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43° Congress of the Italian Society of Hematology

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ABSTRACT BOOK

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

Ancient Greek

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

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

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

Modern English

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BEST ABSTRACTS

BEST-001

THE MOLECULAR HISTORY OF RICHTER SYNDROME

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The mechanisms involved in chronic lymphocytic leukemia (CLL) transformation to Richter syndrome (RS) are poorly understood. We explored intraclonal diversification (ID) of immunoglobulin genes in RS in order to: i) follow the evolutionary history of the RS clone; ii) understand if interactions with antigen play a role in RS transformation. RS (n=11) were clonally related to the paired CLL phase. Cases were scored positive for ID only in the presence of confirmed mutations. Phylogenetic analyses was performed with MEGA4. Most (10/11, 90.9%) clonally related RS directly stem from the original CLL clone observed at the time of CLL diagnosis. One single RS case had a transformation pattern compatible with sequential evolution from a secondary CLL subclone. Once RS transformation had occurred, all secondary CLL subclones disappeared and were substituted by the dominant RS clone with its own descendants. These observations suggest that the transforming genetic lesion of RS might be acquired by a cell belonging to the original CLL clone, rather than being progressively accumulated by later CLL subclones. In agreement with this hypothesis, next generation ultra-deep sequencing (GS Junior, Roche) (median number of reads: 6645; range 2661-8025) documented that RS genetic lesions (eg: TP53 mutations) were already present, though at very subclonal levels (prevalence of individual subclones harboring the index mutation: 0.39-5.8%), in the paired CLL phase up to 6 years before RS transformation. Paired analysis of CLL/RS samples documented that RS transformation was accompanied by selection of a clone that required no ID (4/11, 36.4%) or reduced ID levels (5/11, 45.4%) for interacting with antigen. This observation suggests that the RS clone has no or limited requirement to interact with antigens through further BCR affinity maturation by ID. Independence from antigen at RS transformation might result from the acquisition of new genetic lesions subsidizing BCR activation by antigen. Screening of BCR pathway genes (CD79A, CD79B, CARD11) revealed the acquisition of a mutation within the coiled-coil domain of CARD11 in 1/4 (25.0%) RS that switched off ID at transformation. These data indicate that: i) the RS clone stems from a cell that is already present in the context of the initial CLL clone and gains selective advantage over other CLL subclones; iii) most RS have become independent of antigen stimulation for their sustainment.

BEST-002

SNPS ARRAY KARYOTYPING REVEALS A NOVEL RECURRENT 20P13 AMPLIFICATION IN PRIMARY MYELOFIBROSIS

Visani G[1,*], Sapienza MR[2,*], Isidori A[1], Tripodo C[3], Laginestra MA[2], Righi S[2], Sagramoso Sacchetti CA[2], Gazzola A[2], Mannu C[2], Rossi M[2], De Nictolis M[4], Valentini M[5], Donati M[5], Emiliani R,[5] Alesiani F[6], Paolini S[2], Finelli C[2], Pileri SA[2,§], Piccaluga PP[2,§]

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Background. The molecular pathogenesis of primary myelofibrois (PMF) is largely unknown. To date, a few recurring abnormalities, including JAK2 mutations have been recorded. However, none of them could provide a clear explanation of PMF complex biological and clinical features. Aims. In this study, we performed a high throughput SNPs profiling of PMF cases in order to identify novel recurrent genomic abnormalities. Methods. We studied 20 PMF patients (training set, N=10; test set, N=10) by using the Genome-Wide Human SNP Array 6.0. We isolated DNA from both myeloid (neoplastic) and lymphoid (bona fide non neoplastic) cells, the latter serving as matched controls. Real-time PCR Taq Man Copy Number assay and immunohistochemistry were used for validation purposes. Results. We observed a complex karyotype in all cases, detecting 2470 CNVs. Among them, we recorded all the previously reported lesions (del(5q), del(20q), del(13q), +8, aUPD at 9p24 and abnormalities on chromosome 1). In addition, we identified several novel cryptic lesions. Specifically, we focused on the CNVs recurred at higher frequency in our cohort of patients, thus being candidate to retain a potential pathogenic significance. Remarkably, however, no lesion was consistently present in all the cases; on the other hand, we identified a CNV affecting the cytoband 20p13, occurring in 55% of patients. Specifically, we defined a minimally affected region (MAR), represented by an amplification spanning over 9,911 bps and overlapping the SIRPB1 gene locus. Interestingly, by extending the analysis to the adjacent areas, the cytoband 20p13 turned out to be overall affected in 95% of cases. Remarkably, our results were confirmed in an independent test set and validated at genomic level by real-time PCR and in silico in a large independent series of myeloproliferative diseases. Finally, by immunohistochemistry we found that SIRPB1 protein was over- expressed in the bone marrow of PMF patients carrying the 20p13 amplification. In conclusion, we detected a novel recurrent lesion involving the cytoband 20p13 and the SIRPB1 gene in PMF patients. Future studies are definitely warranted in order to further characterize the genomic lesion and its functional consequences, as well as to assess the possible clinical relevance of such lesion.

BEST-003

DAPSONE AS SALVAGE THERAPY FOR ADULT PATIENTS WITH RELAPSED OR REFRACTORY IMMUNE THROMBOCYTOPENIA.

Zaja F, De Luca S, Marin L, Puglisi S, Mazzucco M, Chiozzotto M, Volpetti S, Fanin R

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Background. Treatment of patients with relapsed or refractor symptomatic immune thrombocytopenia (IT), particularly for those who are not eligible for, refused or failed splenectomy, still lacks a gold standard therapy. Previous studies highlighted the possible therapeutic activity of Dapsone, an antibacterial sulfonamide, in this setting of patients.

Aim. To evaluate the activity and safety of Dapsone salvage therapy in adult patients with IT.

Methods. Consecutive patients treated with Dapsone for relapsed or refractory IT (according to the standardized criteria reported in Blood 2009;113:2386-2393) were retrospectively evaluated. After documentation of normal level of G6PD, patients received oral Dapsone 100 mg/day to be reduced to a minimal active dose in order to maintain a

platelet count of 30 x 109/L or more. Response was evaluated at month 6 from treatment start; parameters of response were the number of patients who achieved overall and complete response (OR and CR, i.e. platelet count of 30 x 109/L and 100 x 109/L ore more, respectively), time to response (TTR) and the response duration (RD).

Results. Twenty patients, median age 51 years (range 27-74 years), were evaluated. Sixteen patients had a primary IT (ITP) while 4 patients had secondary IT, 2 HCV related and 2 with positivity to anti-phospholipid antibodies. All 20 patients previously received treatment with steroids and rituximab; 10 patients received further immune-suppressive therapy and 6 underwent splenectomy. Median baseline platelet count was 19 x 109/L, while the median interval between diagnosis of IT and Dapsone therapy was 46 months (range 2-274 months). OR and CR were 55% and 20%, respectively; median TTR was 1 month. Three out of 4 secondary IT responded to Dapsone. All responders were able to interrupt any other specific anti IT treatment. The median duration of Dapsone therapy was 6 months (range 2-50 months); median RD was 39 months (2-50 months).

None of responders lost response while on treatment. One patient in CR interrupted Dapsone after 9 months and still maintains the response after 36 months. None of the patients interrupted the treatment with Dapsone for toxicity. Three patients showed mild level of haemolysis and 2 mild to moderate level (< 10%) of metahaemoglobinemia.

Conclusions. This single institution experience confirms the high therapeutic activity and good safety profile of Dapsone as salvage therapy with relapsed or refractory IT.

BEST-004

HOSPITAL VERSUS HOME CARE FOR PATIENTS WITH HEMATOLOGICAL MALIGNANCIES IN CURATIVE OR TERMINAL PHASE: COST ANALYSIS AND COST-EFFECTIVENESS STUDY

Cartoni C,[^] Tendas A,[*] Brunetti GA,[^] Meloni E,[§] Niscola P,[*] Efficace F,[°] Minotti C,[^] Finsinger P,[^] Federico V,[^] Cuperli L,[*] Marini MG,[£] Reale L,[£] Martone N,[£] Foà R,[^] Alimena G,[^] De Fabritis P,[*] Mandelli F[§]

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

Aim:to determine and compare the costs and the use of resources according to the phase of disease in patients cared at hospital (H) or at their domicile (D) by a specialized team in partnership between not-for-profit and public health service.

Methods: prospective,observational study. Two groups of patients were analyzed for a maximum period of 6 weeks:i) in curative phase(CF) during non intensive chemotherapy or supportive care for complications; ii) in terminal phase (TP) for palliative care. Use of resources and costs for families were evaluated. A mean weekly cost (MWC) for the provider was built of health professional, laboratory, drugs, laboratory and transfusions costs. To compare the economic advantage between settings of care cost-minimization analysis was performed as well as cost-effective analysis, comparing the relative costs and outcomes(occurring infections).

Results: out of 119 patients,60 were cared at hospital, 59 at home, equally distributed for diagnosis, Hb, neutrohils and platelets levels, with prevalence of TP, older age, co-morbidities, worse performance status and self-efficiency score in the home group (p <.05). Infections occurred more often in the H (54%) than in D group (21%; p <.05). Mean No. of transfusions was similar in both groups. H care was significantly related to a higher MWC (3,534.3 €) and lower cost for families (76.7 €) compared to D care with MWC 1,219.7 €and families cost of 162.6 € (p <.05). At home the highest cost driver was for health providers, at hospital for drugs.

Compared to hospitalization, cost minimization analysis showed at home a weekly 2314.9 €save for the health provider and 85.9 €charge for the family, cost-effectiveness analysis yielded data of saving at home above 5,000 €/prevented infection for patients in CP and more than 12,000 €/prevented infection in TP.

Conclusions: although patients at home were older and in worse clinical conditions, in this study the actual determined standard cost (MWC of 1,219.7€) of D care resulted one-third of the hospital cost regardless of the phase of disease. In this setting home care resulted also cost-effective by saving money because of a lower number of occurring infections. Families of patients in TP have borne a mean weekly extra charge of 162 €

BEST-005

MICROPARTICLE-ASSOCIATED THROMBIN GENERATION AND PROCOAGULANT ACTIVITY ARE INCREASED IN ESSENTIAL THROMBOCYTHEMIA PATIENTS.

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Essential Thrombocythemia (ET) is a chronic myeloproliferative neoplasm characterized by an increased rate of thrombotic complications. A role for circulating microparticles (MP) in the pathogenesis of thrombosis has been recently suggested. A high number of MP of different cellular origin has been described in ET patients. To explore the contribution of plasma MP to the hypercoagulable state of patients with ET, in this study we aimed to characterize the MP functional procoagulant features. We used two different methods: the calibrated automated thrombogram (CAT), to determine the MP-associated thrombin generation (TG), and the P-PPL/1 assay (Stago R&D) to measure the MP-associated procoagulant activity (PCA). Both assays were performed in platelet free plasma (P-FP) obtained from 69 ET patients (24M/45F; 33 carriers of JAK2V617F mutation) and 67 control subjects (32M/35F). In a subgroup of 23 ET patients and 23 controls MP-associated TG and PCA were also determined in MP-free plasma (MP-FP). The results show that P-FP from ET patients generated significantly ($p<0.05$) higher quantity of thrombin compared to controls. Similarly, the MP-associated PCA was significantly increased in ET patients compared to controls ($p<0.05$). This increase was due to the presence of MP, as no TG and little PCA was observed in MP-FP from both patients and controls. The addition of isolated MP to autologous MP-FP restored the TG and PCA of the samples to the original values of P-FP for both assays. TG was significantly ($p<0.05$) increased in the JAK2V617F mutation carriers compared to wild-type subjects, while no significant differences were found for PCA. Significant correlations were found between the PCA by PPL-assay and the different parameters of TG assay [lag-time (R2= 0.414), peak (R2= -0.542), ETP (R2= -0.514)]. In conclusion, our results show that MP-associated TG capacity, as well as PCA, are increased in plasma from ET patients. The highest MP-associated TG was found in JAK-2 mutation carriers, who also are at higher risk for thrombosis compared to wild-type subjects. Our data provide evidence for a contribution of MP to the thrombophilic state of these patients. Prospective studies to evaluate whether MP associated TG and PCA may predict for thrombosis in ET patients are warranted.

BEST-006

HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN -THALASSEMIA MAJOR. DATA ON OVER 3000 TRANSPLANT FROM THE EBMT REGISTRY.

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Hemopoietic allogeneic stem cell transplantation (HSCT) is the only today available widely diffused curative option for -thalassaemia major patients. In the time in which gene therapy seems finally approaching, we analyse HSCT outcome over 20 years of activity of the large Hemoglobinopathy registry of the European Bone Marrow Transplant (EBMT). Data have been analyzed from promise MED-A forms. Here we report the results of the transplant activity reported to the Registry performed in 134 centres from 28 different countries. Three thousand three hundred and fourteen (3314) consecutive first HSCT in patients affected by hemoglobinopathy were registered in over 20 years of activity; the majority of patients were -thalassaemia major and 413 were sickle cell disease patients. Excluding the large Pesaro experience, data were available on a total number of 2415 consecutive first transplants of which

1805 have been performed in -thalassemia. Transplants have been mainly performed in European centres with a trend to increase transplant activity in Asiatic and North African centres (30% in recent years). Median age at HSCT was 6 years (range 0-18). On 1805 HLA identical sibling transplants in -thalassaemia major patients (transplants outside Pesaro experience) overall survival and thalassemia free survival up to 20 years plotted at 88±1% and 76±1%, respectively. To analyze age impact on outcome we stratified patient in 5 groups of age: ≤ 2 years, 2-4 years, 4-8 years, 8-14 years, 14-18 years. Number of patients, overall survival and thalassemia free survival are reported in the table. Survival in the older group (14-18 years) resulted significantly inferior to that of the other age cohorts (p<0.001). Of course in a wide registry study other parameters like the Pesaro classes could not be examined but it clearly resulted significantly better results in patients younger that 14 years old. HLA identical allogeneic HSCT is a available worldwide diffuse high success procedure for children with -thalassaemia major.

	Patients	OS	EFS
<= 2 years	130	0.91±0.03	0.80±0.04
2 - 4 years	337	0.90±0.02	0.74±0.03
4 - 8 years	600	0.90±0.01	0.80±0.02
8 - 14 years	535	0.87±0.02	0.76±0.02
14 - 18 years	203	0.73±0.04	0.67±0.04

Table 1: Overall survival and event free survival in 1805 consecutive transplants performed outside Pesaro experience. Patients are stratified by age.

BEST-007

WHOLE EXOME SEQUENCING REVEALS CONSTANT BRAF MUTATIONS IN HAIRY CELL LEUKEMIA

Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, Pucciarini A, Bigerna B, Pacini R, Wells VA, Sportoletti P, Pettirossi V, Mannucci R, Elliott O, Liso A, Ambrosetti A, Pulsoni A, Forconi F, Trentin L, Semenzato G, Inghirami G, Capponi M, Di Raimondo F, Patti C, Arcaini L, Musto P, Pileri S, Haferlach C, Schnittger S, Pizzolo G, Foà R, Farinelli L, Haferlach T, Pasqualucci L, Rabadan R, Falini B

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Background: Hairy cell leukemia (HCL) is a well-distinct entity of the WHO classification characterized by an indolent course, marked splenomegaly, frequent pancytopenia, and rare circulating tumor cells. However, the underlying genetic lesions causing this disease remain unknown.

Methods: With the aim to discover HCL-associated mutations, we subjected to whole exome sequencing the leukemic and matched normal cells purified from the peripheral blood of one case with HCL (index patient). Findings were subsequently validated by Sanger sequencing in 47 additional patients with HCL.

Results: Whole exome sequencing revealed 5 missense somatic clonal heterozygous mutations that involved the BRAF, CSMD3, SLC5A1, CNTN6 and OR8J1 genes and were confirmed at direct DNA Sanger sequencing. The BRAF mutation in the index patient was predicted to encode the BRAF V600E variant protein. Because the BRAF V600E mutation is oncogenic in other tumors, we focused on this genetic lesion. Direct DNA Sanger sequencing identified the same BRAF mutation in all 47 additional patients analysed (100%). None of the 195 peripheral B-cell lymphomas/leukemias other than HCL that were investigated harbored the BRAF V600E mutation, including 38 cases of splenic marginal zone lymphomas and unclassifiable splenic lymphomas/leukemias. Immunohistology and Western blotting showed that HCL cells express phospho-MEK and phospho-ERK (the immediate downstream targets of the BRAF kinase), pointing to a constitutive activation of the RAF-MEK-ERK mitogen-activated protein kinase pathway in HCL. In vitro treatment of BRAF-mutated primary leukemic cells from 5 HCL patients with PLX-4720, a specific inhibitor of active BRAF, markedly decreased the levels of phosphorylated ERK and MEK. Based on these findings, clinical testing of active BRAF inhibitors are warranted in HCL.

Conclusions: The BRAF V600E mutation qualifies as the disease-defining genetic event in HCL. Our results have important implications for the pathogenesis, diagnosis and targeted therapy of HCL.

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BEST-008

IMPACT OF IMMUNOGENETIC POLYMORPHISMS IN APLASTIC ANEMIAS AND IN MYELODYSPLASTIC SYNDROMES.

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Cytotoxic T lymphocytes and T helper 1 lymphocytes, detected in marrow and blood, are considered the main effector cells of immune-mediated suppression of hematopoiesis in idiopathic aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH) and some types of myelodysplastic syndrome (MDS). We explored whether predisposition to these bone marrow failure syndromes (BMF), is found in killer cell immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) ligand (KIR-L) gene variations or cytokine polymorphisms.

We studied 77 patients with AA, 129 with MDS and 285 healthy controls for the frequencies of KIR-L and KIR genotypes and 22 selected single nucleotide polymorphisms (SNPs) located within 10 cytokine (IL1-alpha, IL1-beta, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-gamma, TNF-alfa, TGF-beta) and 3 cytokine receptor (IL-1R, IL-1RA, IL-4R-alpha) genes.

In AA we found a decreased frequency of inhibitory KIR-2DL3 genes. In MDS, no difference in the frequency of KIR genotype was identified; however, a decreased frequency of 2DL3 was found in hypocellular MDS. Analysis of the KIR genotype in correlation with the corresponding KIR-L profile, revealed a decreased frequency of stimulatory 2DS1/C2 mismatch both in AA and MDS. In AA and MDS cohorts, compared to controls, we found a higher frequency of of TT codon 10 variant and of GG codon 25 variant of TGF-beta gene, consistent with a high secretory phenotype. This relationship was even more pronounced in PNH and hypocellular MDS. We confirm that the hypersecretory genotype T/T at position -874 of INF-gamma gene was overrepresented only in AA and correlates with presence of a PNH clone. Instead, the frequency of G/A polymorphism at position –308 on the TNF-alpha gene promoter, which correlates with higher TNF-alpha production, was found significantly higher only in MDS patients. Moreover, hypocellular MDS was characterized by a higher prevalence of IL-10 GCC/GCC haplotype, which is functionally associated with a low secretory phenotype.

Our findings suggest that alterations in KIR/KIR-L matching, such as increased 3DL2 and decreased 2DS1 mismatch, and in the polymorphisms of TGF-beta1, IFN-gamma, TNF-alpha and IL-10 may account for the propensity to immune-mediated killing of hematopoietic stem cells and/or ineffective hematopoiesis of AA and MDS. Further studies are needed to elucidate whether these immunogenetic traits may be involved in increased risk of developing immune-mediated BMF.

BEST-009

GENETIC VARIATIONS IN ADH1A AND CYP2E1 STRONGLY AFFECTS RESPONSE AND TOXICITY TO A COMBINATION OF GEMTUZUMAB OZOGAMICIN PLUS FLUDARABINE, CYTARABINE, IDARUBICIN IN CD33-POSITIVE ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background: Genetic heterogeneity in drug-metabolizing enzyme and transporter genes affects specific drug-related phenotypes in cancer. In order to investigate a relationship between genetic variation and response in AML, we comprehensively assessed the allele frequencies of 1936 genetic variations of 225 absorption, distribution, metabolism and excretion genes in 94 CD33-positive AML patients younger than 65 years, using the new Affymetrix drug-metabolizing enzyme and transport (DMET Plus) genotyping platform.

Patients and methods: All patients were enrolled in a phase III multicenter clinical trial combining low dose of Gemtuzumab-ozogamicin (GO) with FLAI regimen (Fludarabine, Cytarabine, Idarubicin) as Induction chemotherapy (ClinicalTrials.gov NCT00909168). Cytogenetics, multidrug-resistance phenotype, FLT3 and NPM mutation status, as well as WT1 quantitative expression analyses were performed at diagnosis. Furthermore, high-resolution single nucleotide polymorphism (SNP) array and DMET genotyping analyses (Affymetrix) were also performed.

Results: Of the 94 patients, genotype results were evaluable for 91 cases. The median call rate was 99.48 (range, 96.32-100). Firstly, we tested the association among SNPs and response to the induction cycle (FLAI + GO) comparing the genotyping profile of 80 patients in complete (85%) and partial (3%) remission with that of patients (12%) with no response. A highly significant difference ($p < 0.001$) in the allele frequency of 2 variants, in complete linkage disequilibrium, in the alcohol dehydrogenase enzyme (ADH1A) was found. ADH1A metabolizes the conversion of ethanol to acetaldehyde which is thereafter converted to acetate by ALDH1, which is well known as stem cell marker. These variants were not associated with high risk AML, FLT3 and NPM1 mutations, but strongly influenced response to the induction phase also in a multivariate analysis. Secondly, SNPs were stratified according to liver toxicity and a significant difference in the allele frequency in CYP2E1, involved in the alcohol metabolism was found to be associated with a grade I/II liver toxicity.

Conclusions: Genetic variations in ADH1A and CYP2E1 genes were for the first time identified and associated with response and toxicity in AML patients treated with a combination of GO and FLAI regimen.

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BEST-010

MELPHALAN/PREDNISONE/LENALIDOMIDE (MPR) VERSO MELPHALAN (200 MG/M2) E TRAPIANTO AUTOLOGO (MEL200) IN PAZIENTI CON NUOVA DIAGNOSI DI MIELOMA MULTIPLO (MM): UNO STUDIO DI FASE III.

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Background: High-dose chemotherapy (HDT) with haemopoietic stem-cell improves quality of response and survival in multiple myeloma (MM). In this randomized study we question the role of HDT, when new drugs are incorporated in the conventional treatment or in the autologous transplant (ASCT) setting in newly diagnosed MM patients.

Aims: to compare in a prospective randomized study conventional chemotherapy plus novel agents (MPR) with tandem high-dose melphalan (MEL200) and ASCT in newly diagnosed MM patients younger than 65 years.

Methods: The induction treatment consisted of four 28-day cycles of lenalidomide (25 mg, d1-21) and low-dose dexamethasone (40 mg, d1,8,15,22) (Rd). Patients (N=402) were then randomly assigned to receive MPR [six 28-day cycles of melphalan (0.18 mg/kg d1-4), prednisone (2 mg/kg d1-4) and lenalidomide (10 mg d1-21); pts (N=202)] or MEL200 [tandem melphalan 200 mg/m2 with stem-cell support; (N=200)]. All patients enrolled were stratified according to International Staging System (ISS) (stages 1 and 2 vs stage 3) and age (< 60 vs > 60 years). Primary end point was PFS. Data were analyzed in intention-to-treat.

Results: Response rates were similar between the two groups (MPR vs MEL200): > VGPR (60% vs. 58%, p=0.24) and CR (20% vs. 25% p=0.49). No differences in responses were reported according to cytogenetic abnormalities, such as del13, t(4;14), or t(14;16). After a median follow-up of 20 months, the 18-months PFS was 68% in MPR and 78% in MEL200 (HR=0.58, p=0.006). CR prolonged the 18-months PFS in the MPR (90% vs 66%) and in the MEL200 group (87% vs. 76%). The 18-months OS was similar (91% vs 95%; p=0.073). In the MPR and MEL200 groups, G3-4 neutropenia was 55% vs 89% (p < .001); G3-4 infections were 0% vs 17% (p < .001); G3-4 gastrointestinal toxicity was 0% vs. 21% (p < .001); second tumors were 0.005% in both arms. The incidence of thromboembolic events was similar: 2.44% vs 1.13% (p=0.43).

Conclusions: MEL200 significantly prolonged PFS in comparison to MPR, although toxicities were significantly higher. This is the first report showing a PFS advantage for ASCT in comparison with combinations including novel agents. At present OS is not significantly different in the two groups, but longer follow-up is needed.

BEST-011

LONG-TERM FOLLOW-UP OF ADOPTIVE IMMUNOTHERAPY WITH HAPLOIDENTICAL KIR-L MISMATCHED NATURAL KILLER CELLS AS POST-REMISSION CONSOLIDATION STRATEGY IN ELDERLY HIGH RISK ACUTE MYELOID LEUKEMIA PATIENTS

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Eleven patients with high-risk AML (2 in molecular relapse and 8 in morphological complete remission-CR; median age 62 years (range 53-73)) received highly purified CD56+CD3- NK cells from haploidentical KIR-ligand mismatched donors after fludarabine/cyclophosphamide immunosuppressive chemotherapy, followed by interleukin-2. The median number of infused NK cells was 2.74 x 10e6/Kg. No NK cell-related toxicity, including graft-versus-host disease, was observed. Hematological recovery was comparable to standard chemotherapy. Both patients in molecular relapse achieved molecular CR, which lasted 9 and 4 months. Among 9 patients in morphological CR, 5 patients are disease-free after 42, 46,30, 11 and 8 months (median 30 months; range 8-46), whereas 3 relapsed after 3, 4 and 18 months and then ultimately died due to disease progression.

One patient died during the neutropenic phase due to overwhelming bacteria pneumonia. Seven AML patients, who had achieved CR after the same induction/consolidation therapy and with similar prognostic features as the study patients, were excluded from NK therapy because they did not have KIR-ligand mismatched donors. Interestingly, analysis of clinical outcomes showed that 6/7 patients relapsed at a median of 4 months and died of disease progression, whereas one patient is alive and disease-free after 24 months post autologous stem cell transplant. After infusion, donor NK cells were found in the peripheral blood (PB) of all evaluable patients (peak value on day 10).

They were also detected in the bone marrow (BM) in some cases (peak value on day 5). An association between serum IL-15 concentration and donor chimerism after NK cell infusion was observed. Particularly, the rise in IL-15 serum level was followed by increase in donor chimerism, thus supporting the conclusion that homeostatic IL-15 drives in vivo expansion and survival of adoptively transferred NK cells. Donor-versus-recipient alloreactive NK cells were demonstrated in vivo by the detection of donor-derived NK clones that killed recipient targets, including leukemic blasts. In conclusion, infusion of purified NK cells is feasible in elderly patients with high risk AML.

Adoptively transferred NK cells were alloreactive against recipient cells and might have induced anti-leukemic activity. To test the efficacy of such approach a Phase II clinical trial is currently ongoing at our Institutions.

BEST-012

CHRONIC/RELAPSING BENIGN LYMPHADENOPATHY ASSOCIATED WITH HHV-6B INFECTION: A NEW CLINICO-PATHOLOGIC ENTITY.

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HHV-6 DNA sequences were disclosed in lymph node (LN) tissues of several patients with lymphoid malignancies, but a direct major role of HHV-6 in malignant transformation has so far not been confirmed. By contrast, HHV-6 infection has been associated to either infectious mononucleosis-like syndrome, or to Rosai-Dorfman disease in which viral antigens have been detected by immunohistochimical (IHC) analyses in both histiocytes and follicular dendritic cells (FDC). We investigated whether HHV-6 infection may have a pathogenetic role in benign lymphadenopathies (LAP), which tend to recur without evolving into lymphoma.

We report on 9 patients (median age 45 years, range 18-76 years) with either localized or generalized LAP, in whom the most frequent either reactive or malignant causes of LN enlargement have been repeatedly excluded. Of interest, some of the patients, observed for a median follow-up of 29 months (range 11-157), presented with recurrent LAP (1 to 3 recurrences).

Extensive histological, IHC, serological and molecular examinations were performed in order to study a potential involvement of HHV-6 active infection or reactivation.

A common LN histological pattern at presentation showed florid follicular hyperplasia with concurrent mild paracortical expansion. Three cases showed features consistent with PTGC. IHC analyses revealed HHV-6B positive staining of FDC together with scattered positivity of interfollicular cells. The IHC reactions for both HHV-6A and HHV-6B, performed on further 78 LN tissues from patients with benign LAP induced by other known etiologies, were negative. Serology was suggestive for viral reactivation/reinfection.

The molecular analyses failed to detect HHV-6 viremias in cell-free-serum samples of all the 9 patients with positive HHV-6B IHC staining, while positivity for HHV-6B DNA was disclosed in 7 out of 7 LN tissues studied to date. Constitutional symptoms were absent in all but a 67-year old patient, who developed AITL 11 months later. Of note, this latter patient was the only one, presenting with serological results consistent with primary infection.

We show for the first time that local reactivation/infection of HHV-6B should be considered among the possible causes of chronic/relapsing benign LAP. IHC is the method of choice for investigating the presence of HHV-6 infection in such cases. HHV-6B may indirectly modulate and trigger the proliferation of lymphocytes, by locally affecting FDC and LN microenvironment.

ORAL COMMUNICATIONS

NON-HODGKIN'S LYMPHOMA I

C001

CLINICAL VARIABLES WITH PROGNOSTIC RELEVANCE IN MALT LYMPHOMA PATIENTS TREATED IN THE IELSG-19 INTERNATIONAL MULTICENTER RANDOMISED STUDY

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The IELSG-19 international multicenter study is the largest prospective randomized trial ever conducted in MALT lymphoma patients, comparing chlorambucil alone versus the combination of chlorambucil and rituximab versus the sole rituximab. 454 MALT lymphoma patients (pts) were enrolled from January 2003 to July 2010. Main contributors were the Italian Lymphoma Foundation, the French GELA group, Cancer Research UK, the Catalan Hematology Group and the Oncology Institute of Southern Switzerland. All MALT lymphoma pts with localized disease at any extranodal site who did not respond or were not suitable to local therapy (including H.pylori-negative gastric lymphomas) or those who failed antibiotic therapy were eligible, as well as those with disseminated or multifocal MALT lymphoma. After central histology review 424 pts receiving treatment per protocol were selected for the present analysis. One hundred thirty seven pts were randomized to chlorambucil treatment, 140 pts were randomized to chlorambucil plus rituximab and 147 pts to the rituximab treatment. The primary MALT lymphoma site was the stomach in 179 pts (42.2%), 245 pts (57.8%) had a non-gastric presentation. In 120 pts (30.5%) the lymphoma involved more than 1 extranodal site. Lymph node involvement was present in 154 pts (36.3%); 168 pts (42.6%) had advanced stage (Ann Arbor stage III-IV). The ECOG performance status was 0 in 297 of 393 pts in whom the data was available (70%). According to the international prognostic index (IPI), 66 pts (17%) had intermediate-high risk and only 9 a high risk score (2%). B-symptoms were present in 43 of 294 pts (10.9%) and LDH levels were higher than normal in 42 (10.7%) of 393 pts. The median age was 61 years (range, 26-81). After a median follow-up of 3.2 years, the EFS was 66%, the PFS was 74% and the OS was 94%. At multivariate analysis, among the main clinical characteristics, after adjustment by arm of treatment, the presence of nodal involvement, the presence of B-symptoms, lower levels of serum protein concentration and unfavourable IPI scores were independently associated with shorter EFS and PFS. Analysis of prognostic variables is ongoing to possibly build up a MALT-lymphoma specific prognostic system and better define the clinical features predicting the patient outcome.

C002

SAFETY AND EFFICACY OF 90Y IBRITUMOMAB TIUXETAN (ZEVALIN®) FOR UNTREATED FOLLICULAR NON-HODGKIN'S LYMPHOMA (FL) PATIENTS. ITALIAN MULTICENTRIC TRIAL

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90Y ibritumomab tiuxetan (Zevalin®) combines the targeting advantage of monoclonal antibody with the radiosensitivity of FL. Previous studies showed that Zevalin resulted safe and highly effective in relapsed/refractory indolent NHL, irrespective to prior treatment with rituximab. Based on these results, we designed a multicenter trial to evaluate the safety and the efficacy of "upfront" single-agent Zevalin in FL. The primary endpoint was the incidence of responses in terms of overall remission rate (ORR) and complete remissions (CR). The secondary endpoints were the treatment safety by monitoring hematology and biochemistry parameters as well as adverse events. Fifty patients, with a median age of 59 years (range, 35-81), were treated. Forty-eight percent had bone marrow involvement (<25%) and 14% an elevated LDH. Thirty-four percent of patients had high risk FLIPI. Results: The ORR was 93% (45/48) with a CR rate of 82 % (41/48). By PCR, a Bcl2/IgH rearrangement was detected in 77% of cases at baseline. At the first planned molecular evaluation (week 14), 44% of patients previously PCR-positive converted to PCR-negative. Confirmed molecular responses were observed in 68% (17/25) of assessable patients who had a clinical complete remission. At the last molecular follow-up, 47% of PCR-positive cases at baseline were still in molecular remission. After a median follow up of 24 months, the 2-year EFS for all patients was 85%. As expected, the main toxicity was moderate myelosuppression, with 30% and 26% of patients developing Grade 3/4 neutropenia and thrombocytopenia, respectively. Very few patients required platelets transfusion (4%) or growth factor use (6%). None of the patients experienced grade 3/4 non hematologic toxicity. In conclusion, Zevalin is highly effective and safe treatment for newly diagnosed FL patients. In the next future, the role of radioimmunotherapy - i.e. including optimal sequencing with chemotherapy - should be established in randomized studies.

C003

AN INTERNATIONAL PHASE II STUDY TO INVESTIGATE THE PREVALENCE OF INFECTIOUS AGENTS IN OCULAR ADNEXAL MARGINAL ZONE LYMPHOMA (OAMZL) AND THE EFFICACY OF FIRST-LINE ANTIBIOTIC THERAPY (IELSG#27 TRIAL)

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Background. Chlamydophila psittaci(Cp) infection has been detected in ~80% of Italian patients (pts) with OAMZL. Lymphoma regression has been observed in ~50% of pts after Cp-eradicating therapy with doxycycline (DOXY). Reported studies on this association were retrospective or included both pts with newly diagnosis and relapsed OAMZL. This is the first international prospective phase II trial aimed to elucidate Cp prevalence and DOXY activity as first-line therapy in pts with newly diagnosed OAMZL (ClinicalTrial.gov NCT01010295). Aims: To define the prevalence of Chlamydiae infections, to evaluate bacterial eradication and anti-lymphoma activity of DOXY in OAMZL. METHODS: Pts with stage-IEA OAMZL and measurable lesion were enrolled in the part A, and received DOXY 100 mg bid for 21 days. Pts with other lymphomas, non neoplastic lesions or OAMZL not eligible for part A entered the part B and were treated following local practice (DOXY was an option). Chlamydiae infections were evaluated on diagnostic samples by three different PCR targeting TETR, OmpA, hsp60. Bacterial eradication was tested on conjunctival swabs and peripheral blood mononuclear cells (PBMC) at basal time, at 3 and 12 months after DOXY. RESULTS: From August 2006 to November 2010, 54 pts were enrolled at 6 centers. Cp was detected in biopsies of 32/37 OAMZL (86%) and in 4/7 non-MZL. All cases were negative for C. pneumoniae and C. trachomatis. Among 36 Cp+ pts, infection was detected in 100% of swabs and in 75% of PBMC; no cases with Cp-negative OAMZL were positive in swabs or PBMCs. Among 34 pts entering part A, 28 were evaluable for eradication (swabs in 8, PBMC in one, both in 19). Fourteen pts (50%) achieved Cp eradication; Cp was detected again at one year of follow-up in three of them. All pts completed planned treatment without toxicity. Response was complete in six pts (18%; 95%CI: 5-31%), and partial in 16 (ORR= 65%, 95%CI: 49-81%); 11 had SD and one PD. At a median follow-up of 27 months (range 3-51), 12 pts experienced relapse, with a 3-year PFS of 54±11%. A trend to a higher response rate (82% vs. 53%; p=0.12) and 3-yr PFS (72% vs. 52%; p=0.14) was

observed in eradicated pts. ConclusionsS: Cp infection is frequent in OAMZL. First-line DOXY was associated with lymphoma regression in 65% of pts. However, DOXY failed to eradicate Cp infection in half of pts, with a negative impact on outcome. Further studies to improve antibiotic efficacy are warranted.

C004

THE NF-KB PATHWAY IS TARGETED BY MULTIPLE GENETIC LESIONS IN SPLENIC MARGINAL ZONE LYMPHOMA

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The pathogenesis of splenic marginal zone lymphoma (SMZL) is unknown. The NF-kB pathway may be activated by genetic lesions in lymphoma, and represents an attractive candidate in SMZL. Active NF-kB signaling was documented in SMZL by Western blot for p100/p52 processing (7/12, 58.3%) and by immunohistochemistry for NFKB1 nuclear localization (12/24, 50%). To investigate the molecular basis of NF-kB activation in SMZL, 20 NF-kB genes were screened for mutations and copy number abnormalities in 101 SMZL. Overall, 21/101 (20.7%) SMZL harbored NF-kB mutations. By combining mutations and copy number abnormalities, NF-kB was targeted by molecular lesions in 36/101 (35.6%) SMZL. Mutations distributed in a mutually exclusive fashion, thus representing alternative molecular mechanisms of NF-kB activation, and targeted key regulators of NF-kB signaling, including the TRAF3/MAP3K14/BIRC3 regulatory complex of non-canonical NF-kB signaling, and the IKBKB (positive) and TNFAIP3 (negative) regulators of canonical NF-kB signaling. BIRC3 was mutated in 6/101 (5.9%) SMZL. Mutations comprised frameshift deletions and non-sense mutations truncating the C-terminal RING domain of BIRC3, whose E3 ubiquitin ligase activity is essential for NF-kB signaling suppression via MAP3K14 proteosomal degradation. TRAF3 was mutated in 4/101 (3.9%) SMZL. Mutations comprised frameshift deletions and non-sense mutations that were predicted to cause elimination of the C-terminal MATH domain of TRAF3 that is required for NF-kB signaling suppression via MAP3K14 sequestration into the TRAF3/MAP3K14/BIRC3 regulatory complex. The NF-kB signaling activator MAP3K14 was targeted by missense mutation of the kinase domain or amplification of the locus in 7/101 (6.9%) SMZL. The NF-kB signaling activator IKBKB harbored two recurrent and somatically acquired mutations (K171E and V203I) in 5/101 (4.9%) SMZL. IKBKB mutations located within the kinase domain near the activation loop and led to damaging amino acid substitutions within two highly conserved sites.

The NF-kB signaling inhibitor TNFAIP3 was disrupted by frameshift insertions/deletions in 6/101 (5.9%) SMZL. Our results indicate that the NF-kB pathway is activated and frequently targeted by genetic lesions in SMZL. The novel finding of BIRC3 mutations in SMZL points to a more general role of BIRC3 in marginal zone lymphoma, since BIRC3 is also involved in the t(11;18) translocation of MALT lymphoma.

C005

FLUDARABINE AND MITOXANTRONE FOLLOWED BY YTTRIUM-90 IBRITUMUMAB TIUXETAN IN UNTREATED PATIENTS WITH FOLLICULAR LYMPHOMA. LONG TERM EFFICACY AND TOXICITY RESULTS OF THE FLUMIZ TRIAL

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We previously reported the results of a multicenter non-randomized phase II trial of fludarabine and mitoxantrone plus radioimmunotherapy (RIT) [FLUMIZ (Fludarabine, Mitoxantrone, Zevalin) trial], demostrating that this combination was safe and very effective in untreated patients with follicular non-hodgkin lymphoma. We are now providing long term efficacy and toxicity results of this combination strategy. Sixty-one patients with stage III and IV untreated follicular lymphoma were enrolled between June 2004 and April 2006, at 13 Italian institutions. Briefly, treatment schedule was the following: oral fludarabine 40 mg/m2 on days 1-3, intravenous mitoxantrone 10 mg/m2 on day 1 every 28 days for six cycles, followed by one course of yttrium-90 (90Y)-labelled ibritumumab tiuxetan (Zevalin), which consisted in two weekly infusions of Rituximab 250 mg/m2 followed by a weight based dose of 90Y-ibritumumab tiuxetan. Primary endpoints at the time of the first analysis were complete response and hematological toxic effects, secondary endpoints were overall survival (OS) and progression free survival (PFS). Fifty-seven patients were treated with RIT after the completion of six courses of fludarabine and mitoxantrone (FN) regimen. Four patients were excluded because of disease progression (n=1) and bone marrow infiltration > 25% (n=3) at the end of the FN regimen. Median follow up at the time of the last analysis was 52 months (range 24-75). Five-year PFS was estimated to be 68%, 5-year OS was estimated to be 93.0% . Noteworthy, late hematological side effects such as myelodisplastic syndromes or acute myeloid leukemias have not been observed so far. All patients had a complete hematological recovery after the completion of the sequential treatment. 16 patients relapsed during the follow-up period and 4 patients died due to disease progression. 22 patients (38%) are in first complete remission after more than 4 years of follow-up. All relapsed patients underwent second line chemotherapy and high dose chemotherapy with stem cell rescue was performed in 4 patients. These results confirm the long term efficacy and safety of 6 cycles of fludarabine and mitoxantrone followed by consolidation with 90Y-ibritumumab tiuxetan: the 5-year PFS and OS compare favourably with the results of chemoimmunotherapy alone in untreated follicular lymphoma, with no increased incidence of secondary hematologic malignancies.

C006

FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB IN PATIENTS WITH ADVANCED UNTREATED INDOLENT B-CELL NON-FOLLICULAR LYMPHOMAS: FINAL RESULT OF PHASE TWO STUDY OF FONDAZIONE ITALIANA LINFOMI (FIL)

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Indolent non-follicular non-Hodgkin lymphomas (INFL) are a heterogeneous subset of diseases whose treatment has not been clearly defined. To this end, we evaluated the efficacy and safety of first-line therapy with rituximab, fludarabine and cyclophosphamide (FCR) followed by rituximab maintenance in patients with advanced INFL. This single arm, multicenter, open-label phase II study evaluated the activity of FCR induction immunochemotherapy (rituximab: 375 mg/m^2 i.v. on Day 1 of each cycle and on Days 1 and 14 of cycles 4 and 5; fludarabine: 25 mg/m^2 i.v. on Days 2-4; cyclophosphamide: 250 mg/m^2 i.v. on Days 2-4), every 28 days for six cycles followed by a maintenance phase with four infusions of rituximab (375 mg/m^2 i.v. on Day 1) every two months for responder patients. Forty-seven previously-untreated patients with advanced INFL were enrolled in the study. Among 46 evaluable patients (28 males, median age 59 years), 19 were diagnosed with lymphoplasmacytic lymphoma (LPC), 21 with small lymphocytic lymphoma (SLL) and 6 with nodal marginal zone lymphoma (MZL). The final overall response rate was 78.3% with 65.3% complete remission (CR/CRu) and 13% partial response. After a median follow-up of 40.9 months, FFS and PFS were 90.1%, and OS was 97.4%. The main toxicity was hematological; related grade III-IV neutropenia was observed in 55.3% of patients. FCR induction therapy followed by a short maintenance phase with rituximab is a highly effective regimen with acceptable toxicity for patients with advanced untreated INFL.

MULTIPLE MYELOMA I

C007

POST-TRANSPLANT LENALIDOMIDE CONSOLIDATION - MAINTENANCE THERAPY IN ELDERLY MULTIPLE MYELOMA PATIENTS: UPDATED RESULTS OF A PHASE II TRIAL

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Bortezomib-dexamethasone-doxorubicin (PAD) induction, followed by reduced-intensity autologous transplantation showed to be a safe and effective treatment option in elderly newly diagnosed multiple myeloma (MM) patients. In this analysis, efficacy and safety end-points of post transplant lenalidomide consolidation-maintenance were updated. A hundred and two newly diagnosed patients, aged 65-75 years or younger but considered ineligible for high-dose chemotherapy, were enrolled in this phase II trial. Patients received induction with 4 cycles of PAD, tandem melphalan 100 mg/m^2 and stem-cell support, subsequent consolidation with four 28-day cycles of lenalidomide-prednisone (LP), followed by lenalidomide maintenance (L) until relapse or until tolerated. During LP-L, post-transplant very good partial response (VGPR) rate improved from 82% to 92% and complete response (CR) rate from 38% to 71%. Twenty-one patients upgraded response: 1 patient improved from stable disease (SD) to partial response (PR), 1 from SD to VGPR, 1 from PR to VGPR, 2 from PR to CR, 16 from VGPR to CR. After a median follow-up of 3 years, the 3-year progression-free survival (PFS), time-to-progression and overall survival were 66%, 73% and 85%, respectively. By exploratory analysis stratified by group, the 3-year PFS rate was higher in patients with International Staging System stage I and stage II (74% and 71% respectively) as compared to patients with stage III (30%). 3-year PFS was 81% in patients who achieved CR, 56% in patients who achieved VGPR, and 31% in patients who achieved PR. PFS was similar between patients older or younger than 70 years and between patients with high-risk cytogenetic profiles [presence of del17, t(4;14), or t(14;16)] and patient with standard-risk. LP-L consolidation-maintenance was well tolerated, grade 3–4 adverse events were mainly hematologic [neutropenia (31%) and thrombocytopenia (15%)]. Extra-hematologic toxicities included pneumonia (8%) and cutaneous rash (7%); no treatment-related deaths were reported. LP-L consolidation-maintenance improved post-transplant responses, was well tolerated, and can be considered a safe and effective treatment option for elderly MM patients eligible for reduced-intensity autologous transplant.

C008

PROSPECTIVE ANALYSIS OF THE PROGNOSTIC SIGNIFICANCE OF 18F-FDG PET/CT NEGATIVITY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Aim of the present study was to prospectively analyze the prognostic relevance of PET/CT negativity after induction and ASCT in 192 newly diagnosed MM patients. By study design, all patients were studied with 18F-FDG PET/CT at baseline, after induction treatment, after ASCT, once/year during post-ASCT follow-up and at the time of relapse. At baseline, 44% of the patients showed ≥ 3 focal lesions (FLs), 46% had SUV values > 4.2 and in 6% extramedullary disease (EMD) could be detected. These 3 variables adversely affected 4-year estimates of PFS and OS. Thirty seven percent of the patients were negative after induction, while PET/CT was negative in 65% after 3 months from ASCT. Persistence of severe FDG uptake (SUVmax still > 4.2) after induction predicted for shorter PFS at 4 years (P= 0.004). Complete FDG suppression post-ASCT conferred superior PFS and OS in comparison with persistence of FDG uptake. In particular, 4-year estimates of PFS and OS for negative patients were 66% and 89%, respectively, as compared to 45% and 65% for positive patients (P 0.02 both for PFS and OS). In multivariate analysis, both severe PET/CT involvement at diagnosis (SUV > 4.2 and/or EMD) and persistence of FDG uptake after ASCT were independent predictors of worst PFS (SUV > 4.2= HR: 2.0, 95%CI: 1.13-3.72; EMD= HR: 15.0, 4.0-55.8; FDG uptake after ASCT= HR: 2.12, 1.19-3.77) and OS (EMD= HR: 6.99, 2.28-21.46; FDG uptake after ASCT= HR: 3.57, 1.03-12.39). Forty four percent of the patients clinically relapsed during follow up; 59% of them had a negative PET scan after ASCT. The mean time to relapse was significantly shorter in the patients with positive PET with respect to negative patients (18 vs 27.6 months, p= 0.05). Moreover, the SUV max in positive patients was inversely correlated to the time to relapse (correlation coefficient = -0.7, p<0.003). These results provide demonstration that PET/CT at diagnosis and after treatment is a reliable tool for predicting prognosis in autografted MM patients and to identify patients at different risk and timing of progression. In particular, post-ASCT complete FDG suppression is associated with extended PFS and OS. Based on these data, aims to evaluate MRD should include also imaging techniques such as PET/CT.

C009

THE INVESTIGATION OF GENOMIC PROFILES IN PLASMA CELL LEUKEMIAS BY MEANS OF AN INTEGRATIVE MICROARRAY APPROACH

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Multiple myeloma (MM) is a clonal proliferation of malignant plasma cells (PCs) characterized by a marked genomic instability. Plasma cell leukemia is an aggressive malignancy classified as primary (pPCL) if not progressing from a previous intramedullary MM. To provide insights into the genetic lesions and altered molecular pathways characterizing pPCL, we analyzed highly purified PCs from untreated MM and pPCL patients (multicenter GIMEMA clinical trial) by FISH, gene (Affymetrix Gene 1.0 ST array) and miRNA (Agilent miRNA Microarray V2) expression and genome-wide DNA analyses (Affymetrix 250K Nsp SNP array). Unsupervised analyses of gene and miRNA expression profiles grouped most of pPCLs and MMs in two distinct branches, partially according to the major IgH chromosomal translocations. All c-maf/MAFB translocated MM cases were grouped in pPCL branch. Supervised analysis found 349 differentially expressed genes in 18 pPCLs versus 55 MMs, mostly (79%) up-regulated in pPCLs, and significantly enriched in cell activation, immune system development, programmed cell death, cytoskeleton organization, metabolic processes and transcription regulation functions. Forty-one up-regulated and 36 down-regulated miRNAs were identified in 15 pPCLs vs 39 MMs. Some of the overexpressed miRNAs in pPCL samples may have a particular importance in the context of B cell dyscrasias as suggested by their involvement in B cell development, lymphoproliferative disorders or in sustaining growth of MM cell lines. The genotyping analysis and FISH of 13 pPCLs concordantly detected 13q deletion in 77% and 17p deletion in 58% of cases. The most recurrent copy number alteration specifically identified by SNP-array was 1q gain (61.5%); in addition, losses involving chromosomes 1p (39%), 8p (31%), 14q (39%), 16q (39%) and gains affecting 7q (31%) and 19p (31%) and one amplification at 17q21 (46%) were detected. One case displayed a near tetraploid and another a hyperdiploid karyotype. A correlation between the expression levels and the occurrence of allelic imbalances was identified for 199 genes mainly localized in the previously described altered regions. The same analysis evidenced 23 miRNAs mostly mapping to chromosomes 1p (22%), 13q (26%) and 19 (22%). In conclusion, integrative genomics approach in pPCL allowed the identification of specific gene and miRNA signatures characterizing this more aggressive form of PC dyscrasia and novel genetic lesions associated to expression imbalances.

C010

EFFICACY OF LENALIDOMIDE IN PATIENTS WITH POEMS SYNDROME: A PILOT STUDY

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Introduction: POEMS syndrome is a rare multisystemic disease.Vascular endothelial growth factor (VEGF)correlated with the activity of the disease and it could account for clinical manifestations.

