any kind of cytogenetic response after 6 months of therapy have very little residual probabilities of achieving a complete cytogenetic response (CCyR) later on (4). On this basis, lack of any degree of cytogenetic response at 6 months, lack of a major cytogenetic response (MCyR) at 12 months (< 35% Ph-positive metaphases) and absence of a complete cytogenetic response (CCyR) at 18 months were defined failures. Again, less than partial cytogenetic response (PCyR) at 6 months and less than CCyR at 12 months were defined suboptimal responses and other treatment strategies are justified in these cases.[4]

HR should be evaluated every 2 weeks until a complete HR has been achieved and confirmed, and a conventional cytogenetic examination of marrow cells must be performed at diagnosis and at least every 6 months until a CCyR has been achieved and confirmed, then every 12 months. Once a major molecular response (MMR) has been achieved and confirmed, conventional cytogenetic examination of marrow cells may be performed only when a consistent rise in BCR-ABL transcript level is observed or depending on special circumstances, such as the presence of clonal chromosomal abnormalities in the Ph-negative cell population.

Fluorescence in situ hybridization (FISH) on interphase cells has the potential advantage of evaluating many more cells and of using peripheral blood instead of marrow, but since the data obtained so far are all based on conventional cytogenetics, at the moment FISH has to be considered complementary and not alternative to conventional cytogenetic analysis. FISH is recommended before treatment to identify cases of CML apparently lacking the presence of a Ph-chromosome, to characterize patients with variant translocations and to identify the CML cases bearing deletions on the derivative chromosome 9q+ (3). Finally, in case of imatinib resistance, FISH is important to characterize the few cases presenting BCR-ABL amplification.

Molecular monitoring with RQ-PCR

Given the high rates of CCyR with imatinib and since the achievement of an MMR has prognostic significance and may drive therapeutic decisions, molecular monitoring of BCR-ABL transcript levels by RQ-PCR became the method of choice for monitoring residual disease in patients with CCyR. In the IRIS study, the concept of log reduction from a standardized median baseline derived from untreated patients has been introduced.[3] The standardized baseline was defined by measuring the level of BCR-ABL/BCR in the pooled peripheral blood samples collected before treatment from 30 chronic phase CML patients. The advantage of defining molecular response in these terms is that, once a laboratory has established its baseline level, the results can be expressed on a common scale. In the proposed international scale (IS), the standardized baseline, as established in the IRIS trial, is taken to represent 100% BCR-ABL and a 3-log reduction from this standardized baseline, that corresponds to MMR, is fixed at 0.10% BCR-ABL, while the threshold of CCyR corresponds roughly to 1% BCR-ABL.

The molecular study associated to the IRIS trial offered the first demonstration of the ability of molecular monitoring to evaluate response to tyrosine kinase inhibitor (TKI) therapy.[4] At the 12-month mark approximately 70% of patients treated in the imatinib 400 mg daily arm experienced a CCyR and examining the BCR-ABL transcript levels in these patients a very important finding emerged: a 3-log reduction in BCR-ABL mRNA expression from a median baseline found at diagnosis (which thereafter was defined as a major molecular response, MMR) was obtained in 39% of the cases and the depth of molecular response was significantly associated with progression-free survival (PFS). Indeed, patients without CCyR had a risk for progression (loss of response and a return to chronic phase, or progression to accelerated-phase disease or blast-crisis) of approximately 25%, and their PFS was 72% at a median of 54 months of follow-up. Patients who experienced a CCyR and a less than 3-log reduction in BCR-ABL at 12 months had a PFS of 89%. Patients who experienced a 3-log or greater reduction in BCR-ABL by 12 months had a PFS of 97% (a very significant difference). Another interesting result of the IRIS study was that undetectable BCR-ABL, which was rigorously defined as quantitatively and qualitatively confirmed undetectable BCR-ABL with qualitative nested RT PCR analysis (supposed to be more sensitive than RQ PCR analysis) and was defined Complete Molecular Remission (CMR), was relatively rare, occurring in less than 5% of cases. However, in the vast majority of the patients who achieve MMR, BCR-ABL transcript levels continue to decline at a slow rate and the probability of undetectable BCR-ABL tends to increase during the follow-up, reaching approximately 50% of the patients after 5-6 years of therapy.[12] The patients who do not reach CMR, on long-term follow-up have been reported to have some risks of losing MMR (5). This last observation suggests the frequent presence of a small, but real, reservoir of CML cells that may have the potential to cause disease relapse. CMR needs to be better defined, taking in consideration the degree of sensitivity that usually can be reached in clinical samples. This is likely to correspond to a value between 4 and 5 logs below the median baseline value observed at diagnosis. Therefore, it is likely that in the future CMR will be defined as undetectable BCR-ABL in a sample in which a sensitivity of at least 4,5 log below median baseline at diagnosis can be reached. It is clinically useful to characterize the value of RQ PCR at least every three months in order to verify if the transcripts continue to decline and eventually become undetectable, if the transcripts have reached a stable level or if the transcript numbers tend to increase. Increasing transcript levels are more likely to be associated with BCR-ABL mutations in patients who never reached a MMR, although in some cases also loss of MMR has recently been associated to the emergence of mutated clones.[5]

Stem cell resistance

As discussed, TKIs induce rapid cytogenetic responses in the majority of CML patients in chronic phase but do not eliminate BCR-ABL transcripts in the majority of patients, suggesting persistence of residual disease. These findings, together with the rapid kinetics of relapse in patients who discontinue TKIs, suggest the presence of a reservoir of TKI-resist-

ant leukaemic stem cells.(7) A sub-population of quiescent stem/progenitor cells in CML patients in CP with a primitive phenotype (CD34+38-, Hoechstlow, Pyronin Ylow) able to induce Ph+/BCR-ABL+ reconstitution following transplantation in immunocompromised mice was demonstrated. Furthermore it was shown that these cells are not targeted by TKIs even at high concentrations. A recent study indicates that CML patients treated continuously with IM for 4 years still expressed BCR-ABL in the primitive HSC fraction (CD34+38-).[8] The mechanism for TKI-insensitivity of CML stem cells remains unclear. In addition, the eradication of CML stem cells in patients may be made even more difficult since TKIs exert potent, reversible, anti-proliferative effects on primitive CML cells *in vitro*. These data suggest that TKIs may activate cellular pathways *in vivo* that lead to cell cycle exit and G1 arrest.

Our recent study indicates that TKIs initiate a process in CML stem and progenitor cells that maintains their quiescence and therefore potential resistance to TKIs themselves and to other treatments used in combination with TKIs. Our still unpublished data suggest that the anti-proliferative activity of TKIs against primary CML CD34+ cells is mediated, at least in part, by the re-activation of FOXO1, 3a and 4. In agreement with these data, the recent finding that the FOXO family plays a fundamental role in normal HSC maintenance makes them excellent candidates to explain our observations in leukemia.[9]

New strategies to eradicate minimal residual disease

The ability of imatinib to induce a complete cytogenetic response (CCyR) in most patients, albeit with few CMRs, suggests the opportunity to explore vaccine strategies in a minimal residual disease state.

Fusion peptides from the junctional sequences product of the BCR-ABL fusion have the ability to bind several human leukocyte antigen (HLA) class I and II molecules and to elicit peptide- specific T-cell responses. Such an approach engendered interest in developing this peptide as a possible strategy to induce tumor-specific immune responses in patients who had received treatment with imatinib. Native junction peptides, which are administered in most trials to patients who have CML and minimal residual disease, have induced a specific immune response.

In summary, we present evidence that BCR-ABL peptide vaccination may induce specific T cell responses and molecular improvement in CML, especially in patients who are already responding well to imatinib. Randomised trials are now required to investigate whether peptide vaccination of imatinib responders can improve the molecular control of CML.

Conclusions

In patients treated with imatinib and other TKIs, careful hematologic, cytogenetic and molecular monitoring to evaluate the amount of minimal residual disease is mandatory to ensure that an individual patient receives the proper treatment and to decide if and when a therapy should be changed. New strategies are now under investigation in order to control and reduce the amount of residual Ph positive cells.

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HEMATOPOIETIC STEM CELLS FOR LIVER TISSUE REGENERATION

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Liver regeneration after tissue damage is not dependent on only one class of progenitor/stem cells. At least two resident cell populations have been shown to act as putative SCs. Whereas moderate cell loss is restored by mature hepatocyte self-renewal, more severe liver injury induces the activation of a "facultative" SC compartment located within the smallest branches of the intrahepatic biliary tree, the so-called "oval cells" which give origin to hepatocytes and the bile duct epithelium. Of note, liver oval SC express the antigens CD34 and c-Kit that are strongly associated with the hematopoietic stem/progenitor cell phenotype.

Recently, a third source of liver-repopulating cells has proven to be the BM (reviewed in 1). Petersen et al.transplanted syngeneic male BM cells into lethally irradiated female recipient rats that had been treated with 2-acetylaminofluorene, to inhibit hepatocyte proliferation, and then received hepatic injury by carbon tetrachloride (CCl4) to induce oval cell activation. Donor-derived, Y-chromosome+ oval cells and mature hepatocytes were found 9 and 13 days after liver damage, respectively. Additional evidence of a BM-derived hepatic SCs came from Theise et al. who also used a gender mismatch BM transplantation strategy to track donor-derived SCs into the liver of lethally irradiated mice. This study provided at least two important informations: 1) BM may contribute to normal renewal of liver tissue in absence of obvious damage; 2) regeneration of hepatic tissue could be accomplished by hematopoietic SCs (HSCs) since 200 CD34+Lin- cells produced the same degree of engraftment (1-2% at 6 months) than 20000 unseparated BM cells. Two further studies demonstrated that, also in humans, hepatocytes can derive from BM. Both groups examined the livers of female patients who had received a BM transplant from a male donor and the female livers transplanted into male recipients which had to be removed for recurrent disease. Using in situ hybridization for Y-chromosome, 4 to 43% hepatocytes and 4 to 38% cholangiocytes were found to be of extrahepatic origin.

Taken together, these studies provided the "proof of principle" that BM contributes to liver regeneration. Further investigations were then addressed to important issues such as the functional capacity of marrow-derived hepatic cells, the BM cell type involved in liver tissue differentiation, the molecular mechanisms regulating the recruitment of marrow cells into liver and finally some studies challenged the concept of SC plasticity itself by showing that cell fusion is the main mechanism by which HSCs acquire the function of mature hepatocytes.[1]

Lagasse et al., used BM transplantation in female recipients deficient in the enzyme fumarylacetoacetate hydrolase (FAH-/-), an animal model of fatal hereditary tyrosinemia type I. FAH-deficient mice die after birth unless rescued with NTBC, a compound that prevents the accumulation of toxic metabolites in the tyrosine catabolic pathway. Liver function was restored by transplantation of 106 unfractionated wild-type (FAH+/+) BM cells or by 50 highly purified hematopoietic c-Kit+Thy+-Lin-Sca-1+ SC. Interestingly, only HSCs, and not committed precursors, showed the capacity to differentiate into hepatocytes and to rescue an otherwise lethal metabolic disease. More recently, Wang et al. transplanted purified human hematopoietic CD34+ or CD34+CD38-CD7- SCs into nonobese diabetic-severe combined immunodeficiency (NOD/SCID) and NOD/SCID β2microglobulin (β2M)-null mice. One month after transplant, CCl4 was given to mice to induce severe liver damage and hepatocyte proliferation. Four weeks later, livers were harvested and the expression of mRNA for human albumin and CK19, a marker of bile duct cells, was found. Human albumin was also found in the serum of mice and the results were improved by the administration

of HGF. Taken together, these studies provide a strong evidence that both murine and human HSCs can generate fully functional hepatic tissue. Furthermore, it was demonstrated that even a single CD34+Lin-Sca+ cell can give rise (with variable efficiency) to epithelial cells in different organs of recipient animals, including <1% of biliary cells.

Further studies have shown that different cellular types from the BM or the PB of hematopoietic and non-hematopoietic origin share the capacity to generate hepatocytes. This finding supports the concept that "plasticity" may not be restricted to a unique SC population but, rather, may be a general property of marrow cells that redirect their transcriptional program under appropriate stimuli.[1]

Recently, the mechanisms leading to the homing of HSC to the liver have been investigated.[2] The chemokine SDF-1, which is a pivotal molecule for SCs migration and BM homing, was found to be expressed after irradiation on liver bile duct epithelium of NOD/SCID mice and its expression was upregulated by hepatic CCl4-mediated damage. Human CD34+CD38- HSCs transplanted into immunocompromised mice migrated to the liver whereas the inhibition of SDF-1 receptor CXCR4 abolished their engraftment. HGF and SCF, which are secreted after liver injury, increased the expression of CXCR4 and SDF-1 thus augmenting the recruitment of marrow cells to the liver.[2] This study underlines the potential of CD34+ cells to migrate in response to stress signals in order to repair non-hematopoietic tissue.

The therapeutic potential of selected cytokines and cytokine-mobilized HSCs to migrate to the injured liver and promote tissue repair has been also recently investigated. Acute and chronic liver injury models were induced by injecting CCl4 in C57Bl6 mice and G-CSF was administered to induce SC mobilization. After sex-mismatched BM transplantation into lethally irradiated recipient and treatment with CCl4 ± G-CSF, sry (sex-determining region for Y chromosome) protein was detected by immunohistochemistry in liver section. Double immunohistochemistry for sry and ki-67 protein was used to define the origin of proliferating cells reconstituting liver after injury. In both acute and chronic liver injury model, G-CSF administration ameliorated the histological damage and accelerated the regeneration process. This was associated by a strong survival benefit in G-CSF-treated group versus CCl4 group. The conclusions of the study were that G-CSF treatment significantly improved survival and liver histology in chemical injured mice, predominantly by promoting endogenous repair mechanisms. More recently, G-CSF has been shown capable to promote liver repair, after chemically-induced tissue damage, by improving oval cell proliferation and migration in a rat model.[3] Similar effects were observed when G-CSF was administered to rats undergoing partial hepatectomy. In this model, G-CSF activity was mediated by the down regulation of liver natural killer cells.

Sakaida *et al*.[4] demonstrated that transplantation of BM cells degraded collagen fibers and significantly reduced liver fibrosis with strong expression of metalloproteinases (MMPs), especially MMP-9, related to the migration of BM cells to the inflammatory liver in a model of CCl4-induced liver fibrosis in C57BL6 mice. Reduction in liver fibrosis then translated in improved survival thus introducing a new concept for the therapy of liver cirrhosis. Therefore, G-CSF treatment and HSC reinfusion might offer a novel therapeutic approach for the treatment of acute and chronic liver diseases in humans.

CD133 is a novel antigen expressed on HSCs. CD133+ cells are present in the PB and BM of mobilized and non-mobilized adults, and are capable of long-term reconstitution of human hematopoiesis in immunocompromized mice. CD133 antigen is co-expressed on almost 80% of CD 34+ cells. CD133+cells can be cultured *in vitro* as well as CD34+ or c-kit+ populations, thereby suggesting that CD133+cells share similar growth factor requirements. Taken together, these studies clearly indicate that CD133 represents a cell surface marker for identification of human progenitor/stem cells. Along with their hematopoietic potential, circulating CD133+ SCs give origin to the endothelial lineage and are known to contribute to neoangiogenesis after tissue ischemia and organ regeneration in animal models.[5] Moreover, CD133 antigen is expressed on selected embryonic stem cell lines, human fetal neural SCs with the potential to repair damaged neural tissue, non-hematopoietic adherent cells, and multipotent adult progenitor cells which can differentiate *in vitro* and *in vivo*, at the single cell level, into embryonic stem cell-like cells with visceral mesoderm, neuroectoderm and endoderm characteristics. The multilineage potential of CD133+ cells is further reinforced by the finding that CD133+/CD14+ cells, mobilized into PB of human healthy liver donors, are capable to differentiate, *in vitro*, into liver epithelium.[6] Very recently, Rountree *et al*.[7] demonstrated that murine oval cells with bi-lineage differentiative potential (i.e. hepatocyte and cholangiocyte) express CD133 antigen but not CD45. These putative liver stem cells proliferated and differentiated in response to tissue damage.

CD133+ BM HSC have been already selected and reinfused via portal vein in 3 patients who were scheduled to have major liver resection, to accellerate liver regeneration.[8] The results of that study, demonstrated that infusion of CD133+ cells was well tolerated without side effects. CT-scan volumetry revealed 2.5-fold increase in the mean proliferation rates of left-lateral segments when compared with a control group of patients undergoing the same strategy (i.e. portal venous embolism-PVE) without SC reinfusion. More recently, the Authors upgraded their experience on 13 patients[9] with the same clinical characteristics of the original series. The updated data confirmed the clinical benefit of infusion of autologous CD133+ SCs combined with PVE as compared to PVE alone.

In another study, 5 patients with liver insufficiency were safely mobilized with G-CSF.[10] Circulating adherent CD34+/CD133+ SCs were then shown to express mRNA for genes related to liver, pancreas, heart, muscle and nerve cell differentiation and generated, in vitro, multiple non-hematopoietic cell types. Moreover, this multipotent SC population was reinfused into the portal vein (=3) or the hepatic artery (=2) without side effects, resulting in some clinical improvements.

Thus, the availability of larger numbers of G-CSF-mobilized CD133+ SCs may be a novel strategy to evaluate the role, if any, of adult SCs to ameliorate liver function in patients with liver disease.

Pre-clinical models of liver fibrosis and clinical trials

The therapeutic potential of adult stem/progenitor cells derives mostly from animal data showing their capacity to participate to tissue repair crossing lineage-specific boundaries. Despite the great promise, it is clear that development of novel therapies with cells that repair tissues different from those of origin is not a linear sequence of events and the molecular mechanisms of tissue repair remain a mysterious and complex process.