There are no controlled trials and no established treatment. Lenalidomide has anti-angiogenic activity through inhibition of VEGF and TNF alpha. This study plans to evaluate efficacy of Lenalidomide in POEMS SYNDROME. Patients & method: From 10/09, we started a pilot study with Lenalidomide plus Dexamethasone(RD) in pts pretreated or with newly diagnosed POEMS syndrome.

Lenalidomide 25 mg/day was given for 21 days in association with weekly Dexa 40 mg until tolerated or progression. After first 6 cycle, pts were evaluated for response. 10 pts have been enrolled, 9 men and 1 woman, with median age of 51 yrs (range 45-76). All pts were pretreated and 2 had received transplant; in all pts was detected monoclonal component (MC) (IgAL in 3 pts,IgGL in 6 pts,L light chain in 1) and sensory/motor peripheral neuropathy.

Sclerotic bone lesions was evident in 5 pts, endocrinopathies in 8, skin changes in 8, peripheral edema in 8, organomegaly in 7, lymphoadenophaty in 3, papilledema in 7, thrombocytosis in 3 pts. VEGF serum level was elevated in all patient with a median value of 3428 pg/ml (range 1400-8978) Results: 7 pts are still on treatment and received a median of 10 RD cycles (range 5-18). 5 pts are evaluable for response after 6 cycles. A clinical response, with improvement of all manifestations of disease, was observed in all pts.

Neurological improvement, was evident already after 3 RD cycles, confirmed by nerve conduction studies after 6 cycles. One patient with tetraparesis actually is able to walk and his upper limb strength is normal with improved sensory disturbances. Only in 1 patient MC disappeared. VEGF decreased in all pts: median 3428 pg/ml (range 1400-8978) before treatment to 1500 pg/ml (467-3579). 3 pts discontinued treatment: 1 withdraw consent, 1 drop-out after 4 cycles wit progressive disease and 1 died for pulmonary infection on cycle 1. Lenalidomide reductions was necessary in 2 pts (1 extraematological toxicity and 1 thrombocytopenia) and dexa in 5 pts.

Conclusions: Lenalidomide is efficacy and well tolerated in POEMS syndrome, with rapidly and continuous neurological improvements. Correlation between response and VEGF level was confirmed. At this time no responding pts experienced disease progression. The study accrual is still ongoing.

C011

MOLECULAR REMISSION AFTER BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE (VTD) COMPARED WITH THALIDOMIDE-DEXAMETHASONE (TD) AS CONSOLIDATION THERAPY FOLLOWING DOUBLE AUTOLOGOUS TRANSPLANTATION (ASCT) FOR MULTIPLE MYELOMA (MM): RESULTS OF A QUALITATIVE AND QUANTITATIVE ANALYSIS

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The high rate of complete response (CR) effected by novel agents as induction and consolidation/maintenance therapy in (MM) has renewed interest in the evaluation of minimal residual disease (MRD) after these combined treatment strategies. For this purpose, a useful molecular marker is represented by the IgH rearrangement which can be detected by PCR. We designed a molecular sub-study to the phase 3 GIMEMA trial of VTD vs. TD incorporated into ASCT for newly diagnosed MM. By study design, patients (pts.) randomized at diagnosis to VTD or TD as induction therapy before ASCT received two 35-day cycles of consolidation therapy with VTD or TD starting 3 months after ASCT(s), independently from prior response. Aim of the molecular study was to compare the activity of VTD consolidation with that of TD by qualitative and quantitative PCR analysis. At this time, 94 pts. were included in the study; of these, 70 pts. (36 of which received VTD, while 34 TD consolidation) were analyzed for MRD detection based on the availability of: 1) a molecular marker assessed at diagnosis; 2) both pre- and post-consolidation BM samples. MRD before the start of VTD consolidation therapy was undetectable in 15 out of 35 pts., or 43%; the corresponding value before the start of TD consolidation was 37.5% (or 12 out of 32 pts.). In comparison with the pre-consolidation status, analysis of BM samples collected at months +6 revealed an upgrade in PCR-negativity from 23% to 34% with TD and from 39% to 56% with VTD consolidation (p=0.03, according to McNemar's test). These data were further extended by a Real-time quantitative PCR analysis. In comparison with residual tumor mass at day 0, quantitative PCR analysis of BM samples collected at months +6 revealed a median 1 log reduction in tumor burden with TD vs. a median 4 log reduction with VTD consolidation. By using the Wilcoxon test, the overall reduction in residual tumor burden effected by VTD consolidation therapy was statistically significant (p=0.05). In conclusion, in comparison with TD, VTD consolidation therapy following double ASCT significantly increased the rate of molecular remissions and significantly reduced the burden of residual myeloma cells persisting after ASCT. Additional data on molecular follow-up analyses and comparisons of the prognostic relevance of PCR-negativity with PCR-positivity will be presented during the meeting. *Supported by: Progetto di Ricerca Finalizzata Orientata (to M.C), BolognAIL, Fond. Carisbo.*

C012

PREDICTING UNSUCCESSFUL OR SUB-OPTIMAL CD34+ PERIPHERAL BLOOD STEM CELL (PBSC) COLLECTION IN PATIENTS WITH MULTIPLE MYELOMA (MM) ELIGIBLE FOR AUTOLOGOUS TRANSPLANTATION (AUSCT): A SCORE MODEL DERIVED FROM THE ANALYSIS OF 1337 PATIENTS ENROLLED IN FIVE CONSECUTIVE ITALIAN MULTICENTER CLINICAL TRIALS

Musto P, Grossi A,[*] Sarli E,[**] Leotta F, Baldini L, Corso A, Di Raimondo F, Morabito F, Offidani M, Patriarca F, Petrucci MT, Tosi P, Cavo M, Boccadoro M, Palumbo A

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In order to analyze potential risk factors for unsuccessful/sub-optimal collections of CD34+ PBSC, we evaluated the role of four parameters associated with a possible impairment of PBSC collection in MM: age (greater 60 years), cytopenia at diagnosis (Hb less than 10 g/dl and/or neutrophil count < 1,000 and/or platelet count < 100,000), initial use of lenalidomide and hematological toxicity grade 3-4 during induction therapy. These parameters were evaluated in 1.337 previously untreated MM patients enrolled in five consecutive clinical trials conducted by GIMEMA-MM Italian Network and finalized to AuSCT. In all cases the mobilizing regimen was cyclophosphamide + G-CSF. According to the different studies, induction regimens included TD, VTD, DAV, RD and PAD. Data were retrospectively extracted from two separate databases (Torino and Bologna). Total amounts < 2 and < 5 x 10^6/kg CD34+ PBSC after a single mobilization procedure were considered as "failures" or "sub-optimal results", respectively. Four-hundred-twenty patients (31.4%) did not have any "negative" parameter, while 917 (68.6%) showed at least one of them. Overall, 413 patients (30.9%) had a sub-optimal result, including 256 patients (19.1%) with unsuccessful collection. The use of additional mobilizations or that of marrow rescue improved these data by 5% and 8.5%, respectively. Any single parameter negatively influenced PBSC collection vs absence of parameters (p < 0.000). At multivariate analysis, however, use of lenalidomide (p <0.013) and grade 3-4 hematological toxicity during induction (p <0.000), showed the most powerful negative effects on mobilization. The number of parameters significantly paralleled the inefficacy of procedures, both in terms of absolute PBSC amount or unsuccessful /sub-optimal collections (table). On these basis, we constructed a predictive score where the four parameters were pooled and weighted according to their relevance as single or combined variables, giving the value of 3 for grade 3-4 hematological toxicity, 2 for the use of lenalidomide and 1 each for age > 60 and baseline cytopenia. Statistical analysis (see the Table below) confirmed that this model is a simple and reliable measure for predicting unsuccessful/suboptimal PBSC collections in MM and for planning possible alternative strategies (i.e. the early use of plerixafor) for selected patients.

	N. parameters						p	Score < 3	Score > 3	p
Collections	0	1	2	3	4	Total				
CD34+ < 2 x10^6/kg	55/420	119/615 13.1%	61/237 19.3%	14/56 25.7%	7/9 25%	256 77.8%	<0.000 19.1%	189/1093	67/244 17.3%	< 0.000 27.5%
CD34+ < 5 x10^6/kg	101/420	188/615 24%	93/237 30.6%	23/56 39.2%	8/9 41.1%	413 88.9%	<0.000 30.9%	313/1093	100/244 28.6%	< 0.000 41%
Mean absolute CD34+ x10^6/kg (C.I. 95%)	10.6	8.5 (9.8-11.4)	7.5 (8-9)	6.3 (6-6-8.4)	1.2 (4.8-7.9)	8.8 (0-3.2)	<0.000 (8.5-9.3)			

ACUTE MYELOID LEUKEMIA I

C013

LOW-DOSE LENALIDOMIDE COUPLED WITH LOW-DOSE CYTARABINE INDUCES COMPLETE REMISSION OF ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS WITH UNFAVORABLE CITOGENETICS: PRELIMINARY RESULTS OF A PHASE II MULTICENTRIC STUDY

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We designed a phase II study to assess the antitumor efficacy of the combination regimen with low-dose lenalidomide and low-dose cytarabine in patients with acute myeloid leukemia (AML) aged >70 years. Sixteen patients (median age 76 years, range: 70-80) were consecutively enrolled in the study. Median white blood cell count at diagnosis was 7.5 x109/l (range: 0.59-44x109/l), whereas median haemoglobin was 9.4 g/dl and median platelet count was 44x109/l. Two out of sixteen patients had a normal karyotype, whereas 14/16 presented with an intermediate or unfavourable karyotype. Twelve patients had a de novo AML, whereas 4 patients had a secondary AML (2 after MDS, 1 after a CMPD, 1 after myelofibrosis). All patients received low-dose lenalidomide (10 mg/day orally, days 1-21) and low-dose cytarabine (20mg twice day subcutaneously, days 1-15). Therapy was repeated every 6 weeks, up to 6 cycles Five out of 16 patients died in aplasia while receiving the first induction cycle of therapy, and are not evaluable for response. Eleven patients received at least one cycle of therapy and are evaluable for response. Among these patients, 6/11 (54%) cleared peripheral blood blasts at the end of the second week of the first cycle, with recovery of normal WBC, hemoglobin and platelets values after a median of 33 days (range: 29-42) from the start of chemotherapy. Five out of 6 responding patients are still in morphologic, cytogenetic and FISH CR after 11, 10, 8, 4 and 3 months from the start of therapy, respectively. One patient died after receiving the third cycle of therapy while in CR due to a multi organ failure after an infectious complication. The other 5 patients who completed at least one cycle of therapy did not respond at all and rapidly died due to progressive disease. At present, out of 6/11 (54%) patients evaluable for response that obtained CR, 5/11 (45%) are alive in continuous CR and one died in CR. Notably, all responding patients presented with low blast count and unfavorable cytogenetics at diagnosis. In conclusion, low-dose lenalidomide has clinical activity, when coupled with low-dose cytarabine, in an extremely poor-prognosis subset of AML. Considering the scarce compliance of elderly, frail AML patients to high-dose therapy, the low dose schedule could be particularly profitable, specially for patients with low blast count and unfavorable cytogenetics. The study was registered at EMEA with the EUDRACT number 2008-006790-33.

C014

POLYMORPHISM Q141K OF ABCG2 PROTEIN IS ASSOCIATED WITH POOR OUTCOME IN ACUTE MYELOID LEUKEMIA PATIENTS RECEIVING IDARUBICIN-BASED CHEMOTHERAPY

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Introduction Single nucleotide polymorphisms (SNPs) in drug metabolism and transporters genes can explain the large inter-individual variability in response to therapy. Among SNPs reported in ABCG2 protein, a member of ABC transporter protein associated with negative prognosis in acute myeloid leukemia (AML), Q141K variant is the most frequent in Caucasian population. In vitro data suggest that this polymorphism affects the protein function modifying the resistance profile of many anticancer agents, but in vivo effects are still largely unknown. We evaluated the prevalence of Q141K ABCG2 protein in a series of patients with AML referred to our Division for diagnosis and/and therapy, to assess its potential impact on response to chemotherapy. Methods 163 consecutive patients with non-promyelocytic AML were included. ABCG2 expression was evaluated by flow cytometry; ABCG2 sequence by TaqMan SNP Q141K genotyping assay. 122 patients received chemotherapy according to Institutional protocols: induction course included fludarabine in all cases and idarubicin was the only anthracycline used through all the therapeutic program. Results Q141K ABCG2 was detected in 29/163 (17.8%) patients, similarly to what is reported in the literature. Membrane protein expression was variable among mutated patients, with ABCG2 overexpression in 18/29 (62%) cases. Of the 122 treated patients 72 (59%) attained remission, irrespectively of ABCG2 expression or polymorphism. Relapse occurred in 27/72 (37%) cases; relapse risk was affected by ABCG2 overexpression, CD34 and CD56 positivity. Considering the whole population, a non-statistically significant trend of shorter leukemia free survival (LFS) was observed in Q141K patients. However, stratifying patients in 3 groups (high and low ABCG2 expression and Q141K polymorphism regardless of ABCG2 intensity), mutated patients had a LFS comparable to those with ABCG2 over-expression. Overall survival (OS) was affected by age, CD34 and high ABCG2 expression. Again no differences were observed between wild type or mutated protein in the whole population, but a worse survival in Q141K group emerged after stratification in the 3 groups (p=0.032). Conclusions This is the first observation of the negative impact of Q141K ABCG2 polymorphism on AML outcome. Our data suggest that patient-tailored post induction therapy could be planned in presence of Q141K ABCG2, including drugs not affected or positively affected by the mutated protein.

C015

LEVEL OF MINIMAL RESIDUAL DISEASE (MRD) AND WHITE BLOOD CELL COUNT (WBCC) DISCRIMINATE CATEGORIES OF PATIENTS WITH DIFFERENT OUTCOME AMONG ADULTS WITH FAVOURABLE-RISK ACUTE MYELOID LEUKEMIA

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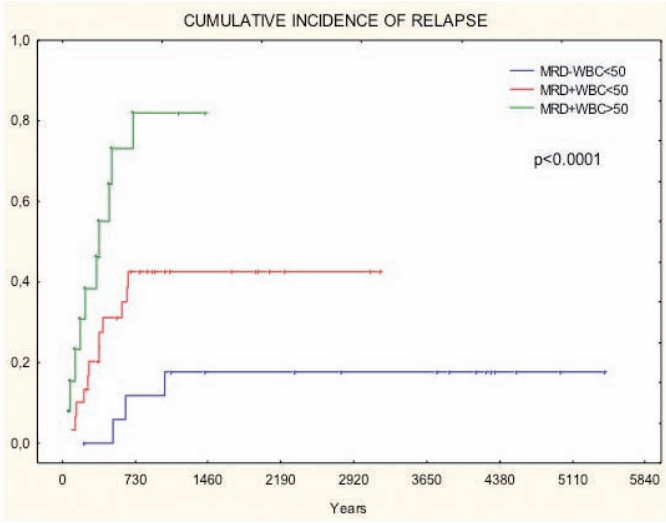
Core binding factor acute myeloid leukaemia (CBF-AML) and AML with mutated Nucleophosmin (NPM) without FLT3-ITD mutation are currently regarded as favourable-risk AML. Recent findings suggest a biological and prognostic heterogeneity of this AML subgroup. The aim of our study was to assess whether MRD detection was able to identify patients with increased risk of relapse. MRD was determined by multiparametric flow cytometry (MPFC) on bone marrow (BM) samples collected at the end of consolidation therapy. The threshold for MRD negativity was set below the level of 3.5x10e-4 residual leukemic cells. We evaluated 61 patients with de novo AML, enrolled sequentially in AML10/AML12 (n=49) and AML17 (n=12) EORTC/GIMEMA randomized trials between 1995 and 2007. Median age was 49 yrs (range 18-75), 12 patients were older than 60 years, 38 were males and 23 females and 48 (78%) had white blood cell count (WBCc) <50 x 10e-9/L. Twenty-seven CBF-AML [20 with t(8;21) and 7 with inv(16)] and 34 NPM-AML were evaluated. Overall 25 patients (41%) relapsed, 3 NPM-AML patients experienced an early relapse after consolidation therapy. After first consolidation, 23 patients underwent AuSCT, 13 AlloSCT and 25 did not receive any transplant procedure: 12 because of age, the remainders due to refusal or medical reasons (2 of 13 were consolidated with high dose ARA-C). MRD positive status after consolidation (MRDpos), age >60 years and WBCc >50 x 10e-9/L were significantly associated to relapse (p=0.01, 0.04 and 0.004, respectively). At 4 years, DFS for patients MRDneg vs MRDpos and with WBCc <50 vs >50 x 10e-9/L was 71% vs 45% and 60% vs 22% (p=0.015 and 0.014, respectively). Accordingly, cumulative incidence of relapse (CIR) at 4 years for patients MRDneg vs MRDpos and with WBCc <50 vs >50 x 10e-9/L was 20% vs 56% and 34% vs 82% (p= 0.003 and 0.001, respectively). Therefore, we identified 3 different groups of patients based on the combination of MRD status after consolidation and WBCc. At 4 years, DFS for MRDneg/WBCc <50 x 10e-9/L, MRDpos/WBCc <50 x 10e-9/L and MRDposWBCc >50 x 10e-9/L was 72%, 56% and 22%, respectively (p=0.007) and CIR at 4 years was 18%, 43% and 82% (p<0.0001). Combined evaluation of WBCc and post-consolidation MRD status enables identification of patients at higher risk of relapse in spite of a favorable risk genetics/cytogenetics profile for whom intensification by AlloSCT should be considered.



C016

WT1 TRANSCRIPTS PREDICT AML RELAPSE AFTER ALLOGENEIC TRANSPLANTATION: KINETICS CALLS FOR A MONTHLY MONITORING IN HIGH RISK PATIENTS

Sala E, Messina C, Tresoldi C, Tassara M, Crotta A, Malato S, Forno B, Giglio F, Vignati A, Mattarucchi R, Corti C, Peccatori J, Ciceri F, Bernardi M

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Introduction: several approaches have been used in AML and MDS patients (pts) in complete remission to detect minimal residual disease (MRD) and predict the risk of relapse. The Wilms' tumor gene 1 (WT1) is over-expressed in > 80% of AML and advanced MDS, making this molecule an ideal marker for MRD monitoring. We analysed the kinetics of WT1 quantitative expression after allogenic hematopoietic stem cell transplantation (HSCT) in pts at high risk of relapse. Aim: to correlate the kinetics of WT1 expression in bone marrow (BM) of AML/MDS pts with relapse occurrence after HSCT. Materials and methods: between 12/2007 and 10/2010, 57 pts (46 AML and 11 MDS) received HSCT (13 MRD, 18 MUD, 26 MMRD) after a myeloablative conditioning. During a median follow-up (FU) of 6 months (2-26), WT1 in BM was quantified using real-time PCR (RQ-PCR), with TaqMan technology on RNA from mononucleated cells. The housekeeping gene ABL was used as control gene, with WT1 level being normalized to 10e4 copies of ABL per sample. WT1 expression was monitored considering as MRD negative values < 180 WT1 per 10e4 ABL. Results: In 19 pts out of 57 (33%) an hematological relapse occurred. At day + 30 after HSCT all 19 pts were in complete remission (CR), 8 pts with BM WT1 < 180 and 11 with BM WT1 > 180. 10 pts out of 19 (53%) showed an increase of BM WT1 levels above 180 at day +160 (median, range 60-710) after HSCT, 40 days (median, range 20-70) before overt relapse; at the same time-point, we documented hematologic and cytogenetic CR and chimerism analysis (Single Tandem Repeat) showed 100-95% donor component. In 6 pts out of 19 (32%) BM WT1 was <180 105 days (median, range 80-130) before relapse; unfortunately, this group skipped 1 or 2 point of FU for medical decision. 3 pts out of 19 (15%) relapsed within 50 days post HSCT and are not informative as only the day 30 BM evaluation was performed. Conclusions: post HSCT kinetics of WT1 in BM can predict relapse in AML/MDS pts. A quantitative increase above the threshold of MRD positivity was detected about one month before hematological AML/MDS relapse, in our pts. Our results suggest that monthly quantitative monitoring of BM WT1 with RQ-PCR is useful to detect early relapse and to drive proper post transplant immunotherapy (e.g. immunosuppression withdrawal or donor lymphocyte infusion) and induce an anti-leukemia effect at an early stage.

C017

ROLE OF DEATH CELL-RECEPTORS PATHWAY IN APOPTOSIS INDUCED BY THE HISTONE DEACETYLASE INHIBITOR SODIUM BUTYRATE IN NPM1-MUTATED AML CELLS

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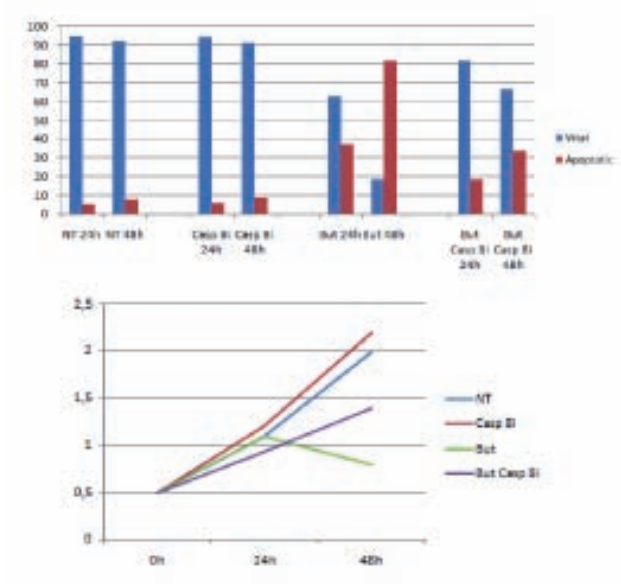
AML carrying NPM1 mutations [Falini B et al, NEJM 2005;352:254-266] accounts for about one-third of adult AML, shows distinctive biological and clinical features [Falini B et al, Blood 2007;109:874-885] and has been included as a provisional entity in the 2008 World Health Organization (WHO) classification of myeloid neoplasms. In spite of the relatively good prognosis of NPM1-mutated AML, there are still cases that show poorer outcome, especially those associated with FLT3-ITD mutation and elderly patient population. Therefore new therapeutic strategies need to be explored. Here, we investigated the effect of sodium butyrate, a short-chain fatty acid which has long been known to be a histone deacetylase inhibitor (HDACi) able to induce maturation in normal and tumor cells, in cellular models of NPM1-mutated AML: i) the OCI/AML3 cell line, previously identified as a human AML cell line carrying cytoplasmic mutated NPM1 in the absence of FLT3-ITD; ii) primary AML cells originated from a patient with NPM1-mutated AML bearing FLT3-ITD mutation (MONT1) and propagated as cell line in NOD/SCID mice; and iii) primary AML cells from 4 NPM1-mutated AML patients at diagnosis. In either cell lines or patients' primary AML cells carrying NPM1 mutation, but not in the U937 or OCI/AML2 cell lines (not harboring NPM1 gene mutation) used as control, growth arrest and pro-apoptotic effects were evident after 24 hrs and marked after 48 hrs of treatment with doses of drug of 0.5-1 mM. In particular, no signs of differentiation were evident at morphological examination of treated cells. Interestingly, induction of apoptosis was associated with activation of caspase-8, suggesting involvement of the death cell receptors pathway. Indeed, flow cytometric analysis showed increased expression of TRAIL-receptor DR5 upon drug treatment. Moreover, concomitant treatment with a specific caspase-8 inhibitor prevented cell growth arrest and markedly reduced apoptosis (Fig 1). Levels of either NPM1 mutant or wild-type protein did not appear significantly affected by treatment with sodium butyrate. Although these preliminary studies in vitro suggest a potential efficacy of drugs with HDACi effects against AML with NPM1 mutation deeper investigations are needed in order to characterize the molecular mechanisms of action responsible for this effect.



C018

FREQUENCIES OF CKIT MUTATIONS DETECTED IN PATIENTS WITH ACUTE MIELOYD LEUKEMIA (AML) CHARACTERIZED BY CORE BINDING FACTOR (CBF) REARRANGEMENTS

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More than 50% of AML patients with CBF rearrangement display cKit mutations, leading to the constitutive activation of the receptor. Indeed, the most common mutations (D816V/Y and N822K) involving ex17 are associated with IL3 independence growth and acceleration of the leukemogenic process. In particular, cKit missense mutations at codon 816 are described in 10-30% of patients with t(8;21) AML and are associated with a poor prognosis, a lower overall survival and event-free survival. We evaluate the presence of cKit ex17 mutations in our cohort of 52 patients with AML at diagnosis [33 Normal Karyotype (NK); 11 inv(16) and 8 t(8;21)] and 15 patients with mastocytosis. In particular, we carried out six ARMS-PCRs to specifically amplify D816Y/V and N822K mutations and PCR products were analyzed by DHPLC. We detected the described cKit mutations in 6% NK AML patients, 27% patients with inv(16), 25% patients with t(8;21) and 60% patients with mastocytosis. In particular, the D816V mutation was detected in 12,5% in patients with t(8;21), in 9% of patients with inv(16) and in 6% of NK AML patients. The D816Y mutation was present in the t(8;21) and inv(16) AML at similar frequency (12,5% and 9%, respectively). In contrast, the N822K substitution was more frequent in patients with inv(16) (18%) than NK AML (3%) or t(8;21) (12,5%). Mastocytosis patients were characterized by D816V/Y mutations in 47% and N822K mutation in 13% of the cases. Clinical data showed that D816V but not D816Y and N822K is correlated with dismal outcome Notably, the only patient with t(8;21) AML showed both N822K and D816Y mutations and did not present any reduction of MRD. In conclusion, we demonstrated that a combination of optimized PCR strategies and DHPLC analysis may be efficiently applied for the detection of cKit mutation in AML patients and could be useful to address selected patients to a synergic approach of targeted drugs (such as Tyrosine Kynase Inhibitors) in combination and conventional chemotherapy.

THALASSEMIAS AND HEMOGLOBINOPATHIES

C019

IL28B SINGLE NUCLEOTIDE POLYMORPHISMS ARE ASSOCIATED WITH SPONTANEOUS VIRUS CLEARANCE, SEVERITY OF LIVER FIBROSIS AND SUSTAINED VIROLOGICAL RESPONSE TO INTERFERON IN THALASSEMIA MAJOR PATIENTS WITH HEPATITIS C VIRUS INFECTION

Di Marco V, Bronte F, Calvaruso V, Capra M, Borsellino Z, Maggio A, Concetta RM, Pitrolo L, Lo Pinto MC, Rizzo M, Fiorenza F, Gerardi C, Grimaudo S, Di Cristina A, Levrero M, Craxì A

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Several studies showed that single nucleotide polymorphisms (SNPs) at the IL28B locus are important determinants in the control of Hepatitis C Virus infection. We assessed in 301 Thalassemia Major patients (18.6% not infected, 32.6% with spontaneous virus clearance, 48.8% with HCV chronic hepatitis) the association between the genotypes of the rs8099917 and rs8099860 SNPs and spontaneous virus clearance, severity of liver fibrosis, and response to Interferon. T/T genotype (OR 2.130; p=0.008) of rs8099917 and C/C genotype (OR 2.425; p=0.001) of rs8099860 SNPs were significantly associated with spontaneous virus clearance. Patients with severe liver fibrosis were older (OR 1.058; p=0.01) and more frequently carried the G/T and G/G genotypes of rs8099917 (OR 3.962; p=0.001) or the C/T and T/T genotypes of rs8099860 (OR 3.494; p=0.005), regardless the liver iron concentration. T/T genotype of rs8099917 (OR 3.014; p=0.03) or C/C genotype of rs12979860 (OR 3.285; p=0.01) SNPs, with age (OR 0.902; p=0.001), female gender (OR 3.418; p=0.01) and virus C genotypes 2 or 3 (OR 4.700; p=0.007) were independently associated with Sustained Virological Response to IFN-monotherapy. In conclusion, the association of IL28B SNPs with HCV related events in Thalassemia Major patients supports the involvement of innate immunity and interferon lambda in the control of HCV infection during the first years of the life.

C020

HEMOGLOBIN RESPONSE TO THALIDOMIDE, HIGH-DOSE INTRAVENOUS I MMUNOGLOBULINS, AND MYCOPHENOLATE MOFETIL IN / -THALASSEMIC HOMOZYGOUS ADULT PATIENT

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A 56 year old -thalassemia homozygous female patient (genotype: °/° - /-) with clinical characteristics of thalassemia intermedia was admitted to our hospital because of severe anemia. She had undergone spleenectomy at the age of 28. The patient could not be treated with transfusions due to post-transfusion hemolysis since 2003. Therefore since that time she was treated with hydroxyurea. With this treatment her hemoglobin levels remained stable at 6-6.5 g/dl until a recent infectious complication and subsequent antibiotic therapy. Following this latter event her hemoglobin level dropped down to a bottom level of 1.8 g/dl. Very severe anemia (hemoglobin < 3 g/dl) lasted more than 2 months despite further hydroxyurea therapy.At admission hemoglobin was 2.6 g/dl almost completely due to fetal hemoglobin (HbF = 98% - HbA2 = 2%); direct and indirect Coombs' test were positive; haptoglobin was barely detectable.Hydroxyurea was stopped and thalidomide, 75 mg/day, was promptly started. Two further blood transfusion attempts were ineffective due to severe post-transfusion hemolysis.Two weeks later, at a level of Hemoglobin 2.2 g/dl, we added to current thalidomide therapy high-dose intravenous poly-specific immunoglobulins (400 mg/kg/day for five consecutive days every three weeks) plus mycophenolate mofetil (500 mg/day) .After few days a slow but progressive increase in hemoglobin levels was observed. At 50 days the patient's

hemoglobin level has reached 6.9 g/dl with evident clinical benefit. This hemoglobin improvement was due by increase of fetal hemoglobin only. Recently an impressive increase of hemoglobin in a thalassemia major patients has been reported by the Monza group after thalidomide therapy. Despite our patient was treated with other immunosuppressive drugs because of the hemolytic anemia this case underline the potential efficacy of thalidomide in this extreme conditions. Further follow up and further studies should discriminate different drugs role in hemoglobin increase.

C021

DONOR'S NK CELLS MAY INFLUENCE THE ENGRAFTMENT IN PEDIATRICS PATIENTS AFTER T-CELL DEPLETED HAPLOIDENTICAL STEM CELL TRANSPLANT FOR THALASSEMIA

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The infusion of alloreactive NK cells in haploidentical transplant 1) ablates recipient T cells which reject the graft, and 2) ablates recipient dendritic cells (DCs) which trigger GvHD, thus protecting from GvHD. NK cell alloreactivity also boosts very rapid rebuilding of donor adaptive immunity to infections. In this study we analysed the potential role of NK cells after haploidentical transplant in beta-thalassemia patients. A total of 11 pediatric patients with beta-thalassemia received T and B cell depleted transplants from their haploidentical mothers. T and B cell depletion was carried out with CD34+ CliniMACS selection (Miltenyi Biotec©) from peripheral blood and bone marrow of donors (the mothers) and resulted in grafts consisting of stem cells and effector cells (NK cells, monocytes) with the addition of bone marrow mononuclear cells (BMMNCs 3 x 10^5/kg of the recipient). To analyse the mechanisms involved in immunological reconstitution post transplant, we analysed T cell subsets by flow cytometry, particularly NK cells at day + 20 and + 60 post transplant. NK cells were among the first lymphocytes to repopulate the peripheral blood, and up to 70% of these cells were CD3-CD56+bright cells. Interestingly, a direct correlation has been observed between the percentages of CD56+CD16+ NK subset and the BM engraftment (in mean 71 ± 21% CD56+CD16+ in the four patients with full engraftment, 27 ± 28% in the three patients with a stable mixed chimerism after BM transplant (70-80% of donor cells) and 1.4 ± 1% in the four patients with rejection). In all the patients the origin of the NK subsets was from the mothers. Day + 60 post transplant we observed an increase of the CD3-CD16+ NK cells (potent cytotoxic effector cells) especially in the patients with full engraftment (in mean 47 ± 20% vs. 28 ± 31% in mixed chimerism). We observed higher percentages of NK subsets just twenty days post transplant in the patients with full engraftment respect the mixed chimerism and the rejection, suggesting a role of donor NK cells on improved engraftment and on prevention of the rejection with the attack of the host lympho-hematopoietic cells. These observations may suggest the importance of NK subsets analyses at the first time of the transplant as an useful parameter for the outcome of the transplant and/or the use of the infusion of alloreactive NK cells especially in haploidentical recipients.

C022

CEREBROVASCULAR EVENTS IN SICKLE CELL DISEASE PATIENTS TREATED WITH HYDROXYUREA

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Stroke and Silent cerebral infarct (SCI) are the most common cause of brain damage in patients with Sickle-Cell- Disease (SCD). Approximately 10% of patients will have overt stroke. The prevalence of SCI is 21.8% in children (6 to 19 years old) with SCD . No recommended treatment has been so far established for the treatment of SCI, although possible benefits of blood transfusion management for SCI have been suggested. Hydroxyurea (HU) is considered to be the most successful drug therapy for severe SCD phenotype. Nevertheless, concerns remain regarding its role in the prevention of cerebrovascular events. We evaluated a population of 168 Sicilian patients with SCD (141 HbS/ß-thalassemia genotype, 27 HbS homozyguos) treated in our center, with MRI of the brain . 42/168 patient (25 %) had SCI and 7/168 (4,1 %) overt stroke. Also we report clinical data of 104 patients with SCD treated with hydroxyurea (HU) (mean age, 36; range, 18-53;) during 11-years mean follow-up. HU at a dosage of 20 mg/kg was started in patients with 3 or more sickle cell crises per year or acute chest syndrome. The dosage was increased by 5 mg/kg if this was judged to be appropriate and safe by the physician. A statistically significant reduction was observed in the number of sickle cell crises (86%, 7.8 ± 6.9 per year, $p < 0.0001$), hospitalizations (2.5 ± 2.9 per year vs. 0.3 ± 1.5, $p < 0.0001$), and days in the hospital (22.4 ± 21.9 per year vs. 0.3 ± 1.5, $p < 0.0001$). In 25 patients was performed MRI before the treatment with HU and then it was repeated periodically during HU therapy (mean follow-up, 11 years) , aimed at the detection of SCI or , eventually, at determining its progression. Progressive cerebral infarcts occurred in 72 % (18 of 25) of HU-treated patients. Notably, 3 overt strokes (1 of 3 relapse strokes) and 15 SCI were observed. . In these patients, neurocognitive deficits were observed. Moreover, stroke and SCI were not related to the HU clinical response. A large silent infarct was observed after 7 years of therapy in one excellent responder patient (from 12 to 0 crises/year). Therefore, HU therapy appear not to prevent these complications. Most likely, factors related to chronic inflammation and vascular wall damage are involved, and a new therapeutic approach is needed.

C023

SICKLE CELL DISEASE AND SICKLE BETA-THALASSEMIA: CLINICAL FEATURES

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BACKGROUND. Sickle Cell Disease (SCD) is one of the most common severe monogenic inherited disorder worldwide. The haemoglobin polymerization, leading to erythrocyte rigidity and vaso-occlusion, is central to the pathophysiology, as chronic anaemia, haemolysis, and vasculopathy are crucial for the clinical outcome. Similar findings are observed in Sickle beta-Thalassemia (SCD-T).

SCD and SCD-T are multisystem diseases, associated with episodes of acute illness and progressive organ damage. Aims. The objective of this retrospective study is to compare the clinical findings of the SCD-T compared to those of SCD in a cohort of patients followed at Hereditary Anemia Centre, Foundation "Ca' Granda" Policlinico, in Milan. METHODS. Mutation analysis of the beta-globin gene was established by direct DNA sequencing. Clinical and haematological features were evaluated by routine tests and physical examination, according to guidelines for SCD follow-up. RESULTS. Forty-three SCD-T and 16 SCD patients were studied; the beta-mutations detected in SCD-T were severe (beta°) in 86% of the patients, moderate (beta+)in 11.5% and mild (beta++) in 2.5%.

The mean age was 38±10.5 years, and the mean follow-up was 20±6 years. In SCD group 5/16 and in SCD-T group 17/43 were male. In SCD-T, the mean of HbF was 10.1±7.1 and in SCD 7.3±5.4, while the total Hb was around 95 g/l similar in both groups.

Comparing SCD-T and SCD patients, there were not statistically significant differences in the prevalence of clinical manifestations, including stroke, leg ulcers, priapism, bone pain crisis, except for splenomegaly (in SCD-T 50 %) and splenectomy (SCD-T 49% splenectomized vs 12.5 % SCD; p-value < 0.001). In SCD 87.5% had functional asplenia. Splenic infarctions were present only in SCD-T patients: 5/11 patients (45%) with splenomegaly and 2/11 patients (18%) with normal spleen (p value < 0.001); no spleen lesions were detected in SCD patients. All the patients were only occasionally transfused and all the patients with normal spleen started blood transfusion in childhood; 18/43 SCD-T patients (41.8%)e 6/16 (37.5%) SCD patients were treated with hydroxyurea initiated in the adult life.

Conclusions. These data suggest that T-SCD patients, particularly those with severe beta mutations have similar clinical course than SCD-patients. T-SCD were more transfused than SCD patients: transfusions prevented splenomegaly and spleen infarct. SCD patients were asplenic.

C024

MATCHED RELATED ALLOGENEIC TRANSPLANTATION IN BETA-THALASSEMIA: RESULTS IN 58 PATIENTS FROM COUNTRIES WITH LIMITED RESOURCES

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Hematopoietic stem cell transplantation (HSCT) from a matched family donor (MFD) offers a hope for cure to beta thalassemia patients (Bthal pts) from countries with limited resources. Between 2005 and 2009, 58 consecutive Bthal pts underwent HSCT from a MFD at our center (56 from an HLA-identical and 2 from a pheno-identical family donor). Median age 8 years (2-17), origin: Lebanon (9), Iraq (19), Palestine (3), Syria (22), Egypt (2), other (3). Two pt were class in I, 28 in class II and 28 in class III. Class I-II pts were conditioned with ivBu-Cy 200 mg/kg (+TT 10 mg/kg if <4 years). Class III pts were conditioned with ivBu-Cy 160 mg/kg-Flu 100 mg/m^2 or, from April 2007, ivBu-Cy160mg/kg-Flu 150 mg/m^2-ATG7.5 mg/kg(Thymoglobuline®) (Chiesa et al, BBMT 2010). GVHD prophylaxis consisted of CyA and short Mtx (+ methylprednisolone for 30 days for pts not receiving ATG). The source of stem cells was unmanipulated BM. Fifty-four pts engrafted donor cells. Four class III pts had primary graft failure, among these 2 died in aplasia and 2 had autologous reconstitution (1 cured by 2ndHSCT, 1 chose medical management). The incidence of acute GvHD II-IV was 8/54 (5 grade II, 2 grade III, 1 grade 4) and mortality 1/54 evaluable pts. Six pts had secondary graft failure and were all rescued by 2ndHSCT. Nine pts developed mild chronic GVHD, among these 2 had vitiligo as only manifestation. Pts were discharged to their country between 6 and 12 mo following HSCT. A follow-up phone contact at a median of 32 months from HSCT (11-61), evidenced that one Iraqi patent experienced late graft rejection and died of cardiovascular failure due to lack of transfusion support. All other pts are alive with 100% Lansky score and have returned to school. With the exception of non-resolved vitiligo, only 2 pts, transplanted from the pheno-identical mother, currently off chronic immunosuppressant, still have signs of chronic GVHD evidenced by xerostomia, atrophy and ulcers of the buccal mucosa. Overall survival is 93% (96% in class I-II, 89% in class III), current thal free survival 91% (96% in class I-II pts and 85% in class III). In conclusion, in Bthal pts from countries with limited resources with a MFD the risk-benefit balance is in favor of HSCT. It is particularly beneficial to offer HSCT from a well MFD to pts with less advanced disease, as the incidence of mortality and graft failure following a myeloablative condition is particularly low.

CHRONIC LYMPHOCYTIC LEUKEMIA I

C025

STEREOTYPED B-CELL RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA: A BIOLOGICAL AND CLINICAL COMPENDIUM FROM A MULTICENTER ITALIAN COHORT

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Background.: Highly homologous B-cell receptors, characterized by non-random combinations of immunoglobulin heavy-chain variable (IGHV) genes and heavy-chain complementarity determining region-3 (HCDR3), are expressed in a recurrent fraction of patients affected by chronic lymphocytic leukemia (CLL). Materials and methods. We investigated the IGHV status of 1131 productive IG rearrangements from a panel of 1126 CLL patients included in a multicenter Italian study group. The analysis of stereotyped subsets was performed based on previously reported criteria (Stamatopoulos et al. Blood 2007). A pair-wise alignment of sequences was adopted in order to discover novel potential subsets (HCDR3 identity > 60%). We correlated the presence and type of HCDR3 stereotyped subsets with the major cytogenetic alterations evaluated by FISH, molecular prognostic factors, and the time to first treatment (TFS) of patients with early stage disease (Binet A). Results. Stereotyped HCDR3 sequences were found in 357 cases (31.7%), 231 of which (64.7%) were unmutated. In addition to the previously described subsets, 31 new putative stereotypes subsets were identified. A significant association between different stereotyped HCDR3 sequences and prognostic factors, such as CD38 and ZAP70 expression, IGHV mutational status and genomic abnormalities was found. In particular, deletion of 17p13 was significantly represented in stereotype subset #1 (5/18;27.8%). Notably, subset #1 was significantly correlated with a substantially reduced TFS compared to other patients, independently of the occurrence of other unfavorable prognostic factors (ZAP-70 and CD38 high expression, UM IGHV and unfavorable cytogenetic lesion, including del(17)(p13). Moreover, subset #2 was strongly associated with deletion of 13q14 (12/13; 92%), subsets #8 and #10 with trisomy 12 (5/7 and 6/7 respectively) whereas subset #4 was mainly characterized by the absence of the common cytogenetic abnormalities (13/23; 56.5%). Conclusions. Our data from a large panel of CLL patients indicate that particular stereotyped HCDR3 sequences are associated with specific cytogenetic lesions and a distinct clinical outcome.

C026

NORMAL CYTOGENETICS AND DELETION 13Q14.3 ARE CHARACTERIZED BY MARKED BIOLOGICAL AND CLINICAL HETEROGENEITY IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Del Principe MI, Rossi FM,[*] Coletta A, Simotti C, Biagi A, Zucchetto A,[*] Cox MC, Maurillo L, Buccisano F, Niscola P, Dal Bo M,[*] Catalano G, Venditti A, de Fabritiis P, Perrotti AP, Amadori S, Gattei V,[*] Del Poeta G

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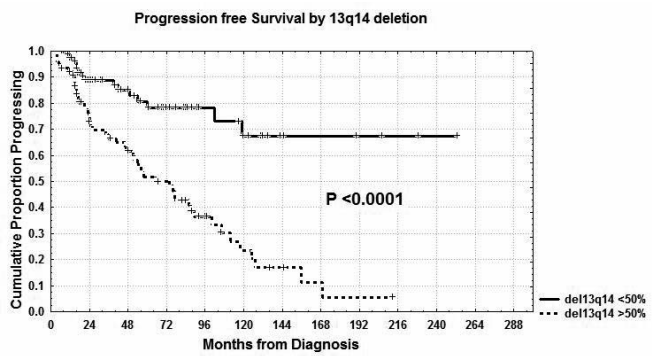
Interphase fluorescent in situ hybridization (I-FISH) with specific probes is used to detect cytogenetic abnormalities in CLL. Moreover, 13q14.3 deletion is a favourable prognostic biomarker when detected as

a sole abnormality, even if a higher percentage of 13q- nuclei was found to be associated with a shorter time to treatment. The primary endpoints of our study were: 1) to determine progression free survival (PFS) and overall survival (OS) within the apparently homogeneous normal karyotype on the basis of ZAP-70, CD38 and IgVH status; 2) to correlate percentages of 13q- nuclei (<50% or >50%) with PFS and OS and 3) to confirm I-FISH as an independent prognostic factor. We investigated 442 pts with a median age of 66 years whose 129 pts had a modified low Rai stage, 294 an intermediate and 19 a high stage. One hundred fifty pts showed a normal karyotype, 160 pts an isolated 13q-, 55 pts trisomy 12, 43 pts 11q deletion, 24 pts 17p deletion and 10 pts other abnormalities. Patients with intermediate/poor cytogenetics (trisomy 12, del11q-, del17p-) showed significant shorter PFS and OS (6% vs 33% at 16 years, P<0.0001 and 47% vs 66% at 16 years, P=0.0003) in comparison with normal and del13q- pts. Therefore, we analysed ZAP-70, CD38 and IgVH status within the normal karyotype. Interestingly, we observed significant longer PFS for ZAP-70 negative pts (58% vs 29% at 10 years, P=0.00001), for CD38 negative pts (52% vs 23% at 10 years, P=0.0004) and for IgVH mutated cases (61% vs 33% at 10 years, P=0.0002). Moreover, pts with isolated 13q- in <50% of nuclei (84 pts) showed a longer PFS and OS (67% vs 6% at 16 years, P<0.0001 and 91% vs 43% at 16 years, P=0.05) compared to those with >50% of nuclei (76 pts). Noteworthy, 43 (57%) of 76 of 13q- >50% pts had received chemotherapy at the time of analysis (P<0.0001). In multivariate analysis of PFS, I-FISH cytogenetics was an independent prognostic factor (P=0.002) together with Rai stages (P=0.007), ZAP-70 (P=0.00003) and IgVH status (P=0.00001). Within del13q- subgroup, multivariate analysis of PFS confirmed the percentage of nuclei (P=0.008) together with IgVH (P=0.0006) and ZAP-70 (P=0.01) as independent prognosticators. Therefore, ZAP-70, CD38 and IgVH are irreplaceable in order to better define prognosis in pts with normal karyotype, probably presenting abnormalities undetectable by FISH, and the percentage of nuclei exhibiting 13q- has to be considered as an important predictor of the outcome in CLL.



C027

PROGRESSIVE TELOMERE SHORTENING OCCURS DURING THE NATURAL HISTORY OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND ASSOCIATES TO OUTCOME.

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Telomere length(TL) at diagnosis has been established as an independent outcome predictor in CLL(Rossi D.2009). However data on TL dynamics over time are scant and anecdotal. Aims were to assess telomere dynamics in the natural history of CLL. This issue has been here addressed on a series of 86 CLL patients(pts) with a median follow-up of 83 months(range:35-284).TL was analyzed as previously described(Rossi D.2009; Ladetto M.2004) at diagnosis and during subsequent phases of CLL history with a median time between determinations of 43 months(range 12-231). Between the determinations 23 pts were treated while 63 were in "watch and wait"(WW).Among WW pts, 15 received treatment after the second TL assessment(median 40 months, range:12-181).Biological, clinical features and detailed clinical history were available in all pts.Telomere loss was calculated in terms of absolute(AL) and yearly loss(YL).Comparisons and treatment-free-survival(TFS) analysis were made using the Mann-Whitney or Wilcoxon t-test and the Kaplan-Meyer method.Telomeres were shorter at follow-up compared to baseline with a median loss of 669bp(range +493 bp,-5874 bp;p<0.001).AL and YL were greater in cases with higher baseline TL while those with short telomeres at diagnosis had only modest additional erosion(p=ns for those in the 25th lowest percentile).AL and YL did not correlate with any clinical parameter with the exception of a positive association with IGHV-MS(p<0.05).We then restricted our analysis to WW pts to assess the impact of telomere-erosion on TFS.As expected the validated cut-off value of 5000bp for baseline TL(Rossi D. 2009) identified a subset of pts(25.3%) with an inferior TFS(median TFS 40 months vs 181 months;p<0.001). Surprisingly, also an YL above the median value(-293bp) was predictive of a poor TFS(median TFS 62 months vs 181months;p<0.01).Pts having at least one of these two features(TL<5000bp;YL>-293bp) had a considerably shorter TFS compared to those with long and stable TL(median TFS 50 months vs not reached;p<0.001).The latter population had indeed only two events for TFS at 107 months and 181 months.The results of the first systematic analysis on TL dynamics in CLL indicate: a)progressive telomere erosion occurs as part of the natural history of CLL;b)YL is more pronounced when baseline TL is higher; c)YL associates to an inferior TFS despite being more common among IGVH-mutated patients; d)pts with long and stable telomeres have an excellent long-term outcome.

C028

AN ITALIAN RETROSPECTIVE STUDY ON THE ROUTINE CLINICAL USE OF LOW-DOSE ALEMTUZUMAB IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: Alemtuzumab given at lower dose has shown a favorable toxicity profile coupled with good results in terms of efficacy in relapsed/refractory chronic lymphocytic leukemia (CLL). Aim: We conducted a multicenter retrospective study on the routine clinical use of low-dose alemtuzumab in relapsed/refractory CLL. Methods: One hundred eight relapsed/refractory CLL patients (pts) from 11 Italian centers were included in the analysis. Low-dose alemtuzumab was defined as a total weekly dose <45 mg and a cumulative dose <600 mg given for up to eighteen weeks; both subcutaneous (SC) and intravenous (IV) administrations were allowed. Results: Median patient age was 68 years (range 43-85), 50 pts (46%) were in Binet stage C, bulky lymph nodes (>5 cm) were present in 17 pts. An unfavorable cytogenetic profile, as presence of del(17p) or del(11q), was detected in 43/97 (44%) pts; CD38 and ZAP70 were expressed by 47/92 (51%) and 48/80 (60%) pts respectively; 33/49 (67%) pts were characterized by an unmutated IGHV configuration. The median number of previous lines of therapy was 2 (1-6); 75% of pts were previously exposed to chlorambucil (60% refractory) and 60% to fludarabine (74% refractory). Four treatment schedules of alemtuzumab were employed; most pts (95%) received a weekly dose of 30 mg either divided into three 10 mg administrations or given as a single dose. In 94 cases, alemtuzumab was given SC. The overall response rate (ORR) was 56%, including 22% of CRs. After a median follow-up of 42.2 months (2.1-91.9), the median OS and PFS were 39 months (95% CI 29.8-58.4) and 19.4 months (95% CI 15.9-23.6), respectively. In univariate analysis, response was inversely associated to lymph node (p=.01) and spleen (p=.02) size, fludarabine-refractoriness (p=.01),

previous treatment lines (p=.01), presence of del(11q) (p=.009). Presence of del(17p) and advanced age (>70 years) were not associated to a worse outcome. Cumulative dose of alemtuzumab administered did not influenced response. Grade 3-4 neutropenia, thrombocytopenia and anemia were reported in 29%, 6% and 6% of pts, respectively. Severe (grade 3-4) infections occurred in 7 pts (7%) during therapy. CMV reactivation was documented in 37 pts (34%), with only 1 case of CMV infection. Conclusions: This retrospective analysis shows that alemtuzumab given at lower doses is a valid and currently used therapeutic option for the treatment of relapsed/refractory CLL, in particular in elderly and frail pts.