Chronic degenerative diseases frequently impair the regeneration process leading to a non functional fibrotic repair, as it occurs in liver. Nowadays, liver failure causes high costs in terms of health care, lost economic productivity, diminished quality of life, and premature death. Thus, it seems of great relevance to design and realize novel treatments to restore the normal architecture and function of the damaged tissue. Although several studies have provided the *proof of principle* that BM contributes to liver repair, more recent evidence suggests that the BM may not play a significant role in the repopulation of hepatic parenchyma after tissue damage and cell fusion seems to be the predominant process. However, alternative mechanisms may explain restoration of tissue function. For instance, it has recently been suggested that BM-derived SCs are able to stimulate the proliferation and activation of resident cells by paracrine secretion of cytokines (feeder effect) or enhance fibrous matrix degradation. Thus, it may well be that BM SCs create a favourable mileu for organ regeneration by the transient supply of still unknown soluble factors. Additional crucial issues still need to be fully addressed such as the long-term functional capacity of marrow-derived hepatic cells, the optimal cell type (i.e. hematopoietic and non-hematopoietic) for liver tissue engraftment, the molecular mechanisms regulating the recruitment of non-hepatic cells into liver and their role for the development/reversal of liver fibrosis, and the alloreactivity and immunomodulatory effect of BM SCs in inflamed tissues. In particular, chronic liver injury is invariably accompanied by progressive fibrosis. In the progression to chronic liver injury/inflammation, hepatic stellate cells and myofibroblasts are major fibrogenic cell types that contribute to collagen accumulation. Several studies have shown the capacity of BM-derived SCs to improve liver fibrosis. Sakaida *et al*.[4] demonstrated that transplantation of BM cells degraded collagen fibers and significantly reduced liver fibrosis with strong expression of MMPs, especially MMP-9, related to the migration of BM cells to the inflammatory liver in a model of CCl4-induced liver fibrosis in C57BL6 mice. Reduction in liver fibrosis then translated in improved survival. Moreover, transplantation of Flk1+ MSCs significantly reduced CCl4-induced chronic liver damage, collagene deposition and fibrosis in a mouse model. Immunofluorescence, RT-PCR and fluorescence in-situ hybridization (FISH) analyses revealed that donor cells engrafted into host liver, had epithelium-like morphology, expressed albumin and ameliorated the fibrogenic effects of CCl4 injury.

Very recently, the therapeutic role of EPCs has been explored in animal models. Ueno et al., demonstrated that ex-vivo expanded CD133+ EPC transplanted in chronically CCl4-treated mice localized in areas of hepatic necrosis and fibrosis where the liver is likely to be ischemic. After 4 weeks of iv administration of EPCc, hepatic fibrosis was dramatically reduced together with the expression of type I collagen, fibronectin and transforming growth factor (TGF)-Beta. Moreover, CD133+ EPCs transplantation improved liver function and enhanced the proliferation of hepatocytes and the regeneration of hepatic sinusoids. The main mechanism of these effects was considered the down-regulation of TGF-Beta by HGF secreted by EPCs. Subsequently, the same approach was proven to be effective in a cirrhotic liver rat model (39). Single and multiple infusion of CD133+ EPC were performed in rats previously treated for 6 weeks with CCl4. SC therapy markedly reduced liver fibrosis with decreased procollagen, fibronectin, TGF-Beta and alfa-smooth muscle actin-positive cells. In addition, RT-PCR analysis showed increased expression of MMP-2, -9 and -13. As a consequence, liver function and overall survival were significantly improved in transplanted rats.

Few pilot clinical trials have been already published addressing the role of BM-derived SCs to improve liver function in chronic disease (reviewed in 11). Terai *et al.*, reinfused a mean of $5.2\pm0.63x10^9$ mononuclear BM cells in 9 cirrhotic patients. Significant improvement of liver function was observed 24 weeks after BM cell transplantation associated with enhanced cell proliferation assessed by liver biopsies. Four additional studies showed the results of transplantation of autologous G-CSF-mobilized CD34+ cells in cirrhotic patients (=22). Overall, the published data suggested the clinical benefit of SC therapy with limited, if any, side effects. Of note, the major limitations of the clinical studies performed so far are related to the limited number of patients enrolled, the different route of administration employed (e.g. hepatic artery, portal vein), the different cell source (e.g. BM, mobilized PB), the use of unselected hematopoietic cells or highly purified SC populations (i.e. CD34+ or CD133+ cells), the different number (within the same trial) of reinfused cells. Thus, the clinical impact of cytokines and cell therapy for patients with end-stage liver function must be investigated in rigorously designed clinical studies.

CD133+ cell populations and liver injury

Whereas CD133+ cell compartment represents a homogeneous population of pluripotent SCs capable of liver tissue regeneration is unclear. For instance, Gehling *et al.*,[6] identified a subset of circulating CD133+/CD14+ cells, mobilized into PB of human healthy liver donors, capable to differentiate, in vitro, into liver epithelium. Conversely, Gordon *et al.*[10] characterized G-CSF-mobilized PB adherent CD34+/CD133+ cells which gave origin, in vitro, to multiple non-hematopoietic cell types including liver cells. Rountree *et al.*[7] demonstrated that murine oval cells, proliferating in response to tissue damage, express CD133 antigen but not the hematopoietic CD45 molecule. On the contrary, Harb *et al.* found that transplantation of BM bi-potent CD133+/CD45+/CD31+ endothelial and hematopoietic progenitor cells were capable to regenerate sinusoid and central vein endothelial cells after the necrotic phase of sinusoidal obstructive syndrome (SOS) in rats. Cell therapy resulted in the remarkable improvement of tissue damage.

As mentioned above, *ex vivo* expanded EPCs, co-expressing the CD133 antigen along with a variety of endothelial markers (Flk-1, Flt-1, Tie-2, CD34), have been transplanted in animal models of liver tissue injury inducing regression of liver fibrosis, increase of liver function and overall survival.

From the clinical standpoint, so far, only a few publications on the application of purified CD133 cells for the regeneration of liver are available. None of the studies reports on further *subsetting* of the applied CD133+ cell population whereas all confirm the feasibility of infusing autologous CD133+ cells for regeneration of the liver associated with some clinical improvements. In summary, according to the literature and our own experience (see below) CD133+ cells enriched from the BM, mobilized PB and cord blood seem to be capable to improve liver fibrosis. However, it is possible that CD133+ cells may be a heterogenous SC population that harbours early and very early SCs with multidirection differention potential, although there is no clear indication of a cell subset preferentially involved in liver tissue repair. Thus, CD133 is widely considered as a valid candidate for tissue regeneration strategies.

Background and pre-clinical model of CD133+ cells infusion in liver cirrhosis

The potential role of BM-derived SCs in patients with end-stage liver disease has been recently addressed in our Center. In the first study,[12] we assessed the PB stem/progenitor cells compartment of 24 adult patients receiving OLT and 13 patients submitted to liver resection. Taken together, phenotypic and functional analyses showed that both early subsets of the endothelial and HSC compartment and more mature committed progenitors were mobilized into PB after OLT. We also demonstrated, for the first time, the release from the BM of liver-committed HSC co-expressing epithelial markers after OLT. Conversely, the longitudinal study performed after surgery showed only the mobilization of total CD34+ cells whereas PB endothelial stem/progenitor cells as well as different subsets of HSCs did not increase in response to liver resection even after removal of 55% of the organ. Thus, our data suggest that SCs recruitment to non-hematopoietic organs may be a relevant physiological process in case of extensive tissue injury and underline the potential role of BM-derived cells for cell therapy of liver diseases.

In another study,[13] we assessed whether G-CSF can be safely administered to patients with liver cirrhosis in order to expand and mobilize BM-derived SCs. Eighteen patients with advanced liver disease were treated with r-hu glycosylated G-CSF. Increasing doses of G-CSF were administered subcutaneously for 7 consecutive days to five cohorts of three patients each, starting from 2 ug/kg/daily. The dose finding phase demonstrated that 15 ug/Kg/day of G-CSF mobilized both CD34+ HSCs and pluripotent CD133+ SCs in patients. Circulating SCs were safely collected by single volume leukapheresis in 3 patients and a mean of 1.3 ± 0.73 and $1.2\pm0.5x10^6$ CD34+and CD133+/Kg, respectively, were cryopreserved. No severe adverse events were observed at any dosage or during leukapheresis. In conclusion, we established that the administration of G-CSF to patients with liver cirrhosis is safe and feasible and allows the mobilization and collection of BM-derived SCs at the dose of 15 ug/kg/day.

Concurrently, we investigated the effects of G-CSF and mobilized HSCs in a rat model of acute liver injury.[14] Acute liver damage was induced by a single intraperitoneal injection of 1.25 mL/Kg of CCl4. Preliminary experiments had shown that this dose causes a moderate-severe, but reversible, liver necrosis of approximately 40-50% of the liver parenchyma. After CCl4 injection, the animals received daily intraperitoneal injection of G-CSF at the dose of 150 mg/kg or vehicle for 4 consecutive days. Another group of rats received daily intraperitoneal injection of G-CSF at the dose of 150 mg/kg or vehicle for 7 consecutive days before CCl4 injection. After the induction of liver damage by CCl4, the rats were treated with G-CSF, at the same dose, or vehicle for additional 2 days. The 7-day pre-treatment period was chosen to guarantee an effective mobilization of BM HSCs into PB. Treated rats were sacrificed under general anaesthesia before and after 2 and 4 days from CCl4 injection to collect blood and liver tissue samples. Our study failed to demonstrate, in rats, the protective role of G-CSF in a model of non lethal, acute liver injury induced by a single-dose of CCl4. Treatment with G-CSF before and/or after intoxication, and associated BM-derived SC mobilization, did not induce either the improvement of tissue damage or the promotion of hepatocyte regeneration. Treatment with G-CSF may be more effective in chronic diseases, although, at present, the data deriving from experimental models of chronic liver injury are very limited and controversial. Alternatively, it may well be that only pluripotent SCs enhance liver regeneration after acute or chronic damage.

To address this issue, we recently established an efficient and well-tolerated protocol to induce liver cirrhosis in mice.[15] C57BL/6N mice received CCl4 sc, intraperitoneally or by inhalation according to three different schedules. Our results demonstrated that short cycles of CCl4 inhalation were the most efficient strategy to induce decompensated cirrhosis in mice with less than 10% mortality rate.[15] Therefore, we used this protocol to investigate the effects of transplantation of human G-CSF-mobilized CD34+ and CD133+ SCs on mice with chronic liver injury and fibrosis. To this end, C57BL/6N mice were divided into four experimental groups: healthy controls; cirrhotic mice without transplantation; cirrhotic mice transplanted with CD133+ cells; cirrhotic mice receiving CD34+ cells. Mice received CCl4 by inhalation for thirteen weeks. CCl4 was delivered (2 L/min) three times a week by short inhalation cycles. At the end of the induction phase, Cyclosporin-A was administered by oral gavages. All mice received a daily dose of 25 mg/Kg starting the day before SCs transplantation and continued until the sac-

rifice.Transplantation was performed by a single intravenous injection (tail vein) of 106 human CD34+ or CD133+ SCs positively selected, by immunomagnetic method, from the leukapheresis products of the three cirrhotic patients enrolled in the previous study (14). After four weeks from transplantation all mice were sacrificed to perform histological and morphological analysis. Samples from liver, lungs, kidney and spleen were fixed in 10% phosphate buffered formalin, embedded in paraffin, and stained with haematoxylin-eosin (H&E). Liver fibrosis was evaluated with Sirius Red staining technique. Our results demonstrate that during the induction of cirrhosis and transplantation no mice showed any symptoms (e.g. pain). At sacrifice, control mice did not show any morphological alteration of liver, kidney, lungs and spleen. Mice treated with CCl4 did not show morphological alterations of lung, kidney and spleen while they showed overt liver cirrhosis. Xenotransplantation of human SCs had no detrimental effects on the morphological aspect of lung, kidney, spleen and liver. However, Sirius Red staining showed a readily appreciable difference in fibrotic tissue between experimental groups: mice transplanted with either CD133+ or CD34+ human cells appear to have less fibrotic septa than mice without SC transplantation suggesting the potential therapeutic role of human SCs on the recovery of liver fibrosis. Of note, we did not find any tumor growth in mice transplanted with human CD133+ cells.

Based on this background, a clinical phase I trial is about to start to assess the safety of the intrahepatic reinfusion of increasing number of autologous highly purified CD133+ cells previously collected and cryopreserved after mobilization with G-CSF and leukapheresis in patients with liver cirrhosis.

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GENE THERAPY IN HEMOGLOBINOPATHIES

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The β-thalassemias are congenital anemias caused by the absent or insufficient production of the beta chain of hemoglobin due to mutations in or near the β-globin gene. This deficiency causes ineffective erythropoiesis and hemolytic anemia, which typically become patent one year after birth. Without treatment, the severe form of the disease, known as beta-thalassemia major or Cooley's anemia, is lethal within the first decade of life. The only curative treatment is allogeneic bone marrow transplantation from a matched, related donor, which carries a low risk of mortality. This option, however, is not available to the majority (70-80%) of thalassemia subjects and because of the greater risk associated with matched-unrelated or mismatched transplants, most subjects have to settle for palliative therapy based on life-long red blood cell transfusion and daily treatment with an iron chelator to eliminate iron burden. While controlling the anemia, transfusion therapy does not correct ineffective erythropoiesis, and exacerbates iron accumulation in a variety of organs, thus the major cause of death in these patients is heart failure due to secondary hemochromatosis.

The genetic correction of autologous hematopoietic stem cells (HSCs) by the transfer of a regulated beta β-globin gene represents a highly attractive alternative treatment because it is potentially curative. The goal of globin gene therapy is to restore the ability of the thalassemia subject's own hematopoietic stem cells to generate red blood cells containing normal hemoglobin which will reverse the ineffective erythropoiesis and correct the inherited anemia.

The beta-globin gene is particular in its stringent transcriptional requirements (transgene expression has to be erythroid-specific, differentiation stage-specific, and highly elevated in comparison to most other genes) and achieving these goals has represented a tremendous obstacle for over a decade with the most difficult issue in identifying a vector genome configuration that sustains durable high-level gene expression in developing erythroblast.

In early studies, oncoretroviral vectors containing the beta-globin gene driven by its own promoter were shown to transduce murine hematopoietic stem cells but globin gene expression was either absent or very low. Even the incorporation of relatively small (less than 1 kilobases) *core* hypersensitive sites (HSs) fragments of the locus control region (LCR) upstream of the human beta-like globin gene locus, generated sub-terapeutical amount of vector transcription and, indeed rendered oncoretroviral vectors unstable during passage in vitro and/or low titers of viral producer cell clones. Systematic elimination of cryptic splice and polyadenilation sites or empiric evaluation of the many potential orientations and order of individual HSs yielded higher titer vector particles that transferred a beta-globin gene but failed to express therapeutic level of globin and in some instances exhibited silencing over time.

Recently, human immunodeficiency virus type-1-based lentiviral (LV) vectors have been emerged as more suitable vectors capable of transferring complex genomes containing extended LCR elements linked to a beta-globin gene. In a pioneering study, Sadelain's group, using a lentiviral vector, named TNS9, that harbored an optimized combination of proximal and distal _-globin transcription control elements, demonstrated that therapeutic levels of globin expression could be achieved in thalassemic mice and could correct the hematological features in mice affected by beta-talassemia intermedia and major (2-3).

TNS9-LV is a replication-defective, HIV-1-derived lentiviral vector encoding the normal human _-globin gene. The human _-globin gene is partially truncated within intron 2 (removing a cryptic polyadenylation site) and is flanked by an extended promoter sequence, the _-globin 3' proximal enhancer, as well as large LCR elements (3.2 kb) spanning HS 2, 3, and 4. Following the initial report by Sadelain's group, several groups have now shown that LV vectors can stably transmit the human beta-globin gene and large elements of the LCR, resulting in therapeutical levels of beta-globin expression for correction of the thalassemia phenotype in animal models of severe hemoglobinopathies.[4-7] Some of these vectors have recently entered the stage of human clinical trials with the start of the first study on the gene therapy of hemoglobinopathies in France in year 2007. A second clinical trial has received RAC approval and is expected to begin next year the United States using *TNS9* LV-derived vector.

However, the level of globin gene expression in these mouse studies ranged from 1.1- to a 3.gram/deciliter rice in hemoglobin per vector

copy/cell (VC), with most groups showing a requirement for multiple VC to achieve therapeutic correction of phenotype.

Safety concerns, related to potential insertional mutagenesis, highlighted by the high incidence of leukemias in the first successful human gene therapy trials,[8,9] are likely to impose a limit to the average number of allowed vector copies per transduced HSC in any approved clinical trial. Common sense, but also informal contacts with FDA committee members, suggests that the optimal vector copy number (VCN), to whom all clinical protocols should aim, should be between 1 and 2 copies per cell. Hence, the vector copy number constraint imposes an improvement in the design of the vector that will ensure levels of expression as much as possible close to the expression of the endogenous globin genes. As in the best vectors published to date the expression has barely reached half the value of the endogenous gene, this is probably due to repressive chromatin position effects (PE); in fact, a critical issue when introducing a transgene into the genome is the great variability or even the total lack of gene expression caused by the integration of the retroviral vector genome into scarcely receptive chromosomal regions. Since the bulk of the mammalian genome is packed into transcriptionally silent heterochromatin, and retroviral vectors integrate relatively randomly in the genome, a significant proportion of insertions occurs in transcriptionally silent regions where they are subject to negative position effects.

This can lead to highly variable expression, with silencing of provirus expression in a significant fraction of clones. Moreover, the sister cells derived from a single clone containing a unique integration event can also be differentiately affected by the surrounding chromatin to varying degrees in terms of clonal expansion, a phenomenon known as position effect variegation (PEV). Using recombinant globin gene LV, PE and PEV has now been observed in several studies despite inclusion of β or α-globin LCR-like elements, known to impart position independent expression. A minimum of three copies of vector integrations per cell are required to have a significant disease correction.[2-7]

Integration of multiple copies of LV vectors, which have a propensity to integrate within or around active genes and carry a strong erythroid enhancer may have safety implications, even though these LV vectors have several built in safety features as compared with the RV vectors used in some recent successful gene therapy trials.[8] In fact, have recently been reported integration of β globin/LCR LV vectors in genes important for hematopoiesis which, if dysregulated by the LCR enhancer, can become potential oncogenes.

These adverse effects on retroviral mediated gene therapy could be overcome to some extent by incorporation of DNA elements into vectors such as chromatin insulators functioning to establish and delimit domains of expression. These elements, first described in Drosophila and found in a wide range of organisms, can influence gene expression because of two experimentally defined properties of positional enhancer blocker and barrier. By the first function, chromatin insulators prevent promoter enhancer interactions only when placed between the two, and in so doing can shield promoters from the influence of neighboring regulatory elements. In addition, by acting as barriers against the propagation of condensed chromatin, insulators may buffer a transgene from chromosomal position effects, limiting the number of vectors necessary for therapeutic effects. Several studies have shown that flanking of the transgene in recombinant vectors with the characterized cHS4 chromatin insulator can reduce the rate and the severity of vector PE, moreover recent evidences suggest that vectors insulated with this element may have a lower propensity to perturb nearby gene expression. Therefore, the use of chromatin insulators is desirable in gene therapy approaches as they have the ability to shield transgenes from the negative influence of chromatin, together with the potential to avoid insertional mutagenesis. We have previously identified an enhancer blocker element, named sns5, at the 3' end of the H2A early histone gene that displays the capability to function as an enhancer blocker in erythroid milieu in that interferes with the interaction between the human beta-globin LCR and gamma-globin promoter and binds erythroid and ubiquitous transcription factors.[9] More recently we demonstrated that this element, exhibits boundary properties, counteracting PE, PEV and extinction of expression when flanking an onco-retroviral vector and binds transcription factors and histone epigenetic modifications that marks active chromatine.[10] Our results suggest that this new insulator is able to maintain a euchromatin state inside the provirus locus with mechanisms common to other characterized insulators and, on the basis of its ability to function as barrier element in erythroid milieu and to bind the erythroid specific factor GATA1 the inclusion of sns5 insulator in viral vectors may be of practical benefit in gene transfer applications, in particular for gene therapy of erythroid disorders such as beta thalassemia.

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LEUKEMIA – MORPHOLOGIC EVALUATION OF THE CELL TYPES

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Proper classification of the type of leukaemias is necessary to make appropriate treatment decisions. Categorization of the leukaemias has been classically done by assessing the morphology of May-Grunwald Giemsa stained specimens under light microscopy. The classfication of the cases is based on the cell type which the malignant clone resembles and the stage of maturation. In table I are listed the most useful criteria in categorization of the blood and bone marrow cells. *Specific* morphologic criteria have been described[1-4] in order to assess the stage of differentiation and the degree of maturation of neoplastic cells. Importantly, there is still no machinery that can replace microscopic examination of bone marrow aspirate and therefore evaluation of bone marrow (and blood) morphology is required for the diagnosis of leukemia.

Although marrow and blood preparation stained with May-Grunwald-Giemsa are the cornerstone of initial evaluation, it is best to be aware of the limits of light microscopy. For example, morphology alone can be ineffective in identifying the lineage of all types of leukaemia as in indifferentiated leukemias. Moreover, major advances have recently occurred in our understanding of the cytogenetics and molecular biology of haematological malignancies, and therefore precise classification requires a combination of morphological, cytochemical, immunologic and genetic features. To this end the WHO classification has been recently revised in order to accommodate new data and to help identify specific clinico-pathological entities.[5] In particular, among the other analyses, flow-cytometry plays an important role in the evaluation the phenotype of hemopoietic cells. The clinical use of this technology has been recently expanded. Several monoclonal antibody recognize biomarkers that identify different cell types. Nevertheless flow-cytometry is a useful method where applied as a supplement to the morphologic classification of leukemias and flow-cytometic determination of blast percentage should not be used as a substitute for visual inspection.

Acute lymphoblastic leukemia

There are limits of light microscopy in performing morphologic diagnosis of acute lymphoblastic leukaemia. The need of flow-cytometry investigation is essential for determination of lineage in acute lymphobalstic leukemia.

Acute myeloid leukemia

Standard in the diagnosis of AML is the cytologic examination of May-Grunwald-Giemsa stained blood and bone marrow smears by light microscopy. The most widely used AML classification was FAB classification that described the degree of differentiation and lineage of leukaemia predominantly based on morphologic criteria.[6] The current WHO AML classification of hematopoietic neoplasm was recently updated.[1] Morphology plays an important role in the diagnosis of WHO category of "Acute myeloid leukaemia not otherwise specified".[1,7] The use of monoclonal antibodies to characterized cell lineage in AML is now commonly performed. However, immunophenotype has not been as useful in AML as it has in the lymphoid leukemias. In general, the cells seen in AML do not express highly specific immunophenotypes; however some subset demonstrate preferential association with some antigens (e.g. AML t(8;21) may express myeloid and lymphoid antigen).

Mixed lineage leukemia

Morphology and immunophenotyping are both essential for diagnosis of this type. Cells in these leukemias express antigens at different stages of differentiation and from more than one cell line. Different combinations may occur; e.g. biphenotypic acute leukemias in which different antigens are expressed on the same cells or bilineage leukemias in wich there are two distinct cell populations, myeloid and lymphoid (Figure 1).

Table 1.

Criteria for morphologic cell type identification
1) cell size
2) nucleus cytoplasm ratio (high or low)
3) Cytoplasm characteristics
a) color of background cytoplasm
b) presence or absence of granules
c) color and size of granules
4) Nuclear characteristics
a) shape
b) colour
c) chromatic pattern
d) presence or absence of nucleoli

From Stierne-Martin E.A., Lotspeich-Steining C.A. and Koeps J.A.: Clinical Hematology. 2d edition; Lippincott Editor (modified).

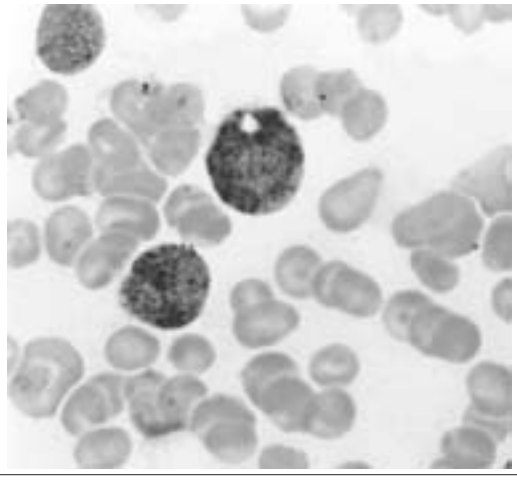

Figure. 1. Acute mixed leukemia. Peroxidase stain (x200). In this case of bilineage leukaemia, there are two distinct cell populations. One showing large peroxidase positive cell (myeloid features), and the other, small cells, displaying lymphoblast morphology (lymphoid lineage demonstrated CD19+, CD10+ phenotype). In addition there are some peroxidase negative neutrophils confirming involvement of myeloid lineage.

The immunophenotyping (positivity of myeloid and lymphoid antigens) should be avaluated according Egil criteria[8] and must be implemented with morphology of the blast cells. In some cases it is very difficult to classify the blasts. In some istances, the morphological examination identifies seemingly different blast cell subpopulations. In others, blast cells of homogenous morphology display an aberrant phenotype in a fraction of the cells.

Cytochemistry in leukemias

Cytochemistry in the past, has been an integral part of the diagnosis process in the identification of cell lineage in patients with leukaemia disorders. However, nowadays cytochemical staining procedures play a smaller role in the diagnostic process, as compared to immunology and molecular biology procedures

No single cytochemical stain result should be used for diagnostic decisions. Nevertheless cytochemistry may play role in selected cases of leukaemia as eosinophilic leukaemia. Normally eosinophilic granules are strictly chloroacetate negative. Abnormal positivity as chloroacetate esterase in eosinophilic granules[5,2] may suggest neoplastic feature of these cells. Combined morpho-cytochemical evaluation may help further in defining the cytology of leukemia cells as in acute promyelocytic leukamia.[9]

In conclusion, light microscopy evaluation of the cells will probably still play an important role in the future of hematology. However strict quantitative and highly reproducible criteria are and will be required in morphological descriptions based on continuously growing knowledge of biology of the various haematologic cell types.

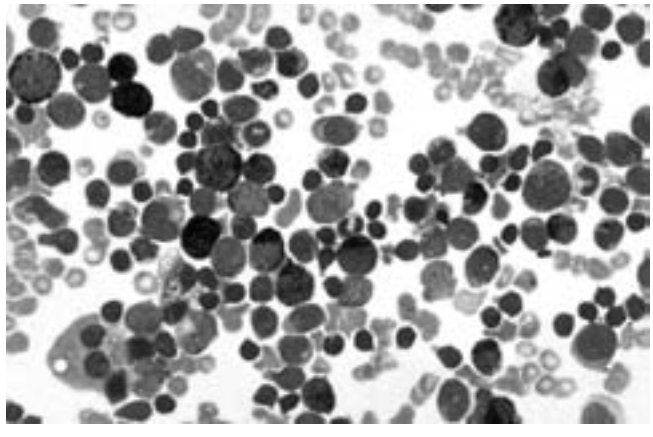

Figure 2. Eosinophilic leukaemia. Chloroacetate stain (x1000). The majority of eosinophilic granules of two cells show intense abnormal chloroacetate positivity. Normal eosinophil display non specific esterase positivity and negative chloroacetate reaction.

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A EUROPEAN APPROACH TO BLOOD CELL IDENTIFICATION

Zini G., Bain B.,[1] Liso V.[2] on behalf of the European Morphology Faculty, ELN WP10

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Microscopic peripheral blood and bone marrow cell evaluation remains crucial in hematological diagnosis. Many factors contribute to the lack of standardization of this diagnostic test, such as differences in the preparation of films, staining, degree of skill in interpretation, definitions and terminology. However the new WHO classification highlights the importance of morphological aspects, quantitative as well as qualitative, for more accurate diagnosis and a better stratification of patients with haematological malignancies, particularly myeloid malignancies and above all myelodysplasia. Morphology is no less important in the diagnosis of benign disorders of red cells, white cells and platelets.

The European LeukemiaNet (ELN) Network of excellence, an EU project funded by the 6th FP, is coordinated by R. Hehlmann and includes 162 participating centers in 33 countries, with more than 1000 researchers and associates. Its major goal is construction of a cooperative network for improvement of leukemia diagnosis, care and research. The Diagnostic Platform (WP10) is focused on Flow Cytometric and Morphological panels, chaired by MC Benè and G Zini. A European Morphology Faculty (EMF) including 28 expert morphologists from 17 European countries has been organized: the criterion for an invitation was that the morphologist was well recognized in this role in his/her own country. The main goals of the EMF are to: harmonize identification of blood and bone marrow cells for a common European morphological diagnostic pathway; take into account specific national skills, competences and methods; provide the patient with the same morphological diagnosis all over Europe.

To achieve these goals, as a first step the EMF created a consensus-based cell library of meaningful blood images identified and named by top level European Morphologists for lineage, maturation stage and terminology and accepted by EMCF, as a valuable tool to train and test European morphologists.

The project started in February 2007: from June 2007 to July 2009, 228 images with 604 labelled blood cells were collected and submitted via internet to all the EMF members: 106 cells for which a full consensus was not reached were collectively discussed, agreed and named during a 2-day meeting which took place in October 2009 in Nancy.

After the meeting all the 604 cells were named with a full consensus.

Moreover two documents were agreed and released: a common European Blood Cell Glossary (EBCG) and a Consensus Statement document with sections involving not only the appropriateness of the morphological cell identification and the choice of the term used, but also those technical aspects useful to harmonize those blood cell images on the net which can be used as a didactic/training aid.

All these data will be organized and submitted for publication.

This interactive session will consider representative disorders of red cells and white cells and will introduce the European Blood Cell Glossary.

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ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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The treatment outcome of Acute Lymphoblastic Leukemia (ALL) is highly successful in pediatric patients, but remarkably less favorable in most adults. Although initial response rates to chemotherapy in adults are almost as high as those seen in children, the chance of relapse and the risk of treatment-related mortality are both considerably higher in adults. In most cooperative adult ALL study groups, only 30-40% of adult ALL usually survive beyond 5 years. Survival largely depends on risk factors such as age, white cell count, time to complete remission (CR), disease immunophenotype, cytogenetics, and molecular abnormalities. Traditionally, these features have been used to identify risk groups with survival probabilities that range from less than 20% to greater than 50%. However, risk models often lack prognostic precision at the level of individual patients. In fact, a considerable proportion of standard-risk (SR) patients treated with standard chemotherapy will eventually relapse (up to 50%), thus determining a 5-year survival rate of 50% or higher for this group. The prognostic model is usually more accurate for the high-risk (HR) group, but even in this case important limitations exist since approximately 20% to 25% of HR patients do not relapse. These limitations explain why the definition of the best post remission therapy (a conventional chemotherapy or a transplant) is still lacking and this field largely remains matter of clinical investigation.

Recent progress of chemotherapy in younger adults

There is good recent evidence that modern intensive chemotherapy programs have improved the outcome of younger adults with ALL. A recent analysis of clinical trials performed by CALGB since 1988 (where only patients with Ph+ ALL were allocated to alloHCT in CR1) shows that the overall survival (OS) of patients <30 years old, including those with high-risk features, was 59% at 3 years.[1] Moreover, retrospective studies focusing on patients with an age ranging between 15-21 years, showed that adolescents and young adults treated with adult ALL protocols have inferior outcomes as compared to patients treated with chemotherapy intensified, pediatric protocols. Having this as a background, the PETHEMA ALL-96 protocol was designed to compare toxicity and efficacy of a pediatric-based protocol in adolescents (age 15-18 years) and young adults (age 19-30 years) with standard-risk (SR) ALL. The overall CR rate was 98% and after a median follow-up of 4.2 years, 6-year event-free survival (EFS) and OS were 61% and 69%, respectively, with no differences between adolescents and young adults.[2] Whether these intensified pediatric protocols may benefit also patients with more advanced age remains to be determined. In particular it remains to be demonstrated whether such a dose intensification can be afforded by most older adults (30-60 years) with tolerable toxicity and similar efficacy.

Allogeneic transplantation in adult ALL (a donor/no donor problem?)

There is no doubt that the high relapse rate in adult ALL can be reduced by allogeneic hematopoietic cell transplantation (alloHCT) which stands as the most active anti leukemic post-remissional therapy for patients in first remission (Figure 1). While this suggests an important role for the graft versus-leukemia effect (GVL) in ALL, the relapse and non-relapse mortality negatively impact on the clinical outcome of alloHCT even when this is performed in first CR. Moreover, the overall toxicity, particularly the graft versus host disease (GVHD), is still very high so that the decision to offer a transplant to an adult ALL patient is neither easy nor automatic. This is why, in general, patients considered to be at high risk for relapse often are considered for HSCT in first CR, while those at lower risk may not be referred until they have relapsed when unfortunately, their chances for cure are very poor. This empirical approach, however, is based on a few studies many of which performed several years ago.

In the early days, a French randomized clinical trial (LALA-87) compared in young adult ALL (age 15-40) the effect of related alloHCT bone marrow transplantation (BMT), with a control group of patients with no suitable sibling donor treated with an autologous transplant or chemotherapy. There was no significant difference in median Disease Free Survival (DFS, 24 vs. 22 months) or OS (51 vs. 30 months; p=0.08) between alloHCT and chemotherapy groups. However, in a subset of high-risk patients, there was a significant benefit for the alloHCT group compared with the chemotherapy group in terms of DFS and OS

(p=0.01).[3] In a subsequent study (LALA 94) Thomas and co-workers analyzed the benefits of a risk-adapted post remission strategy in adult lymphoblastic leukemia (ALL), and re-evaluated stem-cell transplantation (SCT) for high-risk ALL. A total of 922 adult patients entered onto the trial and an improved DFS was observed in high-risk ALL treated with alloHCT in first CR.[4]

On the other hand, other investigators have reported less impressive or negative results. Zhang and coworkers in 1995 reported the long term follow up of adults with ALL in CR1 treated with chemotherapy or alloHCT. This study was a retrospective collaborative effort between the German Multicenter ALL (GMALL) study group and the International Bone Marrow Transplant Registry (IBMTR). Chemotherapy recipients were 484 adults ALL patients treated in two consecutive GMALL trials. Transplant recipients were 234 consecutive recipients of HLA-identical sibling bone marrow transplants for ALL in first remission in 98 centers, worldwide, reporting data to the IBMTR. Fewer relapses but a higher treatment-related mortality were seen with alloHCT than with chemotherapy so that, at 9 years, despite a relapse probability of 66 for chemotherapy vs 30% for alloHCT (p<0.0001), the leukemia-free survival rates were similar (32% vs. 34%, respectively).[5] Comparable results have been obtained also by a multicenter randomized trial conducted by the Spanish group (PETHEMA ALL-93). This study was planned to compare chemotherapy, allogeneic and autologous SCT as post-remission therapy in adults with high-risk ALL. A total of 222 patients entered the trial. Patients in complete remission with an HLA identical family donor were assigned to alloHCT (n=84) and the remaining were randomized to autologous SCT (n=50) or to delayed intensification followed by maintenance chemotherapy (n= 48). By an intent-to-treat analysis no differences were found between patients with or without a donor both in terms of disease-free survival (39% vs. 33%) as well as overall survival (44%, vs. 35%,). No differences were similarly observed when the analysis was made on the basis of the treatment actually performed or for autologous SCT vs. chemotherapy comparisons.[6]

Based on this and other similar studies, a recent evidence-based review of the literature published by investigators of the American Society for Blood and Marrow Transplantation, pointed out that in first complete remission, alloHCT yields outcomes similar to chemotherapy and is not recommended as first choice therapy in standard-risk patients. For high-risk patients, there are no direct comparisons, but most data would suggest an advantage for SCT.[7]

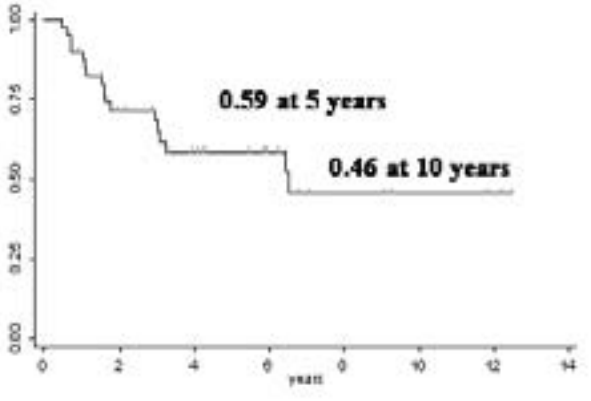

Figure 1. Overall Survival of 41 consecutive adult ALL patients (including 20 Ph+ cases) transplanted at Bergamo center, in first remission.