C029

EXPRESSION OF TELOMERIC PROTEINS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Severe telomere erosion in CLL is an independent predictor of poor outcome(Rossi D.2009).Telomere protection and function are ensured by several proteins(TP):the DNA binding shelterin complex (TRF1, TRF2, TINF2, hRAP1, TPP1,POT1);the telomere elongation proteins (hTERT, hEST1A, DKC1); the telomeres DNA-repair proteins (RPA1, KU80, RAD50, MRE11).The expression of TP has been so far investigated on 42 patients (pts)(Poncet D.2008)showing severe deregulation of different key actors. Aims were to verify previous results and correlate TP expression with: telomere length(TL); clinical features (binet, lymphocytosis); biological features (IgVH mutational status, Zap70, CD38, cytogenetics); treatment-free survival(TFS)(available in 40pts).70 CLL pts characterized in terms of clinical and biological features were studied. Pts evaluable for TFS had a median follow-up of 63 months(range: 2-194).Controls were 11 healthy subjects(HS) and 5 cell lines(CL) of lymphoid tumors.All samples underwent CD19 selection to reach a purity of at least 85%.The expression of the above mentioned TP was investigated while BCL-2,Zap-70,Ki-67 were used as controls proteins (CP).For greater accuracy experiments were done on a 20 CLL and 8 HS using: a)SybrGreen method(Poncet D.2008); b)TaqMan gene expression Assays (Applied Biosystem).Based on superimposal results the latter method was then used in the whole series.Statistical and survival analysis were made using the Mann-Whitney t test and Kaplan-Meyer method. As expected CP were over-expressed in CLL. Expression of TRF1, TINF2, TPP1, POT1, DKC1, RPA1, KU80, RAD50,was significantly higher in CLL compared to HS.Other TP (TRF2, hRAP1, hTERT, hEST1A, MRE11) had comparable expression levels.Compared to CL, CLL under-expressed POT1, hTERT and DKC1.CLL with TL<5000bp were associated positively with TRF2, TPP1, RAD50, MRE11 and negatively with hEST1A.Advanced Binet associated with higher TRF1 and lower hEST1A expression.Trends were found between TP expression and other features.Using the median value as cut-off we noticed a borderline association with an inferior outcome for pts expressing high levels of TRF2(p=0.05) and low level of hEST1A(p<0.05). In CLL we observed a particular pattern of TP expression that might play a role in progressive telomere disruption. The overexpression of proteins recruiting DNA repairing mechanisms might further increase telomere instability and contribute to the formation of telomere dysfunction-induced foci which are known to occur in CLL.

C030

DE NOVO 11Q DELETION IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL HETEROGENEITY AND PROGNOSIS

Marasca R, Maffei R, Martinelli S, Fiorcari S, Bulgarelli J, Debbia G, Rossi D, [1] Francesca R,[2] Rigolin GM,[3] Coluccio V, Zucchini P, Cuneo A,[3] Gattei V,[2] Del Poeta G,[4] Laurenti L,[5] Forconi F,[6] Montillo M,[7] Gaidano G[1]

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Interstitial deletions on the long arm of chromosome 11 (del 11q) occur in 15-20% of all chronic lymphocytic leukemia patients (CLL). To define clinical and biological parameters associated with different clinical outcome in CLL patients with de novo 11q deletion, we retrospectively studied 131 CLL patients, harbouring 11q- before treatment, collected from several Italian Institutions. The mean age at the time of 11q documentation was 66 years (range, 27-88). IGHV unmutated genes, CD38 and ZAP70 positivity were detected in 68%, 32% and 52% of patients respectively. The mean percentage of 11q- nuclei was 52% (range, 6-99) and patients with complex karyotypes (11q deletion plus other FISH abnormalities) represented 71% of our cohort. One hundred eleven patients were assessable for evaluation of time to first therapy (TTFT). Overall, 71/111 (64%) patients have been treated accounting for a median TTFT of 19 months. Factors significantly associated with short TTFT were advanced Binet stage, white blood cell (WBC) count at least 50x109/L, high b2-microglobulin levels, more extensive lymphadenopathy and splenomegaly ($p < 0.001$ in all instances). Patients with sole 11q deletion and patients with complex karyotypes were not different in term of time to treatment. Moreover, patients with at least 25% deleted nuclei experienced shorter TTFT (median TTFT, 14 months in CLL with 11q deleted nuclei > 25% and 40 months in CLL with 11q deleted nuclei < 25%, $p<0.01$). Multivariate analysis identified 4 risk factors independently associated with reduced TTFT: advanced Binet stage (HR 8.0; p=0.0001), 11q- nuclei more than 25% (HR 4.3, p=0.007), high WBC count (HR=2.2, p=0.042) and high b2-microglobulin levels (HR 2.4,p=0.040). Increased frequencies of 13q deletion, IGHV mutated genes and early clinical stages were found in CLL with 11q < 25% of nuclei ($p < 0.05$ in all instances). At the time of analysis, 33 patients (25%) had died with a median overall survival (OS) from 11q- documentation of 116 months. Factors significantly associated with reduced OS on univariate analysis were age at least 60 years, advanced stage, extensive lymphadenopathy, splenomegaly and CD38 positivity. Multivariate analysis identified age at least 60y (HR 8, p=0.003) and splenomegaly (HR=7,$p < 0.0001$) as factors independently associated with reduced OS. In conclusion, CLL patients with 11q deletion exhibit clinical heterogeneity that can be predicted by several clinical and biologic characteristics.

HEMOSTASIS AND THROMBOSIS

C031

ALL-TRANS RETINOIC ACID (ATRA) DOWNREGULATES ACTIVATED FACTOR VII-ANTITHROMBIN COMPLEX (FVIIA-AT) LEVELS IN ACUTE PROMYELOCYTIC LEUKEMIA (APL) PATIENTS

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APL patients present with laboratory signs of disseminated intravascular coagulation. Clinical manifestations include both severe hemorrhage and thrombosis. In this setting, a key role is played by promyelocytic procoagulants, including Tissue Factor (TF). Differentiation therapy with ATRA induces complete remission of >90% patients while rapidly resolving the associated coagulopathy. In this study, we prospectively evaluated, in newly diagnosed APL patients, the plasma levels of FVIIa-AT complex, a new parameter which may reflect the degree of intravascular TF exposure and inhibition. Plasma levels of TFPI, TAT complex and D-dimer, and TF mRNA expression by peripheral mononuclear cells (PBMC), were also evaluated. Fifty-four patients were consecutively enrolled in the AIDA 2000 prospective registry (GIMEMA protocol) of major hemorrhage and thrombosis in APL patients receiving ATRA+Idarubicin: 8 had early major hemorrhages (3 fatal), 3 presented with thrombosis (1 fatal Budd-Chiari syndrome), and 2 developed thrombosis during induction therapy. Blood samples from 26 (F/M= 10/16) of these patients were obtained at the following time intervals: at baseline (before ATRA, D0), and on days 7, 15 and 25 (D7, D15, D25) after starting ATRA. Twenty-five healthy subjects acted as control group. At D0 FVIIa-AT levels were significantly higher in APL compared to controls ($p<0.05$), remained elevated at D7 and D15, but dropped at D25 ($p<0.05$ vs D0). A persistent elevation of TFPI from D0 to D15 was also observed. Differently, TAT and D-dimer levels, significantly elevated at D0 compared to controls, continuously decreased starting from D7 ($p<0.05$ vs D0) until D25. The levels of PBMC TF mRNA were significantly elevated at D0 and were progressively downregulated by ATRA until D25. The elevated FVIIa-AT levels we found in APL may reflect a persistent increase of TF exposure by leukemic cells. However, the parallel decrease of hypercoagulation markers under ATRA therapy, combined with high TFPI levels, suggests that cellular clotting activation at the cellular site is efficiently controlled by FVIIa-TF inhibition by indirect (AT) and direct (TFPI) inhibitors. This study suggests that monitoring FVIIa-AT complex levels is important in understanding the mechanisms of the coagulopathy of APL and might help to identify APL patients with persistent TF/FVIIa activation, who may carry an increased thrombotic risk.

C032

THE OPTIMAL DURATION OF ANTICOAGULANT THERAPY IN PATIENTS WITH CANCER-RELATED DEEP VEIN THROMBOSIS: THE ADVANTAGE OF USING RESIDUAL VEIN THROMBOSIS (THE CANCER-DACUS STUDY)

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Introduction. 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). Objective of the study. 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. Materials and Methods. Patients with active cancer and a first episode of DVT were treated with LMWH for 6 months (the first month at full dosage followed by dose reduction of 25% in the next 5 months). At the end of treatment, they were managed according to RVT findings: those with RVT were randomized to continue anticoagulants for 6 additional months (Group A1) or to stop it (Group A2), while patients without RVT stopped LMWH (Group B). Outcomes were recurrent venous thromboembolism and/or major bleeding; patients were followed up for one year after LMWH discontinuation. Results. Over a period of 36 months, 409 patients were evaluated; 62 were excluded (refusal, need for continuing anticoagulation, etc). In total, 347 were included in the study. RVT was detected in 242 (69.7%) patients; recurrent events occurred in 21.9% of those randomized to discontinue and 14.2% of those who continued LMWH. In patients without RVT (105, 30.3%), recurrent events occurred in 3 cases (2.8%)(Figure). The adjusted Hazard Ratio (HR) for age and sex between RVT groups (Group A2 vs A1) was 1.58 (95% confidence interval [CI], 0.85–2.93; P=.145). The adjusted HR between group A1 versus RVT-negative group (B) was 4.54 (CI 2.3–6.66; P =.028). Five major bleeding events occurred in Group A1 and two events both in Group A2 and B. Overall, 89 (25.6%) patients died due to cancer progression after a median follow-up of 10.2 months after heparin withdrawn. Conclusions.: The Cancer DACUS is the first ever study evaluating an individual marker for assessing duration of anticoagulation in active cancer population. Final results of the study show that absence of RVT identifies a group of patients at low risk for recurrent thrombosis who can safely stop LMWH after 6 months.

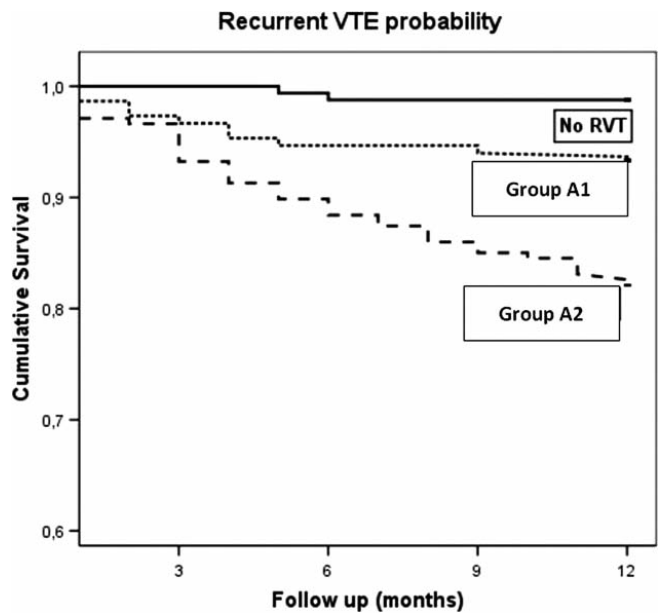

Figure 1. Kaplan-Meyer curve for recurrent events among groups

C033

IDENTIFICATION OF MOLECULAR BASIS OF ANTITHROMBIN DEFICIENCY: CLINICAL FEATURES OF 41 INVESTIGATED PROBANDS AND REPORT OF 16 NOVEL MUTATIONS

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More than 200 mutations of antithrombin (AT) gene cause type I (quantitative) or type II (qualitative) deficiency. Type II is further subdivided into reactive site (RS) or heparin binding site (HBS) or pleiotropic defects. Type II HBS is associated with a low thrombotic risk. DNA of

34 unrelated patients with venous thromboembolism (VTE) and of seven asymptomatic women was sequenced according to Picard et al. (Thromb Haemost 2005;93:57). Twenty-six different mutations were identified in patients with VTE. Four novel (Y260_P352del -two cases, E34X, W307X, R413KfsX20) and five known (C-4X, A94V, R129X –three cases, R132X, R425QfsX8 –two cases) mutations were found in 13 heterozygous patients with type I deficiency; two of them were heterozygous for factor V Leiden (FVL) (R129X) or prothrombin (PT) G20210A (C-4X). Four novel (E205K, E265K, D342G, E377D) and two known (M251I, R393C –five cases) mutations were found in ten heterozygous patients with type II RS deficiency; two of them (E377D, R393C) were heterozygous for FVL. Three known mutations (L270P, A404T, L409P -two cases) were found in four heterozygous patients with pleiotropic deficiency. Two known mutations (P41L, R47C) were found in two heterozygous patients with type II HBS deficiency and additional abnormalities (antiphospholipids in the former case, homozygous FVL in the latter). One novel mutation (E180K) was found in one heterozygous patient with type II HBS deficiency who had first VTE at 77 years of age. Three novel mutations with phenotype to be characterized were found in three heterozygous patients (L81V, R132G, L173R). Finally, one patient with type I deficiency was double heterozygous for two novel mutations, L210PfsX43 and G2R; the father heterozygous for L210PfsX43 had type I deficiency, whereas heterozygous G2R mutation had null effect in the mother. Among the asymptomatic women, three with type I deficiency had two novel (S250IfsX16 and V303CfsX13) and one known (C-4X) heterozygous mutations. Four with type II HBS deficiency had three known heterozygous mutations (R47H, R47C, L99F -two cases); one of them was heterozygous for PTG20210A (L99F). In four pregnant women (one type I and three type II HBS) antithrombotic prophylaxis was tailored according to the phenotype. In conclusion, the identification of the molecular bases of AT deficiency can provide data to understand AT structure-function and give advice for antithrombotic prophylaxis tailored according to the deficiency subtype.

C034

EFFICACY AND SAFETY OF ANTITHROMBOTIC PROPHYLACTIC THERAPY WITH STANDARD DOSE OF LOW-MOLECULAR WEIGHT HEPARIN (LMWH) AFTER ORTHOPEDIC SURGERY IN HEMOPHILIA PATIENTS

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Background. Deep venous thrombosis (DVT) is a common post-operative complication in normal subjects undergoing orthopedic surgery of lower limbs. Therefore, thromboprophylaxis with LMWH or other antithrombotic agents is strongly recommended. On the contrary, LMWH thromboprophylaxis after orthopedic surgery is still controversial in hemophilia patients (pts). Aims. To evaluate the efficacy and safety of post-operative anti-thrombotic therapy with LMWH in severe or moderate hemophilia A and B pts, treated with factor concentrates, undergoing orthopedic surgery of lower limbs. Methods. Twelve pts [severe/moderate hemophilia A: 9/1; severe/moderate hemophilia B: 1/1; median age 36 years (30-60)], underwent orthopedic surgery because of a significant hemophilic arthropathy. The following surgical interventions were performed: 3 knee replacements, 3 ankle replacements, 3 knee synovectomies, 2 ankle synovectomies, 1 femur fracture reduction. Recombinant FVIII or FIX were administered as bolus infusions at the following dosages: 1) joint replacement: 1 hour before surgery (t0): 100 IU Kg-1; from the 12th to 60th hour (t12h t60h) after surgery: 50 IU Kg-1 every 12 hrs; from the 3rd to 7th post-operative day: 40 IU Kg-1, 2 bolus day-1; 2) synovectomy: t0: 80 IU Kg-1; t12h t60h: 40 IU Kg-1, 1 bolus every 12 hrs; from the 3rd to 7th post-operative day: 25 IU Kg-1, 2 bolus day-1; 3) femur fracture reduction: t0: 100 IU Kg-1; from the 12th hour to the 7th post-operative day: 50 IU Kg-1, 1 bolus every 12 hrs, followed by tapering. All patients received post-operatively prophylactic antithrombotic therapy with enoxaparin at a dosage of 50 IU kg-1 day-1; the first administration of LMWH was performed at the 12th hour after surgery and was continued until complete mobilization of the pts (5-7 post-operative days); in case of femur fracture reduction, enoxaparin was given until plaster cast removal, 4 weeks after surgery. Results. Twelve severe/moderate hemophilia A/B patients underwent orthopedic surgery. They were prophylactically treated either with factor concentrates or with LMWH, and no bleeding or thrombotic complications were recorded. Conclusions. Adequate pre- and post-operative replacement therapy, enables the use of LMWH given at standard doses. Hence, thromboprophylaxis with standard doses of LMWH is effective and safe in hemophilia pts.

C035

IN FAMILIES WITH INHERITED THROMBOPHILIA THE RISK OF VENOUS THROMBOEMBOLISM IN THE CARRIERS IS DEPENDENT ON THE CLINICAL PHENOTYPE OF THE PROBAND

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Laboratory screening for inherited thrombophilia is recommended for patients with venous thromboembolism (VTE) or obstetric complications (OC). Universal screening (US) is discouraged, but a growing number of asymptomatic carriers of thrombophilia is identified by inappropriate laboratory testing; whether familial screening should be then pursued is debated. To assess the risk of venous thromboembolism (VTE) among the carriers, we investigated a family cohort of 1,720 relatives of 563 probands with thrombophilia who were evaluated as a result of VTE (n=1,088), premature arterial thrombosis (n=113), obstetric complication (n=257), or universal screening before pregnancy or hormonal contraception or therapy (n=262). In 45 families, the proband had antithrombin (AT), protein C (PC) or S (PS) deficiency; in the remaining 518 families, the proband carried factor V Leiden (FVL) and/or prothrombin G20210A (PTGA). Thrombophilia was detected in 968 relatives (56.2%); a first deep venous thrombosis (DVT) occurred in 44 carriers and 10 non-carriers during 37,688 and 29,548 observation-years from birth, respectively. The risk of DVT among the carriers compared with non-carriers was estimated as a hazard ratio (HR) using a Cox model. In carriers the risk for DVT was increased if the proband had VTE (HR 3.11, 95%CI 1.40-4.67), whereas was not if the proband was diagnosed because of premature arterial thrombosis, obstetric complication, or universal screening. If the proband had VTE and a deficiency of AT, PC or PS, the HR for DVT was 5.14 (95%CI 0.88-10.03) in the carriers overall, and 12.86 (95%CI 2.46-59.90) in those with AT deficiency. If the proband had VTE and factor V Leiden (FVL) and/or prothrombin (PT) 20210A, the HR for DVT was 2.26 (95%CI 0.98-5.10) in the carriers heterozygous for FVL, 0.65 (95%CI 0.17-2.73) in those heterozygous for PT20210A, and 5.54 (95%CI 3.20-187.00) in those homozygous or double heterozygous for FVL and PT20210A. In conclusion, familial screening for inherited thrombophilia is only justified for probands with previous VTE; in such setting, the risk for DVT is highest among relatives with AT deficiency and those homozygous or double heterozygous for FVL and PT20210A.

C036

SEQUENTIAL COMBINED BYPASSING THERAPY IN THE TREATMENT OF UNRESPONSIVE BLEEDING IN PATIENTS WITH ACQUIRED HAEMOPHILIA

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Acquired hemophilia A (AH) is a rare but severe autoimmune bleeding disorder, resulting from the presence of autoantibodies directed against clotting factor VIII (FVIII). The etiology of the disorder remains obscure, although most cases are associated with underlying conditions including pregnancy. An appropriate management of the acute bleeding episodes in patients with high titre inhibitors require the use of bypassing agents. Two bypassing agents are currently available and have been shown to be safe and efficacious in the treatment of bleeding episodes in patients with AH: the activated prothrombin complex concentrate (APCC), FEIBA and the recombinant activated factor VII (rFVIIa). However, whichever treatment is used initially, 10-20% of bleeding episodes cannot be controlled by the use of a single bypassing agent. Sequential combined bypassing therapy (SCBT) has been reported to be successful in the treatment of unresponsive bleeding in patients with haemophilia and high responding inhibitors to FVIII. We report our experience in two females with pregnancy-related AH who experienced severe bleedings and were successfully treated with SCBT. Patient 1 (age 28 y) suffered from a large spontaneous haematoma. She was firstly treated with high doses of rFVIIa (270 µg/kg every 6 h) without any clinical improvement and was shifted to the use of FEIBA (85 U/kg every 8 h) without no further success. SCBT with FEIBA (85 U/Kg every 6 hrs) alternated to rFVIIa (180 mg/Kg every 6 h) but resulted in a stable haemostatic response with improvement of clinical conditions, disappearance of pain, and no further requirement of red cell transfusions. Patient 2 (age 31 y) needed emergency surgery of ovariectomy. She was initially treated with rFVII (270 µg/kg) without effective haemostasis. FEIBA treatment was, therefore, introduced (85 U/kg) without haemostatic efficacy. Sufficient haemostasis was only achieved after introduction of SBCT with alternated injections of FEIBA (80 U/Kg) and rFVIIa (250 µg/kg) every 4-6 h. SCBT was discontinued after 7–14 days. No clinical adverse events were observed, but an increase in D-dimer levels was seen in both patients. We conclude that SCBT is an effective modality of treatment in patients with AH and unresponsive bleeding to a single bypassing agent, even if it represents a salvage treatment due to potential thrombotic risks. Further prospective clinical trials are needed in order to optimize the treatment.

MYELODYSPLASTIC SYNDROMES

C037

EFFECTS OF EXPERIMENTAL OVEREXPRESSION OF MITOCHONDRIAL FERRITIN IN NORMAL HUMAN ERYTHROID PROGENITORS AND IN THE K562 ERYTHROLEUKEMIC CELL LINE

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In refractory anemia with ring sideroblasts (RARS), the iron deposited in the perinuclear mitochondria of ring sideroblasts is present in the form of mitochondrial ferritin (FtMt), which is expressed in the early stages of erythroid differentiation. However, it is unknown whether FtMt overexpression is the cause or the result of mitochondrial iron deposition, and the mechanism of the regulation of FtMt expression remains unclear. Our aim was to investigate the possible influence of experimentally induced FtMt overexpression on erythroid differentiation and its capacity to induce a sideroblastic phenotype using a model system based on normal human hematopoietic progenitors. Lentivirus FtMt-transduced CD34+ bone marrow cells from 7 healthy donors were cultured for 21 days in a liquid medium with a cytokine cocktail according to a well established procedure that allowed the expansion of high numbers of erythroid progenitors and the in vitro production of erythrocytes. At various days samples of cultured cells were removed for biological studies. In addition, we analyzed the effect of the FtMt-transduction on iron metabolism and JAK2/STAT5 pathway activation in K562 erythroleukemic cells. Normal FtMt overexpressing erythroid progenitors were characterized by reduced cytosolic H ferritin content and increased CD71 expression, indicative for cytoplasmic iron depletion, and by an higher apoptotic rate in comparison with the FtMt negative controls (P=0.0001), whereas cell ability to terminally differentiate was not abrogated. The appearance of Perls positive ring granules was noticed in rare late cells, after prolonged iron exposure. Significantly lower levels of STAT5 phosphorylation following Epo stimulation were found in FtMt positive erythroid progenitors compared to FtMt negative cells (P=0.03). In the K562 cell line, FtMt overexpression reduced reactive oxygen species, STAT5 phosphorylation and the anti-apoptotic Bcl-XL transcript, and determined cytosolic iron starvation, whereas the transferrin receptor 1 transcript increased due to the activation of the IRE/IRPs machinery. In conclusion, experimental overexpression of FtMt may modify mitochondrial iron availability and lead to ineffective erythropoiesis, at least in part, through the inhibition of the JAK/STAT pathway, which is central to erythroid differentiation; the antioxidant properties of FtMt might play a role in this inhibition. Thus, our data appear relevant to the pathophysiology of RARS.

C038

IRON CHELATION THERAPY WITH DEFERASIROX IN TRANSFUSION DEPENDENT MYELODYSPLASTIC SYNDROME PATIENTS. REPORT FROM THE PROSPECTIVE MDS0306 GIMEMA TRIAL.

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The recent development of a safe and efficient once daily oral iron chelator (Deferasirox, Exjade) made possible regular chelation therapy in transfusion dependent MDS patients. However the reported clinical experience is limited to selected populations. For this reason the

GIMEMA group developed a phase IIIb prospective trial to test safety and efficacy of Deferasirox in a large population of patients comparable to general MDS population. One hundred and fifty-nine transfusion dependent IPSS low-intermediate1 risk MDS patients were enrolled. Baseline characteristics were (data are expressed as median unless otherwise specified): age 72 years (range 24 – 87); 48 patients were IPSS low risk and 75 Intermediate1; duration of transfusion dependency before treatment was 20 months (12-36) corresponding to 38 (22-70) packed red blood cells transfusions received. Baseline serum ferritin was 2000 ng/ml (1471-3000). Baseline Charlson and CIRS comorbity scores were 1 (0-1) and 0.2 (0.1-0.4), respectively. Patients started treatment with the standard 20 mg/kg Deferasirox but dose adjustments on clinical indications were allowed. Sixty-one patients (49%) prematurely interrupted the study (drop out), 62 (51%) patients completed the planned year of treatment. Serum Ferritin evolution in the 62 patients who completed the protocol showed a statistically significant decrement during the 12 months follow up (P< 0.001, median value of decreased was from 2000 to 1500 ng/ml). In multivariate model for drop out rate, high Charlson co-morbidity score >1 (Odds Ratio 1.45) and duration of transfusion dependency before chelation treatment >24 months (Odds Ratio 0.97) were significant risk factors for drop out. Drops out were mainly related to progression to acute leukemia (10 patients) MDS clinical problem (20 patients), unrelated causes mainly aging related (12 patients) and others causes (six patients). Drug related toxicity was drop out cause in 13 patients (11% of the entire population). Main causes of toxicity related drop out were increase of creatinine and gastro-intestinal disturbance. Preliminary results from the GIMEMA MDS0306 study confirmed feasibility of Deferasirox therapy in transfusion dependent MDS patients. High Charlson co-morbidity score and duration of transfusion dependency before chelation treatment were significant risk factors for drop out. Serum ferritin behavior confirms Deferasirox efficacy. *CLINICALTRIAL.GOV IDENTIFIER NCT00469560*

C039

APPLICATION OF THE NEW COMPREHENSIVE CYTOGENETIC SCORING SYSTEM TO EVALUATE DISEASE OUTCOME IN 631 DE NOVO CONSECUTIVE MDS PATIENTS.

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The present study evaluated whether the new cytogenetic scoring system proposed by Haase et al, EHA 2010 could improve the prognostic stratification of 631 consecutive de novo MDS patients observed at our Institution in the period January 1990-January 2010 and treated with supportive therapy only. There were 249 females and 382 males whose median age was 65.3 yrs (IQR 56.6-72.5). According to WHO, 70 pts (11.1%) were classified as RARS, 122 (19,3%) as RA, 25 (3,9%) as RCMD with ringed sideroblasts (RCMDS), 149 (23,6%) as RCMD, 38 (6,0%) as 5q- syndrome, 9 (1,4%) as unclassifiable MDS (u-MDS), 102 (16,1%) as RAEB-1 and 116 (18,4%) as RAEB-2. According to IPPS, 177 pts (28,1%) were cosidered low-risk MDS, 255 (40,4%) int-1 risk, 139 (22%) int-2 risk and 60 (9,5%) high-risk. Median follow-up was 19.6 months (mo.) (Inter-quartile range, IQR, 7.3-46.3). At the time of the analysis 160 pts (25.3%) had died after a median follow-up of 18.7 mo. (IQR 8.4-38.1) and 137 pts (21.7%) had experienced MDS/AML progression after a median follow-up time of 14.6 mo. (IQR 5.9-34.2). Three-hundreds fifty-three (55.9%) pts presented an abnormal karyotype: 260 (41.2%) carried a single chromosomal defect, 46 (7,3%) carried two defects and 47 (7,4%) >=three defects. Based on the new cytogenetic scoring system, 14 pts (3,8%) were considered very good risk, 392 (62,1%) good risk, 162 (25,6%) intermediate risk, 29 (4,6%) high-risk and 34 (5,3%) very high-risk. Univariate analysis showed that the five cytogenetic categories presented a significant difference in OS. In particular the 2-years OS was 91.0% (95% CI: 50.8-98.7) for the very good-risk category, 87.0% (95% CI: 82.7-90.6) for the good-risk category, 72.0% (95% CI: 63.2-80.0) for the intermediate-risk category, 54.8% (95% CI: 29.7-74.1) for the high-risk category and 9.4 % (95% CI: 0.6-32.9) for the very high-risk category (p<0.0001). Multivariate analyses confirmed these findings (p<0.0001). In addition, the 2-years PFI was 90.9% (95% CI: 50.8-98.6) for the very good-risk category, 79.0% (95% CI: 74.0-83.3) for the good-risk category, 54.7% (95% CI: 45.3-63.2) for the intermediate-risk category, 46.1% (95% CI: 22.7-66.7) for the high-risk category and no patient of the very high-risk category was disease-free at this time point (p<0.0001). Multivariate analysis confirmed these findings (p<0.0001). Thus, the new cytogenetic scoring system is truly effective in improving the prognostic stratification of pts with de novo MDS.

C040

PROGNOSTIC SIGNIFICANCE OF COMORBIDITIES IN MDS PATIENTS TREATED WITH 5-AZACITIDINE

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Myelodysplastic syndromes usualy affect elderly patients, and age is considered a negative prognostic factor per se. Comorbidities presumably impact negatively on overall survival (OS) and quality of life (QoL), and may influence response to therapy. Azacitidine (AZA) treatment also in elderly MDS patients have a significantly longer OS respect to BSC treated patients. We analyze if presence of comorbidities has an impact on survival, response and management of AZA treatment in MDS patients. We evaluated outcome, type of response according to IWG criteria 2006, as well as adverse events (AE) and cause of death. We analyzed 103 MDS outpatients (IPSS: 30% INT1, 49% INT2 and 21% High) treated with AZA 75 mg/mq/day sc for 7 days every 28. Mean age was 69 yrs. 30% of patients were ≥75 yrs and 39% of the latter ≥80 yrs; 71% were male. Patients were evaluated by three different geriatric score: Charlson comorbidity index (CCI)(54 % scored 0, 37% 1-2 and 9% ≥3), Cumulative Illness Rating Scale (CIRS)(37% scored 0, 37 % 1 and 26% ≥2) and Adult Comorbidity Evaluation-27 (ACE-27)(41% scored none, 29% mild, 23 % moderate and 7% severe). We compared the OS of our cohort with that of a diagnosis-and age-matched untreated control group of patients (n=246)(Italian registry of MDS–AISSM) in whom comorbidities had been evaluated by CIRS. Median OS of our cohort was 22 mths. Median OS in patients <75yrs and ≥75yrs was not significantly different. No correlation was present between comorbidity scores, age, sex and IWG response. OS was depending on scores. OS in patients with higher CCI, CIRS, and the ACE-27 was respectively 6.5,10 and 8 mths vs OS in patients with lower CCI, CIRS and ACE-27 was respectively 20, 22 and 22 mths. Hematological or non hematological AE grade III-IV were presented by 34% and 36% of patients, respectively. AEs were uniformly distributed independently from age and comorbidity scores. ORR was 49%, stable disease was obtained in 37%. Evaluation of comorbidities with validated indexes is an useful and easily tool to refine prognostic evaluation and should be include routinely in patient assessment.MDS patients with high scores may be treated with success with AZA, without substantial increase in AE. Nevertheless, comorbidities per se negatively influence OS, QoL and, consequently, the duration and the compliance of AZA treatment.For this reason, careful selection of patients eligible for treatment should take assessment of comorbidities into account

C041

CELL MORPHOLOGY HARMONIZATION IS MANDATORY IN THE DIAGNOSTIC PATHWAY OF MDS: EXPERIENCE OF GROM A COOPERATIVE ITALIAN REGIONAL GROUP.

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Microscopic evaluation of peripheral blood and bone marrow cells remains crucial in haematological diagnosis. The new WHO classification highlights the importance of morphological aspects, quantitative as well as qualitative, for recognition of disease entities and better stratifi-

cation of patients with haematological neoplasm, particularly myeloid neoplasm and above all myelodysplastic syndromes. Agreement on cells and their definition is thus mandatory to uniformly stratify patients. On July 2009 a Cooperative Group named GROM (Gruppo Romano Mielodisplasie), including all hematological Centers of the metropolitan area of Rome was created with the aim to improve the knowledge about epidemiology, comorbidities, infections, diagnostic procedures, therapies and farmaco-economic considerations in a homogeneous area of more than 3 millions people: from January 2010 the group was extended to all the hematological Centers of the Lazio region, that is the 3rd Italian region in terms of number of local residents, more than five millions and half, with a mean age of 42,7 ys and with 19,8% of people older than 65ys. Considering these population data, it is thus very relevant to organize a strongly harmonized morphology group involved into the diagnosis of all the patients referring to the Lazio region. Starting from January 2010 a Working Group on morphology, including morphologists from all the haematological centers of Lazio, was created with the aim to standardize the morphological diagnosis. As for the experience of other international groups on morphology, we used the consensus method, working mainly via internet by exchanging anonymous files and by using the Delphi technique to achieve a definitive, fully agreed, cell name for all the submitted images. Up to now we have discussed and validated the first set of 93 cells: two ad hoc meetings were carried out to discuss those not fully agreed cells. With regard to cell identification, most discrepancies concerned the decision if cells should be considered normal or dysplastic, mainly in the granulocytic series, while disagreement concerning a differentiation stage of a given cell was found for cells of the monocytic lineage. A second round was carried out: most of the cells of a second set of 143 cells were selected according to the main disagreements highlighted during the previous round. Disagreements of this second set of 143 cells will be discussed during a dedicated meeting planned on next June 2011: final results will be available together a list of the main critical points faced during this experience. Flandrin G. Image bank, diagnosis codification and telediagnosis in haematology. Leuk Lymphoma 1997;25:97-109. Luethi U et al. Telehematology: critical determinants for successful implementation. Blood 2004;103:486-8. Abramson N. A picture (in the microscope) is worth a thousand words. Blood 2004;103:367-8. Swerdow SH et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 2008;21. Goasguen JE et al. Morphological evaluation of monocytes and their precursors. Haematologica 2009;94:994-997. Zini G et al., A European consensus report on blood cell identification: terminology utilized and morphological diagnosis concordance among 28 experts from 17 countries within the European LeukemiaNet network WP10, on behalf of the ELN Morphology Faculty. Br J Haematol. 2010;151:359-64.

C042

SIMULTANEOUS DETECTION OF GENOMIC REARRANGEMENTS IN MYELODYSPLASTIC SYNDROMES (MDS) USING THE MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) ASSAY

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Cytogenetic analysis of the bone marrow is indicated in MDS not only for diagnostic purposes, but also to assess individual prognosis and to plan tailored therapy. Conventional cytogenetic (CC) analysis is performed in clinical practice to detect chromosomal abnormalities. A new method has recently been described for the measurement of the gene/chromosome copy number using genomic DNA: Multiplex Ligation-dependent Probe Amplification (MLPA). The purpose of this study was to compare the results of the MLPA assay with the CC data obtained in a series of 47 MDS patients (M: 35, F:12, median age 76 years, range 44-88). In accordance with the WHO classification, 15 cases were classified as RA, 13 as RCMD, 5 as RAEB-1, 8 as RAEB-2, 1 as MDS_U, and 5 as CMML. The MLPA assay contains 61 specific target sequences for chromosome regions commonly involved in MDS: 5q, 5p, 7q, 7p, 8q, 8p, 11q, 12p, 17q, 17p, 20q, 20p and 21q. Our study showed a good correlation between the MLPA and CC results (Table I), since most of the alterations being detected by both techniques. Discrepancies were found in 9 samples (19.15%). MLPA analysis did not detect the presence of a chromosomal (chr.) translocation (sample n°4 and n°42); a chr. deletion and a chr. translocation (sample n°11); a chr. deletion (sample n°15 and n°41); several chr. translocations and deletions (sample n°20); a chr. gain (sample n°27). In fact, the MLPA assay is not able to detect chr. translocations but only chr. loss or gain; it can only analyse the chr. regions commonly involved in MDS (5,7,8,11,12,17,20,21); it can reveal chr. abnormalities providing the percentage of cells carrying the alterations is about 30-35% and it do not show mosaicism. On the other hand, CC analysis did not show a small deletion in 2 samples: a deletion of the MLL gene in 11q23 (sample n°23) and deletion of 7q22 (sample n°37). With CC we also observed a karyotype failure (no metaphases) in 5 samples, while the MLPA assay showed three chr. deletions (sample n°14), but no anomalies in the other 4 samples (25, 29, 32, 39). MLPA proved rapid, cost effective, relatively easy to perform, had high throughput and enabled simultaneous analysis of many samples by automated data processing. MLPA and CC resulted complementary techniques, MLPA being particularly useful in MDS cases with karyotype failure and for identifying small rearrangements.

Sample	MLPA	Karyotype
1	No anomalies	46, XX
2	No anomalies	46, XY
3	No anomalies	46, XY
4	Del 5q	46, XY, Del 5q, t(1;12)
5	Del 7	45, XY, Del 7
6	No anomalies	46, XX
7	No anomalies	46, XY
8	No anomalies	46, XY
9	No anomalies	46, XY
10	Del 5q	46, XY, Del 5q
11	Del 5q, Del 11q23	45, XY, Del 11q23, Del 15, der 5 t(5;15) [16] / 46, XY [4]
12	No anomalies	46, XX
13	Trisomy 8	47, XY, Trisomy 8
14	Del 7q, Del 12p, Del 20q	No metaphases
15	Del 11q23	46, XX, Del 11q23 [5] / 46, XX, Del 9q22-23, Del 11q23 [15]
16	No anomalies	46, XY
17	No anomalies	46, XY
18	No anomalies	46, XY
19	No anomalies	46, XY
20	Del5q, Del 12p, Del17p	42~47, XX, Del 1p34 [3], Del 3 [6], t(4;18) [10], Del 5q [18], der 9 [3], Del 12 [10], t(13q;17q) [3], der 15 [4], Del 20 [3], Del 21 [2], Trisomy 22 [3], +m [10] [cp18] / 46, XX [2]
21	Del 20q, Trisomy 21q, Trisomy 19p, Trisomy 8	48, XY, Trisomy 19, Del 20q, Trisomy 21[12] / 48, XY, Trisomy 8, Trisomy 19, Del 20q[8]
22	No anomalies	46, XY
23	Trisomy 11q23 (MLL gene)	46, XX
24	No anomalies	46, XX
25	No anomalies	No metaphases
26	No anomalies	46, XX
27	No anomalies	46, XY,add(11)(p15)[10] / 46, XY[10]
28	No anomalies	46,XY
29	No anomalies	No metaphases
30	Del5q22-33	46, XY, Del5q15-33[8] / 46, XY[7]
31	No anomalies	46, XY
32	No anomalies	No metaphases
33	Trisomia 11	47, XY, Trisomy 11
34	No anomalies	46, XY
35	No anomalies	46, XY
36	No anomalies	46, XY
37	Del 7q22	46, XY
38	No anomalies	46, XY
39	No anomalies	No metaphases
40	No anomalies	46, XX
41	No anomalies	46, XY[17] 45, X [3]
42	No anomalies	46, XY t(9;14)
43	No anomalies	46, XY
44	No anomalies	46, XX
45	No anomalies	46, XY
46	No anomalies	46, XY
47	No anomalies	46, XY

INFECTIONS

C043

HUMAN HERPESVIRUS-6 REACTIVATION IN 49 HEMATOLOGICAL PATIENTS

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Background: In immunocompromised patients (pts), especially transplant recipients, human herpesvirus-6 (HHV-6) can reactivate and determine various clinical manifestations. Materials and methods: We retrospectively evaluated hematological pts who developed positivity to HHV-6, measured by quantitative PCR. All pts were receiving acyclovir as viral prophylaxis except 5. Results: Since 2009 we observed HHV-6 positivity in 49 pts (median age 54; 30 males), 39 of them received allo-SCT, 2 ASCT, 8 CT. A concomitant CMV positivity was detected in 11/49 pts, while a severe neutropenia in 23/49 pts. Allo-SCT was performed in 38/39 pts from PBSC source; 5 from HLA identical sibling, 3 MUD, 29 haploidentical donor, 2 CB. Among allo-SCT pts 15 had GvHD (13/15 with grade III-IV aGvHD), and 32 were profoundly immunosuppressed. Median time of onset was 41 days (7-625) after allo-SCT. In 25 pts HHV-6 was detected in plasma, median of 19937 cp/mL (34-4524600); 18/25 pts had fever (9 bacterial and 1 fungal infection), 8/25 skin rash, 4/25 worsening of liver function, 5/25 cytopenia; 19/25 pts received allo-SCT, 7 of them had delayed engraftment and 3 engraftment failure. In 9 cases HHV-6 was in bone marrow samples, median 25123 cp/mL (568-904000), 5 had concomitant plasma positivity; 3 pts developed cytopenia; 6/9 pts after allo-SCT, 2 of them had delayed engraftment and 1 engraftment failure. In 11 pts HHV-6 was observed in BAL samples, median 502 cp/mL (57-50211); 9/11 pts had fever (5 bacterial and 1 fungal infection). In 16 pts (15 after allo-SCT, 9 with previous gut aGvHD) HHV-6 was present in gastrointestinal biopsy (13 colorectal, 3 gastric), median 5550 cp/mL (120-163800), 4 had concomitant plasma positivity; 11/16 pts had diarrhoea. HHV-6 was found in cerebrospinal fluid in 3 pts (within 30 days post allo-SCT), median 19454 cp/mL (4508-39250); 2/3 had HHV6 in plasma; 3/3 pts experienced encephalitis, 1/3 seizure and 2/3 abnormal brain MRI; all pts had fever and 1 skin rash. Antiviral therapy was necessary in 23 pts (all received foscarnet except 3) and 13 of them solved the event. Among pts who experienced HHV-6 reactivation, 26/49 pts (53%) died and 20 of them received allo-SCT. Conclusions: HHV6 reactivation determines high morbidity and mortality in haematological pts. Particularly in pts after allo-SCT, is associated with a poor outcome and a regular DNA monitoring is needed to achieve an early identification.

C044

NOSOCOMIAL INFECTIONS IN HEMATOLOGIC PATIENTS: 4-YEAR EPIDEMIOLOGIC SURVEY

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Epidemiologic surveillance of nosocomial infections in cancer patients plays a key role in the characterization of local epidemiology, in the identification of new antimicrobial resistance and in the detection of potential outbreaks. From 2006 to 2009 we conducted a prospective molecular microbiological survey in 540 hematologic patients, employing VIGI@ct® (bioMérieux) and fluorescent amplified length fragment polymorphisms (f-AFLP). Antibiotic susceptibility was performed according to CLSI. Bloodstream infections (BSIs) was documented in 32% of total study population (175/540). BSIs were present in 69% of acute leukemia, in 16% of non-Hodgkin lymphoma, in 5% of myeloma and in 10% of other hematologic diseases. Among the pathogens responsible for BSIs, 117 (67%) were gram-positive: CoNS 63%, enterococci 28%, S. aureus 3%, other 6%; 53 (30%) were gram-negative: E.coli 43%, P.aeruginosa 28%, S maltophilia 13%, KES group 16%; 5 (3%) were fungi. As regard gram-positive, 100% of CoNS and 59% of S. aureus were meticillin resistant, whereas 18% and 50% of enterococci were VRE (VanA-type) and HLAR (High–level Aminoglycoside-Resistance), respectively. As regard gram-negative, resistance to ceftazidime occurred in 16% of E. coli, 46% of KES and 75 % of P. aeruginosa; resistance to quinolones occurred in 75% of E. coli, 53% of KES and 52% of P. aeruginosa; resistance to beta lactamase-inhibitors occurred in 20% of E. coli, 46% of KES and 33% of P. aeruginosa; resistance to carbapenems (meropenem) occurred in 8% of KES, 14% of P. aeruginosa , while no E. coli strain was resistant. In 2008 we recorded one P. aeruginosa pan-resistant strain, sensible only to colistin. In May-September 2008, an unexpected high incidence of VRE was reported. AFLP analysis of these VRE strains documented a genetic correlation between two different groups of isolates: 2 isolates show similar AFLP patterns but different from those of the other 4 genetically related isolates. These data suggest a possible horizontal transmission. After corrective measures no other case of VRE was documented. No gram-negative outbreak was recorded in the same period. Our results showed a new increase of gram-negative aerobic bacilli and a progressive multidrug resistance, which must be taken into account when starting antibiotic therapy. Moreover molecular typing methods are very useful for analysing epidemiology as well as identify clonal relationship and taking additional hospital measures.

C045

IDENTIFICATION AND CHARACTERIZATION OF ASPERGILLUS-SPECIFIC IMMUNE RESPONSES TO DIAGNOSE INVASIVE ASPERGILLOSIS IN HIGH RISK PATIENTS: A MULTICENTER STUDY

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Background: Several drawbacks limits the diagnostic accuracy of non cultural based diagnostic methods (NCBDM) for Invasive Aspergillosis (IA) and affect the mortality for the infection. Studies in mice model of IA and in healthy subjects have shown that Aspergillus-specific T-cells producing interferon-gamma (IFN- -T1) are protective, while Aspergillus-specific T-cells producing interleukin-10 (IL-10-T2) are non-protective to IA. Aims: We have investigated whether the identification of Aspergillus-specific IFN- -T1 and/or IL-10-T2 through an ex-vivo enzyme linked immunospot (ELISPOT) assay may be effective in the diagnosis of IA in high risk patients. Furthermore, in the proven IA patients, we have characterized the specific T-cell responses to 7 recombinant antigens of Aspergillus. Methods: 180 enrolled patients were classified, according the EORTC/MSG criteria, as: 18 proven, 35 probable, 17 possible IA cases and 110 controls. The latter included 86 patients (78.2%) with histological and/or cultural verified infectious/inflammatory/neoplastic diseases, other than IA; and 24 patients (21.8%) without clinical and/or microbiological features of IA. ELISPOT has been performed by using as antigens Aspergillus either conidia or recombinant antigens, namely CRF1p, GEL1p, PEP1p, SOD1p, 1-3 glucan, 1-3 glucan and galactomannan (GM). Results: The patient and sample positivity rates were 94.4%/89.5% in proven, 45.7%/35.3% in probable, 35.3%/50% in possible IA cases and 1.8%/4.5% in the controls, respectively. The sensitivity and specificity of ELISPOT for the diagnosis of IA resulted 94.4% and 98.2%. The positive and the negative predictive values were 89.5% and 99.1%. The efficiency was 97.6%. The positive and likelihood ratios resulted 51.89 and 0.06 (Table 1). In proven IA patients, at the onset of the IA, Aspergillus-specific IFN- -T1 were detected to GEL1p, 1-3 glucan and 1-3 glucan, and were absent to CRF1p, PEP1p and SOD1p. GEL1p and 1-3 glucan resulted the only antigens targeted by FN- - T1 along the entire course of IA. Conclusions: Our findings demonstrate the potential of ELISPOT in the diagnosis of IA, suggesting that it may comple-

ment the other NCBDM, enabling a more consistent diagnosis of IA. Furthermore, the identification of the Aspergillus antigens predominantly targeted by protective IFN- -T1 in proven IA patients, may have possible consequences in designing strategies of either adoptive cell infusion or vaccine therapies.

Table 1A, B. Diagnostic Accuracy of the ELISpot assay for the diagnosis of Invasive Aspergillosis (IA).

A. Assay positivity rates per-patient and per-sample. **B.** Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Efficacy of the assay matching proven IA with Control cases.

A.

Classification	N°of Patients with positive ELIspot: Single positive result	Serial positive result	Patient positivity rate, % (95% CI)	N°of samples tested (n° positive)	Sample positivity rate, % (95% CI)
Proven IA (n = 18)	10	7	**94.4%** (73%-99%)	38 (34)	**89.5%** (75%-97%)
Probable IA (n = 35)	8	8	**45.7%** (29%-63%)	85 (30)	**35.3%** (25%-46%)
Proven/probable IA (n = 53)	18	15	**62.3%** (48%-75%)	123 (64)	**52%** (43%-61%)
Possible IA (n = 17)	3	6	**35.3%** (14%-62%)	34 (17)	**50%** (32%-68%)
Controls (n = 110)	9	0	**1.8%** (0.2%-6.4%)	200 (9)	**4.5%** (2%-8%)

B.

	PROVEN	CONTROLS	
ELISPOT +	17	2	19
ELISPOT -	1*	108	109
Total	18	110	128

C046

INCIDENCE OF HEPATITIS B VIRUS (HBV) REACTIVATION IN NON HODGKIN LYMPHOMA (NHL) OCCULT CARRIERS UNDERGOING CHEMO/IMMUNOTHERAPY WITH OR WITHOUT LAMIVUDINE (LAM) PRIMARY PROPHYLAXIS

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BACKGROUND and AIM: While HVB reactivation prophylaxis with LAM in HBsAg + NHL pts undergoing chemotherapy is of proven benefit (Katz, 2008), no comparative study has yet demonstrated an advantage of LAM prophylaxis in the much larger cohort of HBV occult carriers (HBsAg–/HBcAb+). Their reported chance of developing HBV reactivation during chemotherapy ranges from 0.8 % (Targhetta, 2008) to 6.0 % (Yeo, 2008). It is higher with immunotherapy (RR 13.7, Hui 2006), and may carry an higher risk of liver failure. In this study we compared the risk of HBV reactivation with or without LAM in occult HBV carriers NHL pts treated with chemo+/-monoclonal antibodies (MoAb). PATIENTS and METHODS: We analyzed 285 consecutive NHL pts treated at our center during a 30-months period (7/07–12/09). Histology was DLBC (43 %), follicular (22 %), mantle (10%), T (9%), MALT/marginal (8%), other (8%). Median age was 65 years (16-90), M/F 0.78. HBV reactivation was defined as an > 1 log increase in serum HBV-DNA over baseline level; acute hepatitis as a sudden >5x rise in serum ALT above UNL or >3x above baseline levels if abnormal. A policy of primary LAM prophylaxis (100 mg daily p.o.) in occult HBV carriers has been adopted in the second part of the study period, since 2/2009. LAM has been given from the start until a median of 5 months after the last course of NHL treatment RESULTS: HBV serology was available in 91% of cases, and HBVDNA levels in 14%. Ten of 285 (3.5%) cases were HBsAg + and 87 (30%) HBsAg-/HBcAb+; of these 56 (64%) were HBsAb+. In addition 25 cases were HCV+ (8.8%). Ten of 10 HBsAg+ (100%) and 57/87 (65.5%) HBcAb+ cases received primary LAM prophylaxis, The proportion of HBsAb+ cases receiving (38/56: 68%) or not (19/31: 61%) LAM prophylaxis were similar. HBV-related hepatitis developed in 2/86 pts (2,2+/-3,1%). A mild hepatitis occurred in one HBc+/HBs- DLBCL pt receiving LAM (1/57: 1,7+/-3,4%) and a fatal liver failure in a non prophylaxed HBc+/HBsAb+ pt (1/26: 3,8%+/-7,3%) with DLBCL in remission 5 months after completion of R-CHOP and radioimmunoconjugate consolidation. Reactivation rate in 75 pts treated with MoAb was 2,7+/-3,7%. Conclusions: The HBV reactivation rate in non prophylaxed occult HBV carriers is not high (3,8+/-7,3%), even in patients treated with MoAb, but reactivation may be fatal. A beneficial role of primary LAM prophylaxis needs larger patients cohorts to be formally demonstrated.