However, two large clinical studies recently published by the Medical Research Council/Eastern Cooperative Oncology Group and the Dutch-Belgian Cooperative Trial Group (HOVON) have recently challenged this opinion. In the first study, Goldstone et al reported the outcomes of more than 1900 adult ALL patients who were assigned to allogeneic transplantation if in remission after 2 cycles of induction and if a compatible sibling donor was available. The remaining patients were randomized to chemotherapy for 2.5 years versus an autologous transplantation. A donor versus no-donor analysis has shown that Philadelphia chromosome–negative patients with a donor had an improved 5-year OS (53% vs. 45%, p=0.01), and a significantly lower relapse rate (p< .001). The survival difference was significant in standard-risk patients but not in high-risk patients. Nonetheless, from the flow chart of the study and treatment demographics, it should be noted that from the starting denominator of 1929 patients entering this study, 443 had a donor and only 320 had a transplant in CR1.[8] In the Hovon study, Cornelissen et al. evaluated the outcome of patients with ALL in CR1, according to a sibling donor versus no donor comparison. Patients were eligible if younger than 55 years and in CR1. AlloHCT was performed in 91 of 96 patients with a compatible sibling donor. Cumulative incidences of relapse at 5 years were, respectively, 24 and 55% for patients with a donor versus those without a donor. At 5 years, after alloHCT, the estimated non-relapse mortality was 16% so that the disease-free survival was significantly better in the donor group vs. the no-donor group (60 vs. 42%). Interestingly, the improved outcome was more pronounced in standard-risk patients with a donor, who experienced an overall survival of 69% at 5 years (9).

Allogeneic transplantation in Ph+ ALL: (in the pre and post Tyrosine Kinase Inhibitors era)

Among high risk ALL, a prominent role is played Ph+ patients whose disease is invariably aggressive and with a dismal prognosis. Accordingly, there has been a substantial agreement to identify Ph+ ALL patients under the age of 60 as good candidates for alloHCT. However, even with transplant, the relapse rate for Ph+ ALL is in the range of 40-80%. For patients transplanted in a pre Tyrosine Kinase Inhibitors (TKI) era, the most exciting results have been reported by investigators from City of Hope who have recently updated their 20 years experience using fractionated total body irradiation (FTBI) and high-dose VP16 (with the addition of cyclophosphamide in some patients). With a median follow-up of 6 years the event-free overall survival at 10-year for patients transplanted in CR1 was 48% and 54%, respectively.[10] Even in the contest of a large multicenter randomized clinical trial, alloHCT remains the most effective treatment for this subgroup of patients. In the UKALLXII/ECOG 2993 study, for example, patients younger than 55 years of age achieving CR after standard induction were eligible to sib or matched unrelated donor (MUD) alloHCT while those without a donor or unfit were treated with chemotherapy. At 5 years, the OS was 44% after sib alloHCT, 36% after MUD alloHCT, and 19% after chemotherapy. Of note, however, in this study only one third of patients was actually transplanted and the reasons for patients not receiving HSCT were, in most cases, the age limit or having an early event that prevented transplantation even when a donor was available.[11] With the availability of Imatinib and the other TKI like Dasatinib and Nilotinib, the clinical outcome of Ph+ ALL seems to be changing rapidly for at least two reasons. First, therapy with TKI alone[12] or in combination with chemotherapy[13,14] has significantly improved the number of patients achieving a rapid and robust CR and second, it has reduced the proportion of early relapse that in the past, precluded transplant to a large proportion of patients. In fact, in a preliminary study Lee et al. provided data suggesting that when compared to historical controls, patients receiving a combined chemotherapy and Imatinib treatment before alloHCT a) have a lower relapse rate (4 vs. 41%); b) can proceed to transplant more frequently (86 vs. 52%) and c) have a lower probability of relapse after transplant and a better disease free and overall survival.[15] These results are likely to be reproduced by other experience, including ours (Figure 2).

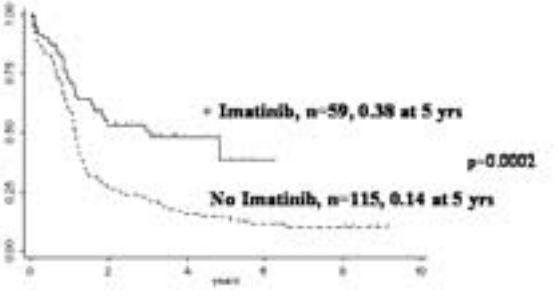

Figure 2. Overall survival of 2 consecutive cohorts of Ph+ adult ALL patients exposed or not to Imatinib during their therapeutic program that could also include alloHCT.

A risk adapted strategy to select patients for alloHCT

Taken together the available evidence deeply challenged the idea to select patients for alloHCT on the basis of simple clinical prognostic factors or the donor availability. In addition, thanks to the new drugs such as the TKI for Ph+ ALL, in the near future it may also be changing the definition of cytogenetic risk factors. All in all there is an urgent need of more precise prognostic definition to make better therapeutic decisions in the individual patient.

In recent years, several studies of childhood and adult ALL identified minimal residual disease (MRD) as an important independent prognostic factor for the duration of CR. MRD can be evaluated at fixed time points during induction and consolidation therapy using flow cytometry or patient-specific molecular probes. There is a close positive association between rapid MRD signal reduction (which is proof of chemo sensitivity) and the duration of CR, independently of the applied treatments. In contrast, patients with persistent MRD almost always relapse. Thus, monitoring MRD may allow us to identify patients whose actual clinical course is unlikely to match the initial risk classification, and could help guide the decision to use SCT or post consolidation maintenance. Such a strategy could spare some HR patients from the toxicity burden of SCT (and the attendant risk of remission death) as well as identify SR cases for whom standard chemotherapy is likely to fail.

Using MRD as the leading risk indicator, we designed an innovative prospective program in which the final treatment protocol was based on MRD study results. The program had 2 distinct phases. The first, phase A, was applicable to all patients, and had the dual aim of eradicating the disease in as many patients as possible while simultaneously allowing the MRD response to be defined. Only patients bearing t(9;22) or t(4;11) translocations could proceed straight to allogeneic SCT. The second, phase B was experimental: treatment depended on MRD status, with maintenance therapy for MRD-negative (MRDneg) patients, and high-dose treatments with SCT for MRD-positive (MRDpos) patients.

We found an outstanding correlation between MRD status and 5-year DFS rate that was 0.75/0.72 in the MRDneg group compared with 0.33/0.14 in MRDpos (p=0.001), regardless of the clinical risk class. Taken together, these findings cast doubt on the indication for SCT as the preferred therapy in unselected patients with ALL in first CR, and call for an alternative MRD-assisted decisional approach. Conversely, outcome was definitely poorer in the MRDpos subset, again with no difference related to the original clinical risk class. Interestingly, a proportion of these patients was effectively rescued by SCT.[16]

Molecular Monitoring of MRD before and after transplant

Several studies have reported the importance of detecting and quantifying minimal residual disease (MRD) status before and after alloHCT, particularly in pediatric patients. Patients with molecularly detectable residual disease before allogeneic HSCT are predicted to have a higher risk of relapse and a poor outcome. In keeping with other experience we also showed that the incidence of leukemia relapse after transplantation is significantly higher in patients with molecularly detectable residual disease before the conditioning regimen. In our patients, the relapse rate is negligible in patients who are in molecular remission before transplantation while almost half of patients relapsed if any level of MRD, no matter the amount, is detectable at this time. By multivariate analysis, the PCR negativity before the conditioning regimen is the best predictor of clinical and molecular complete remission at day 100 after transplantation. This observation highlights the need to deliver therapeutic programs that can achieve molecular remission before transplantation. Another crucial point is the kinetics of MRD clearance since the relapse rate of patients who achieved PCR negativity by day +100 post-transplantation is significantly lower than that among

patients who never achieve or rapidly loose the molecular remission (17). Most importantly, the check point at day +100 rather than earlier time points, should be considered crucial for subsequent therapeutic decisions. In fact, patients not achieving a molecular remission at this time point should be selected for early discontinuation of immune suppression and possibly given infusions of donor lymphocytes or leukemia-specific cytotoxic T-lymphocytes, natural killer cells or molecularly targeted drugs when indicated. Once again, the molecular monitoring of MRD is a crucial tool for therapeutic decision making rather than a prognostic marker.

Conclusion and recommendations

The great complexity of all the involved issues indicates that no homogeneous approach is now possible toward the application of allogeneic SCT in patients with ALL in CR1. The evidence that this is a most effective antileukemic weapon must be tempered by its toxicity (which may cause remission mortality in excess of 10%), by the fact that this is in some way proportional to patient age, and by the awareness that prognosis and response to chemotherapy is now more reliably set on MRD analysis and other biological insights. On the other hand it has to remember that for some very high risk subsets this is the only option for cure, that should be pursued with decision and reducing the transplantation delay to a minimum. Ideally, a first step should be the definition of the patient risk class through a sound subclassification system (which should be obtainable according to standards given by current research protocols). It is likely based on current evidence that a significant proportion of patients (up to one third) be immediately identified as primary candidates to allogeneic SCT therapy, because of clearly adverse cytogenetics such as t(9;22), t(4;11) and other rare abnormalities (low hypodiplody/near triploidy, -7, +8), or other clinico-diagnostic characteristics (WBC count >100, early or mature T-phenotype). The remainder of patients could be managed with a view to confirm the need for an allogeneic SCT only when a suboptimal response to early chemotherapy is proven. This usually means to provide a concomitant MRD study during the first 3-6 months of induction-consolidation therapy, which, in cases deemed to become SCT eligible, will not show an adequate clearing (i.e. disappearance with a sernsitivity level of 10-4 or greater) of the submicroscopic MRD signal, evaluated by either PCR methods or immunophenotype. These studies however require excellent design and supervision, to avoid the bias of inadequate diagnostic and treatment decisions, and may result difficult in the larger cooperative settings. When this is not possible, the decision to allograft rests on clinical grounds alone, but it should preferably not be applied to younger (<50 years) patients with low-intermediate risk features (WBC count <30 for B-lineage, <50-100 for T-lineage, normal cytogenetics or hyperdiploidy, pre-B CD10+ and cortical T phenotype) and it carries the risk of a relatively high remission mortality rate in patients who could be curable otherwise. All in all, the more of these aspects are known the easier the decision becomes, and the final choice should by guided by the indications of ongoing national and international clinical studies and protocols rather than subjectively taken.

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ASCT AS CONSOLIDATION THERAPY ACUTE MYELOID LEUKEMIA

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The optimal consolidation therapy for adults who are in first remission (CR1) of acute myeloid leukemia (AML) after induction chemotherapy and who do not have a histocompatible donor is poorly established. Autologous stem cell transplantation (ASCT) was initially planned in the late 1970s to consolidate remission in patients with AML without compatible siblings or too old for an allogeneic transplantation.[1] Until the mid-1990s, the only source of hematopoietic stem cells (SC) was Bone Marrow (BM) and some teams developed techniques in order to purge the marrow autograft of residual tumor cells that could increase the risk of relapse; many retrospective studies provided evidence in favor of in vitro purging of marrow. However, definitive proof that *in vitro* purging improved clinical outcome was not established in the absence of prospective randomized studies.[2] In 1990, Peripheral Blood Stem Cells (PBSC) collected at the time of recovery from chemotherapy-induced aplasia, after priming with colony stimulating factors (eg: filgrastim, lenograstim, molgramostim) was shown to contain a large number of hematopoietic progenitors (HP) able to reconstitute hematopoiesis. In the last ten years the expanding use of colony stimulating Growth Factors (GF) after chemotherapy, has substantially changed the modalities of performing ASCT in AML: currently Peripheral Blood (PB) is the preferred source of HP in more than 85% of the patients who are autografted for AML. However except for the AML10 study, the main trials published in this setting have been conducted more than 15 years ago, and included mainly AML patients who received the BM source.[3]

The era impact on ASCT outcome in AML adults

Among the main changes occured in the last decades, about the ASCT practice and outcomes, the replacement of the BM source with the PB source has been generally considered one of the main factors which reduced the cots and morbidity, but it has not clearly established if also the non-relapse mortality (NRM) has been lowered thanks to the PBSC coming. In the large trials, reported from a recent methanalysis, NRM has varied from 5% to an unexpected high rate of 12% in the MRC 10 trial.[4] Moreover the high Relapse Incidence (RI) after ASCT, due to the absence of a Graft Versus Leukemia effect (and probably to the possible graft leukemic contamination), still remain an unsolved problem. In the prospective trials evaluating ASCT in AML with the BM source, NRM and RI appeared both relevant in all of them. In the CALGB study RI was 48% and the early NRM 14%; In the EORTC-GIMEMA trial, the 4-year RI was 40%, while NRM was 9%; MRC study reported 37% RI and 12% NRM.[4] After 1998, with the introduction of PBSC, the EORTC-GOMEMA AML10 study evaluated 246 patients, 56% of whom received ASCT after conditioning with Bu-Cy or TBI.: RI was 33% but the NRM was substantially lower (5.5%).

At present, as PBSC have replaced BM as the source for ASCT, the number of older patients candidate for ASCT has considerably expanded: indeed the median age of AML patients undergoing ASCT has increased by one decade. Whether the Stem Cell (SC) source influences the NRM and outcome is still a matter of debate for AML patients receiving ASCT. No prospective trials have been performed, up to now, in order to answer to this crucial question.

The AML patient population receiving ASCT in Europe has dramatically changed over the last decade. In Italy one-third of patients transplanted after 1998 were older than 55 years, while only 11% of those transplanted before 1998 were >55 years old. Similar dramatic changes in the SC source also occurred, with about 80% of patients receiving PBSC after 1998 versus the same percentage of patients receiving BM before 1998. We recently conducted a registry retrospective GITMO survey (preliminary results have been presented at the EBMT Meeting in Florence-Italy, (March 30 – April 2, 2008), analyzing a 20 years data in order to assess NRM and long-term outcomes of ASCT in a large cohort of 2333 adult AML patients. The study outcomes were evaluated by transplant era. We categorized AML patients according to the following criteria: age group: young (<30 years old), intermediate age (between 30 and 55 years old), and older patients (>55 years old); status at ASCT: 1st CR, 2nd CR, or advanced phase disease (>2nd CR); conditioning regimens: patients receiving total body irradiation (TBI) containing regimens, patients receiving Busulfan and patients conditioned with chemotherapy regimens not including either TBI or Busulfan; SC Source: those receiving BM and those receiving PBSC. The main characteristics of the 2333 AML patients are reported in Table 1A. The patients were subgrouped by transplant era (before and after 1998). These two groups were significantly different with regard to: age distribution: fewer young patients received ASCT after 1998. Conventional regimens including TBI and Busulfan were used less during the recent era. With regards to the status of disease: significantly more patients in CR1 received ASCT after 1998. Significantly more patients received PBSC after 1998: 75% versus 16% before 1998. Among the 2333 patients, 2030 received a single ASCT, while 311 patients (13%) received a second rescue transplant. In 207 subjects, ASCT was followed by an allogeneic transplant while 94 patients received a second ASCT. With an overall median follow-up of 616 days [813 (0-8561) before 1998 and 501 (0-3452) after 1998], OS was found to be 37% and 39% before and after 1998, respectively. Although there was a trend in NRM improvement in the recent era, the difference was not statistically significant (11% vs 14%). Overall RI was 48% and did not differ between the two eras (Figure 1 a,b,c).

The role of age and conditioning regimens in AML adults receiving ASCT

Age significantly influences the outcome: our GITMO survey shows that patients older than 55 years had a 23% OS compared to 44% for those younger than 30 years; patients in the intermediate age group (30-55 years) had 36% OS. Patients transplanted during the 1st CR show better OS; however, no difference between the 1st and 2nd CR was found in younger patients (41% vs 47%; p=ns). Conversely only ASCT in the 1st CR resulted in an acceptable outcome in older patients (OS=26%). Among the 149 patients with advanced phase disease (>CR2) that received transplants, OS was 15% (24% for patients <30 years old and 8% for those >55 years old); this unexpected result in younger patients

is not due to an allogeneic rescue or a second ASCT. Regardless of the age groups the NRM was significantly higher in patients receiving ASCT outside of CR1 (Figure 2 a,b,c).

Among the 1742 patients evaluable for the kind of comditioning, a significantly better OS was found for those receiving regimens including TBI or Busulfan. Regimens not including TBI or Busulfan showed the highest (71%) RI. There was no difference between these TBI or Busulfan treated groups (56% vs 61%). No significant difference in overall NRM was observed when different conditioning regimens were compared, although there was a trend toward higher TRM in patients receiving TBI.

Table 1A. Main characteristics of the 2333 AML patients, receiving ASCT and separated in two cohorts according to the transplant era.

	<1998	*1998-2004*	*TOT*	*p*
AML pts	1220	1113	2333	
Patient Age				
<=30yy	345 (28%)	149 (13%)	494 (21%)	
31-55yy	750 (62%)	599 (54%)	1349 (58%)	<0,0001
>55	125 (10%)	365 (33%)	490 (21%)	
Conditioning				
BU-CY	438 (36%)	430(39%)	868 (37%)	
BU-MEL	3 (-) 48 (4%)51 (2%)			
Other without TBI/BU	279 (23%)	202 (18%)	481 (21%)	<0,0001
Including TBI	283(23%)	59 (5%)	342 (15%)	
Unknown	217 (18%)	374 (34%)	591 (25%)	
Disease status				
1 CR	909 (74%)	918 (82%)	1827 (78%)	
2CR	215 (18%)	93 (8%)	308 (13%)	<0,0001
>2CR	85 (7%)	64 (6%)	149 (7%)	
unknown	11 (1%)	38 (4%)	49 (2%)	
Stem cell Source				
BM	971 (80%)	226 (20%)	1197 (51%)	
PBSC	194 (16%)	840 (75%)	1034 (44%)	<0,0001
BM+PBSC	55 (4%)	40 (4%)	95 (4%)	
unk	0	7 (1%)	7 (1%)	

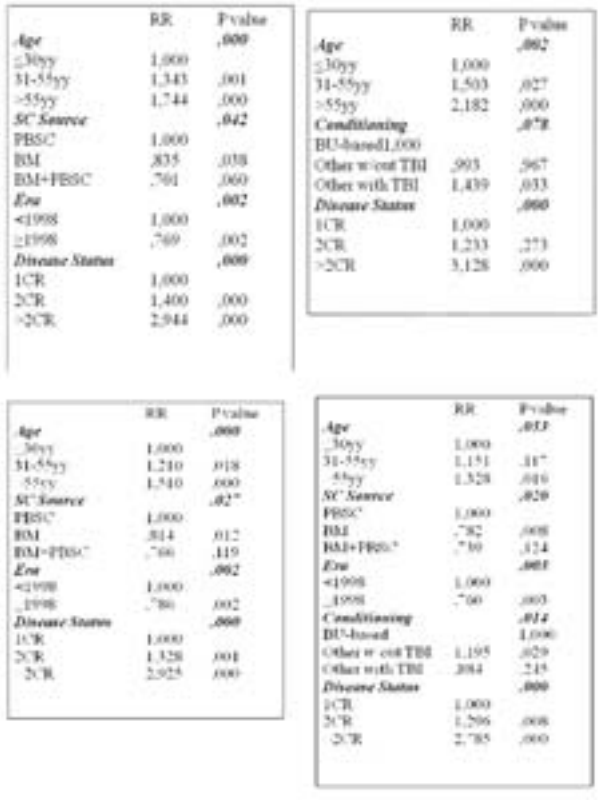
Impact of SC source in AML adults receiving ASCT

Comparisons made between the two groups of patients receiving PBSC (1021) or BM (1056) showed a trend in favor of PBSC for overall NRM. In order to overcome the bias due to the dishomogeneity among the two groups receiving ASCT before or after 1998, and as this dishomogeneity made impossible a correct pair matched analysis, a multivariate analysis was performed for the same variables.

The Multivariate analysis (Table 1b) for the main factors influencing the transplant outcome, confirmed both the negative impact of advanced age and advanced disease status, and the positive impact of Conditioning regimens including Busulfan. BM source was associated with significantly increased OS (RR=0.835; p=0.038), reduced risk (RR=0.782) of relapse and better DFS (RR=0.81) compared to PBSC. Notwithstanding the negative effect of the use of PBSC source, performing ASCT in the recent era is associated with similar NRM, but increased OS (RR=0.77; p=0.002), suggesting that we considerably improved the patient selection and supportive care. These data concerning the negative role of PBSC source have been recently confirmed by the European Cooperative Group for Blood and Marrow Transplantation (EBMT) survey[5] reporting 2,165 patients receiving ASCT (1,607 with PB and 558 with BM respectively) from 1994 to 2006; in a multivariate analysis for patients with AML in CR1, they found that the risk of relapse was significantly greater with PBSC transplantation rather than BM, independent of the interval from CR1 to transplantation. In conclusion these retrospective data suggest that: first, young patients receiving ASCT in advanced phase disease can achieve long term survival in a considerable 24% of cases without an allogeneic rescue; second, the outcome of patients, receiving ASCT in CR2 is not worse than what observed in patients transplanted in CR1; lastly the positive impact of recent era, notwithstanding adverse factors (PBSC and older age), shows a better management and selection of AML patients.

Some recommendations can be proposed based on these data: first, extensive use of PBSC should not be encouraged if it is not associated with strong *in vivo* purging before SC collection; second, conditioning regimens should be Busulfan-based, taking care to avoid TBI and other regimens; third, young patients with advanced disease without a compatible donor can still use ASCT to achieve a relevant (24%) long term survival; fourth, ASCT in CR2 remains a valuable option, since ASCT performed in CR1 yielded comparable results in patients <55 years old, but not in patients >55 years old; fifth, the benefit of ASCT for patients over 55 years old should be carefully evaluated as these patients have twice the risk of NRM and persistently high RI; this matter is still debated and deserves special considerations; up to now very few prospective data are available in this setting.

Table 2b. Multivariate analysis for factors potentially influencing ASCT outcome.



Special issues: ASCT as consolidation therapy in the elderly and pediatric AML patients

The decrease in toxicity has made ASCT a feasible option also in elderly patients: at least 25% of the patients aged 60 to 70 years with AML, can benefit from standard intensive treatment. In some studies the intensification of remission including ASCT was feasible in variable proportion of elderly patients, but it is not clear if this approach could improve the general outcome in overall population. Ferrara[6] evaluated the feasibility of ASCT from 155 consecutive AML patients aged over 60 years (median age 72 years) programmed to receive ASCT. Overall, 90 out of 155 patients (58%) were eligible for aggressive chemotherapy and 45 (50%) achieved CR. Among these, 36 (80%) received consolidation and 32 were monitored for PBSC mobilization. A successful collection was registered in 25/32 patients; 20 patients received ASCT. Median OS was 4 months for the whole population and 19 months for patients actually autografted. Overall, among the 90 patients accrued into intensive chemotherapy only 20 (22%) underwent ASCT, representing only the 13% of the entire patient population. Oriol[7] assessed the proportion of patients over 60 years with de novo AML who qualified for intensive

therapy and determined the feasibility and results of ASCT in 1st CR; 258 patients were registered of whom 135 (52%) enrolled for intensive treatment. The CR rate was 61%; only 27% of the potential candidates underwent ASCT. The probability of 2-year leukemia-free survival (LFS) after consolidation was 39% for these patients and 22% for candidate patients not undergoing ASCT (p=0.07).

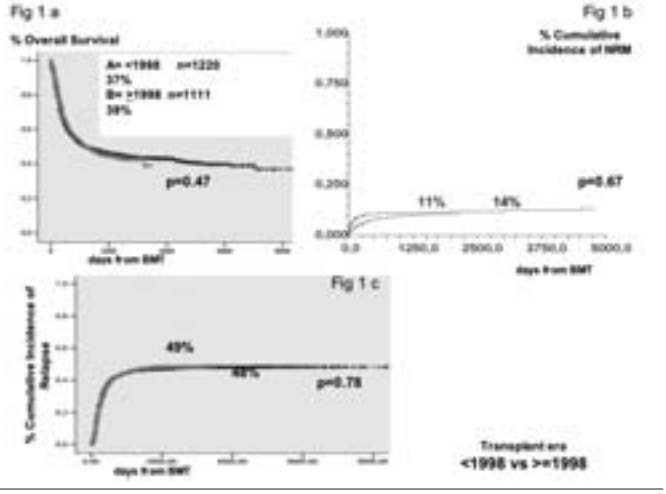


Figure 1. A. OS according to transplant era; in patients receiving one ASCT procedure (2030) OS was 38%; OS was 15% and 22% in those receiving >1 Auto (94) or Auto+Allo (207) respectively. B. Cumulative NRM in 2127 evaluable patients according to transplant era. Death in CR was considered NRM. C. RI according transplant Era; overall RI in 2331 evaluable pts was 48%.

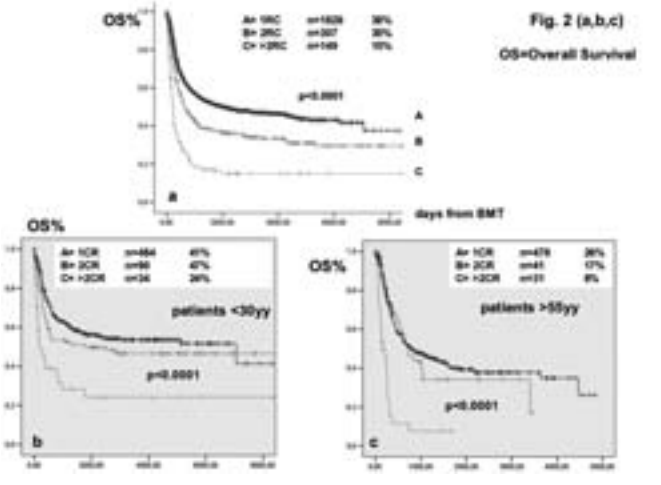


Figure 2(a,b,c): OS according to Disease status at ASCT in all AML patients (a) and according to age: younger (<30 years) patients (b) and older (>55 years) patients (c). The difference between CR1 and CR2 is not significant in patients <30 years.

The GIMEMA –EORTC group (8) conducted a prospective study, aimed to evaluate ASCT in a selected population of non very elderly patients (278 patients aged from 60 to 70 yrs); 159 (57%) achieved CR and 61 were considered fit for ASCT; SC were effectively harvested from 54 patients For the group of 61 patients, the 3-year DFS rate was 21%, while the the 3-year OS rate was 32%. ASCT was performed in 35 patients; after a median follow-up of 5.0 years, the 3-year DFS and OS were 28% and 39%, respectively. We also evaluated ASCT in 42 AML elderly patients, fit for aggressive therapy; as induction therapy they received High Dose Idarubicin plus Aracytin with including Amifostine as cytoprotectant (9). The overall response rate was 83% and 32 patients received intensive consolidation therapy; 15 patients were able to mobilize PBSC and received ASCT. The 5 years OS of the whole population (intention to treat) was 19% with a median follow up of 38 months. Recently we updated this experience to 135 patients with non-M3 AML (median age 71 years). All patients were preliminary evaluated, in order to separate fit patients from frail patients: 79 were fit; 27 (35%) had secondary AML. Among these 79 patients 56 (72%) achieved CR and 52 received the first intensive consolidation course, in order to collect PBSC. In case of mobilization failure patients were allowed to chose between an experimental approach, including low dose Gemtuzumab-Ozogamicin (GO) or a further conventional consolidation course. Among the 52 patients who received intensive consolidation, 42 patients were evaluable for post-remission treatment: 19 patients successfully mobilized SC and received ASCT while 23 did not: 13 of them received GO at the dose of 3 mg/m2 for three times, monthly; the remaining 10 patients refused GO treatment and received further consolidation with chemotherapy or allogeneic transplant from a compatible sibling. With a median follow up of 68 months (range 29-89), 16 patients are alive and in CR, 5 after ASCT, 9 after GO, 1 after CHT, and 1 after RIC. Six-year OS and LFS was 24% and 30% respectively. In conclusion albeit a considerable percentage (about 40%) of selected AML elderly patients is able to mobilize a sufficient amount of PBSC for ASCT, the final percentage of fit elderly patients who receive ASCT is less than 20% of the whole population and the RI is very high (>60%). Although preliminary these data suggest that a late post-consolidation strategy with GO seems very effective and characterized by a better outcome than ASCT alone. It remains to establish if the patients who successfully mobilize PBSC (and consequently able to receive ASCT) have a more aggressive disease (including the possibility to mobilize a large number of leukemic cells) than those who did non mobilize PBSC. Next trials should evaluate both new conditioning regimens and the role of post-ASCT maintenance therapy.

Also in children with AML the role of ASCT as consolidation of 1rst CR is still debated: 3 randomized studies in children with AML in first CR, comparing intensive chemotherapy with ASCT did not show significant differences in OS; 2 trials showed a significant reduction in RI which did not translate in a better OS, due to an increased NRM or to a higher chance of being rescued by a second line treatment. The most recent randomized trial compared 3 aggressive post-remission approaches for children with AML.[10] A total of 652 AML children who achieved remission were eligible for allocation to allogeneic bone marrow transplantation based on matched related donor status (181) or randomization to ASCT (177) or to aggressive high-dose cytarabine-based chemotherapy (179); 115 patients (18%) refused to participate in the postremission phase. Overall NRM was 14% in the allogeneic BMT arm, 5% in the ASCT arm and 4% in the chemotherapy arm. At 8 years actuarial, 54% of all remission patients remain alive. OS by assigned regimen (intent to treat criteria) was: 60% for allogeneic; 48% for ASCT, and 53% for chemotherapy. OS in the allogeneic group was significantly superior to ASCT (p=0.002) and chemotherapy (p=0.05); but differences between chemotherapy and ASCT were not significant (p=0.21).

Conclusions

Although data about the superiority of ASCT in first CR over conventional consolidation chemotherapy are controversial, both in the adult and paediatric population, the decrease in toxicity has made ASCT a feasible option for consolidating CR in younger patients and in a consistent percentage of elderly fit patients who lack an HLA matched donor.

A 45% 5-year OS rate in high-risk (with 31% DFS) and 64% DFS in good-risk patients have been observed in a large cohort of patients receiving ASCT, with a median follow-up of 9.5 years. In other trials, a significant reduction in the RI has been observed in good- and intermediate-risk patients. Conversely, in 2 meta-analyses of 6 trials conducted up to 1996 ASCT was shown to improve LFS by about 25%, without any effect on OS.[11] A recent methanalysis,[4] comparing ASCT with conventional consolidation confirmed that ASCT is associated with DFS improvement , but not in OS, due to a higher NRM. Although allogeneic transplantation was once considered the preferred consolidation therapy for all young patients with AML, recent evidence suggests that this approach should be reserved for patients at the greatest risk of relapse, such as those with unfavorable cytogenetic features. Greater uncertainty exists regarding how AML patients in first CR should be stratified by risk for ASCT. Two retrospective EBMT studies compared ASCT and allogeneic transplants from HLA-identical siblings or unrelated donors. These studies showed higher NRM for allografting and higher RI for ASCT. However, a recent comparison of AML patients with or without a donor suggests that, in the donor group and LFS was significantly better.[12] Nonetheless, retrospective analyses comparing ASCT to matched

unrelated transplant in patients in first CR lacking an HLA identical family donor showed an advantage with the use of ASCT.[13]

In conclusion ASCT can be considered an effective consolidation both for patients lacking a compatible donor and for those who should not be candidated for an allogeneic transplant, due to the low-intermediate risk features of the disease. Compared to the intensive consolidation with high-dose Aracytin, ASCT seems still characterized by higher acute and late toxicity, which counterbalance the lower risk of relapse after ASCT. However there is still room for improvement for the ASCT practice: in particular the use of less toxic Conditioning Regimens, but characterized by more powerful anti-leukemic activity and the chance to evaluate a post-ASCT consolidation with targeted therapy, deserve further large prospective studies.

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ALLOGENEIC STEM CELL TRANSPLANTATION IN MYELODYSPLASTIC SYNDROMES: SOME UNANSWERED QUESTIONS

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Myelodysplastic syndromes (MDS) are clonal disorders of hemopoietic precursors for which allogeneic hemopoietic stem cell transplantation (HSCT) is the only curative option. MDS typically occurs in elderly people with a median age between 60-75 years. They are characterized by peripheral blood cytopenias and a high propensity to evolve in acute leukemia. The course of MDS is variable; the median life expectancy ranges from more than a decade to less than one year. The majority of cases of MDS are idiopathic, although various environmental agents such as age, alcohol, smoking, ionizing radiation, benzene and infections have been indicated as contributing factors. A significant proportion of secondary MDS (s-MDS) are related to prior chemo/ radiotherapy. The risk of s-MDS appears to be significantly increased following previous therapy with alkylating agents; in 90% of cases, s-MDS are associated with chromosome abnormalities involving chromosome 5,7 and complex chromosomal abnormalities. In this setting prognosis is particularly poor. Median survival and probability of developing AML can be predicted based on several scoring systems. In 1997, a group of investigators published an International Prognostic Scoring System (IPSS) based on the analysis of 816 patients classified according to the French-American-British classification (FAB). The median follow-up was 1.9 years. In this study, the percentage of blasts in the marrow, the presence and type of cytogenetic abnormality and the number and degree of cytopenias were correlated with acute myeloid leukemia (AML) progression and survival. The analysis resulted in the identification of four risk groups (low-risk, intermediate-1, intermediate-2, and high-risk) with a median survival ranging from 0.4 to 5.7 years. he IPSS has become the gold standard for risk assessment in MDS. Recently, the World Health Organization (WHO) formulated a new classification, based on clinical and morphological criteria, such as uni- or multi-lineage involvement, cytogenetic abnormalities, and bone marrow blast count.

Malcovati *et al.* proposed a new score system (WPSS) based on the WHO classification, karyotype, and transfusion dependency. The WPSS allows stratifying patients into five categories: very low, low, intermediate, high and very high, predicting a median survival ranging from 8 to136 months. Because of advanced age, the majority of patients with MDS are generally managed with supportive care, which includes transfusional support, antimicrobial therapy, and iron chelation. Other treatments that have been evaluated include hormones, differentiating agents, hematopoietic growth factors, immunosuppressive therapy and low-dose chemotherapy. Recent data on the use of hypomethylating agents seem to suggest an improvement in marrow function and less likelihood of acute transformation. There are controversial data suggesting that in young patients with RAEB or RAEB-t and normal karyotype, intensive chemotherapy may induce long-term survival. None of these modalities has been shown to have curative potential. Until now, HSCT is the only curative treatment. Data from a large registry database and a single center experience including patients of all ages indicate that only approximately one third of patients may be cured (Figure 1 and Table 1). Risk factors influencing the outcome of transplantation include age, disease duration, disease stage at time of transplantation, blasts in the bone marrow, the presence and the type of cytogenetic abnormalities, the source of stem cells, and the intensity of the pre-transplant conditioning. Despite the curative potential of HSCT, this procedure has little influence on MDS population survival because the peak of MDS incidence is over the age of 65. Many questions, such as the timing of transplant, pre-transplant therapy, conditioning intensity, type of donor, and stem cell source, remain unanswered.