C047

PRE-HOSPITAL RISK FACTORS FOR INVASIVE FUNGAL DISEASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS: PRELIMINARY RESULTS FROM THE SEIFEM 2010-STUDY

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Background/Aims: To investigate the potential relationship between pre-hospital exposures to fungal sources and the development of invasive fungal diseases (IFDs) in adult acute myeloid leukemia patients (AMLs). Methods: From January 2010 to March 2011, in 31 Italian participating centers, all consecutive patients (pts) with newly diagnosed AMLs were registered. Information about personal habits and possible environmental exposures were investigated. In particular we collected data about: comorbidities, job, hygienic habits, work and living environment, voluptuary habits (i.e.smoking, alcohol, illicit substances abuse), hobbies, pets. We also included data on other well-known risk factors, such as age, neutropenia, mucosal damages, etc. In order to make our study population very homogeneous, we focuses on pts treated with conventional chemotherapy only. All cases of proven/probable IFDs occurred until the 30th day from the end of first induction were recorded. Results: 593 pts were enrolled in the study; of them, 447 were included in the present analysis and 43 developed a proven/probable IFDs (30 molds and 13 yeasts) (incidence 9.6%). Median age was 61 (range 18-81). Main variables included in the risk analysis have been reported in the table. In particular, at preliminary analysis a significant association with IFDs development was found for performance status (p <0.001), chronic obstructive pulmonary diseases (p 0.04), urinary catheter (p <0.001), neutropenia (<500 neutrophils/µl, > 7 days) (p 0.03). A not significant trend was noted for incidence by gender (males 12% vs females 7%), for diabetes (yes 18%, no 9%), construction sites in the last 3 months to less than 500 meters from home (yes 12%, no 8%), home restructuring in the last 6 months (yes 14%, no 9%). We did not find any association for weight, occupational exposure, geographical origin. For mold infections only, those patients living in a flat resulted to be at higher risk when compared to those living in house with garden (p 0.03). Other variables showing a correlation with the onset of invasive yeast diseases were chronic kidney failure (p 0.006) and liver diseases (p <0.001). Conclusions: several hospital-independent fungal sources emerged at univariate analysis to potentially influence IFDs onset. Inves-

tigation of these factors at time of admission may be helpful in defining patient' risk category and in better targeting prophylactic strategies.

Table: univariate analysis of main risk factors for IFD.

VARIABLES	N° pts	N° cases	p-value
Gender			
males	232	28 (12%)	0.06
females	215	15 (7%)	
Performance status			
0-1	342	22 (6%)	
2-3	97	18 (19%)	<0.001
4	8	3 (38%)	
Diabetes			
No	409	36 (9%)	0.054
Ye	38	7 (18%)	
Chronic kidney failure			
No	434	40 (9%)	0.09
Yes	13	3 (23%)	
COPD			
No	424	38 (9%)	0.04
Yes	23	5 (21%)	
Liver disease			
No	428	39 (9%)	0.08
Yes	19	4 (21%)	
Cigarette smoking			
No	327	31 (9%)	0.8
Yes	120	12 (10%)	
Mucosal damage*			
0	207	17 (8%)	0.34
> 0	240	26 (11%)	
CVC			
No	112	9 (8%)	0.5
Yes	335	34 (10%)	
Urinary catheter			
No	369	24 (7%)	<0.001
Yes	78	19 (24%)	
Neutropenia§			
No	37	0	0.03
Yes	410	43	
Type of house			
flat	248	18 (7%)	0.058
house with garden	199	25 (13%)	
Home restructuring^			
No	398	36 (9%)	0.24
Yes	49	7 (14%)	
Construction sites near home°			
No	310	26 (8%)	0.18
Yes	137	17 (12%)	

Legend:
CVC: central venous catheter; COPD: chronic obstructive pulmonary disease
*: it comprehend oral mucositis, esophagitis, vomiting, diarrhea. Zero means none of them.
§: Neutrophils <500/µl, duration > 7 days.
^: In the last 6 months.
°: in the last 3 months, within 500 meters.

C048

INVASIVE FUNGAL INFECTIONS (IFI) IN LYMPHOPROLIFERATIVE DISORDERS: EPIDEMIOLOGY, OUTCOME AND PROGNOSTIC FACTORS

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Few data about IFI in chronic lymphoid malignancies are available. Our aim is to describe epidemiology, clinical manifestations and outcome of IFI in these patients (pts). We reviewed the records of pts with lymphoproliferative disorders, treated between 2004 and 2010 for probable/proven IFI, according to the revised criteria of EORTC/MSG. We registered 34 probable/proven IFI. Twenty-two pts were affected by lymphoma, 8 by CLL, 3 by Waldenström macroglobulinemia and 1 by hairy cell leukemia. The median age was 57 years (r: 17-71). Twenty-six pts (76%) had progressive/relapsed hematological disease, and 76% were treated with multiple lines of chemotherapy. Risk factors for IFI were: neutropenia (10 pts), previous solid organ transplant (2) or allogeneic HSCT (2), treatment with high dose steroids (3), monoclonal antibodies as rituximab (7) and alemtuzumab (2), nucleosidic analogues (2) or multiple of these factors (8). Incidence of IFI was 2,7%; (moulds 2%, yeasts 0,4%, mixed infections 0,3%). We recorded 6 yeast infections: 5 were documented by cultures (2 C. albicans, 2 C. glabrata and 1 Blastoschyzomices capitatus), and 1 by the microscopic observation of Candida spp in the vitreum. Twenty-five pts developed an invasive mould infection (IMI); 3 of them had a proven pulmonary IMI diagnosed by histology (2 Aspergillus spp, 1 Mucor). Twenty-two pts had a probable IMI: 19 had lung involvement, 2 a sinusal localization, and 1 a pulmonary infection disseminated to brain. Mycological criteria were more often provided by the positivity of the galactomannan antigen (GM, 11 pts) in serum and/or BAL fluid. Cultures resulted positive in 10 cases (5 A. fumigatus, 2 Aspergillus spp, 1 A. flavus, 1 Fusarium and 1 for both Scedosporium and Aspergillus); in 4 of them, both GM and culture positivity from the BAL fluid were present. Finally, we observed 3 mixed IFI by both moulds and yeasts: in 2 pts a proven yeast infection with isolation in the blood culture (1 C. albicans, 1 C. glabrata) was associated to a probable IMI with the positivity of CT scan and GM; in 1 pt autopsy revealed a pneumonia by Candida spp and a disseminated infection by Aspergillus spp. Seven of 34 pts died due to infection, but only in 1 pt IFI was the unique cause of death. IFI attributable mortality rate was 20%. Regarding mortality, the only risk factor was the previous multiple lines of chemotherapy (p 0.04). For some other risk factors a worsening trend on mortality was identified.

HODGKIN'S LYMPHOMA

C049

THE PROGNOSTIC VALUE OF TUMOR AND MICROENVIRONMENT MARKERS IN CLASSICAL HODGKIN LYMPHOMA (CHL) PATIENTS

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Introduction. Early-interim PET-scan (IP) after two ABVD courses was demonstrated to be the most effective predictor of the treatment outcome. So far, many attempts have been made to identify prognostic biologic markers applicable upfront therapy. Purpose of the study. We analyzed the prognostic impact of a series of histological and immunohistochemical parameters on tissue-microarrays from 209 CHLs treated with ABVD ± Rx therapy and compared it to the IP predictive value. We tested proteins encoded by genes shown as prognostically relevant by DNA-microarray studies (STAT1, PCNA, SAP, TOP2A, RRM2, CDC2, MAD2L1, ALDH1A1, CD68, and BCL11a) and the markers previously reported to have prognostic value in conventional studies (CD20, EBER, Bcl-2, p53, CD163, PD1, FOXP3, TIA1, Granzyme B, and Perforin). The molecules were assessed in both neoplastic (HRSC) and microenvironmental cell (MEC) components. Results. The median age was 32 years (range 14-80), the stage IV in 32 patients, III in 50, II in 118, I in 9, constitutional symptoms and bulky disease in 99 (47,4%) in 42 (20.1%) cases, respectively, and the mean FU was 52,3 months (3-93.7). Histopathology review showed: NS-I in 93 cases, NS-II in 37, NS syncytial variant in 8, NS cellular phase in 9, NS-NOS in 10, MC in 29, LD in 3, LR in 1, and CHL-NOS in 16. IP was positive in 37 patients (18%), while treatment failure was recorded in 49(23,4%). In univariate analysis, the factors related to overall survival (OS) were: NS-II/NS syncytial/LD histology (p .0227); Bcl-2 on HRSC and IP (p 0.000); NS-II/NS syncytial/LD histology, p53 on HRSC, PD1 on MC, stage and IP turned out to be significantly related to a worse progression free survival (PFS) (p .0094, 0.000, .0135, .0018, .0194 and 0.000 respectively). In multivariate analysis, including all parameters significant at univariate analysis, BCL2 and IP maintained their prognostic value on OS (p .0121 and 0.000 respectively) and P53, stage and IP on PFS (p .003, .000 and 0.000 respectively). By restricting the analysis to IP negative cases, expression of BCL11a on HRSC and CD163 on MC resulted significantly correlated to treatment failure, and included with IP in a new predictive model; the latter demonstrated a higher capability, than IP alone, to predict the treatment outcome (test misclass error 12,98% vs 13,94%). Conclusion. These findings demonstrate the potential of biomarkers to foresee treatment response in CHL.

C050

THE VIRAL LOAD OF EPSTEIN-BARR VIRUS (EBV)-DNA IN PERIPHERAL BLOOD ASSOCIATES TO BIOLOGICAL AND CLINICAL CHARACTERISTICS IN HODGKIN LYMPHOMA

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The Epstein-Barr virus (EBV) is present in the malignant Hodgkin/Reed-Sternberg (HRS) cells of 20-40% cases of Hodgkin lymphoma (HL) in western countries. Circulating EBV-DNA in peripheral blood is an indicator and biomarker for EBV-associated Hodgkin lymphoma (HL). We further explored associations of EBV-DNA plasma load to biological and clinical characteristics of HL, iincluding new as cell-free circulating DNA and the number of tumor-infiltrating CD68+ macrophages. EBV was detected in peripheral blood compartments (whole blood, plasma, and mononuclear cells) at diagnosis using real-time PCR for the EBNA region (n=93) and in HRS cells using in-situ hybridization for EBER (n=63). Detection of EBV-DNA in plasma had a high specificity (90%), but a relatively low sensitivity (65%) to predict for EBV-association. The viral load was higher in patients with advanced stage disease (p=0.01), older age (p=0.006), in presence of B-symptoms (p=0.001), and IPS score >2 (p=0.007). The presence of EBV in HRS cells and higher plasma EBV-DNA copy numbers correlated to an increased frequency of tumor-infiltrating CD68+ macrophages in lymph node biopsies (p=0.03). Plasma EBV-DNA load correlated to circulating cell-free DNA (r=0.58, p=0.01) and IL-6 levels (r= 0.73, p=0.001), and inversely correlated to lymphocyte counts (r=-0.61, p=0.01) and EBNA1 antibody titers (r=-0.62, p=0.01). The strong correlations of circulating EBV-DNA to parameters of disease activity makes EBV-DNA a meaningful marker for the biological and clinical presentation of EBV-associated HL. Since plasma EBV-DNA may be absent in patients with limited disease, EBV-DNA cannot be regarded as a surrogate marker for EBER, and study of EBV in HRS cells is still the gold standard recommended for screening for EBV-association in HL. The inverse correlations of circulating EBV-DNA to lymphocyte counts and EBNA titers point to a reduction in immune surveillance in EBV-associated HL, favouring the expansion of EBV in HL. Although the presence of EBV-DNA in peripheral blood cannot be regarded as a surrogate marker for EBER, the plasma EBV-DNA load at HL diagnosis is an indicator of disease activity and biological characteristics associated with negative prognosis. Moreover, the inverse correlation to EBNA1 antibody titers and lymphocyte counts may indicate a reduction in immune surveillance, favouring the expansion of EBV-HRS cells in HL.

C051

EARLY POSITIVE FDG-PET SCAN DO NOT CONFIRM ITS PROGNOSTIC IMPACT IN LOCALIZED BULKY DISEASE HODGKIN LYMPHOMA PATIENTS

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Hodgkin's lymphoma is a malignant diseases with the highest rate of cure particularly if diagnosed in early stage.We want to explore 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, 263 localized stage Hodgkin's lymphoma pts were consecutively admitted to 13 Italian hematological centers on behalf of Fondazione Italiana Linfomi (FIL). Pts with stage I-IIA according to Ann Arbor stage, independent of presence of bulky disease were evaluated. Bulky disease pts were the object of our analysis. FDG-PET was mandatory at baseline, after two cycles and at the end of therapy. Mediastinal blood pool activity is recommended as the reference background activity to define PET positivity according to International Harmonization Project (IHP). No treatment variation based only on PET-2 results was allowed. 78 pts presented bulky disease, the median age was 32 years (13-66). The FDG-PET performed after two cycles (PET2) was positive in 18/78 pts (23%): 8 (44%) progressed or relapsed and 10 manteined CR. By contrast 53/60 (88%) pts with a negative PET2 remained in CR. Thus the positive predictive value of a PET2 in bulky disease was very low (44%) and the negative predictive value was 88%. The sensitivity and specificity of PET2 were 53% and 84%, respectively. Radiotherapy was performed in 17 PET2 positive pts, 10 did not fail (59%), one pt with PET2 positivity did not perform radiotherapy and progressed. A FDG-PET was performed at the end of therapeutical program (PET6), all pts (10) with positive PET6 and 5/68 with negative PET6 progressed. In univariate analysis negative FDG-PET performed after two cycles (p .002) and in particular negative PET6 (0.000) were statistically correlated with a better progression free survival. With a median follow-up of 37 months (range 4-103) 73 pts are alive and 63 (81%) are free from progression. The 2-yr PFS probability for PET2 negative and for PET2 positive patients were 92% and 40% respectively (p: .002).

With the IHP interpretation criteria we observed a large number of false positive PET2 in mediastinal bulky early stage Hodgkin disease. For this reason new PET evaluation methods in this subset of pts are mandatory. Moreover in bulky disease pts radiotherapy could permit to overcome the poor prognostic significance.

C052

PROGNOSTIC ROLE OF RESIDUAL CT SCAN MASS IN AGGRESSIVE LYMPHOMA IN PATIENTS WITH PET NEGATIVE AFTER CHEMO+/-RADIOTHERAPY

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Background. According to the revised response criteria (Cheson, 2007),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 FDG avid lymphoma,e.g. Hodgkin(HL) and diffuse large cell non Hodgkin lymphoma (DLCL. Complete remission (CR) is defined as PET negativity independently from the persistence of residual masses on computed tomography(CT scan). Nevertheless, some reports suggested a worse prognosis for patients (pts) with CT scan residual masses despite PET negativity. 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 at the end of treatment program at our institution. The NPV was defined as the proportion of patients without relapse after PET. RESULTS. From February 2004 to February 2009, 256 pts (151 with DLCL and 105 with HL, respectively) achieved CR at PET evaluation. Two hundred ten pts were evaluated after first line treatment program, while 46 pts after salvage therapy program. Residual CT scan mass (PET-/CT+) of at least 2.0 cm in the largest diameter was assessed in 153 pts. One hundred ten had one site with residual mass, and 43 pts at least two. As of March 2011, 48 pts have relapsed. The five years disease-free survival (DSF) for the whole series was 77.5% for PET-/CT- pts and 75.6% for PET- /CT+ (P=0.604. Concerning HL pts, DSF was 89.4% for PET-/CT- pts and 68.7% for PET- /CT + (P=0.053), respectively. The different outcome according to residual masses in HL pts is even more significant by considering the bulk of the masses: in pts with a residual mass larger of 5 cm the DFS was 35.3% vs 78.3% in those with smaller or no residual (HR4.6 2.01;10.54; p<0.001. The number of sites (1 vs >1) of residual mass did not show significative difference. In DLCL pts, among all prognostic factors analyzed,(CT scan+/-, number or size of masses, first vs salvage treatment program) no correlation with DSF or overall survival (OS) emerged. Conclusions. In our study CT scan residual masses in PET-negative HL pts significantly influenced DFS, particularly when residual masses were larger than 5 cm. According to our experience residual disease at CT scan remain an essential prognostic tool even in PET era and suggests the need for additional approach like radiotherapy in this setting of pts.

C053

REGULATORY MYELOID CELLS IN HODGKIN`S LYMPHOMA

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Background In some solid tumors it has been demonstrated that a circulating population of myeloid cells known also as Myeloid Derived Suppressor Cells (MDSC) has the ability to suppress T-cell immune response, thus favouring disease progression. In humans, three different subpopulation of MDSC can be defined: 1) immature im- MDSC (CD11b+, CD13+, CD14-, CD34+, CD45+), 2) neutrophilic-like N-MDSC (CD11b+, CD13+, CD15+, CD14-, Lin-) and 3) monocytic-like regulatory myeloid cells mo-MDSC (CD14+, HLA-DR-). Material and Methods In peripheral blood of 37 Hodgkin lymphoma (HL) patients at diagnosis and after 2 cycles of chemotherapy, we evaluated by flow cytometry circulating levels of MDSC along with T-cell subpopulations, including T-reg (CD4+CD25+FoxP3+), CD4+, CD8+ and their expression of the adhesion molecule CD62L as a marker of their efficient ability to recirculate. Findings were correlated to clinical outcome and interim-PET response (the most important prognostic factor). Results We found that at diagnosis HL patients have higher levels of im- MDSC, N-MDSC but not mo-MDSC when compared to matched for sex and age healthy controls (respectively, p=0.0001 and p=0.006), with return to normal values within the first 2 cycles of chemotherapy in 10 evaluated responders. T-reg were reduced at diagnosis compared to healthy subjects, with an evident increase within the first 2 cycles of chemotherapy, as well. CD62L was significantly reduced in CD4, but not in CD8 T subsets, and correlated with N- MDSC (r=-0.54, p=0.0002) but not im-MDSC. MDSC absolute number and CD62-L expression were not correlated with tumor burden (stage, IPS, presence of bulky disease) or markers of inflammation (ferritin, ESR, C-RP, fibrinogen). In a Kaplan-Meier analysis, the 10 patients (27%) who presented with im-MDSC levels above 4.5 cells/uL at baseline had a higher risk for event (defined as interim PET positivity or relapse within 2 years) than patients with im-MDSC levels below 4.5 cells/uL, with a difference statistically significant in a log-rank test (p = 0.004). As a confirmation of the immunosuppressive abilities of myeloid compartment in HL, we found that myeloid cells from three HL patients were able to suppress PHA stimulated normal lymphocytes more than myeloid cells derived from healthy donors. Conclusion In HL, an increase of MDSC could be responsible for a reduced T- cell activity and has a prognostic value in predicting interim-PET positivity or early relapse.

C054

CONTINUOUS INFUSION CHEMOTHERAPY WITH D-PACE CYCLES IN VERY POOR CHEMOREFRACTORY HODGKIN'S LYMPHOMA PATIENTS

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Patients affected by Hodgkin's lymphoma (HL) who are refractory or relapsed after an autologous stem cell transplant (autoSCT) have a very poor prognosis. Continuous infusion chemotherapy may overcome the chemo-resistance of lymphoma tumour cells. Rituximab is being used to treat HL because it may improve the response to chemotherapy targeting the CD20+ cells of the microenvironment.We reported the results of 29 patients affected by relapsed or refractory HL treated with chemotherapy cycles of D-PACE +/- Rituximab. Treatment consisted of dexamethasone 40 mg ev die as bolus infusion for 4 days with cisplatin 10 mg/ms, adriamycin 10 mg/ms, cyclophosphamide 400 mg/ms, etoposide 40 mg/ms, all given as 24-hour infusion for 4 days. Rituximab was administered on day 1 at the dose of 375 mg/ms in 10 patients. Cycles were delivered monthly. Histological subtypes were as follows: 22 nodular sclerosis, 2 mixed cellularity, 1 lymphocyte depletion, 1 nodular lymphocyte predominant, 3 not determined. At the time of D-PACE the median age was 30,8 (range, 17-53). The majority of the patients (59%) had stage IV disease (n=17). Median number of previous chemotherapy was 3 (range, 1-7). Twenty-two patients (76%) failed a previous autologous stem cell transplant (autoSCT). Thirteen patients (45%) were considered primary chemorefractory as they never achieved complete remission (CR) or CR lasting more than 6 months; 7 patients (24%) were refractory before D-PACE and 9 patients (31%) were chemosensitive. Overall 69% of the patients was chemorefractory at the time of D-PACE. The clinical characteristics of patients who were treated with Rituximab did not differ from all the other patients. The median number of delivered cycles for patient was 2 (range, 1-7). The overall response rate (ORR=CR+partial remission (PR)) was 52% (15/29). Seven patients achieved CR, 8 patients were in partial remission (PR), 3 were in stable disease (SD), and 11 in progressive disease (PD). Eleven of the 15 responding patients underwent allogeneic stem cell transplantation (alloSCT) and 2 an autoSCT. Three patients in PD after D-PACE underwent alloSCT, whereas the others received salvage treatment followed by auto and/or alloSCT in 6 cases. The ORR of the patients treated with R-D-PACE was 40% (2 CR and 2 PR). The ORR was 9/20 (45%) in the refractory group and 6/9 (67%) in chemosensitive patients (p=0.69). Grade 3-4 haematological toxicity occurred in 21/23 (87%) patients. No grade 3-4 non –hematological toxicity was demonstrated. Overall the

median follow-up was 28 months (range, 4-84). Two-years OS and PFS were 65% and 22%, respectively. OS and PFS of CR and PR patients after D-PACE were significantly better than SD and PD patients (OS p=0.0001; PFS p=0.0013). In conclusion, continuous infusion chemotherapy with D-PACE cycles may rescue some very poor prognosis HL patients, including those with chemorefractory and high tumour burden disease and may be able to bridge patients towards transplant procedures.

AUTOLOGOUS TRANSPLANTATION

C055

A NOVEL HIGH-DOSE THERAPY REGIMEN WITH BENDAMUSTINE, ETOPOSIDE, CYTARABINE AND MELPHALAN (BEEAM) FOLLOWED BY AUTOLOGOUS STEM CELL RESCUE IS SAFE AND HIGHLY EFFECTIVE FOR RESISTANT/RELAPSED LYMPHOMA PATIENTS: A PHASE I-II STUDY ON 43 PATIENTS.

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BEAM (Carmustine, etoposide, cytarabine, and melphalan) is the most used conditioning regimen before autologous stem cell transplant (ASCT) in lymphoma patients. However, relapse rate after transplant is still a matter of concern. Therefore, new regimens with a higher efficacy are particularly needed. We designed a phase I-II study to evaluate the safety and the efficacy of increasing doses of Bendamustine for the conditioning regimen to ASCT for resistant/relapsed lymphoma patients. As a biological background, we performed in vitro experiments which showed the synergistic activity of bendamustine with etoposide, aracytin and melphalan in lymphoma cell lines. Forty-three patients (median age 47 years) with resistant/relapsed non-Hodgkin (29) or Hodgkin (15) lymphoma were consecutively enrolled in the study. The new regimen consisted of increasing doses of Bendamustine coupled with fixed doses of Etoposide (200mg/m2/day on days -5 to -2), Cytarabine (400mg/m2 on days -5 to -2) and Melphalan (140 mg/m2 on day -1) (BeEAM regimen). The starting dose of Bendamustine was 160 mg/m2/daily given on days -7 and -6, which was then escalated according to the Fibonacci's increment rule until the onset of severe adverse events and/or the attainment of the expected MTD, but not higher than 200 mg/m2. No patient experienced a dose-limiting toxicity during phase I. Thirty-four additional patients were then treated in the phase II with Bendamustine at a dosage of 200 mg/m^2/day given on days -7 and -6. A median number of 6x10^6 CD34+cells/kg (range 2.4-15.5) was reinfused. All patients engrafted, with a median time to ANC>0.5x10(e)9/l of 10 days. Median times to achieve a platelet count >20x10(e)9/l and >50x10(e)9/l were 13 and 16 days respectively. The 100-day transplant-related mortality was 0%. After a median follow-up of 18 months, 35/43 patients (82%) are in complete remission, whereas 6/43 relapsed and 2/43 did not respond. Disease type (NHL versus HD) and disease status at transplant (chemosensitive versus chemoresistant) significantly influenced disease-free survival (p= 0.01; p=0.007). Remarkably, 4/43 patients achieved the first complete remission after receiving the high-dose therapy with ASCT. In conclusion, the new BeEAM regimen is safe and highly effective for heavily pretreated lymphoma patients. The study was registered at EMEA with the EUDRACT no 2008-002736-15.

C056

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) ON OUTPATIENT BASIS IN 256 PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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ASCT is the standard therapy in relapsed Lymphoma patients and in young Multiple Myeloma patients. In 2001 we started an outpatient program of ASCT, based on an early discharge at day +1, with the following inclusion criteria: WHO Performance Status 0-1, absence of severe comorbidities, availability of a caregiver, estimated time to reach hospital < 40 minutes and good compliance to homing therapy. All patients were analysed according to the intention criteria to perform an early discharge,

with clinical and laboratory surveillance 3 times a week until the complete haematological recovery. The conditioning regimens were: for Multiple Myeloma (MM) patients high dose Melphalan (200 mg/m^2), for Hodgkin Disease (HD) and B-Diffuse Large Cell Lymphoma (B-DLCL) patients standard BEAM. Amifostine 1000 mg was administred before Melphalan in both cases. Patients transplanted until 2004 received Filgrastim 5 µg/day from day +1 while the others received Peg-filgrastim 1 fl at day +1. From 2001 to 2011 a total of 256 patients (170 MM and 86 Lymphomas) underwent to ASCT; 184 patients (72%) were elegible for outpatient program and all patients were discharged at day +1. Haematological reconstitution and clinical outcome after early discharge are showed in the Table below. In MM patients we observed a second hospitalization only in 19 transplants (15%): 10 for infection, 6 for FUO and 3 for mucositis. In univariate analysis the only significative factor affecting 2nd hospitalization was disease status < VGPR. Transplant-Related Mortality (TRM) at day +90 occurred in 2 MM patients (1.6%): 1 for gram negative sepsis and 1 for pneumonia. In lymphoma patients we observed a second hospitalization in 18 transplants (30%): 10 for infection, 4 for FUO and 4 for mucositis, with a TRM at day +90 of 0%. Our experience shows that outpatient ASCT is safe and feasible in patients with haematologic malignancies. We observed an incidence of the 2nd hospitalization lower than observed in previous reports, especially evident in MM patients, maybe due to the use of Amifostine as mucosal cytoprotective agent.

MULTIPLE MYELOMA PATIENTS (# 124)		
	median values	%
Age	62	
Days to ANC > 500 x 10^9/L	10 days	
Days to platelet count > 20.000 x 10^9/L	12 days	
Platelets units transfused	0	
Red cells units transfused	0	
Febrile episodes	30	26%
Mucositis grade 3-4 (WHO)	6	7%
Second hospitalization	19	15%
Days of hospitalization from readmission	7 (1-10)	
Graft failure	0	0%
Transplant related mortality +90	2/117	1.7%

LYMPHOMA PATIENTS (# 60)		
	median values	%
Age	55	
Days to ANC > 500 x 10^9/L	10 days	
Days to platelet count > 20.000 x 10^9/L	12 days	
Platelets units transfused	1	
Red cells units transfused	0	
Febrile episodes	23	39%
Mucositis grade 3-4 (WHO)	6	10%
Second hospitalization	18	30%
Days of hospitalization from readmission	8 (2-23)	
Graft failure	0	0%
Transplant related mortality +90	0	0%

C057

RADIOIMMUNOTHERAPY WITH ^{90}YTTRIUM ZEVALIN AND BEAM (Z-BEAM) AS CONDITIONING REGIMEN FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR THE TREATMENT OF HIGH RISK RELAPSED/RESISTANT B-CELL LYMPHOMA (NHL)

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High dose chemotherapy (HDC) and ASCT is actually considered an effective treatment for relapsed NHL. Standard dose Zevalin (0.4 mCi/kg) combined with conventional BEAM (Z-BEAM) is a promising conditioning 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 2010 29 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 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 at relapse/progression were: 14 refractory and 15 early or multiple relapse; 8 grade I-II FL, 16 PML/DLBCL, 3 MCL, 2 indolent; 6 stage II and 23 stage III-IV; 10 bulky disease and 15 BM+; IPI score 0-2 24 and >2 5 pts; 9 LDH level above normal. 13 pts received one previous therapy and 16 were treated with 2 or more lines before Z-BEAM, all containing Rituximab. Only 5/29 pts received a reducted dose of 0.3 mCi/kg because of low platelets counts. Response status before Zevalin was:14 CR (49%), 9 PR (33%), 3 SD (9%) and 3 PD (9%). At the end of treatment response status is actually available in 26/29 pts: CR 18 (69%), PR 5 (19%) and PD 3 (12%). Median CD34+ cells infused was 7.26x10^6/kg (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 12/29 pts. One pulmonary Aspergillosis and 8 bacteriemia were documented. One experienced an intestinal perforation during aplasia and one cardiac failure was documented in a pt previously treated with cumulative antraciclines doses and mediastinal radiotherapy. With a median follow up of 15 months PFS is 79% and OS is 83%. 4/14 pts after Z-BEAM showed a subsequent progression and 2/15 relapsed: five pts 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 high CR rate with a good PFS. Engraftment and toxicity were not different from standard BEAM. A matched pair analysis to compare this results with those obtained in a previous group treated with standard BEAM without Zevalin in our institution is ongoing.

C058

LONG-TERM OUTCOME OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN 406 NON-M3 ACUTE MYELOID LEUKEMIA (AML) PATIENTS. A SINGLE INSTITUTION STUDY

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We describe the long-term outcome of autologous hematopoietic stem cell transplantation (HST) in 406 non-M3 AML patients, treated at our Institute from 1981 to 2011 and retrospectively identified through the EBMT Registry. The median age was 32 years (range 1-73) and the M/F ratio 229/177. Disease status at transplant was CR1 in 290 cases, CR2 in 74 cases, advanced disease (3rd or subsequent CR, progressive disease) in 42. Autologous rescue was provided with bone marrow (BM) (66%) or peripheral blood stem cells (PBSCs) (34%). Overall survival (OS) at 20 years after HST was 46% and 40% for patients receiving HST in CR1 and CR2, respectively (p: n.s.), while the median survival time is 165 and 30 months from transplantation. Focusing on patients transplanted in CR1 (n. 290), the conditioning regimen mostly employed was Bu-CY (n. 185), while the remaining patients received the BAVC regimen (n. 80) or other schedules (n. 25). The BM/PBSC ratio was 170/120. Engraftment was obtained in 95% of patients. Leukocyte engraftment times were more rapid with the use of PBSC (median 14 days, range 7-33) compared to BM (median 26 days, range 7-99). Cytogenetic data were available for 48% of CR1 patients; of these, at diagnosis 20% had good risk features, 76% had standard risk features and 4% had poor risk features. Molecular analysis showed favorable abnormalities in 30% of the 144 evaluable cases. At 20 years, the actuarial OS and disease-free survival (DFS) estimates are 46% and 45%, respectively. The median follow-up of survivors is 114 months (range 2-274). The non-relapse mortality rate is 27%. Univariate analysis comparing DFS of patients with favorable biologic features with those with standard/high risk features showed a 10-year DFS of 73% vs 28% (p: 0.01). The significant impact on DFS of good risk karyotype/molecular pattern was confirmed in multivariate analysis (p: 0.01). No impact on DFS was observed by stem cell source. This study provides long-term follow-up data in a large series of non-M3 AML patients treated at a single center, and supports the observation that long-term survival is achievable in about 1/2 of patients overall and in about 2/3 of patients with good risk biologic features. In particular, the promising results obtained in this latter group of patients seem to identify a selected population of Ist CR AML patients who are likely to benefit from an autologous transplantation.

C059

LYMPHOMA AND MULTIPLE MYELOMA PATIENTS AUTOTRANSPLANTED WITH PBSC MOBILIZED WITH PLERIXAFOR ADDED TO MOBILIZING CHEMOTHERAPY CAN ACHIEVE RAPID AND LONG TERM ENGRAFTMENT

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Background. Autologous Stem Cell Transplantation (ASCT) is an effective strategy in Multiple Myeloma (MM) and lymphoma patients (pts), but a significant proportion of pts cannot mobilize a sufficient number of CD34+ cells. Plerixafor (P) addition to granulocyte colony-stimulating factor (G-CSF) significantly increases the number of lymphoma and MM pts

mobilizing ≥2x10^6/kg CD34+ cells, the minimum dose safe for ASCT, but there are very few data about the use of P after mobilizing chemotherapy (CHT). We evaluated safety and efficacy of P administration and outcome after ASCT in poor mobilizers (PM) pts, candidated to ASCT, after disease- specific CHT, followed by G-CSF. Patients and Methods: 37 pts (20 lymphoma and 17 MM) received chemotherapy followed by G-CSF. P (0.24 mg/Kg) was administered subcutaneously for up to 3 consecutive days, continuing G-CSF, 9-11 hours before the planned leukapheresis. The pts were either proven PM (failure of a previous mobilization attempt) or predicted PM (presence of ≥1 factor predicting unsuccessful harvest such as: advanced disease, previous extensive radiotherapy or prolonged use of stem cell poisons, advanced age or extensive Bone Marrow (BM) involvement). Results: P administration was safe and no significant adverse events were recorded. We observed a 4 median fold-increase (range: 1.4-32) of the number of circulating CD34+ cells after P as compared to baseline values (from a median of 5 cells/ L, range 1-32, to a median of 32 cells/ L, range 6-201). 27/37 (72%) pts collected > 2x10^6 CD34+ cells/kg in 1-3 leukapheresis and 24 (13 MM, 11 lymphoma) out of 27 pts rescued with P, were transplanted with P-mobilized PBSCs. Median time to Absolute Neutrophils Count (ANC) ≥500/μL was 13 days (range 10-23); for platelet (PLT) ≥20x10^3/mcl and ≥50x10^3/μL it was 15 days (range 9- 88) and 22 days (range 15-180) respectively. With a median follow-up of 396 days after ASCT (range 90-637), 21 patients (12 MM, 9 lymphoma) are alive and evaluable for response: 12 are in complete remission, 5 in partial remission, 4 in progressive disease/relapse; 3 died due to disease progression. No late graft failures have been observed. Conclusions: addition of P to G-CSF after chemotherapy is safe and effective; it allows to rescue, with a safe ASCT, a relevant proportion of Lymphoma and MM pts considered to be PM.

C060

WT1 TRANSCRIPT LEVELS IN AUTOLOGOUS PERIPHERAL BLOOD STEM CELL (PBSC) HARVESTS PREDICT AML RELAPSE AFTER AUTOLOGOUS TRANSPLANTATION (ASCT).

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Introduction: ASCT is a potentially curative option for pts with AML; unfortunately, the relapse rate after ASCT is high and can be due to contamination with leukemic blasts of autologous PBSC collected by leukapheresis (LK), although these procedures are usually performed when pts are in proved complete remission (CR). Thus, identification and quantification of a reliable minimal residual disease marker in collected PBSC could be relevant in determining the relapse risk after ASCT. High levels of WT1 transcripts detected with RQ-PCR in bone marrow and peripheral blood of AML pts in CR predict disease relapse. We retrospectively evaluated the WT1 transcript levels in autologous PBSC of AML pts who received an ASCT in CR to establish the power of this parameter to predict the risk of relapse. Aim: to correlate the quantitative levels of WT1 in autologous PBSC with the relapse incidence in AML pts who received an ASCT in CR, at our Institute. Patients and Methods: 13 pts, all in morphological and genetic CR at the time of PBSC collection and before ASCT. PBSC collection by LK (COBE Spectra cell separator): median CD34+x10^6/kg: 9.32 (3.79-32). RQ-PCR quantification of WT1 was performed in samples of each LK, using TaqMan technology on RNA from mononucleated cells. The control gene was the housekeeping gene ABL, with WT1 level being normalised to 10^4 copies of ABL per sample. Conditioning regimen: treosulfan 42 gr/m^2, fludarabine 150 mg/sqm, cytarabine 10 gr/m^2. Transplant, median CD34+ x10^6/kg: 5.1 (3.3-8.5). Results: at last follow-up 6 (46%) pts have relapsed after ASCT. Median WT1 copies in LK of pts who relapsed or did not relapse were 193,87 (23.47-839.63) and 16.96 (0.59-82.49), respectively. Overall median relapse free survival (RFS) from ASCT was 455 (69-1576) days; median RFS of pts with WT1 copies >90 (n=3) or ≤90 (n=10) was 351 (93-368) and 560 (69-1576) days (log-rank p=0.003), respectively. One patient with a LK WT1 value ≤90 had an extramedullary relapse. Conclusions: these results suggest the possibility to determine a quantitative cut- off level of WT1 transcripts in autologous PBSC, with higher levels being predictive of contamination of LK products with leukemic blasts and predicting an increased relapse risk after ASCT. These data, if confirmed by our ongoing study, will permit us to discriminate pts who can benefit from ASCT from pts who should be addressed to different therapeutic strategies.

CHRONIC MYELOPROLIFERATIVE DISORDERS

C061

BLEEDING IN ESSENTIAL THROMBOCYTHEMIA: ANALYSIS OF INCIDENCE AND RISK FACTORS IN A COHORT OF 565 PATIENTS

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Background. Hemorrhages are potentially severe complications of essential thrombocythemia (ET). Extreme thrombocytosis and previous bleedings are currently considered the main risk factors for bleeding; however, only few data are available. Objectives. We retrospectively analyzed the hemorrhagic events of 565 consecutive ET patients followed for a median time of 7.8 years, with the aim to evaluate: (1) the incidence and the type of the bleeding complications; (2) the correlation between these events and clinical/laboratory data and (3) the therapeutic implications of the study findings Results: Sixty patients (10.5%) had one or more hemorrhages, for a total of 77 hemorrhagic events. Twenty-four major bleeding (grade 3-4 according to WHO criteria) occurred in 23 patients (4%) at ET diagnosis (4) or during follow-up (20). The remaining 53 hemorrhages were minor (cutaneous or mucosal). Overall, 85.6% of the patients received a cytoreductive treatment during the observation time, achieving at least a partial response in 78% of the cases. Antiplatelet drug was administered to 521 patients (92%), with no differences between patients with or without hemorrhages. The incidence rates of total and severe bleeding were 13.4 and 4.8 events per 1000 person-years, respectively. The risk of hemorrhage was higher at diagnosis (0.5%) and progressively decreased during the follow-up. Patients with hemorrhages presented more frequently splenomegaly and displayed lower hemoglobin concentration and higher leukocyte and platelet count (Student's t-test and 2 test). By univariate (log-rank) analysis, splenomegaly baseline (p<0.0001), a history positive for hemorrhages (p=0.005), a platelet count higher than the median value (p=0.003) and higher than 1000x10^9/L (p<0.0001) and a leukocyte count>11x10^9/L (p<0.0001) significantly correlated with subsequent bleedings. JAK2V617F mutational status (qualitative analysis) was not found to correlate with bleeding: however, only 2 patients were homozygous. Analogously, antiplatelet treatment, which was administered to the great majority of the patients, did not appear as a risk factor. By multivariate (Cox) analysis, splenomegaly (HR 2.9, 95% CI 1.5-5.4, p=0.002), platelet count>1000x10^9/L (HR 2.3, 95% CI 1.3-3.9 p=0.003) and leukocyte count>11x10^9/L (HR 1.9, 95% CI 1.1-3.2, p=0.023) retained their prognostic significance. Considering these 3 parameters, patients were stratified in 3 subgroups, characterised by an increasing risk for hemorrhage: low (no risk factors, 339 patients), intermediate (1 risk factor, 160 patients) and high (2 or 3 risk factors, 66 patients). The cumulative risk for haemorrhage at 10 years was 8.2% in the low- risk, 14.4% in the intermediate risk (p=0.22) and 45.8% in the high risk group (p<0.001). When severe hemorrhages were considered separately, only splenomegaly retained its prognostic significance on bleedings. In one case, bleeding was the ultimate cause of death. Conclusions During the course of ET, bleeding represents a relatively rare event, whose incidence decreases over the time. Marked thrombocytosis, leucocytosis and spleen enlargement, particularly when concomitant, identify a cohort of patients at higher hemorrhagic risk. In these cases, the use of antiplatelet treatment should be balanced between the risk of bleeding and the thrombotic risk, which may itself be increased by the same features.

C062

JAK2 EXON 14 SKIPPING IN PATIENTS WITH IDIOPATHIC MYELOFIBROSIS

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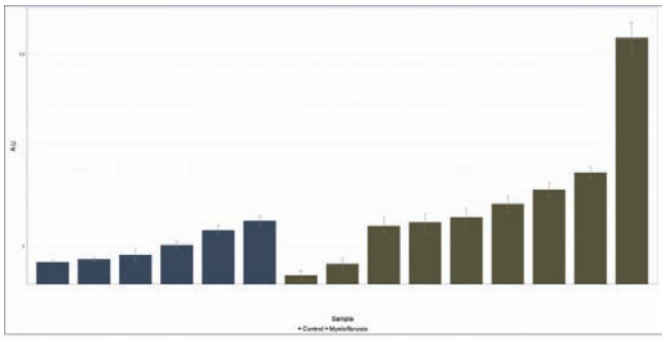
In recent years the diagnosis and treatment of patients with myeloproliferative disorders has focused on the JAK2 gene. Recently, an alternative splicing of JAK2 transcript that leads to the deletion of the entire exon 14 (88bp) has been described (Ma et al. (2010) PLoS ONE, 5, e12165). This mRNA has a stop codon in exon 15 and is translated into a truncated protein that has a deletion within the JH2 pseudokinase

domain and complete deletion of the kinase domain (JH1). The alternative transcript was detected by QF-PCR, in plasma of patients with myeloproliferative neoplasms (58% of patients positive for the V617F mutation and 33% negative) but never in healthy subjects. The proportions of the variant ranging from 2% to 26% compared to the normal transcript. Quantification of the transcript was performed by quantitative fragment length analysis, which is not the most appropriate method for such determination (Cirigliano, V. et al. (2001) Mol. Hum. Reprod., 7, 1001-1006). We investigate mRNA levels of the JAK2 gene and its splice variant lacking exon 14, in granulocytes isolated from peripheral blood of 6 healthy individuals and 9 patients diagnosed with idiopathic myelofibrosis (6 negative and 3 positive for the JAK2 V617F mutation). To overcome the limitations of quantitative fragment length analysis, the levels of the splice variant lacking exon 14 were assessed using a boundary-spanning primer designed on the junction between exons 13 and 15 (Vandenbroucke II, et al. (2001) Nucleic Acids Res., 29:E68-8). The JAK2 V617F allele burden was measured as previously described (Lippert, E. et al. (2006) Blood, 108, 1865-1867). Low levels of the alternative-spliced product was found in all DNA samples from patients and from all healthy individuals, in proportions ranging from 0.2 % to 3% compared to the normal transcript. In 2 patients carrying the JAK2 V617F mutation, the levels were respectively 3 and 11 times higher than in healthy subjects. Our data show that the JAK2 splice variant lacking exon 14 is not peculiar of PMF patients but is detectable also in healthy subjects. The high level of the splice variant found in 2 PMF patients could be due to a pathological increase in the levels of the constitutive variant. These preliminary results suggest that this study may be useful to improve our understanding of how, mutations in JAK2, contribute to the pathological phenotype in this clinical context and in others.



Bar chart representing the quantification of the *JAK2* alternative spliced isoform in 9 MMM patients and 6 healthy controls (quantities are expressed as fold difference relative to the mean of control group)

C063

FREQUENCY OF ASXL1 MUTATIONS IN PRIMARY AND SECONDARY MYELOFIBROSIS

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In patients with Myelofibrosis (MF), genetic lesions other than the JAK2V617F substitution have been recently identified, including ASXL1 truncating mutations, that may contribute to disease development. Aim of this study was to gain insights into the possible role of ASXL1 aberrations in the molecular pathogenesis of primary MF (PMF), and their contribution to the disease progression from Polycythemia Vera (PV) and Essential Thrombocythemia (ET) to MF. Indeed, the whole exon 12 of ASXL1 was directly sequenced in 43 primary (PMF) and 22 secondary MF patients (13 post-PV and 9 post-ET), as well as in 10 polycythemia vera (PV) and 10 essential thrombocytemia (ET) cases. Overall, 19 distinct ASXL1 mutations (10 frameshift, 8 nonsense and 1 missense) were identified in 28/65 (43%) MF patients: 23/43 (53%) with PMF and 5/22 (23%) with secondary MF (3 post-PV and 2 post-ET). Analysis of paired CD3+ T lymphocytes in 14 cases confirmed that all lesions were restricted to the neoplastic myeloid clone. Analysis of sequential samples in 7 ASXL1-mutated PMF patients documented the presence of the same lesion at diagnosis and during follow-up in 6 cases, whereas in one patient the mutation was detectable only during the follow-up. Sequential analysis in 4 secondary MF patients unveiled the presence of the lesion before disease shift to MF in 2 cases; one patient acquired the mutation during the follow-up but before becoming MF, while another proved positive already at the time of PV diagnosis. In our cohort of patients, acquisition of ASXL1 mutations seems to be a rather late event in PV and ET, being detected in 3 out of 4 informative cases several years after first presentation, during the course of chronic-phase MPN. In conclusion, ASXL1 mutations may possibly occur as an early event during the molecular pathogenesis of PMF, whereas they can develop later during the course of the disease in MF arising on a previous PV or ET. Thus, our results suggest a role for these lesions in the molecular progression of the disease.

C064

ANALYSIS OF ASXL1 MUTATIONS IN 215 PATIENTS WITH MYELOFIBROSIS : IMPACT ON DISEASE PHENOTYPE AND PROGNOSIS

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Background. Additional sex comb-like 1 (ASXL1) is a member of Enhancer of Trithorax and Polycomb gene family involved in chromatin modification and retinoic acid pathway regulation. Recent studies identified ASXL1 mutations in various myeloid malignancies such as myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN) and MDS/MPN. Methods. In this study, we investigated ASXL1 mutational status in 215 pts with myelofibrosis (MF) including 152 primary (PMF), 35 post-polycythemia (PPV-) and 28 post essential thrombocythemia (PET-) MF. Somatic mutations of ASXL1 were identified by sequencing exon 12 of whole-genome amplified DNA isolated from granulocytes; all mutations were validated by re-sequencing genomic DNA from the archival sample. Genotype was correlated with clinical presentation and laboratory data. Results. ASXL1 mutations occurred in 31 pts (14.4%) at diagnosis, 25 (16%) with PMF and 6 (9.5%) with PPV/PET-MF. All detected mutations were heterozygous missense changes or frameshift mutations caused by deletion or duplication of nucleotides. Analysis of ASXL1 mutation in sequential samples available in 110 pts showed that ASXL1 mutation can be lost at leukemia transformation (concurrently with loss of JAK2V617F allele; one case) or acquired during disease (3 pts). Forty-eight percent of ASXL1 mutated pts were JAK2 V617F-positive, only one was MPL mutated. EZH2 mutation was more frequent among ASXL1-mutated (20%) than ASXL1 neg pts (5.5%) (p= 0.014). There was no significant difference between ASXL1 mutated and unmutated pts as concerned age, sex distribution, IWG-MRT high-risk category, white cell count, hemoglobin level, platelet count or others hematologic characteristics at diagnosis including splenomegaly and constitutional symptoms. A blast count >1% was more common among ASXL1 mutated pts (60% vs 26.8%; p=0.001). Pts with ASXL1 mutation had worse OS (median: 39.0 months vs 43.8 in ASXL1 unmutated; p=0.035) but the significativity was lost in multivariate analysis. Forty-two percent of ASXL1 mutated pts evolved to AML as compared with 16% of wild-type pts (p= 0.0001). ASXL1 mutation predicted for a shorter leukemia-free survival (LFS) (p=0.047), that was maintained also in multivariable analysis. Conclusions. Exon 12 ASXL1 mutations occur in a sizable proportion of pts with myelofibrosis and represent an independent variable associated with the risk of leukemia transformation.

C065

A RETROSPECTIVE ANALYSIS OF 603 PATIENTS WITH POLYCYTHEMIA VERA (PV) FOLLOWED IN THE LAZIO REGION FROM 1979 TO DATE: DEFINITION OF PROGNOSTIC FACTORS ON THROMBOSIS-FREE SURVIVAL (TFS) AND OVERALL SURVIVAL (OS)

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PV is a myeloproliferative neoplasm characterized by a trilinear marrow expansion and an increased susceptibility to thromboembolic com-

plications. We have collected the data of 604 patients (pts) followed in 11 Hematological centers of our region from 1978 to December 2010. The diagnosis was made according the 1975 PVSG criteria, the WHO 2001 and 2008 criteria, respectively for the diagnoses made until 2000, 2007 and to date. The main epidemiological and clinical features of all pts are reported in the table: Pts number 603 Age (years, median, range) 63 (18-97) Gender, F/M (number, %) 258/345 (42,8/57,2%), WBC (x10^9/L, median, range) 10,0 (6,9-37,3) Hb (g/dL, median, range) 18,2 (10,2-24,8) PLT (x10^9/L, median, range) 437 (343-1790) JAK2 V617F (mutated/all performed,%) 313/376 (83,2%) Quantitative JAK2 V617F (%), median 60,85 (0,3-99,9) Splenomegaly (number,%) 232/580 (40%) Previous thrombosis (all, number,%) 129/594 (21,7%) Arterious /Venous (number,% of all A: 97(75,2%) thrombosis) V:32 (24,8%) Of 603 pts, 67 (11,1%) died, 95 (15,8%) were lost at follow up and 441 (73,1%) are alive at the time of evaluation. The median follow up of living pts was 7,9 years. The thrombotic events during follow-up were 85 (14,3% of 596 valuable pts): the arterious events were 53 (8,9%), the venous were 32 (5,4 %). The rate of thrombosis (patients/year) was 1,75 %. At the univariate analysis, risk factors at diagnosis that resulted statistically significant for Thrombosis-Free Survival (TFS) were: age (> 60 yrs, p =0,0071), WBC (> 10,0 x 10^9/L, p= 0,0012), previous thrombosis (p= 0,0012) and the presence of cardiovascular risk factors (p= 0,033). Hb (>18 g/dL), PLT count (either > 437 x 10^9/L or >1000 x 10^9/L), JAK2 V617F mutated, JAK2V617F allele burden (either > 60,85% or > 50%) did not reach the cut-off value of significance (p<0,05). At the multivariate analysis performed according to the Cox proportional hazards model method, only age (>60 yrs, p= 0,03) and white blood cells (> 10 x 10^9/L, p =0,0139) maintained the significance level. The risk factors for Overall Survival (OS) that reached at univariate analysis the statistical significance were: age >60 yrs, p <0,0001), WBC > 10,0 x 109/L (p =0,0014) and previous thrombosis (p =0,0067). Any of the other variable tested for OS (Hb, PLT, JAK2 V617F mutated, JAK2 V617F allele burden) resulted significant. At the multivariate analysis, age (p <0,0001), WBC count (p = 0,0079) and previous thrombosis (p =0,0173) maintained their prognostic impact on OS. In conclusion, this retrospective analysis on a large cohort of PV pts confirms the prognostic value of age and WBC for both TFS and OS: it will be advisable to evaluate what features could further discriminate thrombotic and survival risk in different age groups (younger vs elderly pts).