Concerns about timing

The best candidates to transplant are young patients with low-risk MDS transplanted early after diagnosis. In this setting of patients, a sibling donor transplant with myeloablative conditioning results in 60-75% of long-term disease free survival (DFS). Registry data on unrelated donor HCT generally show lower survival. There is evidence that relapse rate (RI) and non-relapse mortality (NRM) is high in patients with more advanced disease (Table 1).

Runde et al, in a large retrospective EBMT study, reported a NRM

rate of 29% in patients transplanted within six months from diagnosis compared to 51% for transplants performed 12 months or more after diagnosis. De Witte, in a retrospective study, evaluated the impact of various pre-transplant variables, including disease duration, intensity of the conditioning regimen, type of donor, and year of transplantation on outcome. The study population consisted of 374 patients with refractory anemia. The 4-year survival of patients transplanted with HLA-identical sibling donors and matched unrelated donors was 52% and 50%, respectively; disease duration of >12 months was associated with inferior survival. These results would suggest that transplantation should be performed early after diagnosis. Nevertheless, due to the advanced median age and to the relatively chronic course of low-risk MDS, some Authors are not keen to consider patients with MDS for transplant. The high comorbidity rate in older patients makes the problem particularly important. In 2004, Cutler et al. presented the results of a study based on a Markov decision model aimed at establishing the optimal timing of allogeneic transplantation in MDS. The study was performed on a series of 260 patients who received transplant from a sibling donor compared with a non-transplantation cohort of 186 patients treated with supportive care; the median follow-up was 35.4 months and 11.4 months, respectively. On the basis of this study, a prompt allogeneic transplantation is advisable in patients with intermediate-2 or high-risk MDS, while the best choice for patients with low-risk and intermediate-1 risk MDS is to delay transplant until disease progression. The IPSS score, although of value in identifying high-risk patients shows limits in establishing indications for patients with low-risk and intermediate-1 MDS; critical issues are age, performance status, comorbidities, iron overload, fibrosis, levels of neutropenia, and previous transfusion dependency. , ,

In a recent paper, we have shown that WPSS is more useful than IPSS to predict the post-transplant outcome in MDS. In this study, transfusion dependency was associated with a reduced overall survival (OS) and increased NRM.[9] Choosing the right timing for transplantation is still difficult for patients with low or intermediate-1 MDS. A patient with transfusion-dependency or severe thrombocytopenia or neutropenia, classified as low-risk MDS, should be considered for earlier HSCT. Recent studies have shown that risk factors such as marrow fibrosis, previous transfusions, and iron burden, have an impact on the post-transplant outcome. Therefore, patients with low-risk MDS should be carefully monitored and progression of fibrosis, high levels of body iron, and transfusion requirement should be considered an indication for HSCT.

Table 1

Results according to myeloablative conditioning regimen

Authors (year)	N. cases	Diagnosis	% DFS	% Relapse	% NRM
Runde et al. [11] (1998)	131	RA/RARS RAEB RAEBt tAML	52 34 19 26	13 44 52 50	40 38 60 48
De Witte et al. [7] (2000)	885	RA/RARS CR Non responders	55 44 32	13 30 42	37 37 45
Sierra et al. [8] (2002)	452	MDS	40	23	32
Deeg et al. *[23] (2002)	109	RA/RARS RAEB RAEBT/AML	68-70 45-50	5 33-38	29
Alessandrino et al. [9] (2008)	235	RA RCMD RAEB1 RAEB2	80 57 51 28	9 22 24 56	14 39 38 34

DFS: disease free survival; MDS: myelodysplastic syndrome; R : refractory anemia refractory anemia with ringed syderoblasts ; R EB: refractory anemia with excess R EBt: refractory anemia with excess of blasts in transformation; MDS: myelodysplastic syndrome; RC: refractory cytopenia with multilinear dysplasia; R: Complete remission. NRM: non-relapse mortality. * : conditioning regimen performed by target busulfan and cytoxan

Preventing relapse by cytoreduction before transplant

The current trend in MDS classified according to the WHO classification is to use peripheral blood stem cells avoiding the use of chemotherapy before transplant.[2] This approach does, however, have both advantages and disadvantages. A study reported by de Lima demonstrated that patients in remission had a higher probability of survival than patients who had received front-line transplant. In 2005, Scott et al. failed to demonstrate benefit in patients with MDS who had received a myeloablative transplant in complete remission (CR). The final answer as to whether induction chemotherapy before transplant is useful in preventing post-transplant relapse has still not been found. Patients with MDS treated by chemotherapy in the attempt to achieve CR are inclined to develop late hematopoietic recovery and life-threatening toxicity. In addition, induction chemotherapy may contribute to post-transplant toxicity and mortality: in a Japanese, study the 5-year OS in refractory anemia with excess of blasts in transformation (RAEBt) and secondary AML was 57% for patients who had an up-front transplant and 54% for those who received induction chemotherapy before transplant.

The WHO classification for MDS uses the threshold of 20% of marrow blasts to separate RAEB2 from acute leukemia; therefore, nowadays, patients previously diagnosed according to the FAB classification as having RAEBt should be treated with standard chemotherapy before transplant. For patients with RAEB1 or RAEB 2, the decision to treat or not to treat patients before transplant is still an unanswered question. There is evidence that patients with high blast count or a high-risk cytogenetic profile are more exposed to relapse. In a previous retrospective study, we have shown the same high actuarial relapse rate in patients with RAEB2 and in those with RAEBt/ AML.9 These results would suggest that in patients with > 10% blast cells in the marrow, transplantation should be performed following cytoreduction, namely in those considered for a non-myeloablative or T-depleted transplantation. Nevertheless, in the absence of randomized trials, the use of induction chemotherapy prior to HSCT remains uncertain. The recent use of hypomethylating agents supports the hypothesis to use azacytidine as preparation to HSCT.

Table 2.

Results by reduced intensity conditioning regimen

uthor (year)	Conditioning	N.° Cases	Diagnosis	% NRM	% DFS
Popat et al[35] 2008	Busulfan+Fluda Fluda/Melfalan	89	MDS/MDS- ML	23	46-63 (at 2 years)
Ruutu etal [36] 2008	Treosulfan/Fluda	45	MDS/MDS- ML	15	71 (at 1 year)
lessandrino et al. [25] 2008	Thiotepa/Fluda	50	MDS/MDS- ML	21-45	73-28 (at 5 years)
Kroger et al.[37] 2003	Busulfan/Fluda/ TG	37	R /R EB/R EBt /s ML	12/45	25-31(at 3 years)
Martino et al.[20] 2006	Fluda/TBI 2Gy Fluda/ Ikylant	215	MDS	22	33 (at 3 years)
Lim et al.[38] 2006	Fluda/Busulfan Campath	75	RCMD R EB ML	24 44 21	55 (at 3 years) 18 47

%DFS: probability of disease free survival; % NRM probability relapse mortality; MDS: myelodysplastic syndrome; ML: acute myeloid leukemia; R : refractory anemia ; R RS: refractory anemia with ringed syderoblasts ; R EB: refractory anaemia with excess of blast R EBt: refractory anemia with excess of blasts in transformation; MDS: myelodysplastic syndrome; RC: refractory cytopenia with multilinear dysplasia; R: Complete remission.

Results according to patient age and conditioning

Data from standard-intensity HSCT have shown correlation between patient's age and NRM. In a series of 285 patients with MDS transplanted in Italy until the year 2000, we have identified three groups of patients (aged < 20, 21-45, and 46-65 years); the NRM rate was 17%, 40% and 45%, respectively (EP Alessandrino, unpublished data, 2009). The difference in NRM was noteworthy for patients under 20 years compared with patients 45 years old and older. So far, the threshold of 60 years and a good performance status are the limits for the use of fully myeloablative transplant.

Myeloablative conditioning with busulfan and cyclophosphamide (BUCY) or TBI has been the gold standard for MDS. In the absence of randomized trials, there is no evidence that one regimen is superior to the other; however, conditioning regimens using busulfan (BU) seem to be encouraging for MDS. The best results have been reported in young patients transplanted early after diagnosis having an HLA identical sibling donor. However, a study from Seattle performed in patients aged 55-66 years demonstrated that standard conditioning could be safely offered to patients with good performance status receiving oral busulfan (BU) targeted to plasma concentrations of 800-900 ng/mL. In the attempt to reduce the RI in high-risk patients, some Authors proposed intensifying the preparative regimens by adding to the standard combination BUCY, etoposide or TBI. However, in our experience this approach resulted in an unacceptable early NRM (EP Alessandrino, unpublished data, 1997). Recently, new fully myeloablative combinations have been proposed in the attempt to preserve the cytotoxic effect by a reduced toxicity. Busulfan, thiotepa (TT), melfalan have been associated to fludarabine (FLU) given intravenously over four or five days. De Lima reported results in

22 patients with MDS who were conditioned with FLU at 40 mg/m^2/day and i.v. BU at 130 mg/m^2/day for four consecutive days (BU-FLU). The NRM was 5% at day + 100; the incidence of acute GVHD (grades II-IV) was 25% and 44% for related and unrelated recipients, respectively.[17]

Thiotepa and fludarabine was tested in a series of 50 patients with MDS older than 50 or with comorbidities contraindicating standard conditioning. Patients were followed for a median time of 21 months. NRM at one year was 25%; the 5-year probability of relapse was 27%. The 5-year overall survival was 73% and 28% in low- and high-risk patients, respectively.

De Lima and colleagues compared a non-myeloablative conditioning of fludarabine 120 mg/m^2, plus cytosine arabinoside 4 g/m^2, and idarubicin 36 mg/ m^2 (FAI), with a more intensive regimen of fludarabine 100-150 mg/m^2 and melphalan 140 or 180 mg/m^2 (FM) In this study, the more intensive regimen was associated with a lower incidence of relapse and with a high NRM rate. Nevertheless, there was no significant difference in 3-year overall survival rate which was 30% and 35%, respectively.[18]

In vivo and *in vitro* T depletion have been tested in the setting of standard myeloablative conditioning and also in that of reduced intensity conditioning as well. In a recent paper, Castro Malaspina reported that patients with advanced MDS who attain remission before transplant can achieve long-term survival following a myeloablative T-cell depleted allogeneic HSCT. In this study, the NRM and DFS was 23% and 50% at two years for the responders versus 38% and 15% for patients who had received front-line transplantation .

Busulfan and fludarabine given in association with rabbit antithymocyte globulin (ATG), 4.5 mg/kg, was tested in 70 patients affected with hematologic diseases including MDS who received transplant from a related or an unrelated donor. In this study, the early mortality rate was 2% with related donor transplant and 8% with unrelated transplant. The actuarial probability of survival at two years was 74% for low-risk and 65% for high-risk disease. Ho *et al.* added alemtuzumab to the combination BU-FLU. In this study, 31 patients with excess of myeloblasts had received chemotherapy before transplantation. The NRM was 0% at day + 100 for HLA-identical siblings, and 11% for unrelated donor transplants. The incidence of relapse ranged from 7% to 50% for intermediate-1 to high-risk IPSS groups . Storb tried further reduction in conditioning intensity exploring a true non-myeloablative regimen (NAC) with 200 cGy of TBI and fludarabine 90 mg/m^2; graft failure occurred in 6%, and relapse in 43% of patients. The NRM was 14% at day +100 and 25% at one year. Approximately 20% of patients were surviving at three years. A German study tested a new approach consisting in a cytoreductive chemotherapy given before non-myeloablative conditioning potentiated by the infusion of donor lymphocytes (DLI). The schedule incorporated fludarabine, ara-c, and amsacrine followed by TBI or BU, cytoxan, ATG and late DLI infusion. Favorable promising results have been reported. Reduced intensity conditioning and non-myeloablative regimens have a low toxicity and an acceptable NRM. Nevertheless, the incidence of acute and chronic GVHD remains unmodified while the relapse rate is high in patients with advanced disease or a high-risk cytogenetic profile.

No prospective data are currently available to answer the question as to whether standard regimen is better than RIC or NAC. We assume that patients with distinct comorbidities, a different age and risk-score should be treated with tailored conditioning. The best results in MDS are obtained in patients with low-risk MDS treated with standard conditioning such as BU-CY or with a more promising BU-FLU. The RIC regimens are generally indicated in patients in whom standard conditioning would be contraindicated for age or comorbidities. Many of the reported trials exploring RIC protocols have small sample size and short follow-up. In addition, RIC regimens include a wide range of intensity varying from non-myeloablative conditioning to ablative combinations. There is a large overlap in terms of overall survival and disease free survival between standard myeloablative conditioning and RIC. Naturally, patients receiving RIC are older or with comorbidities. In a large retrospective study comparing standard myeloablative conditioning and RIC, Martino et al. found similar overall survival and relapse free survival . Scott et al. reported similar conclusions in 172 patients with MDS . In a recent report, Luger found RIC and NAC offered no advantage; while early NRM was substantially less with RIC approaches, 5-year NRM was, however, equivalent.

Similar outcomes suggest the need for prospective trials comparing standard myeloablative conditioning and RIC in patients eligible for either approach. At present, patients up to the age of 55 without comorbidity should receive transplant with standard myeloablative conditioning by using related or unrelated donor. Older patients or patients with poor performance status should be enrolled in randomized trials aimed at exploring RIC. Pre-transplant cytoreductive chemotherapy before RIC transplant is advisable in patients with high-risk MDS in whom a high-risk of relapse is generally predictable. In the absence of randomized trials, status of disease, age, comorbidity, type of donor, stem cell source, and probable patient compliance, should be taken into account to optimize the choice.

Is UBC transplantation suitable in adult MDS?

The use of umbilical cord blood (UCB) as alternative source of stem cells is generally well tolerated carrying a reduced risk of acute and chronic GVHD. UCB stem cells are promptly available, making rapid transplant possible in patients with advanced disease for whom a long delay would not be prudent. The major limitation on the use of UCB in adults is the very small numbers of stem cells contained in each individual sample. Recently, with the introduction of the double cord transplants, UCB transplants are becoming more widely used in adults. At present, available data on adult MDS are limited. However, they confirm that UCB transplantation may be a good BMT or PBSCT substitute for patients lacking an HLA-matched sibling donor. In a recent paper, Warlick reports the outcome of 84 transplanted adult patients with MDS. Graft source was related in 47 (56%), unrelated donor marrow in 11 (13%), and unrelated cord blood (UCB) in 26 (31%). In this study the donor sources (PBSC, BM, or UCB) yielded similar outcomes. In a series of 22 MDS patients who received standard ablative conditioning followed by a single UCB transplant, only 4 out of 22 patients relapsed; the actuarial probability of DFS was 76% at four years. These encouraging data suggest a promising role for UCB source for MDS eligible for transplant. This needs to be confirmed by large prospective studies.

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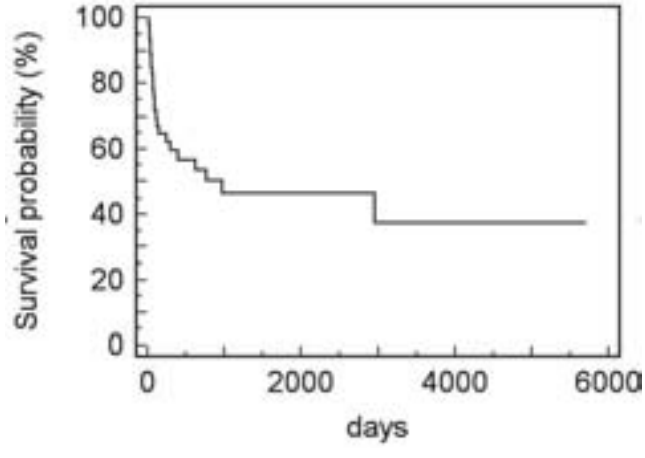

Figure 1. Overall survival of 57 adult patients with MDS submitted to allogeneic stem cell transplantation (BMT Unit, Clinica Ematologica, Fondazione IRCCS Policlinico San Matteo, Pavia, 1989-2007).

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ACUTE AND CHRONIC PH+ LEUKEMIAS: DIFFERENTIAL LEUKEMOGENESIS PATHWAYS TRANSLATE INTO DIFFERENT CLINICAL NEEDS

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Philadelphia (Ph) chromosome - a balanced, reciprocal translocation involving the long arms of chromosomes 9 and 22 is the hallmark of two types of leukemias. Virtually 100% chronic myeloid leukemia (CML) patients are characterized by the presence of Ph chromosome or, in patients with complex or masked translocations and consequently lack the classical Ph chromosome, by the BCR/ABL gene. The Ph chromosomal aberration is not restricted to the CML, but a sizeable subset of acute lymphoid leukemia (ALL) patients may have this abnormality. Hence, despite the presence of a consistent genetic abnormality Ph leukemias display considerable clinical and hematological heterogeneity, the basis of which is only partially understood.

For various reasons, CML is, between the two types of Ph leukemias, probably one of the most comprehensively studied human malignancies. CML was the first human cancer to be associated with a consistent chro-

mosomal abnormality - the Ph chromosome. CML is characterized by distinct clinical phases: most patients present in chronic phase (CP), a phase in which mature granulocytes are still produced, but patients have an increased number of myeloid progenitor cells in the peripheral blood. As the disease progresses, patients enter an accelerated phase (AP) followed by blast crisis (BC), in which hematopoietic differentiation has become arrested and immature blasts accumulate in the bone marrow (BM) and spill into the circulation. The CP is relatively long-lasting, so researchers have the opportunity to study malignant cells with an 'indolent' behavior and to identify the changes associated with transformation to the 'aggressive' phenotype of blast crisis. Furthermore, CML is unusual in that a single genetic lesion occurring in a hematopoietic stem cell generates a fusion oncogene, BCR-ABL, which encodes a protein tyrosine kinase that is necessary and sufficient for cell transformation. The cytoplasmic location of the BCR-ABL oncoprotein allows access to many cellular substrates that are unavailable to the predominantly nuclear ABL protein, determining their phosphorylation and therefore the activation of proliferation and survival pathways. Finally, CML was the first hematological malignancy for which a program of rational drug design yielded an effective targeted molecular therapy (imatinib mesylate), that is now considered the precursor of a new family of anticancer drugs, the tyrosine-kinase inhibitors (TKIs).