C066

CIRCULATING PROANGIOGENIC TIE2-EXPRESSING MONOCYTES (TEMS) IN PATIENTS WITH PH NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background. Patients with Primary Myelofibrosis (PMF) have an enhanced angiogenesis both in the bone marrow and in the spleen. However, little is known on the mechanism producing this phenomenon. The expression of the angiopoietin receptor Tie2 identifies a novel subset of circulating CD14lowCD16+ cells, called Tie2 Expressing Monocytes (TEMs), endowed with marked proangiogenic activity. Besides these markers, TEMs are identified by lack of expression of L-selectin (CD62L) and CCR2, both involved in the inflammatory response. Aims. To assess the frequency of TEMs in peripheral blood (PB) and single cell suspension of splenic tissue of patients with PMF, Policythemia Vera (PV) or Essential Thrombocythemia (ET), and in healthy subjects (CTRLs). Methods. Pre-fibrotic PMF (n=10), fibrotic PMF (n=39), PV or ET (n=10) patients and CTRLs (n=15) were studied. To assess TEMs, 100 l of PB or 2 x105 cells from fresh spleen were stained with APC-conjugated-anti-Tie2, PECy7-conjugated-anti-CD14, FITC-conjugated-anti-CD16, PerCP-conjugated-anti-CCR2, PE-conjugated-anti-L-Selectin. Frequency of circulating CD14lowCD16+ cells was expressed as percentage of total PB mononuclear cells, whereas TEMs were expressed as percentage of circulating or splenic CD14lowCD16+ cells. Data are shown as median (range). Results. The frequency of PB CD14lowCD16+ cells was higher (p<0.01) in patients with pre-fibrotic (2.4%, 0.2-3.7) and fibrotic PMF (1.7%, 0.0-8.0) than in CTRLs (0.9%, 0.4-1.6), while in PV or ET patients it was in the range of CTRLs values (1.2%, 0.4-4.1). The percentage of circulating TEMs (CD14low CD16+ Tie2+ CD62-CCR2- cells) was lower (p=0.02) in patients with fibrotic PMF (5.2%, 0-27.7) than in CTRLs (15.5%, 2.2-28.2), notwithstanding the higher frequency of CD14lowCD16+ cells. TEMs were comparable in patients with pre-fibrotic PMF, PV or ET, and in CTRLs. The percentage of TEMs was higher in splenic cells from PMF patients (n=4) (1.4%, 0.2-8.7) than in CTRLs splenectomised for trauma (n=3) (0%, 0-3). Conclusions. Patients with PMF showed a decreased percentage of circulating TEMs compared to patients with PV or ET and to CTRLs. However, the percentage of TEMs was higher in the spleen of PMF patients than in healthy individuals. Our data suggest that in patients with PMF non-inflammatory circuits may recruit proangiogeneic TEMs to the spleen, supporting pathological angiogenesis, similarly to what has been reported in solid cancer-associated neoangiogenesis.

CYTOGENETICS AND MOLECULAR GENETICS

C067

FISH REVEALS DEFECTS IN UNEXPECTED CHROMOSOMAL REGIONS OF CYTOGENETICALLY NORMAL MDS PATIENTS

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The eighty-nine consecutive chromosomally normal MDS patients analysed in the present study came to our observation in the period January 2005-December 2010. They were thirty-four females and fifty-five males, median age 64 years (range 22-77). Six patients were classified as RARS, 35 as RA, 7 as RCMD, 25 as RAEB-1 and 16 as RAEB-2. Considering IPSS score, 32 patients were considered low-risk, 34 intermediate-1 risk and 17 intermediate-2 risk and 6 as high-risk. Median follow-up was 21 months (range 1-66). At the time of the study no patient has died. Overall, 21 patients experienced disease progression. FISH probes were chosen based on the frequency of their involvement in MDS and their Mb position determined using UCSC genome browser on Human Mar. 2003 assembly. They were obtained from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA), labelled and applied as previously described. 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); RP11-101K5 (4p14); RP11-544H14 (2q33). i-FISH cut-off values were fixed at 10%. An abnormal FISH pattern was revealed in 30 patients (33.7%). A single defect was revealed in 22 patients (73.3%) and more than two defects in 8 (26.7). Nineteen patients (63.3%) presented a 19q13.2 deletion, 7 (23.3%) a deletion of band 14q13.2, 4 (13.3%) a deletion of band 17q11.2, 5 (16.6%) a deletion of band 5q13.1-q13.2, 4 (13.3%) a deletion of band 4p14, 3 (10%) a deletion of band 10q11.22, 3 (10%) a deletion of band 7q34, 2 (0.6%) a deletion of band 10q21.3 and one a deletion of band 2q33.1-q33.2. An abnormal FISH pattern was observed in 1/6 RARS, in 8/35 (22.8%) RA, in 3/7 RCMD, in 10/25 (40%) RAEB-1 and in 8/16 (50%) RAEB-2. Considering the IPSS, at least one defect was observed in 5/32 (15.6%) low-risk, in 14/34 (41.2%) intermediate-1 risk, in 8/17 (47.0%) intermediate-2 risk and in 3/6 (50%) high-risk patients. Disease evolution occurred in 2 RA patients, in 4 RAEB-1 and in 4 RAEB-2 patients with an abnormal FISH pattern. Seven of these patients presented at least two chromosomal deletions. In conclusion our data suggest that FISH: i) reveals novel unexpected chromosomal lesions in about 36% of chromosomally normal MDS patients; ii) these chromosomal lesions mostly consist in gains/losses; iii) an abnormal FISH pattern with more than two deletions seems to correlate with disease progression.

C068

MOLECULAR CHARACTERIZATION OF ACUTE MYELOID LEUKEMIA; A SICILIAN NETWORK EXPERIENCE

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Acute myeloid leukaemia (AML) is a heterogeneous group of haematopoietic malignancies characterized by proliferation and maturation arrest of myeloid blasts in bone marrow and blood. In 25% of cases, specific chromosomal translocations like the t(8;21), inv(16), or t(15;17) are showed and are associated with a good outcome. About half of AMLs have normal karyotype (NK-AML) but are characterized by an heterogeneous molecular abnormalities; adverse prognostic impact include gene mutations of FLT3, WT1, IDH1, DNMT3A and high expression levels of the BAALC, ERG and MN1 genes, whereas favourable prognosis is associated with the presence of mutations in the CEBPA and NPM1 genes. Aims: We planned to perform a regional study to realize a bio-bank of molecular characterized leukemic blasts. All the Haematology Sicilian Centre are involved and the primary objectives are to valuated the incidence of the molecular aberration in our region. Preliminary results on 100 consecutive AML patients enrolled in the study are reported. Methods: Molecular characterization included fusion gene (BCR/ABL, AML1/ETO, CBFB/MYH11, MLL rearrangements); gene mutations (FLT3, NPM1, CEBPA, WT1, IDH1 and IDH2); gene expression (BAALC, ERG, AF1q and MN1). AML1/ETO and CBFB/MYH11 positive leukaemias were also characterized for KIT gene mutation. Results: We identified: 32/100 AML cases (32%) showing NPM1 mutations, 21/100 AML cases (17%) showing FLT3 mutations (19 with FLT3 ITD and 2 with D835), 2/20 AML cases (10%) showing CEBPA mutations, 5/76 AML cases (6,5%) showing WT1 mutations (4 cases on exon 7 and 1 case on exon 9), 2/20 AML cases (10%) showing R132C IDH1 mutations, 1/20 AML cases (5%) showing R172K IDH2 mutations, 4/100 AML cases (4%) showing AML1/ETO fusion gene, one of them showing D816V KIT mutation, 4/100 AML cases (4%) showing CBFB/MYH11 fusion gene, 2/100 AML cases (2%) showing BCR/ABL fusion gene, 3/100 AML cases (3%) showing MLL rearrangements. For gene expression analysis (BAALC, ERG, AF1q and MN1) 50 NK-AML patients were analyzed and divided into quartiles by gene expression levels, cases are scored as low and high expressers using the median expression level. We identified 35/50 patients showing high expression of 2 or more genes. Conclusions: Assessment of these molecular prognostic markers at the presentation of the acute leukaemia allows to determine risk category of most AML patients and may be very helpful to appropriately tailor the aggressiveness of therapy.

C069

OVEREXPRESSION OF THE ILDR1 GENE IN MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDSs) are a heterogeneous group of hematopoietic stem cell disorders characterized by ineffective hematopoiesis and a propensity to progress into acute myeloid leukemia. In de novo MDS, chromosomal lesions consist mainly of unbalanced rearrangements and numerical defects whereas balanced structural rearrangements are rare, being observed in fewer than 5% of patients and their prognostic impact remains unknown. Aims. We aimed to characterize the t(3;11)(q13;q14) rearrangement in MDS at the genomic, molecular and functional level. Methods. One-hundred and fifteen MDS patients at diagnosis were analyzed by conventional cytogenetic analysis. FISH analyses were performed using BAC and fosmid probes selected according to the University of California Santa Cruz database (http://genome.ucsc.edu/). Quantitative real-time PCR (qRT-PCR) experiments were performed using the ABI Prism 7300 Sequence Detection System. Statistical analysis of the relative expression results was performed with the Relative expression software tool (REST). Results. Two (1.7%) cases showed a t(3;11)(q13;q14) translocation. FISH experiments detected the presence of the same breakpoints in both patients. UCSC database querying showed that no known gene was located on the chromosome 11 breakpoint, whereas 3 genes (CD86, ILDR1, and CASR) with known function were mapped next to the chromosome 3 breakpoint. qRT-PCR experiments showed ILDR1 upregulation in the patients by a mean factor of 13.775 (p=0.02). Bioinformatic analysis of the chromosome 11 breakpoint region showed the presence of a promoter (892+)#2 and a CpG island (CpG 172) at a distance of about 220 Kb from the breakpoint region. Conclusions.

We reported a novel t(3;11)(q13;q14) rearrangement associated with overexpression of the immunoglobulin-like domain-containing receptor (ILDR1) gene in MDS patients. We hypothesize that the gene upregulation could be mediated by the juxtaposition of regulatory elements next to the ILDR1 gene as a consequence of the chromosomal translocation. ILDR1 expression has been related to the development and/or progression of cancer, as it has been detected in the transformation of a low grade follicular lymphoma to a high-grade diffuse large B cell lymphoma.

The question whether there is a functional link between the clinical features and the ILDR1 gene dysregulation and whether it may have a potential prognostic significance in MDS remains to be established.

C070

SUCCESSFUL CONTROL OF MOLECULAR PROGRESSION AND HEMATOLOGICAL RELAPSE OF NPM1-MUTATED AML BY 5-AZACYTIDINE (5-AZA)

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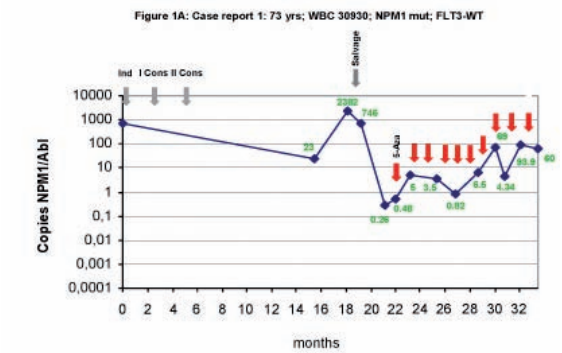
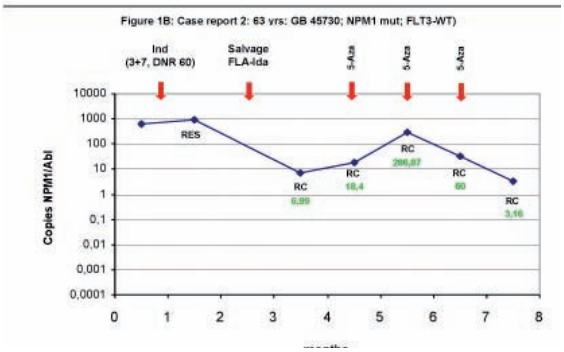
Acute myeloid leukemia (AML) with nucleophosmin (NPM1) gene mutations displays distinctive clinical and biological features which lead to its inclusion as a new provisional entity in the 2008-WHO classification of myeloid neoplasms. Although generally associated with favourable prognosis (in absence of FLT3-ITD mutation), about 50% of patients experience disease relapse. Thus, new therapeutic strategies, likely with low-toxicity, are warranted for the treatment in particular of elderly patients or patients with co-morbidities. Given the proven time stability of NPM1 mutation and the possibility to monitor the minimal residual disease (MRD) by quantitative assessment of NPM1 mutated transcript copies, and the evidence that increasing MRD levels after achieving complete remission (CR) are nearly uniformly followed by rapid overt AML relapse, prevention of overt relapse by MRD-guided therapeutic interventions, the so-called pre-emptive therapy, seems to be a reasonable approach. Recently, Wermke et al. (Leukemia 2010;24:236) successfully treated molecular relapse of NPM1-mutated AML with 5-azacytidine (5-AZA) and here we confirm this finding in two patients from our institution. The first patient we treated was a 73-year old woman in II CR after salvage therapy. Because of age, previously received cycles of chemotherapy and cardiac comorbidity, we decided to try to control MRD of NPM1-mutated AML by cycles of 5-AZA. Interestingly, monthly administered 5-AZA (75 mg/sqm/day, days 1-7) was well-tolerated allowing a good control of the molecular disease and prevention of overt hematological relapse for the following 13 months (Figure 1A).





The second patient was a 63-year old man in I CR after salvage therapy for primary induction failure which was considered un-fit for any further intensive chemotherapy because of pulmonary comorbidity. Interestingly, after an initial increase in NPM1 mutated transcript copies up to 286,87 upon the first dose of 5-AZA, we then assisted at their progressive reduction after the second and third doses to reach the value of 3,16 (Figure 1B). Given these successful experiences, further investigation of this drug, alone or in combination with other compounds, is warranted in NPM1-mutated genetic subtype of AML.

C071

TELOMERE LENGTH MEASUREMENT IN THE CLINICALLY ORIENTED RESEARCH SETTING: A COMPARISON BETWEEN SOUTHERN BLOT(SB), REAL TIME QUANTITATIVE PCR(RQ-PCR)AND FLOW-FISH (FF).

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Telomere length(TL) measurement has a growing role in the characterization of both lymphoid and myeloid disorders.In Chronic Lymphocytic Leukemia (CLL), it has also been validated as an independent outcome predictor(Rossi D.2009).The most widely used approaches are SB, RQ-PCR and FF. All of them have advantages and caveats, but have never been directly compared on large series of samples. Aims were to compare the three methods in a panel of 12 CLL patients(pts) and 46 healthy or free of malignancy disease subjects(HS) in terms of: reproducibility; ability to detect the correlation with age in HS; ability to detect the correlation with VH-mutational status(VH-MS) in CLL; reciprocal concordance of the three methods.SB and FF were performed as previously reported(Ladetto M.2004,Hultdin M.1998).RQ-PCR was performed according to Gil M.L.and Coetzer T.(Mol Biotech2004) which proved the most reliable than the other published methods. All the experiments were performed in duplicate by different operators and showed excellent intra-method reproducibility(SB:r2=0,7 p<0,0001;FF:r2=0,8 p<0,0001;RQ-PCR:r2=0,7 p<0,0001).All the three methods were able to clearly discriminate HS and CLL pts based on the severe telomere erosion described in CLL(SB p<0,001;FF p<0,001;RQ-PCR p=0,0250).In HS a good age correlation was observed using either SB and FF(SB:r2=0,5 p<0,0001;FF:r2=0,42 p<0,0001) which was less evident with RQ-PCR(r2=0,06 p=ns).In CLL pts the well known correlation between TL and VH-mutational status was detected either using SB and FF(SB p<0,001;FF p=0,0159), but not using RQ-PCR.Finally when SB results were correlated with FF an excellent correlation was found(r2=0,4;R Spearman=0,6;p<0,0001).The same did not happen when either SB or FF were compared to RQ-PCR(QPCR vs SB:r2=0,1;R Spearman= 0,19;QPCR vs FF:r2=0,08;R Spearman=0,15).We were unable to improve the performance of RQ-PCR despite extensive tuning-up of the PCR reaction(including variation of primers concentration and thermal profile). FF and SB appear accurate and show a high degree of concordance and their use is fully justified in the clinically oriented research setting.RQ-PCR still remains an attractive option due to its low labor-intensiveness and modest DNA requirements, but the tune-up of the method is difficult and so far we have been unable to generate satisfactory results.This suggest that additional effort is required to make RQ-PCR more user-friendly in order to ensure a broader applicability.

C072

DETECTION OF CENTROSOME AMPLIFICATION INDUCED CHROMOSOME INSTABILITY IN LEUKEMIA CELL LINES.

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Centrosomal abnormalities are a cancer hallmark frequently involved in mitotic spindle defects, chromosome missegregation and aneuploidy. Recently, it has been shown that they constitute the major cause of chromosome instability (CIN) in tumors, through the increased formation of aberrant attachment of centromeres to mitotic spindle. Abnormal centrosome number and structure can be detected at early stages in hematological malignancies, including chronic myeloid leukemia, and correlate with disease progression. The assessment of CIN could be performed by the identification of the increase in karyotype abnormalities over generations or by direct observation of missegregation events. None of the current techniques is sufficient to fully evaluate the mechanisms of CIN, and moreover none of them is really suitable for diagnostic applications. Here we describe an alternative approach to measure CIN based on Cytochalasin-blocked micronucleus assay (CBMN assay). It relies on the detection of once-divided cells, which appear as binucleated due to the action of cytochalasin-B. In the case of CIN phenotype, micronuclei (MNi) can be detected and scored. This biomarker originates from whole chromosomes that lag behind during anaphase division and are likely to be missegregated. K562 cell line was used as a model for CIN in normal condition and under exposure to genotoxic stress, to verify the connection between supernumerary centrosomes and higher chromosome missegregation rate. CBMN assay showed that MNi frequency follows a linear trend both to increasing concentrations or longer exposure time to hydroxyurea. Immunofluorescence assays performed on cytochalasin-blocked cells, demonstrated a clear association between centrosome abnormalities and abnormal nuclear morphology. More than 90% of cells experiencing numerical chromosome instability beared extra centrosomes. FISH analysis was able to detect at the same rate of CBMN assay, both the MNi frequency and chromosome missegregation events, but it gave also more valuable information about chromosome specific missegregation rate and micronuclei content. In conclusion the experiments reported above showed that: 1) CBMN assay can reliably detect CIN in leukemia cells, 2) K562 cell line experiences extensive chromosome instability following exposure to HU, 3) centrosome amplification leads to the chromosomal missegregation events in leukemia cells.

NON-HODGKIN'S LYMPHOMA II

C073

RITUXIMAB, BENDAMUSTINE AND CYTARABINE (R-BAC) IS A VERY ACTIVE REGIMEN IN PATIENTS WITH MANTLE CELL LYMPHOMA NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY OR AUTOLOGOUS TRANSPLANT

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Background: Bendamustine (B) and rituximab (R) in combination have relevant clinical activity in mantle cell lymphoma (MCL), and a favorable toxicity profile. We combined cytarabine, a key drug in the treatment of younger patients with MCL, with B and R (R-BAC) in previously untreated patients with MCL aged >65, and in patients relapsed or refractory to previous immunochemotherapy. Materials and methods: This safety/efficacy phase II study started with a dose-finding stage based on three stepwise dose-escalations of cytarabine. Subsequently, in the treatment stage, patients received 4 to 6 cycles of R 375 mg/m2 on day 1, B 70 mg/m2 on day 2 and 3, cytarabine 800 mg/m2 (fixed as maximum tolerated dose) on Day 2, 3, and 4. Cycles were administered every 4 weeks, mostly as outpatient. The primary objectives were the safety of R-BAC, and assessment of overall and complete response rates (ORR and CRR). This trial was registered at ClinicalTrials.gov (NCT00992134). Results: From june 2009, 33 patients were prospectively enrolled, of whom 19 were previously untreated. Median age was 71 years (range 55-88). Ann Arbor stage was III-IV in 89%, 39% had bulky disease, and MIPI was high in 54%. Cytologic subtype was blastoid in 18%, and mean ki-67 was 25% (range 5-80). Overall, R-BAC was very well tolerated. After 126 administered cycles the dose limiting toxicity was haematological, with 60% and 90% of patients experiencing transient grade 3-4 neutropenia and/or thrombocytopenia, respectively (duration 1 to 5 days). Grade 2 anemia was also frequent, with 60% of patients that were transiently treated with erythropoetin. Hematologic toxicity was significantly more frequent in previously treated patients than in treatment naive. Four patients (12%) had febrile neutropenia with pneumonitis in one. Eleven patients (33%) had grade 1-3 elevation of gamma-GT. No other significant toxicity was observed. None of the patients had alopecia. According to the recently revised PET-including response criteria (Cheson BD et al, JCO 2007), ORR was 96%, and CRR 88% (23 of the 26 patients evaluable for response). One patient had stable disease, and two had partial response (PR). With a median follow-up from the start of therapy of 12 months (range 1-24) two patients have relapsed and died of disease progression. The 1-year progression-free survival was 90%±7%. Conclusions: R-BAC combination is a well tolerated and active regimen in the treatment of older patients with MCL.

C074

INTEGRATED MOLECULAR PROFILING FOR THE DIAGNOSIS AND CLASSIFICATION OF PERIPHERAL T-CELL LYMPHOMAS

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The differential diagnosis among the commonest peripheral T-cell lymphomas (PTCLs) (PTCL not otherwise specified, NOS; angioimmunoblastic T-cell lymphoma, AITL; and anaplastic large cell lymphoma, ALCL) is difficult, the morphologic and phenotypic features being largely overlapping. In addition, recent studies suggested the existence of diverse tumors among PTCLs/NOS, with different cellular derivation and clinical behavior. We performed an integrated profiling of PTCLs to identify molecular signatures able to improve their diagnosis and subclassification. We studied 95 PTCLs, including 73 PTCL/NOS, 12 ALCL (6 ALK+ and 6 ALK-), and 10 AITL, and 16 normal T-cell samples. All tissue samples were formalin-fixed and paraffin embedded (FFPE). Gene expression profiling (GEP) was performed by Illumina Whole Genome DASL Assay, while miRNA profiling was done by TaqMan Array

MicroRNA Cards. Immunohistochemistry (IHC) was used for validation in the same cases. First, we documented the efficiency of GEP from FFPE tissues by comparing the mRNA levels and the presence of the corresponding protein, including expressed (i.e. CD3) and not expressed (i.e. BCL10) molecules. Secondly, we tried to discriminate different PTCLs basing on their GEPs. By dividing a training (N=47) and a test set (N=48), we found 2 signatures able to differentiate PTCL/NOS vs. AITL and PTCL/NOS vs. ALCL ALK- with overall accuracy of 94% and 100% in the test sets, respectively. Interestingly, the identified genes represented relevant functional pathways differentially regulated in the 3 tumor types, including protein kinase cascade, proliferation, and cell cycle. Subsequently, we clustered PTCLs/NOS according to their cellular derivation. Indeed, by studying the molecular signatures of CD4, CD8 and TFH cells, we discriminated 3 PTCL/NOS groups, corresponding to the 3 counterparts. Finally, we identified miRNA differentially expressed in ALCL vs. other PTCLs (N=2), PTCL/NOS vs. AITL (N=7), and PTCLs vs. normal cells (N=106). Notably, their predicted target genes were able to differentiate the samples as the miRNA did, indicating a strong impact of miRNA regulation on the global PTCLs GEP. We successfully generated for the first time GEP and miRNA profiling from routinary FFPE PTCL samples. We identified molecular signatures useful for the clinical practice and, specifically, for the diagnosis of PTCL types and for the prognostic stratification of PTCLs/NOS basing on their cellular counterparts.

C075

THE ADDITION OF LENALIDOMIDE TO RITUXIMAB-CHOP21 IS SAFE AND FEASIBLE IN ELDERLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): RESULTS OF PHASE I REAL07 TRIAL OF ITALIAN LYMPHOMA FOUNDATION (FIL)

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Background. R-CHOP21 is the standard treatment for untreated DLBCL in the elderly, however 30-40% of patients failed. Lenalidomide alone or in combination with Rituximab had a promising role in relapsed/refractory DLBCL. On these basis, FIL is running a prospective multicenter dose finding phase I-II trial aimed to evaluate toxicity and activity of Lenalidomide plus R-CHOP21 (LR-CHOP21) in this setting (NCT00907348). The study was designed with the Continual Reassessment Method; primary end-point for the phase I was to define the Dose Limiting Toxicity (DLT), the maximum dose inducing any grade ≥ 3 non-hematologic toxicity, or a > 15 days delay of planned cycle. Patients and Methods. Inclusion criteria were: age 60-80; untreated CD20+ DLBCL; Ann Arbor stage II/III/IV; International Prognostic Index (IPI) at low-intermediate/intermediate-high/high (LI/IH/H) risk. Treatment plan was: 6 R-CHOP21 in association with Lenalidomide for days 1-14 at the established dose level (5, 10, 15, 20 mg). Phase I was planned to define the Maximum Tolerated Dose (MTD), that is the dose that achieves a DLT in 33% or less patients; at the end of each cohort of three patients, the dose level associated with an updated DLT probability closest to 33% was recommended to be administered to the next cohort. Results. From May 2008 to February 2010, 21 patients were enrolled in the phase I study (Table 1). Patient allocation by Lenalidomide dose was: 5 mg/day in nobody, 10 mg/day in nine patients, 15 mg/day in nine and 20 mg/day in three. DLTs in the first three courses of LR-CHOP21 were recorded in seven patients; these events determined Lenalidomide 15 mg/die as the MTD. Of 115 LR-CHOP21 courses performed, hematological toxicity was mild: grade III/IV thrombocytopenia occurred in 10% of courses, anemia in 4% and neutropenia in 28%. Extra-hematological toxicities were moderate: grade IV increase of CPK in one patient, grade III cardiac in one, grade III neurological in three and grade III infections in four (two pneumonias, one febrile neutropenia with diarrhea and one diarrhea). At the end of six LR-CHOP21, complete remission was achieved in 76% patients. Conclusions. Lenalidomide administered at the dose of 15 mg on days 1-14 of each courses in association with R-CHOP21 is safe and feasible in elderly DLBCL, with promising preliminary efficacy results. The ongoing phase II part of the trial is aimed to test the activity of 15 mg of Lenalidomide in association with R-CHOP21.

Table 1.

Clinical characteristics N=21		Median age 68 years (61-77)	
M/F	57/43 %	Bone marrow	29 %
Stage II/III/IV	19/19/62 %	Extranodal sites ≥ 1	43 %
B Symptoms	52 %	LDH > normal	43 %
PS 0-1/≥2	19/81 %	IPI LI/IH/H risk	24/52/24 %
DLBCL de novo/ shift low grade	95/5 %	B2microgl > normal	57 %

C076

THE OUTCOME OF DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IS NOT PREDICTED BY INTERIM 18-FDG-POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY (PET): FINAL ANALYSIS ON 88 PATIENTS TREATED WITH FIRST-LINE R-CHOP

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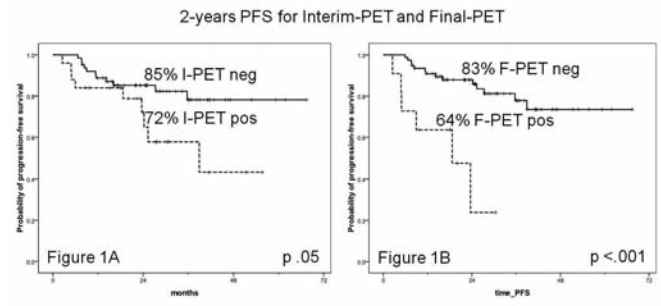
Predictive value of Interim-PET (I-PET) in DLBCL is controversial because interpretation criteria are not standardized and visual analysis by dichotomous evaluation is difficult to apply. Between April 2004 and October 2009 we enrolled 88 first-line DLBCL patients (pts) to determine the predictive value of I-PET and final PET (F-PET) on Progression Free Survival (PFS). All patients were treated with 6-8 R-CHOP never modified by I-PET results. Involved Field radiotherapy to bulky areas was given to 14 pts regardless of PET results. PET scan was performed in all pts at diagnosis, during and at the end of therapy with centrally reviewing according to visual dichotomous response criteria (Ist Consensus Conference, Deauville 2009). Clinical features were: median age 55 years (18-80), stages I-II/III-IV 29/59, IPI score 0-2/3-5 53 and 35. I-PET was performed after 2 R-CHOP in 58 patients, after 3 or 4 in 30. Results: clinical complete response (CR) in 79 pts (90%) and no response in 9 (10%); 63 (72%) were negative (neg) and 25 (28%) positive (pos) at I-PET; 77 (88%) were neg and 11 (12%) pos at F-PET; 15/25 (60%) I-PET pos pts converted at F-PET, but only 1/63 (2%) I-PET neg case had a pos F-PET. The concordance between clinical CR and F-PET negativity was 97% due to 2 false pos scans (biopsied parotid and colorectal carcinoma). With a median follow-up of 26.2 months, 2-year OS and 2-year PFS were 91% and 77% respectively. 2-year PFS rates for I-PET neg vs pos and for F-PET neg vs pos were: I-PET 85% vs 72% (p.0475) (Figure 1A); F-PET 83% vs 64% (p<.001) (Figure1B). In univariate analysis for PFS, high LDH value, ≥ 2 extranodal sites, bone marrow involvement, 3-5 IPI score, I-PET and F-PET positivity were predictors of lower PFS rates. Two independent bivariate analyses by Cox models were tested for PFS. In model 1 only F-PET retained its value compared to I-PET (HR 5.03, 95%CI 1.37-18.43, p=.015 vs 1.27, 95%CI 0.40-4.03, p=.691); in model 2, F-PET (HR 4.54, 95% CI 1.68-12.31) and IPI score (HR 5.36, 95% CI 1.91-15.05, p=.001) remained independent prognostic factors. In conclusion, our results indicate that pos I-PET is not predicted of worse outcome in DLBCL patients treated with first-line R-CHOP. Indeed the majority of these patients achieved a final CR. Conversely, F-PET results strongly correlate with PFS. Larger prospective studies and harmonization of criteria for I-PET reading criteria are needed to better assess the prognostic value of I-PET in DLBCL.



C077

R-CHOP21 VS R-CHOP14 IN 224 DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: RESULTS FROM A RETROSPECTIVE STUDY

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Diffuse large B cell lymphoma (DLBCL) is one of the most common types of non-Hodgkin's lymphoma. R-CHOP21 (C21) is considered the standard therapy but a large number of studies tested R-CHOP14 (C14). The aim of our study was to evaluate retrospectively a cohort of patients (pts) treated with C21 or C14. All pts with diagnosis of DLBCL or follicular grade IIIb lymphoma, treated with curative intent were accrued. From january 2002 to december 2009, 123 pts were treated with C21 and 101 were treated with C14. The two cohorts of pts were balanced for all clinical characteristics a part for age (<65 or >64 years) with more aged pts in C21 arm (p 0.000) and proliferative index evaluated with Mib1 (evaluable in 135 patients 60%) with more proliferative disease (over 80%) in C14 arm (0.003). After induction therapy 164 pts (73%) obtained a complete remission: 82/123 (67%) after C21 and 82/101 (81%) after C14 (p:0.01). After a median period of observation of 36 months 18 pts relapsed, 3 (3%) in the C21 arm and 15 (18%) in the C14 arm. Overall survival (OS) was significantly superior in C14 arm: 68% vs 54% (p:0.04); no difference was observed in progression free survival (PFS) considering the two therapies: 80% vs 75% respectively for C21 and C14. In univariate analysis OS was lower in older pts, advanced stage, symptomatic disease at diagnosis, elevated LDH, bone marrow infiltration, intermediate or high risk IPI and in pts treated with C21; PFS was lower in advanced stage, symptomatic disease, elevated LDH, bone marrow infiltration and intermediate high risk IPI. No differences were reported either in OS or in PFS according to proliferative index. As expected hematological grade III/IV toxicity was more frequent in pts treated with C14, all pts but three (3%) completed the therapy without delay or dose reduction. No differences in extra-hematological toxicity were observed. In conclusion our results confirm that C14 do not improve the results in comparison with standard C21 in the whole lymphoma population. In particular no differences were observed considering proliferative index. The only one data which needs further prospective studies is the higher incidence of CR in C14 arm that does not translate into improved survival due to a major number of relapses. A consolidation therapy in young and high risk patients after C14 therapy could reduce relapses increasing PFS.

C078

THE OCCURRENCE OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS FOLLOWING ORTHOTOPIC LIVER TRANSPLANT CORRELATES WITH THE TYPE OF IMMUNOSUPPRESSION: A SINGLE CENTER SURVEY ON 1,649 PATIENTS

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Introduction. Post-transplant lymphoproliferative disorder (PTLD) is a serious complication in solid organ transplants. The incidence is quite variable, and the role of the immunosuppressive drugs on the risk of PTLD remains to be elucidated. The present study was undertaken in order to evaluate both frequency and risk factors of PTLD in a large series of Orthotopic Liver Transplant (OLT). Patients and Methods. Data have been collected from 1,803 OLT performed in 1,649 patients at the Liver Transplant Center in Torino, Italy, during the period 1990-2008. OLT was performed in patients aged up to 65 yr. old, with 60 pediatric patients and two patients aged over 65 yr. Overall, 1,189 (73.8%) patients received CsA in prevalence, 423 (26.2) had tacrolimus in prevalence. Several parameters were evaluated for possible association with PTLD occurrence, including age, sex, liver disease and HCV state, presence of hepatocellular carcinoma, time elapsed from OLT to PTLD, CsA vs tacrolimus, other drugs for graft rejection. The cumulative incidence of PTLD was determined using the Fine and Gray competing risk regression model. Results. At a median follow-up of 5.8 yrs. (range 0.1-17.5)., 1,298 (78.7%) patients are alive, with a 5-yr Overall Survival projection of 79.5 %. So far, 20 PTLD have been recorded, with a cumulative incidence of 0.94, 1.57 and 3.03% at 5, 10 and 15 yrs, respectively. Median time of PTLD occurrence was 32 mos. (range 2-155) since OLT. On multivariate analysis, the use of tacrolimus vs. CsA was the main factor associated with increased risk of PTLD (SDHR: 2.54, p=0.041). Despite the increased PTLD incidence, the overall risk of death was significantly lower with tacrolimus compared to CSA. An increased PTLD risk was also observed in the 83 patients receiving OKT3 (SDHR: 3.77, p=0.013). None of the other parameters had any significant impact on PTLD development. Treatment of PTLD resulted in good response in most patients and at a median follow up of 4.85 yrs., 17 out of 20 patients (85%) are alive. Conclusions. The overall incidence of PTLD in this large series of OLT is among the lowest reported so far in patients receiving solid organ transplant; the use of tacrolimus is shown as a significant risk factor for PTLD; nevertheless, the addition of tacrolimus significantly increased the life expectancy following OLT; the study confirms the improved outcome of PTLD and the availability of Rituximab likely contributed to the favorable results.

CHRONIC MYELOID LEUKEMIA

C079

THE BCR-ABL FUSION TRANSCRIPT HAS A PROGNOSTIC IMPACT IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB IN EARLY CHRONIC PHASE: A GIMEMA CML WP ANALYSIS

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Background: Chronic myeloid leukemia (CML) is characterized by the BCR-ABL gene. Different types of fusion transcripts can be found: the most frequent are the e13a2 (b2a2) and the e14a2 (b3a2). In the imatinib (IM) era, few data about the prognostic significance of the transcript type in ECP are available: one study suggested that patients with the b2a2 transcript may be more sensitive to IM, while two larger studies suggested that patients with b3a2 transcript may have better responses to IM. No systematic evaluations in large prospective studies have been performed Aim and Methods: To investigate the prognostic influence of the BCR-ABL transcript type in ECP CML treated with IM, we performed an analysis of 3 studies of the GIMEMA CML WP (Clin Trials Gov. NCT00514488, NCT00510926 and observational trial CML/023). Definitions: Major Molecular Response (MMR): BCR-ABL/ABL ratio <0.1% IS; failures: ELN criteria; events: discontinuation for any reason. All the calculations have been made according to the intention-to-treat principle Results: 559 consecutive patients were enrolled. Patients expressing rare transcript types (e1a2 and e19a2) and patients with both b2a2 and b3a2 transcripts were excluded: 493 out of 559 patients were evaluable, 203 (41%) with b2a2 transcript and 290 with b3a2 transcript (59%). The 2 groups were comparable, except for the proportion of patients treated with high-dose IM frontline: 20% (b2a2) and 28% (b3a2), p=0.034. Median observation: 60 months. The rates of either CCgR at 12 months (75% and 79%) and overall CCgR (89% and 88%), were similar in patients with b2a2 and b3a2 transcript. The time to MMR was shorter for patients with b3a2 transcript and the overall estimated probability of MMR was significantly lower for patients with b2a2 transcript (85% vs 90%, p<0.001). The probability of Overall Survival (OS), Progression-Free Survival (PFS), Failure-Free Survival (FFS) and Event-Free Survival (EFS) was 86% and 91% (p=0.064), 82% and 90% (p=0.027), 70% and 76% (p=0.095), 58% and 70% (p=0.027) in patients with b2a2 and b3a2 transcript, respectively. Conclusions: The CCgR rates were comparable; the overall estimated probability of MMR was significantly lower for patients with b2a2 transcript. OS, PFS, FFS, and EFS were lower in patients with b2a2 transcript (PFS and EFS: p< 0.05).The b2a2 transcript is a candidate adverse prognostic factor in ECP CML treated with IM frontline ACKNOWLEDGEMENTS: ELN, COFIN, Bologna University, BolognAIL

C080

SHP1 EXPRESSION ACCOUNTS FOR RESISTANCE TO IMATINIB TREATMENT IN PHILADELPHIA CHROMOSOME-POSITIVE CELLS DERIVED FROM PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Esposito N,[1,2] Quintarelli C,[1,2] Colavita I,[1,2] Sica AR,[1,2] Peluso AL,[1,2,9] Errichiello S,[1,2] De Angelis B,[1,2] Musella F,[1,2] Izzo B,[1,2] Muccioli Casadei G,[1,2] Rinaldi P,[1,2] Cosenza M,[1,2] Pisano I,[1,2] Cacciapuoti V,[1,2] Luciano L,[2] Picardi M,[1,2] Del Vecchio L,[1,2] Buonomo T,[1,2] Hughes TP,[3] Radich JP,[4] Russo D,[5] Branford S,[3] Saglio G,[6] Melo JV,[3] Martinelli R,[1,2] Ruoppolo M,[1,2] Kalebic T,[7] Martinelli G,[8] Pane F[1,2]

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Imatinib (IMA) treatment is considered the standard front-line therapy of CML. However, although the vast majority of patients respond to the therapy, primary or acquired resistance to IMA may occur during treatment. In this study we show that the tumor suppressor tyrosine phosphatase SHP-1 plays a critical role as a resistance determinant of IMA treatment response in both KCL22 Ph+ cell lines (sensitive/KCL22-S and resistant/KCL22-R) and in chronic phase CML patients (CP-CML). Indeed, SHP-1 expression in resistant cell line is significantly lower than it is in sensitive ones, in which co-immunoprecipitation analysis reveals the interaction between SHP-1 and the oncogenic tyrosine phosphatase SHP-2, a positive regulator of RAS/MAPK pathway. SHP-1 ectopic expression in KCL22-R restores both its interaction with SHP-2 and IMA responsiveness; it also decreases SHP-2 activity after IMA treatment. In addition, a low level of SHP-1 in resistant KCL22 cell line is associated with a delocalization of SHP-2 from cytoplasm to cytoplasmic membrane that is reverted after ectopic expression of SHP-1. Consistently, SHP-2 knocking-down in KCL22-R reduces either STAT3 activation or cell viability after IMA exposure. Therefore our data suggest that SHP-1 plays a critical role in BCR-ABL independent IMA resistance modulating the activation signals that SHP-2 receives from both BCR/ABL and membrane receptor tyrosine kinases. The role of SHP-1 as a determinant of IMA sensitivity has been further confirmed in 60 consecutive untreated CML patients, whose SHP-1 mRNA levels were significantly lower in case of IMA treatment failure (p<0.0001). In addition, we evaluated the role of SHP-1 as a predictor of Major Molecular Response (MMR) in 99 newly-diagnosed CML patients enrolled into the TOPS trial investigating 400mg versus 800mg IMA. In particular, patients who do or don't achieve MMR by 12 months show significantly different mRNA levels of SHP1 (7.9±4.0 vs. 5.9±3.4; p=0.01). Indeed, statistical analysis shown that a value of 4.1 or more in SHP-1 is associated with almost 2-fold odds of achieving MMR by 12 months (OR=2.0; 95% CI=1.2, 3.4; p=0.01). In conclusion, we suggest that SHP-1 could be a new biological indicator at baseline of IMA sensitivity in CML patients.

C081

THE ACTIVITY OF TYROSIN KINASE INHIBITORS ON T CELL PROLIFERATION AND ACTIVATION

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TCR / lymphocytes carrying clonal T-cell receptor rearrangements has been observed in CML patients at the time of diagnosis. Whereas the same clone persisted at low levels in most imatinib-treated patients, this clone may markedly expand in 30-40% of dasatinib-treated patients, resulting in absolute lymphocytosis. In particular, the TCR delta clones were confined to / (+) T- or natural killer-cell compartments and the TCR clones to CD4(+)/CD8(+) (+) fractions. In addition, Imatinib treatment has been used to treat sclerodermatous chronic GVHD (cGVHD), since inhibiting transforming growth factor (TGF) and PDGF pathways in fibroblasts. Thus, the functional importance of TKIs in the leukemia immune surveillance require further evaluations, also in the view that besides to the BCR-ABL target oncoprotein, TKIs have off-target effect on other kinases (e.g. c-KIT, TEC, SRC), some of which have physiological functions in immune responses. Thus, we evaluate the activity of clinical relevant dose of TKIs [Imatinib (range 40-5000 nM), Dasatinib

(range 0.8-100 nM) and Nilotinib (range 15-2000 nM)] on T cell proliferation (by CFSE flow cytometry analysis) and function (by INF ELISPOT assay). In particular, T cells were obtained either by OKT3/CD28 beads (OKT3 T cells) or antigen presenting cells loaded with a specific peptide (peptide-specific CTL) and in vitro cultered in the presence of TKIs for 72hr. We demonstrated that among the three main TKIs used in therapy, only Dasatinib inhibits T cell proliferation in dose dependent manner in 10 out of 10 treated T cell lines isolated from healthy donors. Indeed, cell proliferation was significantly inhibited by 10nM of Dasatinib in 48%±5% of OKT3 T cells and 55%±2% of peptide-specific CTL. At the same time, Dasatinib inhibits also T cell functionality, since we demonstrated a reduction of INF production when CTLs were stimulated by specific peptides in the presence of 25nM of Dasatinib (105±5 spot forming cells/105 T cells) respect to untreated CTLs (660±5 spot forming cells/105 T cells). In contrast, Imatinib and Nilotinib did not affect either OKT3 T cell or peptide-specific CTLs at the indicated dose range. In conclusion, our data demonstrated that Dasatinib, but not Imatinib or Nilotinib, may interfere with in vitro expansion of T cells and may have an immune modulating effect.

C082

AT THE TIME OF DIAGNOSIS HIGH SOKAL RISK CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) PATIENTS CAN HARBOR BCR-ABL KINASE MUTATIONS ASSOCIATED WITH A GREATER LIKELIHOOD OF PROGRESSION AND SHORTER SURVIVAL.

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With this report, we aimed to assess in how many patients with CP-CML BCR-ABL KD mutations are already detectable at the time of diagnosis and whether the presence of these mutations detection correlates with subsequent response to imatinib. Fifty-three CP patients had sequential samples evaluable at diagnosis for BCR-ABL KD mutation analyses in Genoa. Mutations were identified by direct sequencing (DS) with BidDye Terminator v 1.1. cycle sequencing kit and analyzed with a 3130 ABI capillary electrophoresis system. In 9/19 (47%) high risk patients we identified the following KD mutations before imatinib treatment: Y253C, S265R, E255K, F359Y,N374S, R332L and E255V (in 3 cases). Three out of 9 patients progressed under imatinib to advanced phase and died of blastic evolution at 23, 33, 69 months from diagnosis despite nilotinib and/or dasatinib treatment. Two of them maintained the same mutation found at the time of diagnosis (Y253C and E255K) also during the follow up; the last patient with S265R mutation achieved the complete disappearance of this mutation under imatinib but soon after he acquired E255L mutation, refractory also to nilotinib. The other 6 patients are alive: 1 patient with E255V mutation is now in CMR after allografting (this patient achieved MMR under imatinib but subsequently progressed also under nilotinib and dasatinib); 3 patients are in CCyR; 1 patient is in CMR and 1 patient is in MMR. Except one patient, who loose MMR and primarily N374S mutation under imatinib and he developed a second mutation (H396R) under the follow up, in all others the mutations found at the time of diagnosis disappeared under imatinib. Differently from high Sokal risk patients, 1/6 patients with intermediate Sokal risk only carried KD mutations at diagnosis (D363G). The mutation disappeared during the follow up and he is alive in MMR 46 months after imatinib. None of the 28 low Sokal risk patients carried KD mutations at diagnosis. In conclusion, we can reasonably hypothesize that the mutations Y253C and E255K, when found at the time of diagnosis, are resistant mutations. The fact that mutations were more often identified in patients in the high Sokal risk group, supports the hypothesis that the probability of developing a mutation is related to the basic biology of the disease rather than being merely a random event.

C083

SEVEN YEAR-EXPERIENCE OF BCR-ABL MUTATION ANALYSIS IN PHILADELPHIA-CHROMOSOME POSITIVE (PH+) PATIENTS ON IMATINIB (IM) OR 2ND-GENERATION TYROSINE KINASE INHIBITORS (TKIS): BY THE GIMEMA CML WORKING PARTY

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We reviewed the database recording the Bcr-Abl mutation analyses performed in our lab from January 2004 to January 2011 (3285 tests in 1439 pts) in order to: i) assess how the clinical relevance of Bcr-Abl mutations has changed from the IM to the 2nd-generation TKI era; ii) understand how the role of mutation analysis in routine clinical management of pts has evolved over the years; iii) elucidate how frequently physicians may expect to find mutations in specific settings. Since 2006, mutation analysis of CP CML pts on IM has usually been triggered by failure or suboptimal response according to ELN definitions. Only 41/142 (29%) failures and 19/222 (10%) suboptimal responses harbored mutations; likelihood of a positive test varied across subcategories, indicating different contribution of mutations to different types of 'resistance'. In particular, no mutations were detected in any of the 42 CCyR patients who failed to achieve MMR at 18 months. Over time, more and more physicians asked for mutation analysis of their CP CML pts because of a Bcr-Abl transcript increase at a single RQ-PCR test; mutations were detected in 0/26 pts who experienced <1-log increase without loss of MMR, 0/41 pts who experienced ≥1-log increase without loss of MMR, 1/36 (3%) pts who experienced <1-log increase with loss of MMR and 2/41 (5%) patients who experienced ≥1-log increase with loss of MMR but not CCyR – indicating that only loss of MMR is a reasonable trigger for mutation analysis. The 25 additional pts analyzed after a transcript increase confirmed by 2 subsequent RQ-PCR assessments were negative for mutations as well. Among IM-resistant CML pts receiving a 2nd-generation TKI (dasatinib, nilotinib), failures according to the provisional ELN criteria were more frequently associated with mutations (57%) than suboptimal responses (21%); in addition, 71% of patients who lost a previously achieved response were positive for a mutation. Thus, mutations are more frequent in this population, but their contribution to different types of 'resistance' differs, similarly to what can be observed in pts on IM. The ten most frequent mutations in CML and Ph+ ALL pts resistant to dasatinib/nilotinib included T315I(30%), F317L(16%), Y253H(16%), F359V(7%), V299L(7%), E255K(6%), E255V(5%), F359I(4%), T315A(3%), F359C(2%) – detected either alone (56% of pts), or combined (29%), or together with other mutations (15%). Additional data will be presented. Supported by ELN, AIL, AIRC, PRIN, RFO

C084

EVALUATION OF RESIDUAL CD34+/PH+ STEM CELLS IN CHRONIC MYELOID LEUKEMIA PATIENTS IN COMPLETE CYTOGENETIC RESPONSE DURING FRONT LINE NILOTINIB THERAPY

Defina M,[1] Ippoliti M,[1] Abruzzese E,[2] Castagnetti F,[3] Tiribelli M,[4] Rosti G,[3] Breccia M,[5] Trawinska MM,[2] Crupi R,[1] Aprile L,[1] Salvucci M,[6] Baratè C,[7] Gozzini A,[8] Rondoni M,[1] Zaccaria A,[6] Alimena G,[5] Santini V,[8] Specchia G,[9] Fanin R,[4] Gozzetti A,[1] Baccarani M,[3] Lauria F,[1] Bocchia M[1]

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Despite its high efficacy in chronic myeloid leukemia (CML), discontinuation of imatinib is associated with disease relapse probably due to the persistence of resistant leukemic stem cells. On this regard it has been reported that BCR-ABL positive progenitor cells can still be detected in patients in long lasting complete cytogenetic response (CCyR). Compared to imatinib, nilotinib appears to eradicate more rapidly the bulk of CML cells, inducing high and fast rate of CCyR and major molecular response (MMolR) (78% CCyR and 52% MMolR at 3 months). However nilotinib didn't appear to be more effective in eliminating in vitro CML progenitors than imatinib. Up to date no data evaluating the persistence of Ph+ stem cells in early chronic phase CML patients during first line treatment with nilotinib have been reported. We investigated the presence of residual CD34+/Ph+ cells in 24 CML patients in CCyR during first line nilotinib treatment (GIMEMA CML0307 and CAMN107A2303 trials). Bone marrow purified CD34+ cells were evaluated for BCR-ABL fusion gene by fluorescent in situ hybridization (FISH) analysis. At the time of residual CD34+/Ph+ evaluation the median time of nilotinib treatment was 22 months (range 9-30). All patients were in CCyR for a median time of 22 months (range 6-29); 20/24 patients (83%) were in MMolR for a median time of 22 months (range 1-27), 1/24 patients (4%) was in complete cytogenetic response (CMolR) since 12 months, while 3/24 patients (13%) didn't achieve yet a MMolR. In 15/24 (63%) patients FISH analysis of purified CD34+ cells was performed on at least 100 nuclei (median 100, range 100-300), in 5/24 (21%) patients the median number of nuclei analyzed was 75, (range 60-86), and in 4/24 (16%) patients FISH was not performed due to insufficient CD34+ nuclei. Remarkably, we documented residual CD34+/Ph+ cells only in 1/20 (5%) evaluated patients. Of note, in this patient 140 CD34+ interphase nuclei were analyzed and only 1 was found bcr-abl positive (0.7%). These results quite differ from what we previously found in imatinib treated patients where residual leukemic CD34+ cells were still detectable in 14/31 (45%) patients in stable CCyR for a median of 39 months. Despite the limited number of patients studied, this novel evidence may support the better short term clinical results observed with nilotinib as first line treatment in CML.

ALLOGENEIC TRANSPLANTATION

C085

ALGORITHM BASED ON KIR GENOTYPE FOR DONOR SELECTION IN UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THALASSEMIA

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Hematopoietic stem cell transplantation (HSCT) is widely recognized as having the potential to cure beta-thalassemia major but is still burdened by a mortality rate, ranging from 6 to 12% in the different patient cohorts. Graft-versus-host disease (GvHD) is the main cause of morbidity and mortality. Another 12% of patients reject the allograft despite the use of intensive conditioning regimens. Donor-recipient matching for human leukocyte antigens is a critical step in the donor selection procedure but alone is insufficient to fully prevent the complications of HSCT. Other immunogenetic factors, especially killer immunoglobulin-like receptors (KIRs), are involved in the development of GvHD and other post-HSCT complications. Different haplotypes carry different numbers of KIR genes. Based on their gene content, two broad groups of KIR haplotypes have been defined. Group A haplotypes contain 6 inhibitory KIR genes and a single activating KIR gene (2DS4) whereas Group B haplotypes contain various combinations of both activating and inhibitory KIR genes. About 60-70% of individuals homozygous for KIR Haplotype A are homozygous for the 2DS4-deletion variants KIR2DS4*003-*009 which means that they completely lack functional activating KIRs on the surface of natural killer (NK) cells. The detection of deletion variants of KIR2DS4 is important because it makes it possible to distinguish between individuals who either completely lack or express one or more activating KIRs on NK cells. In a cohort of 114 thalassemia patients undergoing unrelated HSCT, we observed that the lack of activating KIRs on donor NK cells significantly increased the risk of GvHD [hazard risk (HR) 4.1, 95% CI 1.7-10.1, P=0.002] and transplantation-related mortality (HR 4.7, 95% CI 1.6-14.2, P=0.01). The risk of GvHD furthermore increased when recipients heterozygous for HLA-C KIR ligand groups C1 and C2 were transplanted from donors completely lacking activating KIRs (HR 5.3, 95% CI 1.7-16.5, P=0.004). Conversely, the risk of rejection reached its peak when the recipient was homozygous for the C2 HLA-KIR ligand group and the donor carried 2 or more activating KIRs (HR 6.8, 95% CI 1.9-24.4, P=0.005). By interpolating the number of donor activating KIRs with the HLA-C KIR ligands of the recipient we obtained an innovative algorithm for the selection of unrelated donors for thalassemia patients, capable of significantly reducing the risk of post-transplantation complications.

C086

INTENTION-TO-TREAT ANALYSIS OF 410 PATIENTS IN NEED FOR ALLOGENEIC STEM-CELL TRANSPLANTATION: THE IMPACT OF HLA-MISMATCHED DONORS SOURCE IN A COMPREHENSIVE TREATMENT ALGORITHM

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Background. Allogeneic transplantation of haemopoietic stem cells (allo-SCT) from an HLA- matched related (MRD) or unrelated donor (MUD) is a curative option for patients (pts) with high-risk hematological disease (HRHD). Our policy is to offer an haplo- SCT to adult pts lacking a matched donor in the appropriate time according to clinical indications (www.leukemianet.org www.ebmt.org). This policy is inte-

grated in ongoing protocols for primary disease (www.nilg.it www.gitil.net) Methods. Here we report the intention-to-treat (ITT) analysis of allo-SCT in all consecutive HRHD patients. Results. Between Jan-2004 and July-2010, 410 pts (age 48y, range 15-76, 91 pts over-60y; male 264) received indication to allo-SCT. In acute leukemias the indication to allo-SCT was given in 210/246 pts (85%). Eighty-nine pts (22%) received a transplant from a MRD; 190 pts (46%) activated a MUD search; 84 pts (20% of total pts, 44% of MUD searching) received a MUD transplant; 11 pts (3%-6%) received a UCB. Overall, 149 pts received an haplo-SCT (36%): 42 after MUD research, 107 up-front. The overall survival (OS) analysis in ITT for the entire population is 51% at 1 year and 39% at 3y. OS in pts transplanted in CR is 74% at 1y and 57% at 3y, OS in pts transplanted in PD is 29% and 21% (p<0.0001) – figure 1a. Interestingly, the OS according to donor source (MRD vs MUD vs haplo-SCT) is comparable (p = ns) in pts transplanted in CR – figure 1b. In patients with acute leukemia, 1-year OS is 51% (77% in CR, 20% in PD pts). The outcome analysis per donor source is comparable (p=ns) within CR setting. Conclusion. In ITT analysis, 81% of candidate pts received an allo-SCT as a potential immunotherapy. Significantly, outcome achieved in pts transplanted in CR is comparable within matched and mismatched donors. These results confirm the value of a comprehensive treatment algorithm within an integrated operational framework from hematological diagnosis and treatment to transplantation procedures from all available sources.

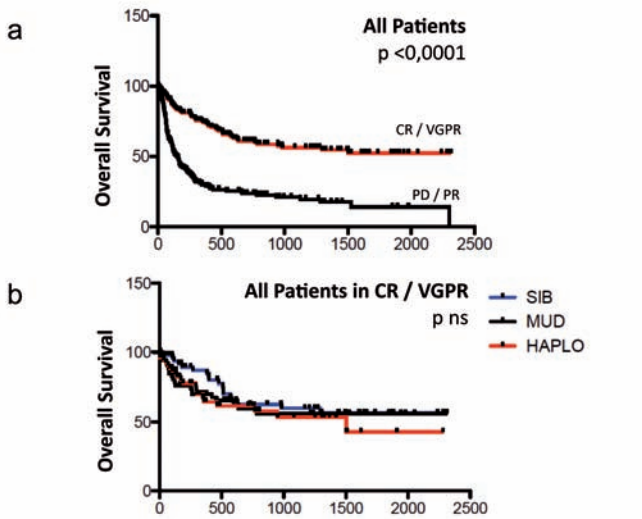

Figure 1: Clinical outcome in intention-to-treat analysis
Kaplan-Meier estimate of overall survival in the intention-to-treat population:
a) all patients in analysis, without distinction for donor source,
b) all patients in complete remission or very good partial remission at transplantation with distinction for donor source.

C087

ALTERATIONS IN THE GENOMIC AND TRANSCRIPTIONAL PROFILE OF LEUKEMIA IN RESPONSE TO IMMUNE PRESSURE BY ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: TRACKING IMMUNOEDITING BY HIGH-THROUGHPUT TECHNIQUES

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The curative potential of allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) against acute myeloid leukemia relies to a large extent on its potent immunotherapeutic effect. Nonetheless, relapse after transplantation remains a major concern. Our group demonstrated that in approximately 40% of clinical relapses after HLA-haploidentical HSCT, de novo genomic loss of the mismatched HLA occurs in acute myeloid leukemia (AML) cells which thereby eliminate the target of alloreactive donor T cells and grant leukemia an effective strategy for immune evasion (Vago et al, N Engl J Med, 2009). To identify novel targets of leukemia immunoediting and provide a rationale for targeted consolidation therapies after transplantation, in the present study we compared by high throughput techniques the genomic and transcriptional profile of AML blasts harvested at relapse after allo-HSCT with those of the original leukemia at diagnosis. To date, we performed single nucleotide polymorphism (SNP) profiling of 10 paired AML samples taken before and after allo-HSCT. In 7/10 relapses (70%), we found the de novo occurrence of genomic alterations in genes relevant to leukemic transformation (WT1, FLT-3, MLL). Newly acquired uniparental disomy was the most frequent de novo alteration (40% - 6/15), involving in 5 cases the region encompassing the FLT-3 gene on chromosome 13. Moreover, by SNP arrays we demonstrated in 5/10 (50%) of the cases the selection of a single leukemic clone at relapse from an oligoclonal population at diagnosis, further supporting in vivo evolution of leukemia upon immune pressure. Five of the paired diagnosis/relapse leukemic samples were also analyzed for their gene expression profile, and an enrichment analysis was performed using the Gene Onthology program. In 3/5 cases, the majority of deregulated genes were immune-related, including costimulatory molecules, tumor antigens, cytokines, and chemokines. Interestingly, in a case of relapse after HLA-haploidentical HSCT in which no genomic loss of HLA occurred, we could document a specific down-regulation of the HLA class II presentation pathway at the time of post-transplantation relapse, which was recovered after transfer of the leukemic blasts into immunodeficient NOD/SCID mice, suggesting plasticity of this transcriptional mechanism of immune evasion. Taken together, our data evidence that both genomic and transcriptional alterations contribute to leukemia immune evasion after allo-HSCT.

C088

A PHARMACOGENETIC APPROACH TO INTERINDIVIDUAL VARIABILITY AT LOCI CONTROLLING GLUTATHION HOMEOSTASIS: IMPACT ON TRANSPLANT RELATED MORTALITY (TRM) AND OVERALL SURVIVAL (OS) IN PATIENTS RECEIVING AN ALLOGENEIC HSCT AFTER A BUSULFAN-BASED CONDITIONING REGIMEN

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Busulfan is the most widely used drug for allogeneic conditioning regimens. Busulfan metabolism depends on liver glutathion (GSH) availability. A number of loci controls liver GSH synthesis and consumption, occurring during drug conjugation and oxidative stress response. Here we report on a study population of 331 consecutive patients who received an allogeneic HSCT for haematological malignancies at Institute of Hematology "Seràgnoli" from 2004 onwards. 185 patients received busulfan in the conditioning regimen, mostly at myeloablative intensity, while 146 received a TBI based regimen or a reduced intensity conditioning regimen non busulfan-based; clinical variables were similarly distributed in the two groups. The impact of polymorphisms at loci involved in GSH balancing on overall survival (OS) and transplant related mortality (TRM) was tested. A total of 35 polymorphisms (32 SNPs and 3 insertion/deletions) at 15 candidate genes were analysed by high throughput mass array Sequenom TM platform or by DHPLC. We found that a C to G rs2180314 SNP at Glutahione Transferase A2 (GSTA2) locus (Codon 112 which leads to a Ser to Thr aminoacidic transition) impacts OS and TRM in the whole population (CC vs G-carriers: HR=1.604, 95%CI=1.081-2.381, p=0.019 for OS and HR=1.992, 95%CI=1.100-3.609, p=0.023 for TRM). Such an effect was particularly evident in patients who received busulfan (CC vs G-carriers: HR=2.438, 95%CI=1.446-4.108, p=0.0008 for OS and HR=4.580, 95%CI=2.005-10.461, p=0.0003 for TRM). No effect was present in the

group not receiving busulfan. The polymorphism at microsomial GST-1 promoter (rs7970208) also affects OS and TRM, although to a lesser extent (AA vs G-carriers: HR=1.405, 95%CI=1.076-1.835, p=0.012 for TRM and HR=1.255, 95%CI =1.050-1.499, p=0.012 for OS). These data point out that genetic variability at loci controlling GSH balancing may affect allogeneic HSCT outcome in busulfan treated patients.

C089

RAPAMYCIN-MYCOPHENOLATE-ATG AS A NEW PLATFORM FOR GVHD PROPHYLAXIS IN T-CELL REPLETED UNMANIPULATED HAPLOIDENTICAL PERIPHERAL STEM CELL TRANSPLANTATION: RESULTS IN 79 PATIENTS.

Daniela C,[1] Forcina A,[2] Bernardi M,[1] Crocchiolo R,[1] Messina C,[1] Noviello M,[2] Lupo Stanghellini MT,[1] Marktel S,[1] Assanelli A,[1] Mastaglio S,[1] Battaglia M,[3] Ferraro A,[3] Rossini S,[4] Roncarolo MG,[5] Bondanza A,[2] Bonini C,[2] Ciceri F,[1] Peccatori J[1]

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Background and aim: Rapamycin, in contrast to calcineurin inhibitors, allows T regulatory cell (T-regs) proliferation while inhibits effector T cell expansion. We investigated the safety of infusion of T-cell repleted unmanipulated PBSC from haploidentical donor with a combination of Rapamycin, Mycophenolate and ATG as GvHD prophylaxis, to preserve early T-regs function (TrRaMM study, Eudract 2007-5477-54). Patients and Methods: Since 2007, seventy-nine patients (pts) underwent stem cell transplantation (SCT) for high risk leukemias and lymphomas. Median age was 47 years (range 14-69). At transplantation 10 pts were in early phase, and 69 in advanced phase. Median comorbidity index score was 2. The conditioning regimen included Treosulfan (14 g/m2 for 3 days), Fludarabine (30 mg/m2 for 5 days) and an in-vivo T and B-cell depletion by ATG-Fresenius (10 mg/kg for 3 times) and Rituximab (a single 500 mg dose). All pts received allogeneic unmanipulated PBSC from an HLA-haploidentical related donor. GvHD prophylaxis consisted of Rapamycin (target level 8-15 ng/ml, till day +60) and MMF (15 mg/kg tid till day +30). Results: All patients engrafted and all but eight were in disease remission at first marrow evaluation on day +30. 1-year cumulative incidence of grade 2-4, grade 3-4 aGvHD and cGvHD were 22, 9 and 21% respectively. 1-year cumulative incidence of TRM and relapse incidence were 25% and 44% respectively. After a median follow-up of 391 days, 1-year OS was 41%. Immunoreconstitution was fast and sustained with a median 221 circulating CD3+cells/μL (range 43-1690) from day 30. The immunoreconstitution was polarized towards central memory (CD45RA-CD62L+ cells 32.7%), IL-2 producing cells (IL-2+ cells 26.2%). We detected high levels of CD4+CD25+CD127-FOXP3+ T-regulatory cells (up to 30% of circulating CD4+ T lymphocytes) starting from day 30. These cells were able to suppress in vitro proliferation of autologous effector cells demonstrating to be regulatory T cells. Conclusions: Rapamycin-Mycophenolate-ATG are effective as GvHD prevention in T-cell replete unmanipulated haploidentical peripheral SCT and are associated with an early T-cell immunoreconstitution characterized by the in-vivo expansion of early-differentiated T cells and T-regs. Further studies are warranted to gain insight on the role of Rapamycin as platform for exploitation of T-regs in allogeneic HSCT from mismatched donors.

C090

ACUTE GRAFT VERSUS HOST DISEASE II-IV: HAS OUTCOME IMPROVED OVER THE PAST TWO DECADES?

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Aim of the study: To assess whether outcome of patient with graft versus host disease (GvHD) grade II-IV has improved between 1990 and 2010 Patients: We analyzed 1465 patients grafted 1990-2010, of whom 579 developed grade II-IV acute GvHD ; donors were HLA identical siblings (n=339) or alternative donors (n=240) Methods. Cumulative incidence of acute GvHD grade II-IV in the 2 time periods was assessed. Then univariate and multivariate analysis were used to assess risk factors for non relapse mortality (NRM) in the cohort of patients with acute GvHD. Actuarial survival was also determined. Incidence of GvHD II-IV. The overall cumulative incidence of aGvHD II-IV was 40% with a NRM of 40% (compared to 26% for patients with GvHD grade 0-I, p<0.00001) . When stratified for transplant era, the incidence of GvD II-IV was 49% in 1990-2000 vs 29% in 2001-2010 (p<0.00001): this was achieved despite the increase in alternative donors (from 28% to 54%, p<0.001) and a greater proportion of patients over 50 (7% vs 35% p<0.001). The incidence of GvHD II-IV was reduced in matched siblings (45% vs 31%, p=<0.0001) but more so in alternative donors (61% vs 28%, p<0.0001). NRM for GvHD II-IV. For patients with GvHD grade II-IV , in univariate analysis NRM was predicted by donor age 35 years (34% vs 45%, p=.007) year of transplant 2000 (45% vs 32% , P=0.002) and donor type (sibling vs alternative donor) (34% vs 48%, p=0.006). In multivariate analysis NRM was predicted by donor age (p=.01, RR=.1.39 for donors >35 years old) , recipient age (p=.02; RR 1.37 for patients >35 years old) , disease phase (p=.004, RR =1.47 for advaced disease), transplant era (p=0.01, RR=.69 for years 2000-2010) donor type (p<0.00001, RR 1.95 for alternative donors) and recipient gender (p=0.002 and RR .65 for females) . Survival: The overall actuarial 1 year survival for patients with GvHD II-IV is 58%, compared to 67% for GvHD 0-I (p=0.001). For patients with GvHD II-IV survival was 57% in 1990-2000 and 60% in 2001-2010 (p=0.8). Conclusion: The incidence of acute GvHD II-IV has been significantly reduced over the past two decades. After correcting for patient and disease characteristics, 1 year survival has remained comparable before and after year 2000 This study suggests that progress has been made in preventing but not in treating acute GvHD.

ACUTE LYMPHOBLASTIC LEUKEMIA

C091

LOSS OF CDKN2A GENE IS A POOR PROGNOSTIC MARKER IN ADULT BCR-ABL1 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS

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Loss of CDKN2A, encoding p16/INK4A and p14/ARF tumor suppressors, has been associated with poorer survival and higher leukemia-initiating-cell-frequency in xenograft models (Notta F et al. Nature 2011). Recently, using genome-wide single nucleotide polymorphisms arrays and gene candidate deep exon sequencing, we identified CDKN2A deletions in 29% (29/101) adult newly diagnosed Ph+ ALL patients and in 47% relapsed cases. Accordingly, here we aimed to investigate the functional consequences of genomic deletions and their prognostic value. By quantitative real-time PCR a significant difference in the expression of CDKN2A was observed among cases with heterozygous (p = 0.04) and homozygous (p = 0.01) loss compared to normal cases, suggesting that deletions lead to CDKN2A haploinsufficiency. In order to investigate their clinical implications, data were collected from 81 patients (median follow-up: 25.2 months- range 2.1-148.1; median age at diagnosis: 54 years- range, 18-76; CDKN2A loss in 29, 36%, cases). 72 patients (89%) were enrolled in the GIMEMA clinical trials (12 patients in GIMEMA LAL0201-B protocol, 13 in LAL2000 and 47 in LAL1205 protocols), while 9 patients (11%) were enrolled into institutional protocols. By univariate analysis a shorter overall survival (OS) and disease-free survival (DFS) were found in patients with CDKN2A deletion compared with those wild-type (OS: 27.7 v 38.2 months, respectively, p = 0.0206; DFS: 10.1 vs 56.1 months, respectively, p = 0.0010). Moreover, a higher cumulative incidence of relapse for patients with CDKN2A deletion (73.3 vs 38.1; p = 0.0014) was also recognized. The multivariate analysis confirmed the negative prognostic impact of CDKN2A deletion on DFS (p = 0051). These results show that there are genetically distinct Ph+ ALL patients with a different risk of leukemia relapse and that testing for CDKN2A alterations at diagnosis may help in risk stratification. Furthermore, since the loss of CDKN2A eliminates the critical tumor surveillance mechanism and allows proliferation, cell growth and tumor formation by the action of MDM2 and CDK4/6, attractive drugs to prevent disease recurrence could be represented by the inhibitors of MDM2 and CDK4/CDK6. These drugs are currently under investigation in in vitro studies by our group. *Supported by: ELN, AIL, AIRC, Fondazione Del Monte di Bologna e Ravenna, FIRB 2006, PRIN 2008, Ateneo RFO grants, Project of integreted program, Programma di Ricerca Regione – Università 2007 – 2009.*

C092

EDITING HUMAN LYMPHOCYTE SPECIFICITY FOR SAFE AND EFFECTIVE ADOPTIVE IMMUNOTHERAPY OF LEUKEMIA

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The transfer of high-avidity T-cell receptor (TCR) genes isolated from rare tumor- specific lymphocytes into polyclonal T cells is an attractive strategy for cancer immunotherapy. However, TCR gene transfer into mature lymphocytes results in competition for surface expression and inappropriate pairing between the exogenous and endogenous TCR chains, resulting in suboptimal activity and potentially harmful unpredicted specificities. To address these limitations, we developed a novel strategy based on zinc finger nucleases (ZFNs) that allows for the first time the editing of T cell specificity at the DNA level, by combining the disruption of the endogenous TCR chain genes with the transfer of a tumor-specific TCR. We designed ZFNs promoting the disruption of both endogenous TCR alpha and beta chain genes. ZFN-treated lymphocytes lacked CD3/TCR surface expression, were stable in culture with IL-7 and IL-15 for up to 60 days, and were permissive to further genetic manipulation. As a model tumor-specific TCR, we selected an HLA-A2 restricted, codon-optimized cysteine-modified TCR specific for the Wilms' tumor antigen 1 (WT1). For a complete editing of T cell specificity, we established a protocol that sequentially disrupted the endogenous TCR chains with high efficiency (averages: 36% and 18%) and was followed by lentiviral transfer of the WT1-specific TCR chains (average efficiencies: 65% and 25%). This procedure resulted in a population of TCR-edited lymphocytes expressing only the tumor- specific TCR that, in the absence of competition, was expressed at high and physiological levels. Upon lentiviral transfer of WT1-specific TCR, these TCR-edited cells were easily expanded to near-purity and proved superior to matched cells undergoing conventional TCR gene transfer in specific antigen recognition, including killing of primary leukemias. Together, these data demonstrate complete editing of T-cell specificity at the genetic level to generate high-avidity tumor specific T cells.

C093

IMATINIB AND CHEMOTHERAPY FOLLOWED BY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IMPROVE THE CLINICAL OUTCOME OF ADULT PATIENTS WITH PH+ ALL

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Background Tyrosine kinase inhibitors (TKI) seem to be changing the clinical outcome of adult patients with Philadelphia positive Acute Lymphoblastic Leukemia (Ph+ ALL) since these drugs may increase the proportion of patients undergoing alloHSCT by reducing early relapses. Nonetheless, the complications of the transplant may still offset the benefit of the procedure. Aim of the study To evaluate the role of Imatinib and molecular monitoring of MRD on the clinical outcome of adult Ph+ ALL. Patients Adult patients with Ph+ ALL (n= 100) were enrolled into NILG protocol 09/00 (ClinicalTrial.gov: NCT00358072). From February 2003, 65 patients received Imatinib during induction/consolidation (600 mg/d) (IM+ group, Bassan et al.: JCO, 2010). Fifty-eight patients received alloHSCT from a sibling, unrelated or haplo donor (n= 33, 24, 1), while 33 received a conventional treatment (24 chemotherapy, 9 autologous transplant) (with or without Imatinib). The median age was 46. The conditioning regimen was myeloablative in 46 and reduced intensity in 9. The stem cell source was the BM in 20 and the PB in 38. Results The addition of Imatinib to chemotherapy during induction consolidation increased the proportion of patients who achieved CR (93% vs 80%, p= 0.05) and who had the opportunity to undergo alloHSCT (66% vs 43%, p= 0.02). With a median follow-up of 1.5 years, the overall survival (OS) at 5 years was 35% for the whole cohort, 39% in the IM+ group and 23% in the IM-, respectively (p=0.007). At 5 years, the OS of patients receiving alloHSCT was 46% vs 21% of the others (p= 0.0001). Transplant related mortality at 4 years was 23%, no matter whether Imatinib was given or not any time during treatment. The cumulative incidence of relapse (CIR) of patients undergoing alloHSCT was 32% in the IM+ vs 57% in the IM- group. The CIR of alloHSCT patients was 19% for patients who proved MRD negative at conditioning vs 51% for MRD positive at any level in the BM or PB (p= 0.04). The disease free survival

of MRD negative was 67% vs 42% of MRD positive (p=0.06). Conclusions Imatinib combined to chemotherapy improves the outcome of Ph+ ALL, reduces the relapse probability of patients undergoing alloHSCT and is associated with a better disease free and overall survival of these latter patients. Finally, alloHSCT either from a sibling or unrelated donor remains the best post remissional treatment of Ph+ adult ALL patients receiving Imatinib and chemotherapy during induction/consolidation.

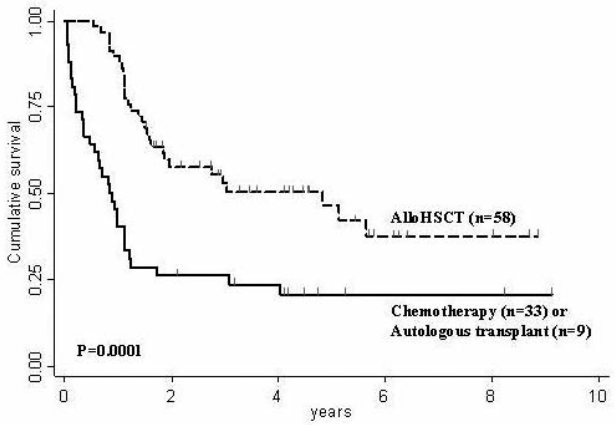

Fig.1 Overall survival according to post remissional treatment

C094

THE IMPACT OF A 7-DAY PREDNISONE (PDN) PRETREATMENT IN ADULT (LESS THEN 60Y) ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) THERAPY: RESULTS OF LAL2000 VS. LAL0496.

Annino L, Paoloni F, Fazi P, Vitale A, Meloni G, Caraci MR, Tedeschi A, Fioritoni G, Ferrara F, Marino C, Carluccio P, Bonifacio M, Foà R, Vignetti M, Mandelli F, Amadori S on the behalf of the GIMEMA Group

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Early cytoreduction represents a cornerstone in adult ALL treatment strategy, several approaches either with single agent or polychemotherapy have been applied to obtain this goal. In the ALL0288 GIMEMA study, PDN pre-treatment response was firstly proved as an independent powerful factor on CR achievement and DFS in adult ALL. 7d-PDN pre-treatment was reintroduced in LAL2000 study and even the same LAL0496 whole therapeutic plan (induction and post-CR treatment) was maintained. The aim of present study was to analyze the impact of PDN pre-treatment addition to a similar induction and post-CR treatment. From April 1996 to December 2005, 1017- 584 males, median age 32.5 y, (range 14.0-60.1 y)- adult ALL were consecutively enrolled in the LAL0496 study- 482 pts, median age 30.5 y-, and LAL 2000- 535 pts, median age 34.8 y-, respectively. The median follow-up of the whole population was 58.3 mos (range 0.3-152.6). As initial WBC count, immunophenotype incidence, there were no significant differences between the two studies; BCR/ABL rearrangement occurred in 25% and 29.57% of patients in LAL0496 and LAL2000, respectively. In LAL 2000, at PDN pre-treatment response (as blast cell < 1000 at d.0) evaluation, 384 (75.89%) resulted PDN-responders, 122 (24.11%) no-responders. Treatment results as CR rate, 6y O.S, and DFS are listed in table I. In LAL2000, PDN-responder patients median OS and DFS were 34.2 and 24.1 vs. 20.4 and 10.2 of no responders, and 23.4 and 19.2 mos in LAL0496 patients (p=0.0221 and p=0.0178), respectively. Furthermore median DFS in B and T PDN-responders was 24.3, 22.1 vs. 9.1 and 11.6 mos in no responders respectively (p=0.0554). In multivariate analysis, CR achievement, was influenced by protocol–in favour of LAL2000-, age and immunophenotype (p=0.0269, p < 0.0001 and p=0.0002, respectively); OS by protocol age and WBC count (p=0.004, p < 0.0001 and p < 0.0001, respectively). DFS was influenced by age and WBC count (p < 0.0001, p < 0.0001, respectively). In LAL2000 PDN response confirmed to be a powerful factor on CR rate, OS and DFS. Furthermore it demonstrated to overcome the impact of immunophenotype on DFS.

Protocol	Induction	Consolidation	Maintenance	pts.N.	PDN response	CR	6y %OS	6y %DFS
ALL0496	P+V+HD DNR +L-Asp	VP-16+CA ± Cy ± Cy	6-MP+MTX+ Reinduction V-Cy/V-DNR (36 mths)	482		78.63%	32.0%	31.4
				535	PDN resp.	88.02%	34.5%	34.2%
					PDN no resp.	63.11%		
ALL2000 7dPDN								

Table 1

C095

COMBINED INHIBITION OF THE BCL-2 AND MTOR PATHWAYS: IN VITRO PRO-APOPTOTIC EFFECTS IN ACUTE LYMPHOID LEUKEMIA

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Deregulation of the apoptotic machinery and aberrant activation of the mTOR signalling pathway are frequently described in acute lymphoid leukaemia (ALL) cells, playing a pathogenetic role in the processes of aberrant proliferation and chemoresistance. In this study, we thus evaluated the effects of the combined inhibition of both Bcl-2 and mTOR signalling, by using the BH3 mimetic inhibitor ABT-737 (kindly provided by Abbott Lab.) and the mTOR inhibitor CCI-779, respectively. ABT-737 induced cytotoxic effects on the MOLT4 cells characterized by a low Mcl-1 expression (IC50=198nM). In contrast, CEM-S, CEM-R, and JURKAT proved resistant (IC50>5.4uM); interestingly, they all displaying Mcl-1 overexpression. When the effect of CCI-779 was explored on these cell lines, only a minor cytotoxic effect was observed (IC-50 between 0.5μM-28.2μM). We then investigated the drug combination activity on the resistant cell line phenotype (JURKAT cells); a significant (p<0.01) increase in the level of of apoptosis (51% ± 5.5 in the presence of CCI-779 1000nM + ABT-737 1000nM) compared to the single agents (22.9% ± 3.5 and 7.7% ± 3.4 in the presence of ABT-737 and CCI- 779, respectively) was recorded. A similar activity was observed on the CEM-R cell line. WB (Western blot) analysis revealed that in both cell lines exposure to CCI-779, particularly in combination with ABT-737, induced a decrease in Mcl-1. However, the observed Mcl-1 down-regulation was not found in the other resistant phenotype (CEM-S). Moreover, interfering Mcl-1 expression by RNAi we did not revert the resistant phenotype in the CEM-S cell line. Primary ALL blasts, obtained from 18 patients were in the majority of the cases highly sensitive to ABT-737 (50nM); in 9 samples, the combination with CCI-779 (5000nM) showed additive or synergistic effects on apoptosis induction.

Resistance to ABT-737 was found in one sample; interestingly, the exposure of this resistant sample to the ABT-737 and CCI-779 combination, induced Mcl-1 down-regulation and induction of apoptosis (57.3% in the presence of ABT-737 50nM + CCI-779 5000nM, compared to 14.1% and 18.8% in the presence of ABT-737 and CCI-779, respectively). In summary, ABT-737 is a highly pro-apoptotic agent in ALL cells; the combined use of the mTOR inhibitor CCI-779 may revert some ABT-737 resistant phenotypes and these effects are associated with Mcl-1 down-regulation.

C096

MULTIPLEX PCR TO RAPIDLY IDENTIFY IKZF1 (IKAROS) GENE BREAKPOINTS IN BCR-ABL1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

Ferrari A,[1] Iacobucci I,[1] Lonetti A,[1] Papayannidis C,[1] Abbenante MC,[1] Trino S,[1] Cattina F,[1] Guadagnuolo V,[1] Paolini S,[1] Parisi S,[1] Baccarani M,[1] Martinelli G[1]

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Background: Deletions of IKZF1 gene, encoding for a regulator of lymphocyte differentiation, occur in more than 80% of adult patients (pts) with Ph+ ALL and significantly affect the prognosis. The deletions either involve the entire IKZF1 locus, resulting in loss of function, or delete an internal subset of exons, resulting in the expression of dominant negative isoforms. To better stratify Ph+ ALL pts according to IKZF1 status, we set up, validate and assess the routine applicability of a IKZF1 deletion screening strategy based on a multiplex-PCR. Patients and methods: We studied 87 adult BCR-ABL1+ ALL pts. For each type of common IKZF1 deletion (D4-7, D2-7, D4-8) an appropriate pair of primers will be designed using Primer 3 (http://frodo.wi.mit.edu/primer3/). Their ability to efficiently amplify the deletion was tested. PCR amplifications was performed using 50 ng genomic DNA as template for each reaction and a FastStart Taq DNA Polymerase (Roche Diagnostics). For each patient 3 amplicons were generated and sequenced (amplicon A for D4-7, amplicon B for D2-7 and amplicon C for D4-8). Moreover, a multiplex amplification strategy was assessed and a forth amplicon was generated and sequenced (forward primers were localized on intron 1 and 3, the reverse ones in the intron 7 and at the end of the gene, after the exon 8). The size of amplicons depends on the positions of the breakpoints in the IKZF1 gene and on the number of nucleotides added at the conjunction. It was in the range of 450-600 nucleotides. A positive control was used for each run. Results: On 87 pts previously analyzed by SNPs array, we identify IKZF1 deletions in 69/89 (78%) samples. Deletions were: 51 D4-7, 12 D2-7 and 4 D4-8; in 2 cases the deletion was extended to all gene, and 2 pts presented two breakpoints simultaneously. Multiplex PCR and subsequent sequencing were performed for most common deletions on 40 pts confirming previous results and indentifying the precise genomic positions of breakpoints and the nucleotides added at the conjunction. This rearrangement has been used to strictly monitor Minimal Residual Disease (MDR) during the follow-up and at relapse to confirm the clonal fidelity. Conclusions: We set-up a method rapid sensitive and with less amount of DNA sample, to screen Ph+ ALL pts at diagnosis and to monitor MRD during the treatment. Supported by: European LeukemiaNet, AIL, AIRC, FIRB 2006, Fondazione del Monte di Bologna e Ravenna, Strategico di Ateneo, GIMEMA Onlus.

CHRONIC LYMPHOCYTIC LEUKEMIA II

C097

TRANSCRIPT LEVEL OF PROAPOPTOTIC P66SHC REGULATOR, IS AN INDEPENDENT PROGNOSTICATOR OF SURVIVAL AND CHEMOREFRACTORINESS IN CLL

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p66shc is a pro-apoptotic protein that induces B-cell death by B-cell receptor signaling inhibition. p66Shc levels are lower in CLL than in normal B-cells, and U-CLL have lower levels than M-CLL. Aims of this study were to investigate the prognostic significance of p66shc in relation to time to first treatment (TTFT), overall survival (OS) and chemorefractoriness. p66shc transcript levels were measured by Real-time PCR in 143 CLL patients. P66shc transcript levels in a B-cell pool of 11 healthy donors were given the standard reference value of 1,00. p66shc levels were investigated for any association with i) clinical and biological prognostic parameters at diagnosis ii) TTFT (of patients diagnosed in stage 0) and OS (of all patients) and iii) refractoriness and time to refractoriness to Fludarabine and/or Alkilators. Rai stage was 0 in 94/143 (65,7%). Sixty-five/143 (54,6%) CLL used U-IGHV. TP53 disrupture by mutations and/or deletion was found in 12/143 (8,5%). Interquartile Median p66shc level was 0,335 (range 0,202-0,600). Based on the best cut-off (0,375 by ROC analysis and Youden's index, death as state variable), 63/143 (44,1%) patients had high p66shc (p66hi) and 80/143 (55,9%) low p66shc (p66lo) levels. A significant association was found between p66lo status and U-IGHV (18/63, 28,6% p66hi vs 47/80, 58,8% p66low, p<.001), CD38+ve (p=.002), ZAP70+ve (p=.02), trisomy 12 (p=.01), del17p (p=.01) or overall TP53 disrupture (p=.009). p66lo status also associated with shorter TTFT of stage 0 CLL (median 245 in p66hi vs 65 months in p66low, p<.001). and with shorter OS (median OS not reached in p66hi vs 112 months in p66low, p<.001). By multivariate Cox-analyses (covariates of TTFT: CD38+ve, ZAP70+ve and U-IGHV; covariates of OS: U-IGHV and del17p), p66lo scored as independent prognostic factors for TTFT (95%-CI 1,1-2,9, HR=1,7, p=.04) and for OS (95%-CI 1,1-7,9, HR=2,9, p=.03). Remarkably, p66lo associated with shorter time-to-fludarabine-refractoriness (median not reached in p66hi vs 125 months in p66low, p=.02) and time-to-alkilator-refractoriness (median 245 in p66hi vs 60 months in p66low, p=.008). Interestingly, 10/16 (62,5%) fludarabine-resistant and 19/32 (59,3%) alkilator-resistant patients scored p66lo in the absence of TP53 disrupture. These data suggest that p66shc plays an important (independent) role in defining outcome and chemorefractoriness risk in CLL patients.

C098

PROLIFERATION CENTRES IN CHRONIC LYMPHOCYTIC LEUKEMIA: CORRELATION WITH CYTOGENETIC AND CLINICOBIOLOGICAL FEATURES IN 183 PATIENTS ANALYZED ON TISSUE MICROARRAYS.

Ciccone M,[1] Agostinelli C,[2] Rigolin GM,[1] Piccaluga PP,[2] Cavazzini F,[1] Righi S,[2] Sista MT,[2] Sofritti O,[1] Rizzotto L,[1] Sabattini E,[2] Fioritoni G,[3] Falorio S,[3] Stelitano C,[4] Olivieri A,[5] Attolico I,[5] Brugiatelli M,[6] Zinzani PL,[7] Pileri SA,[2] Cuneo A[1]

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Introduction In CLL recurrent cytogenetic abnormalities may identify specific disease entities and be associated with distinct prognosis. FISH on paraffin-embedded fixed tissues (PEFT) may be used to study cytogenetics in CLL lymph-node where the "proliferation center" (PC) is usually evident. Aims a) to analyze the sensitivity and reproducibility of FISH on PEFT,b) to correlate the biological prognostic markers to PC extension;c) to estimate the frequency of chromosome lesions on lymph-node samples. Methods 183 CLL consecutive cases (49% untreat-

ed, 51% treated with a median of 2 lines) were submitted to lymph-node biopsy in the presence of progressive disease requiring treatment with adenopathies ≥ 3 cm. The cases, arranged in 5 tissue macro-arrays (TMAs), were analyzed by immunohistochemistry (CD38, MIB-1, ZAP-70) and by FISH for deletions of 11q23/ATM, 13q14 and 17p13/p53; for trisomy 12 and translocations of the IgH gene. Cytogenetic features were correlated with the histologic pattern and 2 groups were identified:1) PCs-rich group including CLL cases with confluent and large PCs and 2) typical group with scattered, well-distinct PCs. Results 75 cases (40.9%) were classified as PCs-rich, 108 cases (59.1%) were classified as typical. Complete FISH data were obtained in 101 cases (55.1%), 75 of which (78.2%) displayed at least one chromosomal aberration. The incidence of each aberration using a hierarchical classification was: 17p-15.6%, 11q- 24.7%, 14q32 translocations 30.8%, +12 19.5% and 13q-36.7%. The PCs-rich group was associated with advanced stage, 17p-, 14q32/IgH translocation, +12, ≥ 2 cytogenetic lesions and CD38+. The median survival from the time of tissue biopsy for PCs-rich and typical groups was 11 and 64 months, respectively (p=0.0048). Other parameters predicting for a shorter survival at univariate analysis were: ≥2 cytogenetic lesions, unfavourable cytogenetic abnormalities (17p- and/or 11q-) and advanced stage at biopsy. The PCs-rich pattern was the only predictive factor of an inferior survival at multivariate analysis (p=0.033). Conclusions FISH on TMAs is a reproducible and a feasible tool for a rapid screening of cytogenetic abnormalities in large number of cases under homogeneous experimental conditions. Our findings establish an association between cytogenetic profile and the amount of PCs in CLL and show that this histopathologic characteristic is of value for risk assessment in patients with clinically significant adenopathy.

C099

FLUDARABINE, CYCLOPHOSPHAMIDE AND LENALIDOMIDE (FCL)FOR PREVIOUSLY TREATED PATIENTS WITH CLL. GIMEMA STUDY CLL0606

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Herein, we report the preliminary results of a prospective phase I/II study with lenalidomide combined with fludarabine and cyclophosphamide (FC) in patients with Chronic Lymphocytic Leukemia (CLL) relapsed or refractory after no more than 2 previous treatments. The schedule was designed with the rationale that the association of lenalidomide with FC might result in an increased activity while preserving the immune function. The first part of the study (phase I) was addressed at defining the maximum tolerated dose (MTD) of lenalidomide given in combination with FC and the second part (phase II) at evaluating the efficacy of FC given in combination with the MTD of lenalidomide. Treatment consists of 6 monthly courses of FC (F, fudarabine: 30 mg/m2 iv and C, cyclophosphamide: 250 mg/m2, days 1-3) combined with 14 consecutive days of lenalidomide (days 1-14). In the first phase of the study, focused on defining the MTD of lenalidomide given with FC, a 3+3 patient design has been used and 3 steps were planned: 5, 10 and 15 mg of lenalidomide. In the second phase of the study FC was given in combination with lenalidomide escalated to reach the MTD. Patients received prophylaxis of granulocytopenia , of thromboembolic events (low molecular weight heparin), of TLS (oral hydration, allopurinol). So far, 13 patients with a median age of 65 years, all but 1 previously treated with the FC/FCR regimens, have been included in the study. Adverse genetic features were recorded in 7 cases. A DLT was recorded in 2 of 3 patients who received 10 mg of lenalidomide (pneumonia; grade 3 liver toxicity). Five mg of lenalidomide was defined as the MTD of lenalidomide given in combination with FC and this was the schedule administered to the patients included in the ongoing second part of the study. We had 4 patients with grade 2 or more non hematologic toxicity (grade 2 fatigue, 1; grade 2 transient skin rash, 1; grade 3 transient liver toxicity, 1 and pneumonia,1). Transient grade 3-4 neutropenia was observed in 46% of patients, a mild, grade 1,TFR in 1 while no TLS or thrombosis were recorded. No treatment-related deaths were observed. Seven patients responded (58%; CR, 3; PR, 4). The preliminary results of this ongoing protocol suggest that FCL, may be considered a promising investigational regimen with acceptable toxicity in patients who have failed a prior fludarabine-based treatment.

C100

INTERACTION WITH ENDOTHELIAL CELLS INDUCED MODULATION OF GENE EXPRESSION PROFILE, SURVIVAL AND DRUG RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS

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Chronic lymphocytic leukemic B cells (CLL) reside in close contact with activated endothelial cells (EC) in infiltrated tissues. Here, we investigate the interactions between EC and CLL cells by co-culturing purified leukemic cells on HUVEC monolayer (HC). Then, we detected CLL viability by flow cytometry and performed whole-genome high density microarrays. We found that endothelial cells protected CLL from spontaneous apoptosis. After 48h, increased number of live CLL cells was present in HC condition (59.7 +/- 4.2%) compared to CLL alone (22.9 +/- 5.1%) ($p < 0.0001$). Moreover, the endothelial cell layer decreased the in vitro sensitivity of CLL cells to Fludarabine-induced apoptotic cell death. Physical contact with EC is essential for protection to apoptosis. The insertion of a microporous membrane or blocking adhesion with anti-CD106 and anti-CD18 antibodies abrogated the apoptosis protection. Conversely, a reduction of apoptosis was also measured in CLL cultured in HC conditioned medium, implying that survival is partially mediated by soluble factors. Then, we compared gene expression profiles (GEP) between CLL cultured in contact with EC layer and CLL at baseline. Overall 1,944 genes were found to be modulated ($FC > 2$, $p < 0.05$). We found that CLL cells in HC showed a 22.6-fold increase of CCL2 (p=0.0032) and a 6.5-fold increase of PDGFC (p=0.0051). Other soluble factors up-regulated in HC condition were VEGFC (FC=9.4, p=0.0061), ANGTL4 (FC=8.6, p=0.015), ENG (FC=4.0, p=0.025), EDN1 (FC=9.2, p=0.0061), AMOTL2 (FC=4.3, p=0.019) and THBS-1 (FC=45.1, p=0.0004) as well as the metalloproteases MMP2 (FC=8.3, p=0.02) and MMP14 (FC=3.0, p=0.039). The GEP data were confirmed by evaluating the secreted levels of soluble factors in conditioned medium collected after 48h- HC culture. In addition, CLL cells on endothelial layer maintained or increased the expression levels of Bcl2, Bcl2A1, BIRC3/c-IAP2 and BIRC5/Survivin compared to CLL at baseline. Of interest, the Angiopoietin-2 receptor Tie2 mRNA was found to be increased in CLL cells in co-culture (FC=10.7, p=0.017). We confirmed GEP data by flow cytometry finding a 2-fold and a 4.3-fold increase of percentage of Tie2+CLL cells at 48h and 72h in HC. Our study supports the notion that EC are major players in CLL-infiltrated microenvironments by creating a vicious cycle of cooperation that strongly sustains leukemic cell survival, protects CLL from drug-induced apoptosis and widely modifies CLL phenotype.

C101

CLINICAL SIGNIFICANCE OF SOLUBLE CD23 IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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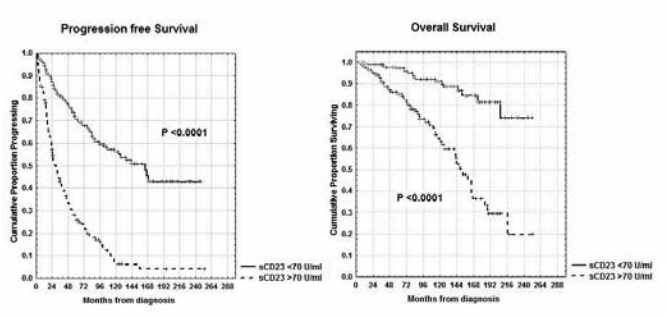
Soluble CD23 (sCD23), a protein derived from the cleavage of the CD23 membrane molecule, is selectively elevated in the serum of CLL pts, reflecting the tumor burden and disease activity, mainly at early clinical stages. The today availability of lumiliximab, an anti-CD23 monoclonal antibody, prompted us to retrospectively evaluate the prognostic impact of sCD23 in a large series of CLL pts. The primary endpoints of our study were: 1) to determine progression free survival (PFS) and overall survival (OS) upon sCD23; 2) to correlate sCD23 with Rai stages, lymphocyte doubling time (LDT), beta-2 microglobulin (B2M), CD38, CD49d, ZAP-70, FISH cytogenetics and IgVH and 3) to validate sCD23 as independent prognostic factor. We investigated 475 pts, median age 66 years, whose 149 had a modified low Rai stage, 303 an intermediate and 23 a high stage. SCD23 was determined by an immunoenzymatic assay, fixing the cut-off of positivity >70 U/ml and sCD23+ pts were 165/475 (35%). SCD23<70 U/ml was associated (P<0.0001) with low Rai stage (131/149), LDT>12 months (285/386) and B2M<2.2 mg/dl (207/245). Significant associations were found between sCD23>70 U/ml and ZAP-70>20% (111/197; P<0.0001) or CD38>30% (71/116; P<0.0001) or CD49d >30% (68/156; P=0.0006). There were significant correlations between sCD23>70 U/ml and IgVH unmutated status (354 cases, 73/118; P<0.0001). There was a correlation between normal karyotype/13q- deletion and sCD23<70 U/ml (337 cases, 195/244; P<0.0001). With regard to clinical outcome, 120 (73%) of 165 of the sCD23+ pts had received chemotherapy at the time of analysis (P<0.0001). Moreover, shorter PFS and OS were observed in sCD23+ patients (4% vs 43% at 16 years, P<0.0001 and 20% vs 74% at 20 years, P<0.0001). Interestingly, sCD23>70 U/ml showed a shorter PFS within low Rai stage (40% vs 70% at 10 years, P=0.01). Of note, pts with sCD23<70 U/ml showed a longer PFS both within the IgVH unmutated subgroup (118 pts, 20% vs 5% at 8 years, P=0.003) and within the IgVH mutated subgroup (236 pts, 54% vs 14% at 14 years, P<0.0001). In multivariate analysis of PFS, B2M (P=0.001), ZAP-70 (P=0.0002), sCD23 (P=0.00002), Rai stages (P=0.004) and IgVH (P=0.004) were independent prognostic factors. We demonstrated that sCD23 was able to improve the historical prognostic ability of the IgVH status and therefore it should be considered a robust prognosticator in CLL having to be necessarily added in a complete and up-to-date scoring prognostic system.



C102

STEREOTYPED B-CELL RECEPTOR (BCR) IS ASSOCIATED WITH IMMUNE THROMBOCYTOPENIA IN CHRONIC LYMPHOCYTIC LEUKEMIA.

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Background. Chronic lymphocytic leukemia (CLL) is frequently complicated during its course by autoimmune disorders, primarily autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP). The pathogenesis of these immune complications and the biological and molecular characteristics of the CLL patients showing them are still unclear. Aim. To investigate the biological/molecular profile and the B cell receptor (BCR) conformation of CLL patients (pts) that develop ITP included in a retrospective cohort from two major Italian Institutions. Methods. Of 463 CLL pts with available IGHV mutational status, we identified 37 patients that developed ITP during a median follow- up of 51 months (range 1-130). Overall, 199 (43%) were unmutated (UM) and 133 (28.7%) harbored a stereotyped HCDR3. FISH analyses for the major cytogenetic aberrations and ZAP-70 and CD38 expression data were available in 326 (70.4%), 343 (74%) and 411 (88.8%) pts, respectively. Diagnosis of ITP was performed according to previously described criteria (Visco et al., Blood 2008). The identification of stereotyped subsets was based on HCDR3 identity >60% (Stamatopoulos et al., Blood 2007). Results. Of the 37 patients with ITP, twenty-nine (78.4%) were UM and 18 (48.6%) carried a stereotyped HCDR3. Unfavorable FISH lesions (del(11q) and/or del(17p)) were present in 10/23 (43.5%) pts with ITP, while ZAP-70 and CD38 were positive in 20/27 (74.1%) and 15/33 (45.5%) pts, respectively. IGHV mutational status (p<.0001), stereotyped HCDR3 (p=.0137), ZAP-70 expression (p=.028), and unfavorable FISH lesions (p=.0017) were all significantly associated with occurrence of ITP. Considering the most frequent stereotyped HCDR3 associated with CLL, we observed that subsets #1 (IGHV1-5-7/IGHD6-19/IGHJ4; 16/16 UM) and #7 (IGHV1-69 or IGHV3-30/IGHD3-3/IGHJ6; 13/13 UM) were significantly associated with ITP development (p=.005 and p=.002 respectively). Considering only UM pts, subset #7 retained a significant association with ITP development (p=.01). Conclusions. In our series of CLL pts, ITP development confirmed its association with a UM IGHV conformation and elevated ZAP-70 expression. In addition, we observed a strong association of ITP development with stereotyped HCDR3 subsets #1 and #7, along with unfavorable FISH lesions. These data suggest that peculiar BCR conformations may be involved in the pathogenesis of ITP secondary to CLL.