The introduction of TKIs for the treatment of chronic myeloid leukaemia has had a profound and beneficial effect on this disease, which previously had a median survival of 5–7 years. Imatinib moved rapidly from phase I and II trials to a phase III randomised controlled trial (International Randomised Study of Interferon versus ST1571 [IRIS]) in which it was compared with interferon alfa plus cytosine arabinoside (IFN-ara-C). Complete haematological responses and complete cytogenetic remissions were 95% and 94%, respectively, for imatinib, compared with 55% and 8.5% for IFN-ara-C. Progression-free survival at 18 months was 96.7% for imatinib compared with 91.5% for IFN-ara-C. These early findings led to accelerated regulatory approval of imatinib for all phases of CML and the drug became first-line treatment for most patients. At 60 months, the estimated rate of event-free survival was 83% in imatinib recipients, and 93% had not progressed to accelerated/blast-phase CML. The efficacy of imatinib has been confirmed in a recent intention-to-treat analysis of 204 consecutive patients with newly diagnosed CML-CP who received standard-dose imatinib for a median of 38 months, most of them outside of the IRIS trial setting. Follow-up was available for all patients, giving a cumulative CCyR rate of 77% and a projected five-year event-free survival rate of 81% (de Lavallade et al, J Clin Oncol 2008) .

Imatinib also induces responses in a significant percentage of patients with AP or BP CML, although these tend to be transient. In phase II studies in patients with AP (600 mg/day) or BP (majority of patients [86%] received 600 mg/day), sustained CHR rates were 37% and 15%, MCyR rates were 28% and 16%, and CCyR rates were 19% and 7%, respectively. These clinical results support the notion that BCR-ABL must be important for the continued maintenance of the neoplastic phenotype, even in the advanced phase, and that a selection pressure favors the continued activity of the oncoprotein. In fact, increased BCR-ABL expression is likely to contribute to the phenotype of advanced phase disease, as studies using cell line models of CP and BC CML indicate that the oncoprotein exerts dose-dependent effects on growth factor dependence, clonogenicity, migration and the rate at which cells develop resistance to imatinib. Other factors, however, that could affect differentiation arrest and the inappropriate reactivation of self-renewal capacity are important in disease evolution in CML. CML is a good example of a cancer in which the transition from mature, terminally differentiated cells to immature, undifferentiated cells can be observed in the malignant clone. This differentiation arrest implies pathological interference with differentiation 'programmes' involving the targeted activation of tissue-specific genes by transcription factors. Such interference may be activated by oncogene products, as has been demonstrated for the suppression of the transcription factor CEBP alfa by BCR-ABL, or by mutations or gene translocations that result in the formation of dominant-negative transcription factors, such as AML1–EVI1 or NUP98–HOXA9 fusion genes, which have been described in a few isolated cases of myeloid BC. Another aspect of the maturation arrest in BC is the question of whether the transformed subclone originates from a cell that is at a distinct differentiation stage from that which gives rise to CP. Thus, disease progression in CML may originate in more committed precursors than had been previously supposed, as myeloid BC has been reported to involve the granulocyte-macrophage progenitor (GMP) 'pool' rather than the haematopoietic stem cell pool. The self-renewal of GMPs requires the activation of the _-catenin pathway.

One of the most remarkable and intriguing results of the treatment of CML in chronic phase with imatinib is the low rate of progression to advanced phase or BC (2% at 5 years follow-up) in patients who achieve and maintain a complete cytogenetic response within the first 12–18 months after starting the drug treatment. The biological basis of this phenomenon is unknown, but is a matter of great interest and speculation. Genomic instability may be based on alterations of the mechanisms involved in genome surveying for DNA damages and of those responsible for repairing these lesions; it is likely that similar failures of genome surveillance and DNA repair may contribute to the genomic instability of all human cancers. It has been proposed that BCR-ABL induces mutations in genes responsible for maintaining genomic integrity, and that such mutations function as "amplifiers of a genetically unstable phenotype". This could explain the occurrence of the non-random chromosomal abnormalities that characterize CML progression. The most frequent are trisomy 8 (33%), an additional Ph chromosome (30%), isochromosome 17 (20%), trisomy 19 (12%), loss of the Y chromosome (8% of males), trisomy 21 (7%) and monosomy 7 (5%)51. These changes have been used as markers of disease progression, but may not necessarily be causal agents of transformation.

As BCR-ABL increases the level of genomic instability, continuous inhibition of its kinase activity by imatinib should lead to a decreased risk of mutations in general, including in genes that can trigger the blast crisis process. Moreover, although imatinib may be unable to kill the leukemic stem cell, it may drastically reduce its rate of proliferation and self-renewal, driving it to a deep and prolonged quiescence where the chances of DNA breaks and mis-repair are lower in the absence of DNA replication. Another possible mechanism relies on the evidence that the cell of origin of BC may not be a 'true' stem cell, but rather a more committed granulocyte-macrophage progenitor (GMP), and it has been shown that imatinib can eliminate a large proportion of these cells, reducing the population at risk of blastic transformation. An additional aspect of the 5-year clinical trial follow-up study was the observation that the rate of disease progression is not only low, but seems to decrease with time under successful treatment, and the reasons for this are not entirely clear. As the emergence of a subclone of leukemic cells with mutations in the kinase domain of BCR-ABL is the main cause of relapse in patients treated with imatinib, it is reasonable to suppose that the chances of this happening at any time during treatment depend largely on the size of the mutant sub-clone at the start of therapy and on its proliferation rate. Therefore, assuming that both the original (non-mutated) and the mutant BCR-ABL clones have a similar doubling time, it could be predicted that the highest risk for a mutant clone to become dominant and lead to relapse and disease progression occurs within the first years of therapy. A longer follow-up of chronic phase patients treated up-front with imatinib should confirm whether such a trend for a continuous decrease in the risk of disease evolution is statistically significant.

Close molecular monitoring of the blood of patients treated with imatinib or other TKIs demonstrated that a 3- to 4-log reduction in BCR-ABL expression strongly correlated with the ability to achieve long-term remissions. However, most of the patients have low-level persistent BCR-ABL transcripts and relapse on discontinuation of the drug, thus indicating that treatment spares a Ph cell sub-fraction with long term repopulating capability, that may be able to originate the progression of disease. Human cells with long-term engraftment potential were enriched for CD34+ primitive HCS characterized by CD90 expression. Closer analysis of transplantable and long-term culture initiating Ph+ cell subpopulations of CD34+ cells demonstrated predominance of quiescent G0 cells. Phenotypically primitive CD34+CD38- cells from the long-term culture initiating cells of chronic phase CML patients had a propensity to differentiate along the myeloid lineage on long-term engraftment in immunocompromised mice and probably constitute the leukemia-initiating cells thought to be resistant to chemotherapy and targeted therapy. Some studies indicate that specific molecular factors such as promyelocytic leukaemia protein (PML) tumour suppressor protein may have a critical role in haematopoietic stem cell maintenance of CML patients. More recently, it has been shown that constitutively active Smoothened, an essential component of the of Hedgehog (Hh) signalling pathway, augments CML stem cell number. Therefore, Hh pathway activity is required for maintenance of normal and neoplastic stem

cells of the haematopoietic system and raise the possibility that the drug resistance and disease recurrence after imatinib treatment of CML might be avoided by targeting this essential stem cell maintenance pathway. As already mentioned, the more common causes of TKI resistance have been clonal evolution present in up to a quarter of patients on disease progression and mutations of the BCR-ABL. The cells harboring previously mentioned resistance mechanisms are less likely to arise in early chronic phase of the disease and may have preexisted at a more advanced diagnostic stage, but more potent TKIs failed to act on the signaling or the behavior of these CML progenitors. Taken together, these observations suggest that new strategies will be needed to eliminate CML.

The Ph chromosome encodes defines a subgroup of ALL with a particularly unfavorable prognosis. The reasons for the aggressive nature of Ph ALL are still under investigation and have not yet been elucidated. In a large survey recently completed by the GIMEMA group on a large cohort of acute Ph leukemias, 75% of ALL patients and 66% of lymphoid blast crisis CML patients, but none of the patients with myeloid blast crisis, showed homozygous or heterozygous deletions in the IKZF1 gene. IKZF1 encodes the Ikaros protein, a ZnF transcription factor, required for lymphoid lineage differentiation, proliferation, and function. Ikaros contains two separate regions with Zinc Finger domains. Isoforms that lack the N-terminal ZnFs are unable to bind transcriptional targets normally but retain the Carboxy-terminal ZnFs and the ability to dimerise and act as dominant negative inhibitors of Ikaros function. Ikaros transgenic and mutant mouse models have clearly demonstrated the important role of Ikaros in both normal hematopoiesis and tumor suppression. An elevated frequency of genomic aberrations could be directly caused by an abnormally high incidence of DNA double-strand breaks. In normal cells, DNA lesions are detected and repaired by sophisticated physiologic machinery and a system of cell cycle checkpoints, preventing cells that have sustained DNA damage from proliferating further. In Ph cells from ALL patients, however, the already mentioned role of the BCR-ABL oncoprotein to promote DNA damages may be one of the factors involved in the onset of these deletions at the IKZF1 gene. However, sophisticated DNA analysis along the breakpoint cluster regions suggesting that IKZF1 deletions could arise from aberrant RAG-mediated recombination. Consistent with this hypothesis, cells from ALL or lymphoid blast crisis patients, but not those from chronic phase CML patients, were found to contain high levels of activation-induced cytidine deaminase (AID). Indeed, ALL cells are derived from B cell precursors in most cases and typically carry rearranged immunoglobulin heavy chain (IGH) variable (V) region genes devoid of somatic mutations. Somatic hypermutation is restricted to mature germinal center B cells and depends on AID. It was also demonstrated that AID expression in CML cells promotes overall genetic instability by hypermutation of tumor suppressor (including IKZF1) and DNA repair genes. Importantly, these finndings uncover a causative role of AID activity in the acquisition of BCR-ABL mutations leading to Imatinib resistance, thus providing a rationale for the rapid development of drug resistance and disease progression in ALL and lymphoid blast crisis patients.

In conclusion, therapeutic development in CML represents a success story for modern medicine. Patients diagnosed with CML today are expected to have a substantially longer survival than patients diagnosed 20 or even 10 years ago. Despite this, disease eradication and further improvements in prognosis of CML remain high on the agenda. On the other hand, treatment of the advanced phase of Ph leukemias including ALL still remain problematic despite the central role of BCR/ABL oncoprotein in the pathogenesis and the possibility to target its activity by first and second generation TKIs. However, additional factors play important roles in sustaining viability and growth of the Ph cells in the advanced phases and causing the observed rapid loss of TKI treatment response in these patients. Hopefully, the new molecular findings might open the way to the discovery of new and more effective treatment strategies also for these patients.

MYELODISPLASTIC SYNDROMES/MYELOPROLIFERATIVE NEOPLASMS: FROM BIOLOGY TO CLINICAL ASPECTS

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Myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPNs) are rare myeloid malignancies characterized by the presence of both dysplastic and proliferative features [Table 1]. They have been recognized as a separate group of diseases for the first time in 2001 by the WHO classification of Tumors of the Hematopoietic and Lymphoid Tissues.[1] This new category incorporated chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia, *BCR/ABL1*-negative (aCML), together with the less well defined unclassifiable forms of MDS/MPNs (MDS/MPN-U), including refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) as a provisional entity.

Because no significant progresses have been made over the last few years in understanding molecular pathogenesis of MDS/MPNs, the 2008 revision of the WHO classification of myeloid neoplasms contained little changes with regard to this disease category.[2] In particular, some cases of CMML with eosinophilia were relocated to the "myeloid/lymphoid neoplasms with eosinophilia and platelet-derived growth factor receptor-, (PDGFRB) rearrangement" category, while for RARS-T the platelet threshold to define thrombocytosis was lowered from 600 to 450 x 10^9/L and the presence of proliferating large megakaryocytes in the marrow resembling those in ET or PMF was added as a required diagnostic criteria.

Taken together, MDS/MPNs represent disease entities with high heterogeneity of clinical and hematologic features, varying from predominantly myelodysplastic to predominantly myeloproliferative forms, which entail different prognosis and demand different medical management. As it has been well expounded in a recent review by Orazi and Germing, the lack of known distinctive genetic features brings about the diagnosis of specific MDS/MPN subtypes to be ascertained by the integration of bone marrow and peripheral blood morphology with other laboratory and clinical findings.[3]

Among MDS/MPNs, CMML and JMML represent the main disorders, respectively, in elderly and in childhood age.

Chronic myelomonocytic leukemia (CMML)

The WHO classification did not make any significant changes in the original FAB criteria for the diagnosis of CMML, which are listed in Table 1. In addition, because in this disease entity a higher proportion of blasts has always been unanimously recognized as being associated to a more unfavorable prognosis, the WHO separated CMML into 2 prognostic subcategories, CMML-1 and CMML-2, depending on the number of blasts in the blood and bone marrow (Table 1).[1]

Having classified CMML among MDS/MPNs, the WHO virtually abolished the distinction between a "dysplastic" (MD-CMML) and a "proliferative" (MP-CMML) variant of the disease, originally proposed by the FAB group in 1994 on the basis of the arbitrary chosen threshold of 13x10^9/L WBC in the blood4. Nonetheless, individual patients necessitate different treatments according to the clinically predominating dysplastic or proliferative manifestations; moreover, MP-CMML are most often related to aberrancies in the RAS/MAPK signalling pathways or, in a minority of cases, to the presence of the $JAK2^{V617F}$ activating mutation, whereas MD-CMML are characterized by a higher frequency of cytogenetic abnormalities. Therefore, even though different etiology and pathogenesis have not been demonstrated so far, from a practical point of view the MD- versus MP- distinction represents a meaningful tool both for clinical management and for further scientific investigations.

The natural course of CMML is highly heterogeneous, with patient life expectancy varying from a few months to numerous years. Besides marrow blasts, several disease- and patient-related variables have been recognized as having significant association with survival length in various retrospective studies, and a few scoring systems have been proposed to estimate the risk of death in patients with CMML. The MD Anderson Prognostic Score (MDAPS), which was developed from the analysis of a large cohort of well-defined patients with CMML, included hemoglobin <12 g/dL, the presence of circulating immature WBCs, absolute blood

lymphocyte count greater than 2.5x10^9/L, and marrow blasts 10% or more as prognostic factors significantly associated with shorter survival.[5] These parameters were incorporated into a scoring system (Table 2) that stratified patients into four groups with statistically different median survival.[5]

Although not included in the scoring system, age, along with any significant comorbidity, should always be taken into consideration when seeking best disease management in individual patients with CMML.[6]

With regard to the biology, a clear understanding of the mechanisms underlying pathogenesis of CMML is still lacking; nonetheless, among hematological malignancies CMML has the highest frequency of RAS point mutations, which are detected in a higher proportion of patients with the MP- than with the MD-variant and have been shown to associate with most unfavorable prognostic factors and with shorter survival. Overall, although not specific for CMML, point mutations in codons 12, 13, and 61 of the N- or K-RAS oncogenes are present in 20% to 40% of patients. Indeed, RAS mutations cause the constitutional activation of the RAS proteins by diminishing RAS-associated GTPase activity and locking RAS in the active state (RAS-bound GTP), with consequent dysregulation of the intracellular raf-MEK-ERK signaling pathways and uncontrolled cell proliferation.[7]

Presence of the V617F somatic mutation of the gene coding for JAK2, which confers constitutive activation to the related nonreceptor protein tyrosine kinase, has been reported in up to 13% of patients with CMML, mostly affected by a proliferative variant of the disease. In view of the rapid ongoing development of molecules selectively inhibiting JAK2 as a new potential therapeutic option, molecular screening for presence of the JAK2^{V617F} mutation in patients with CMML could soon become important for treatment decision-making, although the therapeutic window for JAK2 inhibitors might not require mutant over wild-type selectivity.[7]

It is worth mentioning the possible evolution of dysplastic into proliferative variant of CMML, characterized by progressive increase of WBC count during the disease course. Indeed, though difficult to assess, acquisition of RAS, JAK2 or, sporadically, also of FLT3-ITD genetic aberrations, might function as a secondary event that contributes to disease progression and transformation. Therefore, molecular screening and monitoring over time for mutations, especially of the RAS gene family, represent important analyses in CMML diagnostic work-up and in patients follow-up.[6]

Other biological findings recently identified as possibly relevant for CMML pathogenesis in occasional cases include other molecular abnormalities associated with activation of the Ras pathway, such as mutations of the PTPN11 gene (observed in a large proportion of JMML patients), leading to disregulation of SHP-2 phosphatase activity and increased RAS downstream signaling, and RUNXI alterations, the latter being not mutually exclusive with RAS mutations.