MULTIPLE MYELOMA II

C103

MULTICENTER PHASE II STUDY OF LENALIDOMIDE AND PREDNISONE (RP) FOLLOWED BY LENALIDOMIDE, MELPHALAN AND PREDNISONE (MPR) IN NEWLY DIAGNOSED ELDERLY UNFIT MULTIPLE MYELOMA PATIENTS

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The combination of Melphalan-Prednisone-Lenalidomide (MPR) has shown promising results in elderly newly diagnosed myeloma patients. Hematological toxicity remains the main adverse event with standard MPR doses. A different approach has been designed to reduce toxicity in elderly unfit patients: induction with lenalidomide and prednisone (RP) precedes consolidation with MPR and maintenance with Lenalidomide alone. Unfit patients with newly diagnosed symptomatic myeloma older than 65 years were enrolled in a two-stage phase II clinical trial designed according to Bryant and Day method. No exclusion criteria were included in the protocol, to avoid the selection of fit elderly subjects only. Patients with low blood count, abnormal performance status, hepatic, renal, cardiac or pulmonary functions were enrolled. Patients received 4 RP courses (Lenalidomide 25 mg/day for 21 days every 4 weeks, plus Prednisone 50 mg three times/week for 4 months) followed by 6 MPR cycles (Melphalan 2 mg and Prednisone 50 mg three times/week, plus Lenalidomide 10-15 mg/day for 21 days every 4 weeks) and maintenance with RP (Lenalidomide 10 mg/day for 21 days abd Prednisone 25 mg three times/week until PD). Two different dose-levels of Lenalidomide were tested in combination with MP: 15 mg (dose-level 1) and 10 mg (dose-level 2). Each cohort included 12 patients, with additional 22 patients enrolled at dose-level 2. Forty-six patients (median age 75, range 65-88) were enrolled. Forty-five patients were evaluable after a median follow-up of 19.8 months. During RP induction, the most frequent grade 4 hematological adverse events were neutropenia (9%) and anemia (2%), no grade 4 thrombocytopenia was observed. During MPR consolidation no increase in grade 4 adverse events was registered, incidence of neutropenia was 11%, while no grade 4 anemia and thrombocytopenia were observed. Non-hematological toxicities were more frequent during RP cycles and reduced during MPR cycles (cutaneous rash and infections). Discontinuation rate was higher during induction (13% vs 5%). After RP induction, at least partial response (PR) rate was 76%, at least very good partial response (VGPR) was 16%. During MPR consolidation, PR rate increase to 80%, including 27% of patients who achieved at least a VGPR. In conclusion induction with RP followed by consolidation with MPR showed a manageable safety profile with a reduced the risk of severe haematological toxicity in unfit elderly myeloma patients.

C104

INDUCTION AND CONSOLIDATION THERAPY WITH BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE COMPARED WITH THALIDOMIDE-DEXAMETHASONE INCORPORATED INTO DOUBLE AUTOLOGOUS TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: RESULTS FROM A RANDOMIZED PHASE 3 STUDY

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We prospectively compared thalidomide-dexamethasone (TD) with bortezomib-TD (VTD) as induction before, and consolidation after, double autotransplantation (ASCT) in 236 and 238 patients with newly diagnosed multiple myeloma (MM). CR-nCR rate was significantly higher in the VTD arm compared with the TD arm after all treatment phases, including 3-cycles induction (31% vs 11%, p<0.0001), double ASCT (55% vs 41%, p=0.002) and 2-cycles consolidation (62% vs 45%, p=0.001). Overall, VTD consolidation resulted in 11% improvement in the rate of CR (McNemar test: P=0.005) and upgraded the rate of molecular remission up to the unprecedented value of 64% (McNemar test: P=0.007). The probabilities to complete induction and receive subsequent ASCT were 96% and 92% in the VTD arm, and 89% and 82% in the TD arm. With a median follow-up of 3 years, VTD induction and consolidation plus double ASCT was superior to TD and double ASCT in terms of reduced risk of relapse (HR=0.60; P=0.007) and extended PFS (3-year estimates: 68% vs 56%, respectively; P=0.005). Superior PFS with VTD and ASCT was maintained across all subgroups of patients with poor prognosis, including those carrying t(4;14) ± del(17p) (HR=0.50; P=0.01). In particular, the poor prognosis related to the presence of t(4;14) was completely overcome in the VTD arm (3-year estimates of PFS: 69% vs 74% for t(4;14) negative patients). A multivariate analysis performed in the overall population showed that a low 2-microglobulin level (HR=0.47, P<0,0001), absence of t(4;14) ± del(17p) (HR=0.71, P=0,002), randomization to VTD arm (HR=0.63, P=0.01) and achievement of CR-nCR (HR=0.98, P=0.01) were the most important and independent variables correlated with extended PFS. OS curves seemed to initially diverge after 40 months (44-month projected rates: 84% vs 74% for VTD and TD arms, respectively), although the difference was not statistically significant. In conclusion, in comparison with TD: 1) VTD induction affected a 3-fold increase in the rate of CR-nCR and emerged as a new standard of care for maximizing the degree and speedy of tumor reduction in preparation for ASCT; 2) VTD consolidation affected significantly higher rates of CR and molecular remissions; 3) double ASCT incorporating VTD as induction and consolidation resulted in significantly longer PFS, a benefit confirmed in a multivariate regression analysis and maintained in the subgroup of patients with high-risk cytogenetic profile.

C105

SEROTONIN STORED IN PLATELETS OF MULTIPLE MYELOMA PATIENTS REFLECTS THE ANGIOGENESIS SWITCH ASSOCIATED WITH BONE LESIONS

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Background While MM patients with evidence of osteolytic lesions have been shown to have elevated concentrations of serum serotonin the consequences of this elevation have not been previously studied in the bone itself. Methods We retrospectively measured bone remodeling signal pathway perturbations in 15 bone marrow core biopsies from patients diagnosed with MM and correlated this with the presence of osteolytic bone disease. We also measured circulating serotonin levels by ELISA in a pilot set of MM patients (n=20), in both serum and platelets isolated from peripheral blood to confirm observations obtained by RPMA. Finally, we collected platelets from 19 peripheral blood of subjects affected of monoclonal gammopathy (5 MGUS, 14 MM at different clinical stages and treatment with or without biphoshonates). Samples were lysed, protein extracted, printed and stained on Reverse-phase protein microarrays (RPMAs). In this last independent test, RPMAs were used to quantitatively map 45 cell signaling pathway endpoints, including oxygen sensors and transmembrane receptors proteins, in order to provide quantitative information regarding post- translational modifications (e.g. phosphorylation, cleavage, acetylation) and/or total cell signaling kinase levels. Results Bone marrow core biopsies exhibited significant elevation of Serotonin, RANK, MMP-11, TNF , TNF-R1, and Ezrin Tyr353 by RPMA in patients with osteolytic lesions compared to patients without evidence of bone disease. Cytokines IL-1 , IL-6, and IL- 10 were also significantly elevated in the bone marrow cores of patients with bone disease. Free circulating serotonin in MM sera was elevated compared MGUS and healthy controls. In the platelets we found a positive correlation between the content of serotonin and HIF-1-alpha (r=0.74, p=0.0005). Both analytes decreased after Zoledronic Acid 4 mg i.v. treatment for at least 3 months (respectively p=0.0014 and p=0.0317), suggesting that the treatment

modulate these angiogenesis related platelet proteins. The anti-angiogenic molecule PEDF was not related to serotonin levels and it was not modulated by Zoledronic Acid. Conclusions Our data support the role of the peripheral 5-HT system to mediate signaling cascades related to the pathogenesis of MM- induced bone disease. This insight could provide strategies for reducing osteolysis with agents that regulate local or systemic serotonin, either alone or in combination with other molecular targeted inhibitors.

C106

THE ANTI-MYELOMA EFFECT OF LENALIDOMIDE IS ENHANCED AFTER ALLOGENEIC TRANSPLANTATION

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Lenalidomide (Len), a highly effective drug against multiple myeloma (MM), acts through several mechanisms, such as a direct cytotoxic effect, anti-angiogenesis, microenvironment modifications, and immunomodulation. The latter property is particularly interesting in the setting of allogeneic hematopoietic stem cell transplantation (Allo-HSCT), since Len may interact favourably with the effector cells of graft-versus-myeloma (GVM) effect. Preliminary results from retrospective studies on heterogeneous patient populations suggested that Len is also effective when given after Allo-HSCT. In order to verify this observation, we conducted a case-matched analysis of Len after autologous stem cell transplantation (Auto-HSCT) versus Allo-HSCT. The hypothesis is that Len may be more potent when administered after Allo-HSCT. In this retrospective study, the matching criterion was represented by the number of treatment lines received before Len. In an attempt to uniform the treatment regimens, an intra-centre matching was required. We collected data from 39 patients in each group. Baseline characteristics between Auto and Allo patients were similar, except for age at diagnosis (53 years, range 39-70, in Auto patients; 47 years, range 29-61, in Allo patients). The median number of previous lines of treatment was 3 (range 1-6) for both groups.Twenty-one of 39 (54%) Allo patients received HSCT after the first line. Thirty-two (82%) Auto and 35 (90%) Allo patients received bortezomib in previous lines. Similarly, 34 (87%) Auto and 12 (54%) Allo patients were previously treated with thalidomide. Len was always administered with dexamethasone. Best responses in evaluable patients were for Auto and Allo patients as follows: 0 vs. 3 CR, 6 vs. 8 VGPR, 11 vs. 11 PR, 6 vs. 6 SD, 9 vs. 7 PD. Time to the best response was 4 months for both groups. With a median follow-up of 11.5 months (range 1-39), 1 year and 2 year progression-free survival were 41% and 6% for Auto patients, and 52% and 44% for Allo patients (p=0.03), respectively. Two years overall survival was 48% for Auto and 75% for Allo patients (p=0.03). Similar results were observed regardless of previous thalidomide treatment. No unexpected toxicities were reported. Two (10%) patients had a worsening of a pre-existent extensive chronic GVHD. In conclusion, our study support the hypothesis that Len is synergistic with the GVM effect, still retaining a favorable toxicity profile.

C107

THE RECONSTRUCTION OF TRANSCRIPTIONAL REGULATORY NETWORKS REVEALS CRITICAL GENES WHICH HAVE IMPLICATIONS FOR CLINICAL OUTCOME OF MULTIPLE MYELOMA

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Background: The combined use of microarray technologies and bioinformatics analysis has improved our understanding of biological complexity of multiple myeloma (MM). In contrast, the application of the same technology in attempt to predict clinical outcome has been less successful with the identification of heterogeneous molecular signatures. Aim: This approach was aimed at the identification of robust and reproducible signatures associated with prognosis across independent datasets. Methods: We have reconstructed gene regulatory networks in a panel of 1883 samples from MM patients profiled on Affymetrix platform, derived from seven publicly available gene expression sets. The transcriptional networks were reconstructed using ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks). Critical analysis of network components was applied to identify genes playing an essential role in transcriptional networks, which are conserved between datasets, and proportional hazard models were used to evaluate the association of each gene with outcome. The correlation with overall survival was tested in three of the seven datasets for which clinical data were available. Results: The network critical analysis revealed that i) CCND1 and CCND2 were the most critical genes; ii) among the top critical genes CCND2, AIF1 and BLNK had the largest number of connections shared among the datasets; and iii) robust gene signatures with prognostic power were derived from the most critical transcripts and from shared primary neighbors of the most connected nodes. In particular, a "critical-gene" model, comprising FAM53B, KIF21B, WHSC1 and TMPO, and a "neighbor-gene" model, comprising BLNK shared neighbors CSGALNACT1 and SLC7A7, predicted survival in all datasets with follow-up information. Conclusions: The reconstruction of gene regulatory networks in a large panel of primary tumors suggested novel molecular mechanisms central to MM biology and identified specific genes with prognostic importance.

C108

BORTEZOMIB AND THALIDOMIDE INDUCED NEUROPATHY: A CLINICAL AND GENETIC STUDY

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Peripheral neuropathy (PN) is an important complication of multiple myeloma (MM) and its incidence has increased further after the introduction of new drugs such as thalidomide and bortezomib. The phase 3 GIMEMA trial of thalidomide-dexamethasone (TD) versus bortezomib-thalidomide-dexamethasone (VTD) as induction therapy before, and consolidation therapy after double autologous stem-cell transplantation for newly diagnosed MM, demonstrated that VTD significantly improves response and progression free survival (PFS). This is a subanalysis of the GIMEMA trial aimed to assess frequency, reversibility, risk factors and molecular markers associated with treatment emergent PN. We analyzed 474 patients, 236 assigned to VTD and 243 to TD. Occurrence of PN was higher in VTD compared to TD and in particular: grade ≥1 55% (n: 130) vs 22% (n: 53) (p<0.001), grade ≥2 35% (n: 83) vs 10% (n: 24) (p<0.001) and grade ≥3 15% (n: 35) vs 2.5% (n: 6) (p<0.001), respectively. The median time to onset of PN was 40 days on VTD and 46 days on TD and most of the events occurred during the induction phase (63% on VTD and 66% on TD). PN was reversible and resolved in 89% of patients on VTD and 92% on TD within a median time of 65 and 46 days (p 0.02), respectively. Rates of response and PFS were not affected by PN development. By univariate analysis, patients baseline characteristics, including age, MM subtype, stage and cytogenetic abnormalities, did not influence the development of PN in both arms. Moreover, gene expression profiles (GEP) from pre-treatment CD138+ cells fraction were analyzed for 129 patients on VTD, 65 patients with and 64 patients without treatment emergent PN. Treatment emergent PN was characterized by 2270 differentially expressed genes (p<0.05, one-way ANOVA, Partek Genomics Suite 6.4). In patients experiencing PN genes involved in the development and function of the nervous system resulted significantly up-regulated (e.g. PAX3, HOXA1, SOX10, GLI3), whereas genes involved in Ca++ signalling (e.g. NCX1) and synaptic transmission (e.g. GRIK4) were overall down-regulated. In conclusions the combination of the two neurotoxic agents thalidomide and bortezomib was associated with an higher incidence of PN compared with thalidomide alone, nevertheless PN resulted totally reversible in the majority of patients and it didn't affect response or PFS. Finally, GEP results suggest an interaction between myeloma related factors and development of treatment induced PN.

ACUTE MYELOID LEUKEMIA II

C109

A SIMPLE PROGNOSTIC SCORING SYSTEM FOR NEWLY DIAGNOSED CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA: RETROSPECTIVE ANALYSIS OF 530 PATIENTS.

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Objectives: The main objective of this study was to generate a simple prognostic index score (PIS) for cytogenetically normal acute myeloid leukemias (CN-AMLs). Methods: We retrospectively analysed the data of 337 patients newly diagnosed with CN-AMLs, aged less than 65 years (training set). The PIS was calculated by totalling up the score derived from the regression coefficients of each clinical variable, that was significantly associated with prognosis by multivariate analysis. The patients of the training set were stratified into three groups: low, intermediate and high-risk. This PIS was then validated on 193 patients with newly diagnosed CN-AMLs, aged less than 65 years, and enrolled in the GIMEMA LAM99p clinical trial (validation set). Results: The variables that were independent prognostic factors for EFS and OS in the training set were: age > 50 yrs, secondary AML and WBC > 20 x 10^9/L. The median EFS was 25, 12, and 7 months in the low-, intermediate- and high-risk group (p<0.0001), respectively. The median OS was not reached in the low-risk group and was 19 and 10 months in the intermediate- and high-risk group (p<0.0001). In the validation set, the median EFS was 66, 16, and 3 months in the low-, intermediate-, and high-risk group, respectively (p<0.0001). The median OS was 66, 16, and 5 months in the low-, intermediate-, and high-risk group, respectively (p<0.0001). Conclusions: This simple PIS may be useful for clinical decision-making in CN-AMLs and may be prospectively integrated and refined with the newest biological markers which at present are not routinely assessed and still need prognostic validation.

C110

HIGH DOSE CYTARABINE AND CLOFARABINE IN RELAPSED OR REFRACTORY AML PATIENTS

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Clofarabine has been shown to be effective in AML patients, either as single agent or, mainly, in association with cytarabine. On the basis of these reports, we conducted a preliminary study combining clofarabine and cytarabine in AML patients who relapsed or failed to respond to at least two induction therapies. We treated 47 patients affected by relapsed/refractory AML with a regimen including clofarabine at 22,5 mg/m2 daily on days 1-5, followed after three hours by cytarabine at 1 gr/m2 daily on days 1-5. Ten patients received a further consolidation cycle with clofarabine at 22,5 mg/m2 and cytarabine at 1 gr/m2 day 1-4. Among the 47 patients, 14 were in first relapse, 13 in second or third relapse, 20 with resistant disease. The mean age was 50,5 years (range 21-71 years). 24/47 (51%) patients achieved a complete remission, 5/47

(10.5%) a partial response, 14/47 (29%) had a resistant disease, 4/47 (8.5%) died of complications during the aplastic phase. The most frequent non haematologic adverse events were vomiting, diarrhea, transient liver toxicity (3/47 grade 3-4), febrile neutropenia (22/47), infections microbiologically documented (13/47 bacterial infection, 1/47 fungal pneumonia). 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 24 days), except in a patient (female, 34 years old, relapsed after ABMT) that experienced an unexpected, irreversible aplasia after the consolidation course, complicated by an unusual HHV6 reactivation. Among the 24 responding patients 13 underwent allogeneic bone marrow transplantation. In 14 patients complete remission duration was shorter than 12 months, whereas 10 patients experienced a longer complete remission duration. These very preliminary results suggest that that clofa-ARA-C regimen is effective in this particularly poor prognosis category of patients, representing a potential "bridge" toward bone marrow transplant procedures. Safety data were consistent with previously reported salvage therapies. Further studies and a longer follow up are warranted.

C111

VALUE OF NPM GENE MUTATIONS IN RESPONSE ASSESSMENT AND MINIMAL RESIDUAL DISEASE EVALUATION IN DENOVO CYTOGENETICALLY NORMAL NPM+ AML

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Background and aims: for minimal residual disease (MRD) evaluation the study of NPM gene mutations is not routinely performed and WT1 gene expression is considered the standard method for all patients. We evaluated NPM gene mutations as a possible indicator of quality of response and as marker of MRD, in comparison with WT1. Patients and methods: 158 bone marrow samples of 24 consecutive normal karyotype NPM+ denovo AML patients (median age 56 years, range 42-71) treated between May 2004 and June 2010 and achieving CR after two courses of conventional chemotherapy were studied. A four-color flow cytometric analysis was used to perform immunophenotype (IF). NPM A and B DNA mutations and WT1 expression were studied by a quantitative Real Time PCR. Results. In 18 out of 22 patients in whom IF was done at response evaluation the flow cytometric analyses did not detect the clonal population observed at diagnosis (IF-CR, 81%). In 14/24 patients samples were negative for NPM mutations (NPM neg CR, 58%). Relapses in NPM neg CR patients and in those with persistence of NPM mutations were 7/14 (50%) and 10/10 (100%), respectively (p 0.03). Patients with at least two consecutive NPM negative samples had a lower relapse rate compared to the other patients [5/12 (41%) vs 12/12 (100%); $p < 0.01$]. In the follow up study the reappearance of NPM mutations was always followed by haematological relapse with a median interval of 5 months (range 2-6 months). The median interval between WT1 increase and haematological relapse was 1 month (range 0-5). The median DFS in patients who achieved two consecutive negative NPM determinations and those who did not were 13 months (range 7-69 months) and 6 months (range 1-10 months), respectively. All the seven patients who are alive and disease free had obtained a confirmed NPM negative CR (DFS 22 months, range 11-69). Conclusions: our preliminary analysis shows that the achievement of at least two consecutive negative NPM determinations is associated with prolonged haematological remissions. In the follow up of molecular CR patients the reappearance of NPM gene mutations is always followed by clinical relapse and may be considered an earlier relapse-marker than WT1 increase. Monitoring NPM gene mutations at response evaluation and in the follow up might help in defining subgroups of patients with high relapse risk and therefore likely to benefit of an early allogeneic stem cell transplant.

	N(%)	Relapses (%)	p (for relapses)	DFS months (range)
Haematol CR	24/24 (100)	17/24 (70)		9 (1-69)
IF neg CR	18/22 (81)	11/18 (61)		10 (1-69)
NPM neg CR	14/24 (58)	7/14 (50)	0,03	12 (6-69)
NPM pos CR	10/24 (42)	10/10 (100)		6 (1-9)
Pts with confirmed* NPM neg CR	12/24 (50)	5/12 (41)	< 0.01	13 (7-69)
Pts without confirmed* NPM neg CR	12/24 (50)	12/12 (100)		6 (1-10)

*confirmed = at least 2 consecutive NPM negative samples

C112

BLASTIC PLASMACYTOID DENDRITIC CELL LEUKEMIA: PRELIMINARY RESULTS OF A RETROSPECTIVE ITALIAN MULTICENTRIC STUDY

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Aims: To evaluate the clinical features, the prognostic factors, and the efficacy of treatments in patients (pts) with Blastic Plasmacytoid Dendritic Cell Leukemia (BPDCL) collected among GIMEMA centers. Methods: A retrospective multicenter study was carried out between January 2005 and December 2010 in 28 Italian hematology divisions.

Results: According to WHO2008 classification 49 evaluable cases of BPDCL were collected (M/F 38/11; median age 51 yo, range 20-81). At diagnosis the median bone marrow infiltration was 90% with a poor residual bone marrow function, as documented by low hemoglobin level (median value 6.2 g/dL) and platelet count (median count 23x10^9/L); 42 pts (86%) had peculiar skin lesions, while lymph nodes and/or spleen involvements were documented in 25 cases (51%), and extramedullary disease in 8 (16%).

In 29 pts (59%) cytogenetic study was performed, revealing an unfavourable karyotype in 11. Forty-five pts received an acute leukemia-like induction therapy (4 died early), consisting of antracycline/cytarabine (AML-like regimen) in 33 (73%), and of dexamethasone/vincristine/cyclophosphamide/metotrexate (ALL-like regimen) in 12 (27%); overall 9 pts (20%) underwent allo-HSCT. Complete (CR) or partial remission (PR) after induction therapy was achieved in 21 and 6 pts respectively (overall response rate 60%). It were registered 12 CR and 4 PR after AML-like regimen, and 9 CR and 2 PR after ALL-like regimen, with a significant advantage for ALL-like chemotherapy (p=0.01). The median overall survival (OS) was 8 months (range 0.2-60); 6 months (range 0.2-60) and 12 months (range 1.8-31) in pts received AML-like regimen and ALL-like regimen respectively (p=0.06). In HSCT-pts the median OS was 31 months (range 3-60), with a significant advantage with respect to non-transplanted pts (median 6 months, range 0.2-26, p=0.002). In pts obtaining a complete remission, the median DFS was 9 months (range 3-60); among them 18 relapsed, after a median time to diagnosis of 4 months (range 1-9).

Conclusions: BPDCL is a rare but clinically aggressive hematopoietic neoplasia, preferentially involving skin and bone marrow and behaving similar to high-risk acute leukemia. Initial response to ALL-like induction chemotherapy is good, but relapse occurred rapidly after a median time of only 4 months; allo-HSCT performed in first remission may lead to long-term survival and disease control in selected cases, but more data are needed to confirm these results.

C113

HIGHER BODY MASS INDEX AT DIAGNOSIS IS ASSOCIATED WITH INCREASED RISK OF DIFFERENTIATION SYNDROME AND RELAPSE IN ACUTE PROMYELOCYTIC LEUKEMIA

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The association between obesity and outcome of acute promyelocytic leukemia (APL) patients is still not defined. We investigated whether increased body mass index (BMI) might have a role in the outcome of APL patients. The study population includes 144 patients consecutively diagnosed as having APL between January 1993 and December 2005 at the " Sapienza" University of Rome and treated with AIDA or AIDA2000 schedule. Diagnosis was confirmed for all patients at molecular level. Patient demographic information, weight and height, treatments received, events and survival outcome data were collected. In particular the diagnosis of definitively present retinoic acid syndrome (RAS) was clinically established by the presence of at least three of the following signs weight gain, respiratory distress, unexplained fever, interstitial pulmonary infiltrates, pleural or pericardial effusions, according to Frankel et al. There were 66 males and 78 females, median age 39.3 years. Seventy-five patients were aged more than 40 years at diagnosis. Twenty-four patients out of 144 were classified as having variant type according to FAB classification and 83 patients have BCR1 type of transcript. BMI was defined as the individual's body weight divided by the square of him or her height: we stratified 4 categories of patients, according to height and weight at the time of diagnosis: underweight (BMI< 18.5), normal weight (BMI 18.5-<24.9), overweight (BMI 25->29.9) and obese (BMI ≥30). BMI calculation revealed that 64% and 15% of patients aged > 40 years at diagnosis were overweight and obese, respectively. Twenty-one patients (14.5%) experienced RAS during induction therapy according to reported criteria: we evaluated in multivariable logistic regression model clinico-pathological features at baseline possibly associated to an increased risk of RAS and we identified as the significant factor only BMI (p=0.014, OR 7.2, 95% CI 1.5-34.9). After a median follow-up time of 7.1 years a significant association between BMI and 5-year cumulative incidence of relapse (CIR) was detected: 31.6% (95% CI 22.7%-43.8%) in overweight/obese patients compared to 11.2% (95% CI 5.3%-23.8%) in underweight/normal weight patients (p=0.029). Multivariable analysis for hazard of relapse after the end of induction confirmed that BMI was significantly associated with probability of relapse (overweight/obese vs under/normal weight patients 2.45, 95% CI 1.00-5.99, p=0.049). In conclusion, increased BMI at diagnosis is associated to higher risk of RAS and relapse in APL.

C114

ITALIAN NETWORK ON SECONDARY LEUKEMIAS: PRELIMINARY DATA.

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Background: In 2001, the World Health Organization (WHO) recognized therapy-related myeloid malignancies (t-MN) as a distinct entity. At present, about 10% of all AML patients have a previous history of exposure to chemotherapy and/or radiation for a primary malignancy or autoimmune disease. In 2009, we initiated through a Web-database an epidemiological registry, with the purpose of collecting t-MN observed in 39 Italian Hematological or Oncological Divisions. Demographic and clinical information on individuals with t-MN were included in the database whose access was restricted to selected users and was password-protected. Matherial and methods: Between May 2009 and January 2011 a total of 148 patients observed in 12 of 39 Centers [59 males and 89 females; median age 61 years (range 24-88 years)] with secondary leukemia were registered in the web-database. Among these, 130 cases were t-MN arising after chemo or radiotherapy for a primary malignancy, while in 18 cases leukemia represented a secondary cancer in patients not receiving chemo or radiotherapy for primary malignancy. Results: The primary malignancy (PM) was a hematological neoplasm In 51 cases (34,5%), and a solid tumors in 97 cases (65,5%). Thirteen patients (9%) had a history of two or more previous cancers. Among hematological malignancies, the most frequent PM were lymphoproliferative diseases (37/51 cases), while breast cancer (41/97 cases) was the most frequent primary solid tumor. In particular, hematological PM were: 37 lymphoprolipherative diseases (23 Non Hodgkin and 13 Hodgkin lymphoma, 1 chronic lymphocytic leukemia); 7 Multiple myeloma; 6 myeloproliferative neoplasms (3 Myelofibrosis; 3 polycitemia vera); 1 Acute lymphoblastic leukemia. Sites of primary solid tumors were: 41 Breast; 22 Urogenital (11 prostate); 10 Colo-rectal; 6 Lung; 4 Thyroid; 10 others (3 CNS; 3 skin, 3 oropharynx; 1 sarcoma); 4 unknown. One-hundred-eight patients had previously received chemotherapy for their primary malignancy, associated to radiotherapy in 43 cases. RT represented the only primary treatment in 22 cases. Median latency between PM and t-MN was 4.9 years (range 0.2-48). No differences were observed in the median latency between t-MN after lymphoprolipherative diseases or after breast cancer (p 0.48). According to morphology, t-MN were classified as 122 AML, 24 MDS and 2 ALL. Kariotype was available for 77 patients and was unfavorable in 45 patients. A recurrent chromosomal translocation in only 6 patients [3 were t(8;21), 2 were t(15;17) and 1 was inv16]. Ninety-six patients received chemotherapy for t-MN, while the hypomethylating drug 5-Azacitidine was administered to 18 patients. Twenty patients underwent bone marrow transplantation (17 allogeneic and 3 autologous).

MYELODYSPLASTIC SYNDROMES II

C115

ROLE OF BCL2L10 METHYLATION AND TET2 MUTATIONS IN HIGHER RISK MYELODYSPLASTIC SYNDROMES TREATED WITH 5-AZACYTIDINE

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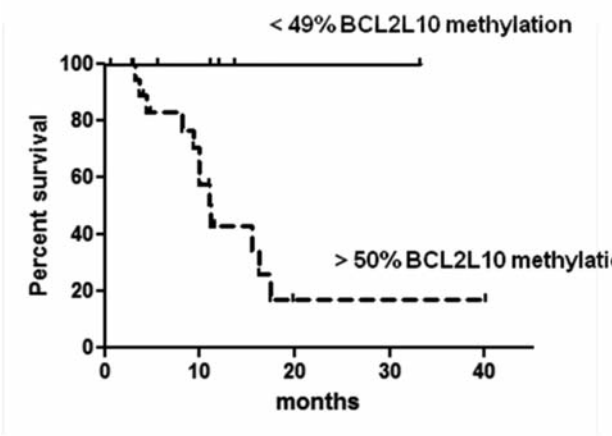
Epigenetic gene regulation plays a critical role in myelodysplastic syndromes (MDS), but the association between mutations of the epigenetic enzyme TET2, methylation profile and response to 5-azacytidine has still to be defined. We studied 38 higher risk MDS patients treated with 5-azacytidine and valproic acid, according to the multicenter clinical trial MDS0205. Treatment response was evaluated in 32 patients. The validation group included 27 higher risk MDS patients treated with 5-Azacytidine alone. Using a PCR array for 22 genes (RUNX1, FOXO3, TET2, PTEN, DUSP1, EZH2, DAPK1, TWIST1, HOXA9, PNPLA8, NRCAM, GLCCI1, CDH1, KLF5, OLIG1, OLIG2, BIK, BCL6, BCL2L10, TP53, ASXL1 and SPARC) and the principal component analysis, we identified four methylation patterns which explained about 65% variability of methylation in higher-risk MDS. TET2 mutations in coding exons 3-11 were present in 12 of 38 patients (32%) with 5 frameshift, 3 nonsense, 6 missense (2 recurrent and 4 putative) mutations, and 2 putative polymorphisms not annotated in NCBI SNPs database. TET2 mutations were more frequent in in CMML (3 of 4 patients, 75%) versus RAEB/RAEB-t (9 of 34 patients, 27%, p=0.08), but there were no associations with gender, IPSS score or karyotype. TET2 mutations did not have any impact on the methylation rate of the 22 genes analyzed. Five of the eleven TET2-mutated patients (46%) responded to epigenetic treatment, while only 5 of the 21 patients (24%) with wild-type TET2 gene responded, but this difference was not statistically significant (p=0.2). Median overall survival was 14.4 months, without any difference between TET-2 mutated or unmutated patients (log-rank p=0.9). On the other hand, a significant impact on patients' overall survival was exerted by the third methylation component, including the genes NRCAM, BCL6, TP53, and BCL2L10. In particular, methylation of the apoptosis gene BCL2L10 was significantly associated to decreased survival, independently from IPSS. These results were validated in an independent cohort of 27 higher-risk MDS with BCL2L10 methylation predicting inferior 5-azacytidine response and survival (p=0.047 and 0.04, respectively). Our data indicate that the methylation pattern, and in particular methylation of BCL2L10, may predict survival in higher-risk MDS treated with epigenetic treatment, while TET2 mutations did not impact on prognosis and methylation in our patients.



C116

ACTIVATION OF COMPENSATORY SURVIVAL PATHWAYS AFTER TREATMENT WITH 5-AZACYTIDINE IN HIGH-RISK MDS

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Background 5-azacytidine (5-AZA) is a DNA methyltransferase inhibitor used in treatment of high-risk MDS, that is able to delay the progression to AML and improve clinical outcomes. Methods By reverse-phase protein microarray (RPMA), we analyzed bone marrow mononuclear cells from 19 patients affected of high risk MDS, treated for 4 months with 5-azacytidine (median age 71 years, M/F=12/5). Treatment consisted of 4 cycles of 100mg flat dose for 7 days+21 days of wash-out. In 7 cases the sample after 4 cycles of therapy was matched to the sample collected before the therapy start. RPMA was used to quantitatively map 45 cell signaling pathway endpoints, including survival, proliferation, drug resistance, apoptosis, and autophagy. Results All patients were evaluable for response one month after the 4th cycle. Three patients were refractory, progressing to AML under treatment and 1 was a late responder (documented response after 7cycles). All other patients experienced hematologic improvement. We found that, after 4 cycles of 5-AZA, three main signaling pathways were increased and did not correlate with the response: 1) pro-survival signaling: PLC-y-1-Tyr783 (p=0.0017), and its upstream regulators Src-Tyr416, c-Abl-Tyr735 (p=0.002) and downstream target STAT5Tyr694 (p=0.0017) were increased, without affecting proliferative pathways, such as AKT activation status on Ser473 and Thr308 or mTORSer2448. 2) Autophagy: ATG5, Beclin 1 and LC3B were significantly elevated after treatment (p values respectively <0.0001, 0.0056 and 0.0124). Activation of the autophagy pathway occurred downstream of mTOR, since mTORSer2448, AktSer473, AktThr308, ERKThr202Tyr204 (and in general proliferation markers) were not affected. 3) Restore of cell cycle G1-S checkpoint: c-Abl-Tyr735, Rb- Ser608, Rb-Ser780Chk1-Ser345, p53, p53Ser15, and in particular HDAC1, HDAC3 were significantly elevated (p<0.001) Conclusion After 5-AZA treatment we observed in the bone marrow a change in signaling pathways that can be informative for further studies. In particular, autophagy activation can be considered an escape pathway promoting survival in neoplastic cells and the observation that 5-AZA does not affect proliferative pathways, suggests the possibility to combine it with anti-proliferative agents, such as Rapamycin or RAD001. In addition, the increase of HDAC1 and HDAC3 provides the molecular rationale for the development of a combination of 5-azacytidine with HDAC inhibitors.

C117

USE OF THE CUMULATIVE ILLNESS RATING SCALE FOR GERIATRICS (CIRS-G) IN A COHORT OF 1065 PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES. A SURVEY FROM THE MDS PIEDMONT REGISTRY.

Messa E, Gioia D, Bertassello C, Allione B, Bonferroni M, Cametti G, Cilloni D, Crisà E, Falco P, Ferrero D, Freilone R, Lunghi M, Salvi F, Tonso A, Saglio G, Levis A. On behalf of the MDS Piedmont Registry

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BACKGROUND: Comorbidity evaluation is a critical issue for the management of neoplastic patients (pts) and different Comoribidity Indexes (CI) have been proposed. One of the most useful in geriatric oncology is the Cumulative Illness Rating Scale of Geriatrics (CIRS-G). CI evaluation can be extremely important in myelodysplastic syndromes (MDS), due to the advanced age of these pts. Which CI is more suitable in this setting is still under debate and recently a new score (MDS-CI) has been developed by Della Porta et al. Aims: Aim of our study is to test the usefulness of the conventional and easy to apply CIRS-G score among a cohort of MDS pts enrolled in the MDS Piedmont Registry. PATIENTS AND METHODS: CIRS-G scale evaluation was prospectively recorded at diagnosis from 1999 to 2010 in 1065 (629 males, 436 females) cases. Its role in affecting Overall Survival (OS) was calculated either considering the whole population or stratifying pts according to IPSS score. RESULTS: 956 pts were diagnosed according to WHO classification: 202 RA, 70 RARS, 165 RAEB-1, 130 MDS-U, 140 RAEB-2, 219 RCMD and 30 5q- syndromes. Moreover, 81 CMML, 26 RAEB-t and 2 atypic chronic myeloid leukaemia pts were included in the analysis.

None of the 14 individual items of the CIRS-G CI showed, when applied alone, a statistically significant value sufficient to predict OS. Pts were therefore stratified into 4 groups according to global CIRS-G scale as follows: Score 1: no comorbidity or one or more grade 1 (348 pts); Score 2: up to 3 grade 2 (480 pts); Score 3: more than 3 grade 2 or one grade 3 (85 pts); Score 4: a grade 3 coexisting with at least a grade 2 or more than one grade 3 or at least one grade 4 (152 pts). Time to Leukemic Evolution (LE) was not affected by any CIRS-G. At the opposite CIRS-G score significantly affected OS ($p<0.01$), even if there was only a slightly difference between score 3 and 4, very close to a statistically significant level ($p=0.06$), probably due to a low number of patients in these two groups. Moreover, stratifying pts for IPSS, the prognostic role for OS of CIRS-G score was highly evident for Low/Int-1 risk pts, while it failed to be significant in Int-2/High risk group. Conclusion In low and Int-1 IPSS pts the CI assessment based on the CIRS-G score can be a valid tool in clinical practice for a better prognostic evaluation, as already suggested by other recently published works based on Sorror, Charlston or MDS-CI comorbidity scales.

C118

MDS-SPECIFIC COMORBIDITY INDEX (MDS-CI) IS ABLE TO IDENTIFY OVERALL SURVIVAL DIFFERENCES IN MYELODYSPLASTIC SYNDROME PATIENTS

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MDS-specific comorbidity index (MDS-CI) is a specific score reported by the Pavia group for myelodysplastic syndromes (MDS) patients. This score is a time-dependent index developed for predicting the effect of comorbidities on outcome of this category of patients. We applied this score on a total of 450 MDS patients with comorbidities recorded at the time of diagnosis and extracted from medical staff for the analysis. All patients were consecutively diagnosed and followed at our institute in a period between January 1992 and December 2006. Statistical analysis was carried out using SPSS software; survival was defined as survival from the time of diagnosis to last contact or death for any cause. Median age of the whole population was 69 years (range 21-88), with a prevalence of male sex (ratio m/f 1.6). Overall, we found the presence of one or more comorbidities in 94% of the examined patients. The most common comorbidities were cardiac disorders observed in 40% of patients, followed by diabetes with organ damage recorded in 22 cases. Application of MDS-CI score identified 300 patients with score 0, 55 patients with score 1, 80 patients with score 2 and 15 patients with score >2. We found significant differences in OS according to MDS-CI stratification: from 38 months for low risk patients (score 0) to 22 months for high-risk patients (score >2, $p=0.02$). We then evaluated the prognostic effect of MDS-CI on patients stratified according to WPSS prognostic index. WPSS application was possible in 330 of 450 patients who entered the analysis (73%), due to cytogenetic availability of data: we identified 112 patients with very low/low risk (34%), 137 patients with intermediate risk (41.5%) and 81 patients with high/very high risk (24.5%).

As reported also in the article by Della Porta and colleagues1 we assessed prognostic relevance of comorbidities in very low/low risk WPSS patients, intermediate and high/very high-risk patients. We found in the first category a significant difference in OS stratification: from 48.5 months for patients with score 0 to 20.4 months for patients with score >2 ($p=0.002$). In WPSS intermediate risk we found similar significant OS differences: from 32.3 months for patients with score 0 to 18.3 months for patients with score 2 ($p=0.001$). Conversely, we did not find significant differences in WPSS high/very high-risk patients (table 1). We also found significant correlations between presence of comorbidities at baseline, as stratified with MDS-CI, and risk of non-leukemic death: from 22% in patients without comorbidities to 75% in patients with score >2 according to MDS-CI ($p=0.002$). Our analysis showed the efficacy of MDS-CI on a large series of MDS consecutively seen, diagnosed and followed in a single center.

Our analysis also strengthens the results reported by Della Porta and colleagues1, because the value of comorbidities was confirmed in a cohort of patients similar to that observed in their original paper1, in terms of median age (69 years in our series vs 66 years in the Pavia series), but with higher percentage of patients with intermediate WPSS risk (41.5% in our series vs 18% in the Pavia population).

MDS-CI is a very valid tool capable to differentiate MDS patients with very low/low and intermediate WPSS risk in terms of OS and non-leukemic death risk.

C119

GLOBAL DNA METHYLATION IN MDS BONE MARROW MPO-POSITIVE AND CD34-POSITIVE CELLS CORRELATES WITH IPSS

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Epigenetic aberrations are now well recognized as very frequent and also as early events in the process of malignant transformation. The clinical responses of MDS to drugs that reverse aberrant hypermethylation, such as 5-aza-2-deoxycytidine and 5-azacytidine, suggest that aberrant hypermethylation plays a causative role. We investigated global DNA methylation by immunohistochemistry in bone marrow trephine biopsy specimens in a cohort of 132 MDS patients comprising all subgroups. Results were compared to an age-matched control group of 47 healthy subjects and to a group of de novo (36) and secondary (20) AML patients. We applied a double staining procedure to discriminate the cell lineage of positive cells. Immunohistochemistry was performed on paraffin-embedded sections using anti-5-methylcytosine/5mc antibody. Scoring of immunohistochemistry was evaluated with a four-points scale for both the number of positive tumor cells and their intensity of immunoreactivity. Double immunostainings were performed on histological section for nuclear 5-methylcytosine/5mc and one of four cytoplasmic/cell membrane markers (CD34 for precursors, MPO for myeloid cells, Glycophorin-C for erythroid cells, Factor VIII for megakaryocytes) by using EnVision™ G|2 Doublestain System, Rabbit/Mouse (Dakocytomation): cells showing double stainings showed brown nuclei and red cytoplasms or cell membranes. Normal bone marrows showed a low number of cells reactive for 5mc: with double stainings they were recognised as intermediate myeloid MPO-reactive and early erythroid glycophorin-C-reactive precursors accounting for less than 10% of the entire series. Compared to normal bone marrows, MDS and AML cases showed respectively a moderate and a marked increase of positivity for 5mc. Primary AML were characterized by the highest percentage of 5mc+/CD34+ and 5mc+/MPO+ cells; 5mc+/glycophorin-C+ cells were few in this group of cases. Unilineage and multilineage MDS without excess of blasts showed a mild increase of 5mc+/MPO+ and a significant increase of 5mc+/glycophorin-C+ precursors compared to normal bone marrows. MDS with excess of blasts exhibited a slight increase of 5mc+/CD34+ precursors compared to MDS without blasts and normal bone marrows. Differences were statistically significant between AML and MDS cases and between AML and normal marrows. In MDS the 5mc+/CD34+ and 5mc+/MPO+ cell percentage correlated significantly with the risk score according to the International Prognostic Scoring System. Future studies have to analyse whether the determination of global methylation levels may serve as a new predictive marker for therapy response.

C120

SELECTION BY SEVERE HYPOXIA OF REPOPULATING PROGENITOR CELLS IN PRIMARY MDS BONE MARROW CELL CULTURE

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Myelodysplastic Syndromes (MDS) are clonal disorders.However, whether the transforming event occurs in a myeloid committed cell or in earlier progenitor (stem cell?) it is still not ascertained. Evidence have been accumulated in both senses,but certainly,MDS initiating cells must be capable of sufficient repopulating capacity to perpetuate the disease. Objectives: To evaluate the repopulating ability of hypoxia selected cells in primary MDS bone marrow cultures and characterize the "stemness" of MDS maintaining cells. We evaluated 13 patients with different WHO subtypes of MDS (3 RCMD,4 RAEB-I,2 AL/post MDS,3 RA,1

CMML).Mononuclear bone marrow cells were isolated after gradient centrifugation and grown in RPMI 1640 medium supplemented with 20% FBS and a cocktail of cytokines (TPO, FLT3-L,SCF,IL-3).Cells were incubated and selected in Ruskin Concept 400 anaerobic incubator, in severe hypoxia conditions by flushing with a performed gas mixture (0,3 % O2,5%CO2,95% N2). Cells were cultured under hypoxic conditions for 10-13 days (LC1) and daily counted (Trypan blue). The stem and progenitor cell potential of these cultures at different times of incubation was explored by transferring cells to growth-permissive secondary cultures in normoxia (LC2),with SCF,G-CSF,IL-6,IL-3,according to the Culture-Repopulating Ability assay methodology (Leukemia, 14:735-9, 2000).The phenotype of hypoxia selected cells was evaluated by determining the expression of CD34,CD38,CD117,CD133 and the frequency of early progenitor cells CD34pos CD38neg CD133pos was compared to that present before hypoxic culture and to the Culture–Repopulating ability.The hypoxic culture system allowed selection of a minute cell population: in 13/13 cases viable cell number was decreased of one log after 10-13 days.In five cases we observed,after hypoxia selection a reduction of CD34pos cells (decrease of one log in all cases).This population was enriched with CD133pos, CD117neg and CD38pos cells in 4/5 cases and decreased in 1/5 cases.Only 2/13 cases showed a significant repopulating ability at day 17 of LC2. In the other 11/11 cases, repopulating ability was apparently absent.Surprisingly, MDS cases presenting blasts in the bone marrow did not show more repopulating cells after selective hypoxic conditions.Although our results are preliminary, we demonstrate that it is possible to select by severe hypoxic conditions primary MDS progenitor cells endowed with repopulating ability.

POSTERS

LYMPHOMAS I

P001

PATIENTS WITH B-CELL NON HODGKIN LYMPHOMA SHOW INCREASED FREQUENCIES OF REGULATORY T CELLS AND CD8+ T-CELL EXPANSIONS

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Although most non Hodgkin lymphomas (NHL) take origin from the B-cell lineage, several studies suggest that any impairment involving the different branches of the immune system may play a role in their pathogenesis. In order to explore the possible impact that the degree of activation of the T-cell immune system and the balance among different T-cell populations may have on the NHL pathogenesis, we analysed the TCR repertoire and the distribution of different T-cell subsets -including regulatory T-cells (Treg)- in patients with NHL. Our study was based on a flow cytometric analysis performed on the peripheral blood of 15 patients (6 with indolent NHL and 9 with DLBCL) and 15 age-matched controls. We first determined the frequency of CD3+, CD4+, CD8+ and CD16-56+ T-cells. Treg were then identified by considering the CD4+ cell fraction with a very high (>2 log) expression of CD25 and by a very low (<2 log) expression of CD127, as well as expressing FoxP3 and CD152. TCR repertoire analysis was based on a panel of 24 beta variable (BV) family-specific antibodies. We first showed that patients had reduced frequencies of CD16+ CD56+ natural killer cells (14% vs 21%) when compared with normal controls, while CD3+, CD4+ and CD8+ frequencies were similar. Patients also showed a higher frequency of Treg than controls (mean 2.35% vs 1.14%), although this increase was mainly confined to patients with DLBCL (mean 2.79%) rather than in patients with indolent NHL (mean 1.70%). Finally we determined the frequency of expanded T-cell subpopulations expressing the same TCR BV subfamilies, showing in patients and controls a similar frequency of expansions in CD4+ cells (1,3% vs 1,5%), besides an increased frequency of CD8+ expansions in patients (5,7% vs 3,2%). When we looked at the possible influence of several disease-related factors, such as WHO lymphoma subtype, IPI score, presence of constitutional symptoms, bone marrow involvement and stage, only a diagnosis of DLBCL rather than indolent NHL was associated with a trend towards an increased frequency of CD8+ lymphocyte expansions (6,2% vs 4,8%). Our preliminary data suggest that the T-cell branch of the immune system in patients with NHL show features which can be distinguished from those observed in normal controls. In particular, NHL patients seem to show an increased degree of activation of the TCR repertoire along with a higher frequency of Treg, which are both even more pronounced in patients with aggressive NHL.

P002

ONLY RITUXIMAB IN THE EARLY TREATMENT OF INDOLENT LYMPHOMAS RELAPSED AFTER AUTOTRANSPLANTATION

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In the last years the indolent 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, 23 indolent lymphoma; 15 follicular; 7 mantle cells and 1 marginal lymphoma. 13/23 (56%) patients have relapsed by a median PFS of 12 months (range 3-87).All patients received strict follow-up with CT and PET and were treated early. 9/13 (70%) patients relapsed (8 follicular and 1 mantle cell lymphoma) were treated with 4 weekly doses of rituximab 375 mg/m for 1 month and then re-evaluated, if CR have started maintenance with rituximab 375 mg/m every 2 months for 2 years. 7/9 patients (80%) revaluated after 4 weekly doses have documented the CR and began rituximab maintenance. All patients who responded had a follicular lymphoma. With a median follow-up of 36 months 6/7 (85%) patients are in CR and in 4/9 (44%) we have documented grade IV hematologic toxicity (neutrophils<500 mm3) quickly resolved with G-CSF treatment. In conclusion, for patients with follicular lymphoma,a strict follow-up may to consent a rapid treatment in relapsed patients after the autologous transplantation and 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.

P003

R-COMP-14 IN ELDERLY CARDIOPATHIC PATIENTS WITH POOR-RISK DIFFUSE LARGE B-CELL LYMPHOMA

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Elderly patients with diffuse large B-cell lymphoma (DLBCL) and a poor prognostic profile according to the International Prognostic Index (IPI), were suggested to benefit from time-intensification of the standard three-weekly CHOP-21, with or without rituximab (R). While waiting for conclusive results of ongoing randomized trials, application of the biweekly R-CHOP (R-CHOP-14) program to high-risk elderly patients with DLBCL represents a therapeutic standard for some cooperative groups and a possible option in the clinical practice. Delivery of densified R-CHOP in older patients with heart-related morbidities, may result however challenging because of the acute, early and late cardiotoxicities of doxorubicin. We investigated the use of liposome-encapsulated formulations of doxorubicin in elderly cadiopathic patients as a strategy to minimize cardiac side effects and favor a more selective drug uptake by lymphoma cells (R-COMP-14). A total of 208 courses were delivered, with close cardiac monitoring, to 41 patients (median age:73 years r,62-82; 37% >75 years) at a median interval of 15.6 days (r,13-29); 67% completed all six scheduled courses. Response rate was 73%, with 68% complete responses (CR); 4-year disease-free survival (DFS) and time to treatment failure (TTF) were 72% and 49%, respectively. Failures were due to early death (n=3), therapy discontinuations (no-response n=2; toxicity n=6), relapse (n=6) and death in CR (n=3). Incidence of cardiac grade 3-5 adverse events was 7/41 (17%; 95% CI:8-31%). Time to progression and overall survival at 4-years were 77% and 67%, respectively. R-COMP-14 is feasible and ensures a substantial DFS to poor-risk DLBCL patients who would have been denied anthracycline-based treatment due to cardiac morbidity. Further studies are warranted to confirm the safety and effectiveness of polichemotherapy with liposomal anthracycline in NHL frail patients.