Worth mentioning, aberrant methylation and impaired expression of the p15(INK4b) cell cycle regulatory gene, which was correlated with increased expression of DNA methyltransferase (DNMT) 3A, has been reported as a recurrent epigenetic abnormality in CMML.[7]

As far as cytogenetics are concerned, the majority of patients with CMML have normal karyotype. Most common abnormalities are monosomy 7 and trisomy 8. The t(5;12)(q33;p13) chromosomal aberration, which results in TEL (ETV6)/PDGFRB fusion gene, has been shown to lead to myelomonocytic cell proliferation in CMML via constitutively activated tyrosine kinase. Subsequently, other fusion partners of PDGFRB, all leading to constitutive activation of the tyrosine kinase activity, have been reported in CMML; in general, - due to their potential therapeutic relevance - the occurrence of other specific translocations involving PDGFRB must be sought using molecular polymerase chain reaction-based screening, because of the possibility of a cryptic translocation involving the 5q33 region. Although occurring only sporadically in patients with CMML, these aberrations are often associated with conspicuous eosinophilia. Indeed, according to the 2008 WHO revised classification, all cases of CMML with eosinophilia should be investigated for the possible PDGFRB abnormality and, if it is found, the case should be further classified as a "myeloid/lymphoid neoplasm with eosinophilia associated with PDGFRB rearrangement".[2]

Juvenile myelomonocytic leukemia (JMML)

Juvenile myelomonocytic leukemia (JMML) is a rare mixed myelodysplastic/myeloproliferative aggressive clonal hemopoietic stem cell disorder of early childhood, mainly characterized by proliferation of the neutrophil and monocytic lineages. Although characterized by a poor prognosis in most cases, in recent years major progresses have been made in understanding its pathogenesis.

Minimal diagnostic criteria for JMML, as adopted by the International JMML Working Group8, are reported in Table 3.

Patients with JMML tipically present with severe hepatosplenomegaly, leukocytosis with absolute monocytosis, anemia and thrombocytopenia. Clinical course is frequently complicated by infiltration of various non hemopoietic tissues (skin, lung, gut) by leukemic cells, even though transformation into acute leukemia occurs in less than 20% of cases. Poor prognostic factors include age less than 2 years, low platelet count and elevated fetal hemoglobin levels.

Dysregulated hematopoiesis in JMML is characterized by an *in vitro* spontaneous proliferation of granulocyte-macrophage colonies (GM-CFCs) from bone marrow or peripheral blood cells, which is the result of an acquired selective hypersensitivity to granulocyte-macrophage colony stimulating factor (GM-CSF) by leukemic progenitor cells.

At the molecular level, unlike in CMML, JMML pathogenesis is extensively known. In particular, the close connection between RAS signalling pathways and JMML pathogenesis has been elucidated thank to the frequent association of this rare hematological malignancy with two inherited genetic disorders: neurofibromatosis type 1 and Noonan syndrome. In fact, children with neurofibromatosis type 1, who are deficient in one of the two neurofibromatosis type 1 gene (NF1) alleles, have a 500-fold increased probability of developing JMML or other myeloid disorders and 11% of children with JMML have a clinical diagnosis of neurofibromatosis.[9] The NF1 protein (neurofibromin) functions as a GTPase-activating protein and negatively regulates RAS. It has been demonstrated that 10-25% of patients with JMML acquire a second event to their remaining normal allele, for example loss of heterozygosity, which leads to the lack of neurofibromin and subsequent constitutive activation of the RAS pathway by increasing intracellular levels of RAS-GTP.[9]

Noonan syndrome is an inherited dominant somatic disorder characterized by short stature, heart malformation and a characteristic configuration of facial features. Missense mutations in the PTPN11 gene that encodes the non-receptor protein tyrosine phosphatase SHP-2 are detected in approximately 50% of cases with Noonan syndrome. On the other hand, somatic mutations in PTPN11 are observed in up to 35% of JMML patients, generally mutually exclusive with RAS or NF1 mutations. SHP-2 protein is expressed at high levels in hematopoietic cells, and relays signals from haematopoietic growth factor receptors including the GM-CSF receptor to the RAS pathway. Therefore, by modifying the active-inactive protein switching, PTPN11 mutations induce increased enzymatic activity and cell proliferation.[9]

Finally, with regard to the RAS gene family oncogenic aberrations, somatic point mutations of N- or K-RAS are seen in approximately 20-25% of JMML patients and are usually mutually exclusive of patients with NF1 and PTPN11 mutations.[9]

Furthermore, very recently, by means of single nucleotide polymorphism arrays, CBL mutations have been identified in 10-15% of patients with JMML, being mutually exclusive with PTPN11, K- and N-RAS, and NF1 mutations.[10] Cells from these patients displayed identical GM-CSF hypersensitivity- STAT5 signaling activation as other known RAS pathway mutations, as well as mutual exclusivity indicated that leukemia-associated CBL mutations result in hyperactive Ras proteins.[10]

Altogether, approximately 90% of JMML cases harbor one of four mutually exclusive mutations leading to GM-CSF hypersensitivity and activated RAS/STAT5 signaling, including direct oncogenic RAS mutations (approximately 20%), NF1 inactivating mutations (approximately 15-25%), protein tyrosine phosphatase, non-receptor type 11 (PTPN11) (SHP-2) mutations (approximately 35%), or CBL mutations (approximately 10-15%).

On the contrary, mutations common in other myeloid diseases such as FLT3 and JAK2 are hardly ever found in JMML.

As in CMML, also in JMML cytogenetic abnormalities are infrequent, with almost two-thirds of patients showing a normal karyotype. Abnormalities of chromosome 7, including its monosomy, represent the most frequent aberration and are seen in 25-30% of patients. Other karyotipic alterations occur in only 5-10% of JMML patients, especially involving chromosomes 3 and 8, and no specific cytogenetic abnormalities have been identified.[9]

In conclusion, MDS/MPNs represent a heterogeneous group of malignan.

Table 1. WHO diagnostic criteria for CMML.[1]

Persistent peripheral blood monocytosis greater than $1x10^9$/L
No Philadelphia-chromosome or BCR/ABL fusion gene
Fewer than 20% blasts* in the blood or bone marrow
Dysplasia in one or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are present and:
an acquired, clonal cytogenetic abnormality is present in the marrow cells, or the monocytosis has been persistent for at least 3 months and all other causes of monocytosis have been excluded.
CMML-1: blasts < 5% in the blood and < 10% in the marrow
CMML-2: blasts 5-19% in the blood and 10-19% in the marrow, or if Auer rods are present
Quando siano presenti I criteri sopracitati e inoltre vi sia la presenza di un numero di eosinofili > $1.5x10^9$/L nel sangue periferico, la diagnosi deve essere quella di LMMC-1 o LMMC-2 con eosinofilia.

**blasts encompass myeloblasts, monoblats, and promonocytes.*

Table 2. Median survival of 190 CMML patients according to the MDAPS.[5]

Risk group	*Score*	*Median survival (months)*
Low	0-1	24
Intermediate-1	2	15
Intermediate-2	3	8
High	4	5

Table 3. Minimal diagnostic criteria for JMML8.

Category	*Item*
Suggestive clinical characteristics	Hepatosplenomegaly
	Lymphoadenopathy
	Pallor
	Fever
	Skin rash
Laboratory criteria (all have to be fullfilled)	Peripheral blood monocyte count > $1x10^9$/L
	Absence of Philadelphia-chromosome or BCR-ABL gene rearrangement
	Bone marrow blasts less than 20%.
Other criteria (at least 2 have to be fullfilled)	Increased hemoglobin F (corrected for age)
	Immature myeloid precursors on the peripheral blood smear
	White blood cell count > $1x10^9$/L
	Clonal cytogenetic abnormalities (including monosomy 7)
	GM-CSF hypersensitivity of myeloid progenitors (*in vitro*)

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THE LONG-TERM OUTCOME OF HIGH-RISK FOLLICULAR LYMPHOMA PATIENTS RECEIVING INTENSIVE THERAPY WITH AUTOGRAFT AS PRIMARY TREATMENT: RESULTS FROM THREE CONSECUTIVE PROSPECTIVE TRIALS PERFORMED IN ITALY OVER THE PAST 18 YEARS

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High-dose (hd) chemotherapy followed by hematopoietic stem cell autografting has been widely employed as salvage or front-line treatment for high-risk follicular lymphoma (FCL).[1] This approach is now less frequently used, due to the complexity and the toxicity of the procedure for a neoplasm that can often be handled for long periods of time combining the anti-CD20 monoclonal antibody rituximab with conventional chemotherapy.[2] Nevertheless, favorable results have been reported in the past with intensified treatments aimed at maximal tumor reduction in young patients with FL and poor prognostic features. In particular, it has been proposed that an extensive cytoreduction may represent a valuable step to improve the overall outcome of FL and to eventually achieve disease eradication. The most relevant observations come from the studies on molecular monitoring of minimal residual disease (MRD). It has been reported that molecular remission (MR) can be often achieved following hd-chemotherapy and autograft with either purged or unmanipulated autologous stem cells.[3,4] Patients achieving post-transplant MR have a much lower relapse rate compared to patients with persistent PCR positivity.

The studies regarding MRD in FL have been pioneered by the Dana-Farber Cancer Institute group.[3] Their experience was mainly based on patients receiving autografting as salvage treatment. Besides the high rates of complete remission (CR) achieved, the long-term findings from Dana Farber Cancer Institute showed that MR was associated with an extremely low relapse rate and a > 80% projected freedom from relapse at 12 years (5). Subsequent studies have shown that MR can be achieved in a sizeable proportion of FL following intensive chemotherapy alone, with no need for ex-vivo purging procedures.[4] The intensified high-dose sequential chemotherapy (i-HDS) regimen, employed by several groups in Italy, is among the most effective hd-schedules for FL. The i-HDS program was developed several years ago and included the collection of peripheral blood progenitor cells (PBPC) following a prolonged chemotherapeutic debulking in order to obtain an in-vivo purging effect and to avoid the laborious and erratic ex vivo purging procedure.[6,7]

In the original, single-center experience with i-HDS in FL, PCR-negative harvests were collected in a high proportion of patients and approximately half of the patients achieved persistent clinical and molecular remission following autologous transplantation.[4,6] A multicenter, prospective trial was then launched in 1996 by 20 hematological Centers affiliated to the Gruppo Italiano Trapianto di Midollo Osseo (GITMO) to evaluate applicability and efficacy of the i-HDS regimen in the multicenter setting. The results were similar to those observed in the previous single-center pilot trial.[8,9] Both studies showed that an ex vivo purging-free autografting procedure is feasible with limited early toxicity. Moreover, high rates of both CR and MR were achieved, with persistent molecular remissions in a good proportion of patients. Subsequently, the anti-CD20 rituximab was added to the program, in order to increase the *in vivo* purging process and to further improve the therapeutic efficacy of the intensive treatments.[10] Thus, a second multicenter trial was launched, involving both GITMO and the IIL (Intergruppo Italiano Linfomi) Centers. In this instance, an open-label, randomized phase III trial was performed, comparing the rituximab-supplemented version

of HDS (R-HDS) with six CHOP supplemented by an identical number of rituximab courses (CHOP-R) in high-risk FL patients. High risk was defined according to the aaIPI score (≥2) or the IIL score (≥3). The randomized trial confirmed that achieving MR is critical for effective disease control, regardless of which treatment is used.[11] Moreover, R-HDS proved to be highly effective, ensuring superior disease control and molecular outcome than CHOP-R, although the overall survival curves were similar for both treatment arms.

At present, three consecutive trials have been concluded using the hd-sequential chemotherapy schedule and autograft, without and with rituximab, as first-line therapy for advanced-stage FL patients, aged less than <60 yrs. Recently, an update of the long-term outcome of the patients enrolled in these three consecutive trials has been performed. Aim of the study was to verify whether FL patients with advanced-stage presentation may experience prolonged survival if treated at diagnosis with an intensive approach. Ultimately, the study addressed the question on whether autologous transplantation in FL may possess a curative potential in this otherwise incurable disease. Long-term results with 10 years of median follow-up are here presented.

Patients and treatment schedules

The HDS programs employed have been previously described in details. Briefly, the original i-HDS schedule consists of an intensive debulking (2 APO courses +/- 2 DHAP courses) followed by the high-dose phase, including the sequential administration of etoposide (2 g/sqm), methotrexate (MTX) (8 g/m^2) and cyclophosphamide (CY) (7 g/sqm). PBPC collection is scheduled after the last course to maximize the *in vivo purging effect* operated by high-dose chemotherapy. A chemotherapy-free interval of 30 days was scheduled prior to hd-CTX 7gr/ m2, to allow optimal PBPC mobilization. Three hd-Dexametasone courses (Dexametasone at 40 mg/day for four consecutive days) were administered every 10 days during this interval.[6] The final auto-SCT concluded the program, two conditioning regimen have been employed, either the BEAM schedule or, most often, the Mitoxantrone/L-PAM combination (12). In the most recent schedule employed for the third, randomized trial, rituximab was included in place of MTX. In details, 2 rituximab doses were administered before CY, and two more doses after CY. Patients in partial response (PR) or who remained PCR-positive received two additional rituximab courses at the end of the program (11). Radiotherapy (30–36 Gy) was allowed on bulky sites or on residual masses, approximately two months after the end of HDS.

As summarized in Table 1, the first trial was a single Center phase II study exploring both feasibility and efficacy of the HDS program as first line therapy in advanced-stage indolent lymphoma. The subsequent multicenter phase 2 trial was then started at national level, within the GITMO group, to verify the efficacy of HDS in advanced-stage FL in a multicenter setting. Patients should have received no previous chemotherapy or extended-field radiotherapy and have one or more of the following adverse prognostic features: bulky disease (greater than 5 cm), high serum LDH, disease related compression symptoms, systemic "B" symptoms, ECOG performance status ≥ 2 or bone marrow (BM) invasion greater than 20%. Lastly, the muticenter phase 3 study was performed together with GITMO and IIL, comparing R-HDS vs. CHOP-R. Again, eligible patients were chemotherapy- or extended-field radiotherapy-free. High-risk disease was defined by an aaIPI ≥2 or an IIL score ≥3. Only patients enrolled in the R-HDS arm are considered in the present survey.

Overall, 186 patients have been treated with HDS, updated results have been obtained for 168 of them (see Table 1). They all had a diagnosis of FL (grade 1-2: 71%) and presented with advanced stage, their median age was 48 yrs., LDH was elevated in 48%, BM involved in 77%.

Updated long-term outcome of 168 HDS-treated FL patients

140 patients out of 168 (83%) attained Complete Remission (CR); there were 6 early toxic deaths (3.6%); 8 patients had Partial Remission (4.8%) and 14 had no response (8.3%), soon followed by disease progression. So far, 14 patients (8.3%) developed secondary myelodysplasia or acute leukemia (sMDS/AL), and 7 patients (4.2%) had a secondary solid neoplasia. As of July 2008, 50 of 168 patients died, due to: i. early toxicity (6 patients, 3.6%); ii. disease progression (25 patients, 15%); iii. second neoplasia (12 patients, 7.1%); iv. other causes (7 patients, 4.2%). Thus, at a median follow-up of 10 yrs., 118 patients (70.2%) are alive, and 80 (48%) are in their 1st continuous CR (CCR), and most of them are also in molecular remission. Details of long-term outcome according to the type of study protocol are reported in Table 2. The actuarial OS curve for the whole series of 168 patients indicates that the median survival has not yet been reached at a median follow-up of 10 yrs.. According to life table analysis, the cumulative proportion of surviving patients is projected to 63% at 15 and 20 yrs. Similarly, the cumulative proportion of patients surviving disease-free is projected to 47% at 15 and 20 yrs.(life table). The latest relapse has been recorded at 8 yrs. since HDS. At present, 50 patients (30%) are in their 1st CCR between 8 and 16 yrs after HDS.

Table 1. Main characteristics of the three prospective trials with HDS in Follicular Lymphoma.

Study Protocol	*period of enrollment*	*active Centers*	*references of the study*	*No. of patients enrolled*	*No. (%) of patients with outcome updated*[2]
Single Center – phase II trial	1991-1998	1	4, 6, 7	26	26 (100)
GITMO 1996 – phase II trial	1996-1999	20	8, 9	92	79 (86)
GITMO/IIL randomized trial[1]	2000-2005	30	11	68	63 (93)
			all patients	186	167 (90%)

[1]only patients of the R-HDS treatment arm are considered; [2]updated follow-up obtained as of July 2009.

Table 2. Long-term outcome of 167 Follicular Lymphoma patients entered in the prospective trials with HDS.

Study Protocol	*Updated patients n=*	*Patients alive n=(%)*	*Median follow-up (yrs.)*	*Patients alive in 1st CCR n=(%)*	*median follow-up (yrs.)*
Single Center – phase II trial	26	20 (77)	13.8	15	13.6
GITMO 1996 – phase II trial	79	51 (65)	10.9	30	10.0
GITMO/IIL randomized trial	63	47 (75)	6.1	35	5.1
all patients	168	118 (71)	9.8	80 (48)	8.7

Appendix. The following Investigators and Institutions contributed to the trials over the last 18 years and to data collection:

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Conclusions

Several considerations can be drawn from this recent survey on the long-term outcome of advanced stage FL treated upfront with the intensive HDS regimen. First of all, the study has been made possible due to the attention and the willingness of many Italian Centers to participate in this broad effort aimed to update the outcome of several patients treated over the past 18 years. This has allowed to obtain unique information on the clinical outcome of a large series of FL patients, followed for a median of 10 years. With such a long follow-up, we can now conclude that advanced-stage FL receiving HDS with autograft as primary treatment do have a prolonged survival, with a median survival not yet reached. This compares favorably with the historical survival expectancy of analogous patient populations managed with conventional chemotherapy in the pre-rituximab era.[13] Despite the intensive treatment, the main cause of death was disease progression, followed by deaths due to both early and late toxic side effects. The most relevant finding is that approximately half of the patients are long-term survivors without any sign of disease recurrence. This suggests that a prolonged survival in absence of any sign of disease and possibly the disease eradication should be pursued also in advanced-stage FL. It is now well established that achievement of MR following both conventional and intensive chemotherapy and autografting is predictive for a prolonged disease-free survival, while persistent PCR positivity is associated to a high relapse risk.[4,5,11,14–15] The present survey documents that the extensive tumor reduction and the possible achievement of MR following primary treatment translate into a prolonged survival, often in continuous CR. Recent observations indicate that the addition of rituximab to HDS may further increase the efficacy of the program allowing the achievement of durable CR in a very high proportion of patients.[16] Future studies, with prolonged follow-up, will verify whether these therapeutic goals may be achieved with chemo-immunotherapeutic schemes at least as effective but less toxic and laborious than HDS program with autograft.

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