P004

PREDICTIVITY OF CARDIOLOGY BIOCHEMICAL MARKERS AND LIPOSOMAL ANTHRACYCLINE IN UNTREATED PATIENTS AFFECTED BY NON-HODGKIN'S LYMPHOMA

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Background The toxicity of anthracyclines is a well know limiting factor in the treatment of patients with lymphoma. The pre-existing heart disease limits its use, while in patients with known risk factors and in patients with unknown risks could induce the onset. Patients and Methods In the period 2008-2010 were analyzed prospectively 40 patients with newly diagnosed non-Hodgkin lymphoma by a serial samples of pro-BNP and Troponin I, before each cycle of chemotherapy inclusive anthracycline, at day +1 by the execution, at the end of therapy and during the follow up at 3 and 12 months. Before therapy and at the end, was assessed left ventricular ejection fraction by echocardiography. On the basis of risk factors have been identified two groups (Table I). Patient's age or history of heart disease was classified as high risk and received the CHOP scheme with the replacement of doxorubicin with MYOCET (non-pegylated liposomal doxorubicin). While the second group performed the standard therapy with CHOP scheme. Both groups received a total of 6 cycles every 3 weeks. Results 32 out of 40 patients

achieved a complete remission, 7 patients a partial remission and only one has progressed. At 1 year, 31 patients are alive and well. The PRO-BNP increased during the cycles of therapy in 35 to 40 (Table II) and troponin I is increased in 3 patients (all in the group treated with conventional CHOP). At 3 months and 1 year 2 out of 40 patients have pro-BNP increased while no one with increased troponin I. At 1 year after the treatment in the group treated with CHOP-MYOCET 7 patients showed an impairment (not life treathing) of cardiac disease, while in the group treated with conventional anthracyclines three patients developed a new severe heart disease. Conclusions The PRO-BNP in our study did not show a prediction as it increases in almost all patients. Appeared the most promising Troponin I, although given the small sample size will require more study. The use of the anthracycline liposomal MYOCET confirmed its efficacy and tolerance without inducing new heart disease in a group of patients at high risk for heart disease and ancient age.

Table 1.

CHOP-MYOCET vs CHOP N° 25/40 vs 15/40;
M/F 8/17 vs 9/6;
MEDIAN AGE 74 (36-84)vs 62 (33-76);
HEART DISEASE OF ALL GRADES 11/25 vs 4/15;
MEDIAN TOTAL DOSE OF ANTHRACYCLINE: 480 mg (320-640)vs 540 mg (320-600)

Table 2.

CHOP-MYOCET vs CHOP CR 19/25 vs 13/15; PR 5/25 vs 2/15; PD 0/25 vs 1/15;
INCREASE OF PRO-BNP DURING CYCLES 23/25 vs 12/15;
INCREASE OF TROPONINA I IN CYCLES 0/25 vs 3/15;
INCREASE PRO-BNP TO 3 MONTH 1/25 vs 1/15;
GAP E.F ≥ 10% AT THE END OF THERAPIES 0/25 vs 0/15;
LIVE AT 1 YEAR 20/25 vs 11/15;
DEVELOPMENT OF HEART AT 1 YEAR IN ALL GRADES 0/25 vs 3/15;
INCOMING OR SEVERE HEART DISEASE 7/25 vs 0/15

P005

ROLE OF CONSOLIDATIVE AUTOLOGOUS STEM CELL TRANSPLANTATION IN T-CELL LYMPHOMA PATIENTS: A SINGLE INSTITUTION RETROSPECTIVE ANALYSIS

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T-cell lymphomas represent a rare and heterogeneous group of malignancies, exhibiting a poor outcome after conventional chemotherapy, so that a T-phenotype is regarded as a negative prognostic factor. Previously reported retrospective studies have shown that affected patients may improve treatment outcomes if consolidated with autologous stem cell transplantation (ASCT). Our clinical database was retrospectively reviewed; patients with T-cell lymphoma who underwent ASCT, either as a front-line or at disease relapse/progression were considered eligible for evaluation. 34 patients (22 males, 12 females; median age 40.74 years) received a consolidative ASCT after a median number of 2 (range: 1-11) previous lines of therapy. 14 patients had a peripheral T-cell lymphoma, not otherwise specified, 6 an anaplastic large cell lymphoma (ALK-negative in 2 cases), 6 a lymphoblastic lymphoma, 3 an angioimmunoblastic lymphoma and 2 a cutaneous anaplastic CD30-positive large cell lymphoma. The remaining three cases were a mycosis fungoides, a T/NK nasal type lymphoma and a pleomorphic lymphoma (according to Kiel's classification), respectively. 28 patients presented with stage IV disease, with bone marrow involvement in 8 cases and B symptoms in 17 cases. 14 patients were transplanted in complete response (CR), 3 in partial response (PR) and 16 with progressive disease (PD). Disease status was unavailable for 1 patient. After transplantation, 24 patients achieved a CR, 4 were in PR, 1 had stable disease and 5 progressed. Overall response rate was 82.36%, with a complete response rate of 70.59%. 13 patients (38.24%) could improve their best response after transplantation; a CR was obtained in 10 cases (29.41%). 13 patients, all in response after ASCT, experienced disease relapse: 2 of them received an allogeneic bone marrow transplantation, and 1 underwent a tandem ASCT-allogeneic transplant. At a median follow-up of 4.13 (range 0.92-15.03) years, 16 patients are still alive, 14 in CR and 2 in PR. 17 patients died, mainly for disease progression (14 cases). 10-year overall survival from ASCT is 31.90%, with a median of 4.92 years. ASCT appears effective as consolidation treatment for responding patients, as well as a feasible salvage therapy for those who relapse or progress. A high improvement of response rate is documented, along with a consistent amount of continuous CR and long-term survival rates.

P006

SHORT-COURSE OF CHEMO-IMMUNOTHERAPY FOLLOWED BY RADIOIMMUNOTHERAPY IN UNTREATED LYMPHOMA PATIENTS: EXPERIENCE OF TWO TRIALS' LONG TERM FOLLOW UP

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Two prospective, single-arm, multicenter phase II trials were conducted to evaluate the efficacy and safety of treatment with 90Y-ibritumomab tiuxetan following a short-course of rituximab-containing regimen in untreated aggressive and indolent non Hodgkin's lymphoma (NHL) patients. Fifty-five elderly (≥60 years) patients with diffuse large B-cell lymphoma (DLBCL) were treated with four cycles of R-CHOP (rituximab, cyclophosphamide, adriamycin, vincristine and prednisone). The patients achieving at least a partial remission (PR) received a single administration of 90Y-ibritumumab tiuxetan (90Y-IT) 6 to 10 weeks later. We also treated fifty-five patients with intermediate/high-risk follicular NHL with four cycles of RFN (rituximab, fludarabine and mitoxantrone) followed by a single administration of 90Y-IT as consolidation 8 to 14 weeks after chemioimmunotherapy. Forty-eight DLBCL patients completed the entire treatment with an overall response rate (ORR) of 80%, including 73% complete remissions (CR) and 7% PR. In particular, after radioimmunotherapy, 16 patients improved their remission status achieving CR. At last follow up forty-one patients are alive: 20 of them are in CR, 4 in PR and 10 in progressive disease (PD). Seven patients cannot be evaluated (lost). The 4-years overall survival is 83.1% and the 4-years progression-free survival is 47.6%. Fifty-one patients with follicular lymphoma received 90Y-IT achieving an ORR of 96% (89% CRs). At the last follow up 45 patients are alive with 26 CR, 4 PR, 11 PD. Four patients cannot be evaluated. The 4-years overall survival is 95% and the 4-years progression-free survival is 50.7%. Regarding the safety profile of 90Y-IT in the two regimens, toxicity consisted of grade 3 to 4 hematologic toxicity, mainly neutropenia and thrombocytopenia. No extra-hematologic adverse events were reported. These studies confirmed that a short-course of rituximab-containing regimen followed by radioimmunotherapy can be considered an effective, feasible and tolerable treatment in both aggressive and indolent non Hodgkin's lymphoma untreated patients. Noteworthy, the results were maintained in the long term follow up.

P007

PRIMARY EXTRA-NODAL MARGINAL-ZONE B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) OF THE LUNG: A RETROSPECTIVE STUDY ON 32 PATIENTS.

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The clinical presentation of extra-nodal marginal-zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) varies according to the lymphoma location (stomach, intestine, lung, thyroid, salivary glands, skin, orbit or breast). MALT lymphomas generally show an indolent behavior and a favorable outcome. MALT lymphoma is a rare disease particularly when occurring in the lungs, few data are present in literature. We report the experience of our institute: in the period between 1992 and 2009, 32 (13 females and 19 males) patients had diagnosis of

MALT lymphoma of the lung. With a median age of 65.8 years, 30 patients were stage IE and only 2 patients had a stage IV; furthermore, 5 patients presented with extra-pulmonary involvement. Fifteen patients were treated with a fludarabine-containing regimen (with/without rituximab) therapy (FN, fludarabine and mitoxantrone), 9 with CHOP (cyclophosphamide, adriamycin, vincristine and prednisone) or CHOP-like regimen, 2 with rituximab as single agent, 4 patients were treated by surgery only and 2 patients were never treated. Seventeen (56.7%) patients achieved complete response (CR) after treatment, 7 (23.3%) showed a partial response. Among the 17 CR patients, 12 were treated with fludarabine containing regimen, 3 with CHOP-like chemotherapy and 2 with surgery. Only 2 (6.7%) patients relapsed after 15 and 30 months, respectively. Particularly, among fludarabine-containing regimen patients, the CR rate was 80% and only 1 patient relapsed. Our data confirm a favorable outcome for patients with MALT lymphoma of the lung presenting a 84.4% 18 year-progression-free survival. Heterogeneity of therapies is still present, but fludarabine-containing regimen potentially represents the best front-line treatment for this specific lymphoma patients.

P008

PET-DRIVEN RADIOTHERAPY IN EARLY STAGE, LOW RISK DIFFUSE LARGE B CELL LYMPHOMAS (DLBCL)

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Background: Radiotherapy (RT) in limited stage DLBCL is historically a matter of debate. Rituximab (R) and dose-dense/dose-intense chemotherapy have improved the prognosis of these patients (pts). FDG PET is an essential tool to define complete remission(CR). Thus, in 2002 we hypothesized that RT could be spared in pts with non bulky stage I-II DLBCL and negative PET after abbreviated intensified R-CHOP treatment (RICHOP). Materials and Methods: Since 2002, we treated early stage DLBCL with RICHOP regimen: R 375 mg/mq, cyclophosphamide 1750 mg/mq, doxorubicin 75 mg/mq, vincristine 1.4 mg/mq, and prednisone 100 mg d 1-5 of each 14-day courses, GCSF day 7 -day 12. Pts were re-staged after 3 courses with PET: 1) pts with non bulky (< 10 cm) mass and negative PET concluded the treatment program, 2) pts with positive PET received radiotherapy (36Gy involved field); 3) pts with bulky disease received two further R-ICHOP cycles and RT(36 Gy on the bulk)regardless of PET result. Results: Twenty pts are evaluable. Main clinical characteristics: M/F 11/9, median age 46 years (range 19-61), Ann Arbor stage I/II 14/6, bulky mass 5, IPI 0/1 16/4 , LDH ratio </>1 16/4 , extranodal disease 11.Fourteen patients received three and six five RICHOP courses (five for bulky disease and one for treating physician's decision) . Post-chemotherapy PET was negative in all pts . With a median follow up of4 .6 years , one pt with previous stage II,IPI 0 non irradiated disease has relapsed after 19 months with extended stage IV disease including previous sites. The RICHOP regimen showed an acceptable toxicity with one hospital admission for management of grade III supraventricular tachycardia. Grade IV neutropenia complicated 100% of courses and in eight pts febrile neutropenia was registered. Among 69 evaluable courses, two required red blood cells transfusion. Thrombocytopenia never exceeded grade II. A total of 11 courses were delayed or given at reduced dose, and dose-intensity analysis will be performed. Conclusions: PET-oriented policy seems to correctly drive decision on whether or not to irradiated limited stage aggressive lymphomas. Negative PET after R-CT is now reported to overcome the prognostic significance of bulky masses . Our data suggest confirmation in larger series.

P009

RITUXIMAB COMBINED WITH MACOP-B REGIMEN AND MEDIASTINAL RADIOTHERAPY IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY.

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Third-generation MACOP-B (doxurubicine, cyclophosphamide, vincristine, bleomycin, methotrexate and prednisone) regimen in combination with involved field mediastinal radiotherapy seems to improve lymphoma-free survival of primary mediastinal large B-cell lymphoma (PMLBCL). Recently, the superiority of rituximab-CHOP (cyclophosphamide, adriamycin, vincristine and prednisone) over CHOP-like regimens has been demonstrated in elderly and younger low-risk diffuse large B-cell lymphoma patients. Between November 2000 and May 2010, 60 (33 females and 27 males) previously untreated PMLBCL patients were diagnosed and treated at our institute. Forthy-five patients were stage II, 5 patients were stage III and 10 stage IV. At diagnosis, 23 patients presented B symptoms and 10 had high LDH level. Fifty-nine (98.3%) patients had bulky disease presentation. The first line treatment included a combination of a third-generation chemotherapy regimen (MACOP-B), concurrent rituximab (4 global administration) and involved field mediastinal radiotherapy. All patients were evaluated by CT scan and PET total body. Thirthy-one (51.7%) patients achieved a complete response (CR) and 19 (31.7%) obtained a partial response after the MACOP-B plus rituximab. After radiotherapy, the final CR rate was 76.7% (46/60 patients). At a median follow-up of 3 years, among the 46 patients who obtained a CR, 3 patients relapsed after 2, 9 and 26 months, respectively. Projected overall survival was 83.2% at 10 years; the disease free survival (DFS) curve of the 46 patients who achieved CR was 89.1% at 9 years.In PMLBCL patients, combined modality treatment using MACOP-B regimen plus rituximab induces a high remission rate, with patients having a greater than 80% chance of surviving relapse-free at 10 years.

P010

RADIOTHERAPY (RT) DOES NOT PROLONG LYMPHOMA SPECIFIC SURVIVAL (LSS) IN EARLY STAGE DIFFUSE LARGE B-CELL LYMPHOMA OF THE WALDEYER'S RING (WR-DLBCL) IN COMPLETE REMISSION (CR) AFTER ANTHRACYCLIN CONTAINING CHEMOTHERAPY (CHT)

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WR-DLBCL is a rare entity of extranodal non-Hodgkin lymphoma (NHL). Due to its low incidence, only scarce data regarding the different treatment options is available. Especially in early stage disease (stage I/II) it is not clear, if the addition of radiotherapy (RT) does provide a survival advantage or if it leads only to excessive toxicity, compromising the quality of life. In order to investigate the impact of additional radiotherapy in early stage disease patients in CR after CHT, the International Extranodal Lymphoma Study Group (IELSG) performed this retrospective analysis. From 1985 to 2005, 14 international cancer centers of the IELSG included in the study 184 consecutive patients having the above mentioned characteristics. 62 (34%) underwent CHT alone, while 122 (66%) received additional RT (CHT+RT). Except for advanced age (>60 years), which prevailed in the CHT group, clinical characteristics were comparable between both treatment groups and overall with those reported in previous trials. Thirty-six patients (20%) experienced relapse: 11 (19%) in the CHT group and 25 (20%) in the CHT-RT group. After a median follow up of 4.5 years (range 0.16-18 yrs), forty-seven patients in the CHT group (76%) were alive (45 in CR) vs. 97 (80%; all in CR) in the other group. While the disease-related death was comparable between the two groups (7, 11% in CHT vs. 17, 14% in CHT-RT), the percentage of death due to unrelated causes was nearly twice among CHT patients (8, 13% in CHT vs. 7, 6% in CHT-RT). Overall, 5-year OS

and LSS were 80% and 86%. In the CHT group 5-year OS was 67% compared to 87% in the other group (p=0.061), while 5-year LSS was 81% and 88% (p=0.558), respectively. In order to verify whether the observed survival differences in univariate analysis were independent from other clinical parameters, a multivariate analysis for OS and LSS including age (≤60 vs. >60 years, ECOG-PS score ≤1 vs. >1, elevated vs. normal LDH serum level, bulky disease (≥10 cm), and consolidation with RT, was performed. In all three models, RT was not a significant prognostic factor, suggesting that the differences in univariate analysis were due to a higher prevalence of elderly patients in the CHT group. In conclusion, the addition of RT does not prolong survival in early stage WR-DLBCL in CR after anthracycline containing chemotherapy. These results need to be confirmed in prospective randomized trials.

P011

IN VITRO AND IN VIVO MODEL OF EBV-POSITIVE NON-HODGKIN PLASMABLASTIC LYMPHOMA WITH FOCAL PLASMACYTIC DIFFERENTIATION

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Introduction. Diffuse large B cell lymphoma(DLBCL)represents a group of aggressive lymphoid neoplasms with morphological variants.Plasmablastic lymphoma has been recently classified as a clinical-pathological variant with plasmablastic features and terminal B-cell differentiation. The lack of representative cell lines and animal models is a major impairment in understanding the biology of this disease. We established VR09 cell line, a new plasmablastic lymphoma cell line with plasmacytic differentiation and tested its tumorigenic capacity in vivo. Methods. Mononuclear cells from human DLBCL bone marrow, were seeded in RPMI 10% FBS medium. Cells showed spontaneous proliferative capacity and a cell line was established after months of continuous culture. Cells were evaluated by flow cytometry, immunohistochemistry, molecular biology and fluorescence in situ hybridization(FISH). Cells were inoculated subcutaneously into 6 immunodeficient Rag2-/- -chain-/- mice. Subcutaneously-growing tumors were evaluated by immunohistochemistry and FISH.Disaggregated cells from masses were cultured again to confirm their biological features and proliferative capacity. Results. Cells in suspension formed large clumps with round shape.They appeared morphologically as medium/large-size cells with plasmablastic/plasmacytic appareance. When subcutaneously injected in mice,VR09 cells grew as a spherical tumor and maintained their proliferative capacity once disaggregated from mass and cultured. Evaluated by flow cytometry and immunohistochemistry,cells displayed the phenotype of plasmablastic lymphoma with secretory differentiation (CD19+ CD3- CD5- CD20+ CD79a+ CD79b+/- CD138+ CD38+ cycline D1- Ki6780% IgM+ IgD+ MUM1+ MDNA+ CD10- CD22+ CD23+ CD43+ K+, - Bcl2+ Bcl6-).The same markers were detected on the tissue of growing tumors.FISH analysis revealed the trysomy of chromosome 12 and the negativity for the MYC rearrangement both in VR09 cell line and in tumors.EBER probe detected episomal EBV genome in both VR09 cell line and tumors.Genetic analysis in VR09 cell line showed the somatic hypermutation in the VH region,the expression of Card 11 and CD79 genes, and wild type p53. Conclusions. We have established and characterized a new plasmablastic EBV-positive cell line with focal plasmacytic differentiation and with tumorigenic potential in immunodeficient mice. VR09 cell line represents a model for in vitro and in vivo studies regarding pathogenesis and drug sensitivity.

P012

BORTEZOMIB FOR THE TREATMENT OF HEAVILY PRETREATED NON-HODGKIN'S LYMPHOMA PATIENTS. A MULTICENTER RETROSPECTIVE STUDY.

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To retrospectively assess the efficacy of Bortezomib, 53 (16 females and 37 males) relapsed or refractory lymphoma patients were enrolled in 9 Italian centres of Haematology. At diagnosis, the median age was 59.8 years (range: 25-76); 2 patients had disease's stage I, 9 stage II, 13 stage III and 27 presented with stage IV. Fifty percent of patients had abnormal LDH level and bone marrow involvement. The median number of treatments before bortezomib was 3 (range: 1-6). The diagnoses were: 31 mantle cell lymphomas, 12 follicular lymphomas, 5 diffuse large B-cell lymphomas, 5 indolent non-follicular lymphomas (small lymphocytic, marginal, lymphoplasmacytic), 1 Hodgkin's lymphoma. Bortezomib therapy was scheduled at a dosage of 1.3 mg/m2 (weekly or biweekly) for 4-6 cycles. Forty-four patients were evaluable for response: 10 (22.7%) had a complete remission (CR), 15 (34.1%) obtained a partial response (PR) with an overall response rate (ORR) of 56.8% and the remaining 19 patients were non-responder. According to histotype, we observed an ORR of 55.2% in mantle cell lymphoma subset (7 CRs and 9 PRs), an ORR of 60.0% (2 CRs and 4 PRs) among follicular lymphoma patients, 50.0% of ORR (1 CR and 1 PR) in the indolent non-follicular lymphomas and the Hodgkin's lymphoma patient had a PR. Projected overall survival was 36% at 4 years; the progression-free survival curve of the 25 patients who achieved at least a PR was 55.5% at 4 years. Extra-haematological toxicity was really mild and peripheral neuropathy occurred in only 5 (9.4%) patients; haematological toxicity was grade 3-4 thrombocytopenia in 11 patients (20.8%) and grade 3-4 neutropenia in only 2 patients (3.8%).

In summary, this retrospective study shows that treatment with bortezomib is a safe and effective regimen in a subset of heavily pretreated lymphoma patients.

P013

THERAPEUTIC AND MOBILIZING ACTIVITY OF A VINORELBINE,IFOSFAMIDE AND CYTARABINE REGIMEN (VIHA) AS SALVAGE REGIMEN IN RESISTANT/RELAPSE NON HODGKIN LYMPHOMA NHL)

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Background:The identification of active regimens sharing clinical and mobilizing activity are warranted NHL patients (pts)at high risk at onset or at relapse, as induction therapy before peripheral blood stem cell transplantation(PBSCT. Vinorelbine (VNR,ifosfamide(IFX), and cytarabine(ARA-C)are of proved efficacy in this setting. METHODS: From November 1999 to September 2008, 115 pts underwent the VIHA regimen: VNR 25 mg/mq day 3, IFX 2500 mg/mq days 1-3, and ARA-C 2 gm/mq bid days 2-3. Pts older than 60 years, were given the same regimen at 75% of doses. A total of 4 cycles was repeated every 21 days with G-CSF support from day 7 to day 12 or up to the apheresis. Mobilization was performed from the 3rd cycle, in patients withat least partial remission (PR), achieved after cycle 2. All cases with at least stable disease (SD) at the end of VIHA induction and with a CD34+ cell collection of >1.5 x 10<6/Kg were then candidated to PBSCT. Results: Main clinical characteristics: median age 47(with 36 pts older 60 years), aggressive histology 73, indolent histology 42, primary refractory disease 44, stage III or IV 42, bone marrow involvement 20. Seventy-seven pts had received at least two lines of chemotherapy before VIHA. Sixty-seven patients (59%) obtained an objective response (OR) with 48(42%) of these obtained complete remission (CR) according to CT-scan criteria. Seventy-four patients entered PBSCT and 41 did not for the following reasons: 15 due to unplanned PBSCT, 13 pts failed mobilization, one for toxic deaths, and the others for disease progression. With a median follow-up of 47 months, 4-year progression free survival (PFS) and overall survival (OS) are 30% and 39%, respectively. In univariate analysis, only the histologic category (aggressive vs indolent) was a predictive factor of response (p.001) and survival (p<.0001. In multivariate analysis, only

elevated IPI at relapsed (> 2) was a significant negative predictor. Interestingly, there was no difference in OR between patients treated with full dose or reduced dose VIHA. As concerns stem cell mobilization, in 80 mobilized pts, median number of CD34+ cells collected was 7.1 x 106/Kg (range 1.5-45) after a median of 2 (1-3) apheretic procedures. Among more than 240 VIHA cycles analyzed for haematological toxicity 86% of full dose VIHA required platelet transfusions and 50% RBC transfusions, as compared to 46% (p<.0001),and 34% (p0.03), of reduced dose VIHA, respectively. Febrile neutropenia complicated 27% of all cycles and there were 19 documented infections. Thirty-five per cent required hospitalization for documented infections or febrile neutropenia was necessary in 35% of full-dose VIHA cycles and in 16% of reduced VIHA cycles,(p.01.There was only one death-related therapy. Conclusions: VIHA shows clinical and mobilizing activity comparable to better known salvage chemotherapy regimens; otherwise our data suggest to use as well the reduced dose regimen, which shares clinical activity and lower toxicity profile. The high CD34 mobilizing activity is notheworthy.

P014

TUMOR-TARGETED IMMUNOLIPOSOMAL NANOSYSTEMS TO DELIVER EITHER CIDOFOVIR OR ANTINEOPLASTIC SIRNA AGAINST PRIMARY EFFUSION LYMPHOMA (PEL).

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Therapeutic applications of siRNA-mediated gene silencing appear to be highly dependent on the use of pharmaco-technologic carrier systems, able to protect siRNAs from rapid degradation upon administration, as well as to specifically deliver them to target cells. Actually, while siRNA-expressing viral vectors are burdened with safety concerns for their clinical use, the development of modified liposomal nanocarriers may represent a feasible option to harness the therapeutic potential of targeted antineoplastic siRNAs. Recently, we have successfully developed and characterized novel liposomal nanosystems to increase delivery and efficacy of Cidofovir (an anti-herpesviral nucleotide analogue, also showing antitumor activity) against PEL cell lines, demonstrating a significant improvement of the antineoplastic effect, especially at lower drug doses (less than 0.05 mM). Thus, we modified such liposomes in PEL-specific immunoliposomes (ILs) (PEGilated nanovescicles made of cationic/neutral lipids, engineered with anti-CD138 moAb on their surface), aiming to efficiently encapsulate siRNAs and deliver them into PEL cell lines (highly expressing CD138 membrane protein). Our preliminary data showed that single liposomal treatments with specific siRNAs against Blimp1 (Prdm1), which is a master transcription factor in PEL (a plasmablast/pre-plasmacell lymphoma, consistently Bcl-6 neg, Blimp1 pos), were able to induce a dose-dependent (50-200nM) inhibition of Blimp1 production (as assessed by Blimp1 mRNA and protein levels using RT-PCR and Western Blot, respectively), and this was strongly associated with enhanced cell death (more than 80%, using Annexin V/PI test). In particular, we observed a massive reduction of PEL viability (mean viable cells 8%, range 3-15%) as soon as 48-72 hours after treatment with 100nM anti-Blimp1 siRNAs. Interestingly, these data may resemble those described in multiple myeloma cell lines, after transduction with lentiviral vector constitutively expressing anti-Blimp1 shRNAs. Further studies on PEL murine models are now warranted to assess the efficacy and toxicity profile of in-vivo treatment with PEL-specific ILs, loaded with either Cidofovir or anti-Blimp1 siRNAs. The rational combined development of tumor-targeted ILs with siRNA-mediated inhibition of relevant genes for tumor proliferation and survival, as suggested here in PEL model, may provide novel promising approaches of antineoplastic target therapy.

P015

ENDOSCOPIC ULTRASONOGRAPHY IN GASTRIC LYMPHOMAS: APPRAISAL ON RELIABILITY IN LONG-TERM FOLLOW-UP

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Background: The importance of endoscopic ultrasonography (EUS) in defining the loco-regional staging at diagnosis and in assessing the response evaluation in gastric lymphomas is generally accepted but its reliability in follow-up management has not been clearly validated. Patients and methods: 23 patients with primary gastric lymphoma treated with a stomach-conservative approach were retrospectively analyzed. Twelve of them were affected by MALT lymphoma, eight by diffuse large-B-cell lymphoma (DLBCL) and three by high-grade lymphoma with low grade component. Treatment was given according to histotype and stage of disease. Evaluation during follow-up was performed by means of endoscopy with biopsy (E-Bx) and EUS, according to validated guidelines and clinical judgment. Results: A total of 120 matched evaluations with both EUS and E-Bx were evaluated at a median follow-up of 87 months ranged between 9.5 and 166 months. Survival analysis, expressed as Progression-Free Survival (PFS) and Disease-Free Survival (DFS), showed a strict relationship between persistence of EUS abnormalities and clinical outcome in patients with MALT lymphoma (p=0.0079; p=0.02), but not in patients with high-grade lymphoma. Conclusions: The dependability of EUS in follow-up management of gastric lymphomas is questionable. We have shown, albeit with the limits of a retrospective analysis, that it is not reliable in high-grade lymphomas, where most patients maintained a complete remission even with persistent EUS abnormalities. On the contrary, in MALT lymphoma, the role of EUS during the follow up period could be more important since we have noticed that, in a prolonged follow-up time, patients with persistence of EUS abnormality, have a high risk of relapse.

P016

RITUXIMAB PLUS HYPERCVAD ALTERNATING WITH HIGH DOSE METHOTREXATE AND CYTARABINE (R-HCVAD-AM) FOR PATIENTS WITH NEWLY DIAGNOSED MANTLE CELL LYMPHOMA (MCL). A MULTICENTER TRIAL FROM GISL.

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Background . R-HCVAD-AM has been tested in patients with newly diagnosed MCL with promising results (Romaguera et al. JCO 2005). In 2005 the Gruppo Italiano Studio Linfomi (GISL) started a phase II multicenter study investigating clinical activity and toxicity of R-HCVAD-AM in a similar group of patients. Patients and Methods. Histologically confirmed MCL, age ≤ 70 years and adequate organ function were main inclusion criteria. Chemotherapy consisted of 2 different alternating blocks (A and B), for a total of 4 cycles as originally reported by Romaguera et al. Only patients achieving partial response (PR) were to be addressed to HDC followed by ASCT. Results. Sixty-three patients were enrolled and 60 were eligible. Median age was 57 yrs (22 to 66), 75% were males and 93% were in stage III-IV. Sixty %, 33% and 7% were classified at low-, intermediate- or high-risk according to MIPI, respectively. Only 21 patients (35%) completed the 4 cycles and 3 patients died during therapy. Treatment was discontinued due to AEs (19), unsatisfactory response (4), physician's decision (16). Overall response rate (ORR) according to intention to treat (ITT) analisys was 83%, including 43 patients achieving a CR and 7 patients showing a PR. ORR rose to 98%, according to "per protocol analisys" (PPA) on 51 patients with assessable response. Grade 3-4 toxicity has been recorded: neutropenia occurred in 94% of patients, thrombocytopenia in 69%, anemia in 44%, infections in 50%, and other non-haematological toxicity in 21% of patients. After a median follow-up of 46 months (range 1–72), 28

patients had a failure, 17 had progressive disease, and 15 died. Considering ITT, the estimated 5-year OS, PFS and FFS rates were 73% (95% CI 59% to 83%), 61% (95%CI 45-73%) and 46% (95% CI 33% to 59%), respectively. MIPI score maintained the prognostic value when applied at the 60 patients enrolled in our trial; the estimated OS at 5-yr were 89%, 80% and 24% for low, intermediate and high risk score respectively (log-rank P<0.001). Conclusions. This multicentric trial confirms that R-HCVAD-AM is an active regimen for the initial treatment of patients with MCL, but is affected by significant toxicity that may limit its use outside well trained centres. This regimen should be further investigated to define the optimal strategy also considering the availability of new drugs and the role of maintenance therapies.

P017

OUTCOME OF ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PROSPECTIVELY IDENTIFIED BY COMPREHENSIVE GERIATRIC ASSESSMENT (CGA). RESULTS FROM A STUDY OF THE FONDAZIONE ITALIANA LINFOMI (FIL).

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Introduction. In 2003 the IIL started a clinical research program to prospectively identify frail versus fit elderly patients with DLBCL and to tailor treatment intensity according to patients' status. Patients and methods. Patients older than 65 years with newly diagnosed stage II-IV DLBCL were defined as "unfit" or "frail" in case of age > 80 years, impairment of Activity of Daily living (ADL) scale (score <6), three or more grade 3 or one grade 4 comorbidities, and the presence of geriatric syndrome. Fit patients were addressed to a randomized trial comparing R-CHOP vs R-miniCEOP while unfit patients were to be treated according to physician judgment. Results. From 2003 to 2006, 334 elderly patient with DLBCL were prospectively registered in the study and underwent CGA assessment; 235 were considered fit and were then registered in the randomized trial. According to CGA, the remaining 99 patients were classified as "frail". Comparing frail vs. fit patients the two groups only differed in terms of age. Fit patients were randomized to receive R-miniCEOP (114) or R-CHOP (110). Overall, the rate of complete remission was 70% (P=0.466). After a median follow-up of 42 months (5 to 81), 5-year EFS rates were 46% (95% CI, 36%-55%) and 48% (95% CI, 37%-58%) for R-miniCEOP and R-CHOP, respectively (P = 0.538). The 5-year Overall Survival (OS) rates were 63% (95% CI, 52-72%) and 62% (95% CI, 50-71%) for R-miniCEOP and R-CHOP (P = 0.702). Treatment of unfit patients consisted of several different regimens; interestingly 67% received doxorubicin-containing regimens, 19% received combination without doxorubicin, and the remaining 14% were treated with single agent chemotherapy, or radiotherapy alone. Combination chemotherapy was associated with rituximab in 39% of cases. Overall, 62 patients died resulting in a 5-year OS of 28%. The outcome of frail patients was poorer than that of "fit" patients, as demonstrated by an HR of 3.03 (IC95% 2.17- 4.23; P<0.001). Frail patients had a poorer outcome compared with the "fit" ones also if they were treated with rituximab containing regimen (HR 2.34 IC95% 1.43–3.83; P=0.001). Conclusions. Treatment of frail patients with DLBCL is largely unsatisfactory also if a treatment with curative intent is adopted. CGA is a valid tool to prospectively identify frail subjects among elderly patients with DLBCL. Regimens containing rituximab seem to improve the outcome but clinical trials specifically addressed to this population are warranted.

P018

BENDAMUSTINE AND CYTARABINE ARE STRONGLY SYNERGISTIC IN INDUCING APOPTOSIS OF SEVERAL B- AND T-CELL LEUKEMIA/LYMPHOMA CELLS AND CELL LINES

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Background Bendamustine is a bifunctional agent that acts as an alkylating agent as well as a purine analogue with relevant clinical activity in several non-Hodgkin's lymphomas. We have previously reported a striking additive cytotoxic effect of the combination of bendamustine and cytarabine compared with the two drugs alone in two mantle cell lymphoma cell lines. Here we tested this combination in different B- and T-cell leukemia/lymphoma cells and cell lines. Design and Methods Cell lines of follicular lymphoma (DOHH-2), chronic lymphocytic leukemia/lymphoma (EHEB), diffuse large B-cell lymphoma (SU-DHL-4) and T-cell lymphoma (JURKAT and KARPAS) were exposed to the two single drugs or combined with them simultaneously and consecutively. Additionally, we analyzed peripheral blood lymphocytes from 2 patients with chronic lymphocytic leukemia (CLL) with a disease prevalence of 80%. One of them had 17p deletion. Apoptosis was measured with the 7-AAD test, cell proliferation/metabolic activity was measured using the tetrazolium-based assays and mitochondrial damage was evaluated with the JC-1 dye. The combination index (CI) was used to assess the synergy between the drugs. Results were compared with the effects obtained with the two drugs on two MCL cell lines (JEKO-1 and GRANTA-519). Results Bendamustine as single drug exhibited a strong cytotoxic effect on all cell lines and all CLL lymphocytes that was dose- and time-dependent. Both T-cell lymphoma and SU-DHL-4 cells were instead resistant to the drug. The addition of cytarabine to bendamustine in a consecutive schedule significantly improved the cytotoxic effect compared with the single incubations of the two drugs, similarly to what observed on MCL cell lines (Figure 1). Of note, the apoptotic rate of CLL B-cells also with 17p deletion was relevant and significantly improved when bendamustine and cytarabine were administered consecutively (p<0.01, Figure 1). Similarly, the diffuse large B-cell lymphoma SU-DHL-4 cells, the lymphoblastic T-cell lymphoma JURKAT, and the CD30+ anaplastic large T-cell lymphoma KARPAS cells resulted extremely sensitive to the consecutive incubation of the two drugs. A significant improvement of cell mortality compared with the drugs alone was observed (Figure 1), that resulted in a highly synergistic CI (<0.01). Conclusion Bendamustine and cytarabine are highly synergistic on B- and T-cell lymphoma cells and cell lines, similarly to MCL. The drug combination overcomes the resistance to the single agents and looks very promising also in 17p deletion CLL B-cells.

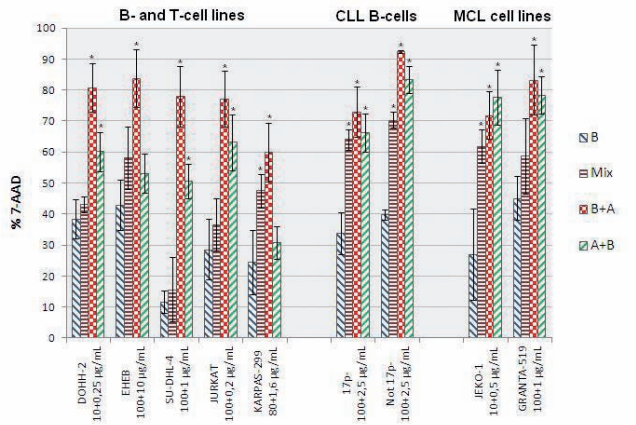

Figure 1. Apoptotic effect (% of 7-AAD+ cells) of bendamustine as single drug (B) and in combination with cytarabine (A). Mix: simultaneous incubation; B+A and A+B: consecutive incubations. Each experiment for cell lines was performed four times in duplicate. Experiments on CLL B-cells of patients were performed in duplicate. *: significant versus B. 17p- or Not 17p- histograms represent the percentage of cell death observed in the peripheral blood lymphocytes of one patient with this cytogenetic abnormality or without the deletion, respectively. JEKO-1 and GRANTA, the MCL cell lines, were used as comparison. Doses of B+A are listed under each cell type.

P019

FOLLICULAR LYMPHOMA STAGE I/II : ROLE OF MOLECULAR MONITORING IN PATIENTS TREATED WITH LOCAL RADIOTHERAPY +/- RITUXIMAB

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Background: Conventional treatment of stage I-II follicular lymphoma (FL) is local radiotherapy (RT), which allows eradication of the disease in about 50% of patients. Very few data are available on the role of anti-CD20 MoAb and of minimal residual disease (MRD) evaluation in this setting. Methods: 41 consecutive patients with a confirmed diagnosis of stage I/II FL were investigated by PCR in order to identify the presence of Bcl2 rearranged cells in the bone marrow (BM) and/or peripheral blood (PB). All patients were treated with involved field RT (36 Gy). Subsequently, MRD was evaluated every 6 months in patients positive at baseline. Results: PCR analysis revealed Bcl2 rearranged cells in 24/41 patients (58.5%) at presentation. After irradiation of the sole site of the disease, Bcl2 rearranged cells disappeared in 15 of the 24 (62.5%) patients positive at baseline; in 8 (19.5%) MRD was positive, while 1 patient refused the test. After a median follow up of 50 months, 5 patients (12.2%) had a clinical relapse. MRD evaluation demonstrated that: • 17/41 patients were Bcl2- at the basal evaluation; only 1 of these had a clinical relapse as mantle cell lymphoma. • Of the 15 patients positive at baseline and who became negative after RT, 3 have had a molecular relapse during the follow-up, leading in one case to an overt clinical relapse. • Of the 8 patients persistently Bcl2 positive after radiotherapy, 3 had a clinical relapse. Rituximab (375 mg/m2 x 4) was administered to 5 patients with a persistently positive Bcl2 after RT: 3 of them became Bcl2 negative. Discussion: Viable Bcl2+ cells can be demonstrated in the BM and/or PB of the majority of stage I-II FL patients (despite a negative BM biopsy). Irradiation of the sole nodal/extranodal disease sites allows disappearance of Bcl2+ cells in the majority of previously positive patients (62.5%). Pre-treatment Bcl2 BM and/or PB evaluation has a prognostic role: no clinical relapses were observed in Bcl2 negative cases at baseline except for one patient, relapsed as mantle cell lymphoma. MRD evaluation has a prognostic role: among 32 Bcl2 negative patients after treatment, 2 relapses (6.2%) were observed (1 as MCL), while among 8 Bcl2 positive patients after treatment 3 relapses (37.5%, P=0.046) were observed. Prognosis of early stage FL treated with local RT ± rituximab is excellent: only 5 patients have so far relapsed at a median follow up of 50 months.

P020

BENDAMUSTINE PLUS RITUXIMAB (BE-R) IN LYMPHOPROLIFERATIVE DISORDERS: HIGH APPLICABILTY PROFILE IN HEAVILY PRE-TREATED OLDER PATIENTS.

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Background: Bendamustine is a purine analogue chemically related to alkylating agents with a proved activity/safety and no cross resistance with respect to cyclophosphamide. In the aim to evaluate: 1) the efficacy, the rapidity of response and safety of Be-R we applied this schedule in heavily pre-treated NHL pts. Patients and methods:At our center from February 2008 to January 2011 27 pts-16 males-median age 75 (52-80) entered in this study. 9 were CLL, 3 FL, 4 LPL, 3 MZL, 6 DLBCL and 2 MCL. 9 pts had extra-nodal disease. In 7 pts HBV antibodies such as HbcAb (1) and HbcAb+ HbSAb(6) were detected. 2 pts were HCV positive. 17 pts had multiple comorbidities as cardiopathy (11), diabetes(4), polineuropathy (1), concomitant neoplasia (2), BPCO (3), renal insufficiency (2). 16 pts received Be-R as second line and in 11 pts as >= third line therapy. The treatment including Rituximab 375 mg/mq on day 0, Bendamustine 90 mg/mq day 1-2, Prednisone 40 mg/mq day 1-5, given every 21 days for 6 cycles. In pts with HBV antibodies prophylaxis with lamivudine 100 mg daily was employed. Treatment response evaluation by CT scan was done repeated early after third cycle and then after sixth. All pts received treatment on out-patient basis. Results: As of April 2011 22 pts completed 6 cycles, 1 pt is to date in therapy, 3 pts completed 3, 3 and 4 cycles, respectively for not-responsive disease (NR), 1 is lost to follow-up after second cycle. In 9 pt R was discontinued because of anaphylactic reaction. At 3th cycle 26 were evaluable, of these 23 (88%) achieved a response (2 CR, 6 VGPR and 15 PR >50%). At treatment completion 22/26 pts (84%) obtained a response: 7 CR, 9 VGPR and 6 PR. Median response duration was 9 mos (4-30). Median OS from diagnosis is 74 mos (16-156). The schedule was well tolerated: WHO grade >= 2 haematological toxicity has been recorded in 12 pts. 2 pts had perineal HSV which promptly resolves with acyclovir therapeutic dosages, 1 pt had pneumonia and 2 pts had re-exacerbation of pre-existing chronic bronchitis. None pt had Hepatitis virus re-activation. Conclusions: In our experience Be-R schedule proved to be good therapeutic approach in elderly heavily pre-treated pts. Overall response rate was 84%, but it is noteworthy that the majority of pts achieved an early response (ORR at 3rd cycle: 88%). Finally it is outline that none pt experienced severe non haematological toxicity with therapy discontinuation.

P021

COAGULATION DISORDERS IN ELDERLY PATIENTS WITH NON HODGKIN'S LYMPHOMA.

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Acquired hemophilia is a rare coagulation disorder characterized by autoantibodies against circulating coagulation factor, frequently against factor VIII. Patients often have not history of bleeding disorder and present spontaneous hemorrhage, an isolated prolonged aPTT and PT and antibodies against coagulation factors. It has an incidence of 0,2-1 cases/million/year. This condition may be associated in 50% of cases with autoimmune disease, solid tumor, lymphoproliferative disorders and pregnancy. We describe our experience with two patients with indolent non-Hodgkin's lymphoma who showed isolated prolonged aPTT and PT. Case one. A 72-year-old man referred to our Institution because of recurrent epistaxis and abnormalities of coagulation tests PT INR 2.5, a PTT ratio 2.73. No previous personal or family history of bleeding disorders, or recent surgery and new drug intake, were reported. Spleen enlargement, with a large focal lesion, and pancytopenia (Leukocytes 2800/µL; Haemoglobin 9 g/dL; Platelets 82,000/µL)were observed. Laboratory test showed a reduction of Factor VIII activity, Factor II activity, Factor V activity, Factor VII activity, Factor IX activity, Factor activity X and Factor activity XI (FVIII:C 16%; FII: 44%; FV:8%; FVII: 11%; FIX: 10%; FX:30%; FXI:27%) and appearance of antibodies against many coagulation factors. The bone marrow showed a lymphoid infiltrate. Fluorodeoxyglucose F-18 positron emission tomography whole-body scan revealed abnormally high uptake in the spleen and in a slightly enlargement supraclavear lymphonode. Fine needle aspiration biopsy at this site, covered by 60 µg/Kg recombinant Factor VIIa injections, enabled the diagnosis of non-Hodgkin's lymphoma (CD5-, CD22+, lamba+). The patient made six administration of chemotherapy with cyclophosfamide, vincristine, epirubicin and prednisolone (CEOP) and achieved complete remission. We observed that the end of treatment, coagulation parameters and factor activity returned normally. Case two: A 62 year-old female came to our observation for lymphoadenopathy, hepatoplenomegaly, anemia and lymphocitosis. The patient didn't present any personal or family bleeding disorders and didn't take drugs. Laboratory tests showed abnormalities of coagulation: PT INR 3.26, aPTT ratio 4.92. They also showed a reduction of factor VIII activity, Factor II activity, Factor VII activity, Factor IX activity, Factor activity X and Factor activity XI (FVIII:C: 2.3%; FII: 32%; FVII: 47%; FIX: 1%; FX:43%; FXI:1%) and appearance of antibodies against many coagulation factors. Fluorodeoxyglucose F-18 positron emission tomography whole-body scan revealed increased uptake at axillary and inguinal lymphonodes and at spleen. The bone marrow showed a lymphoid infiltrate and enabled the diagnosis of non-Hodgkin's lymphoma (CD5-, CD22+, lamba+). The patient made two administration of chemotherapy with Rituximab, fludarabine. After second administration of chemotherapy, was observed a slow normalization of PT and aPTT. Two patients underwent to maintenance therapy with Rituximab in order to prevent the emergence of lymphoproliferative clone. In both cases there was a correction of the PT and aPTT after the first cycle chemotherapy.In these patients the onset of an acquired coagulation disorder can be used as diagnostic and prognostic marker of derangement of the immunologic network due to an underlying, apparently indolent, lymphoprolipherative disease.

P022

HEPATITIS B REACTIVATION IN PATIENTS WITH NON HODGKIN LYMPHOMA CD 20 + IN MAINTENANCE THERAPY WITH RITUXIMAB

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Background Anti CD20 antibody (Rituximab) based chemotherapy regimens increase the HBV reactivation risk although sporadic HBV reactivation cases are reported in patients on maintenance with Rituximab single therapy too. We evaluated how many HBV reactivation occurred among patients Hepatitis B core antigen positive (HBcAB +) and Hepatitis B surface antigen negative (HBsAg-) who received Rituximab single therapy during maintenance. Aims The aim of this study is to assess the prevalence of HBV reactivation among patients HBcAb +/ HBsAg – during the maintenance therapy with Rituximab. METHODS In our Unit, 56 patients with non Hodgkin Lymphoma CD20+ received maintenance therapy with Rituximab (schedule: 375 mg/mq every 3 months for 2 years) from January 2007 to March 2011. 42% (25/56) of patients were treated with R-CHOP regimen; 57% of patients were treated with R-FN regimen. None of these patients received prophylactic therapy with lamivudine during induction or maintenance. All the patients were given blood tests for HBV (HBsAg; HBsAb; HBeAg; HBeAb; HBcAb) before starting maintenance therapy and liver function tests before each administration of Rituximab. Results 28% of the patients (15/56) were HBcAb positive. 42% of the patients (25/56) completed the maintenance treatment and 28% of them are HBcAb positive (7/25): in one of these patients occurred the HBV reactivation (median follow up: 15 months). 55% of the patients (31/56) are still in therapy with Rituximab and 25% of them are HBcAb positive (8/31): even in these patients has occurred to date the HBV reactivation. Conclusions In patients HBcAb +/ HBsAg – treated with Rituximab in single therapy is indicated the prophylaxis with lamivudine. In our observational study the HBcAb +/ HBsAg patients didn't receive prophylactic therapy with lamivudine during the maintenance therapy with Rituximab and the HBV reacitivation occurred in one patient HBcAb+/HBsAg- three months after the end of the maintenance therapy (1/15). More ambitious prospective studies are required to establish the clinical utility of prophylactic therapy with lamivudine during the maintenance therapy with Rituximab.

P023

MACOP-B WITH OR WITHOUT RITUXIMAB PLUS INVOLVED MEDIASTINAL RADIATION THERAPY (IFRT) FOR PRIMARY MEDIASTINAL B CELL LYMPHOMA (PMBCL): LONG TERM RESULTS AND LATE TOXICITY AT SINGLE INSTITUTION.

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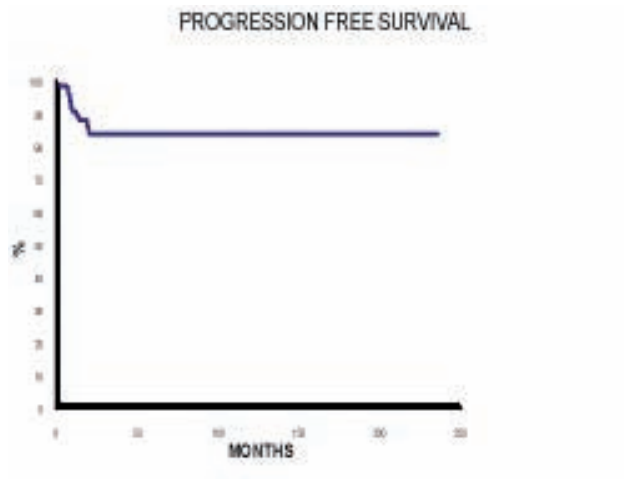
Background: The combination of chemotherapy (CHT) together with mediastinal IFRT is considered the standard treatment in PMBCL patients (pts). The more intensive third generation regimens, such as MACOP-B, have improved survival over standard CHOP but the introduction of Rituximab has removed this difference. The need of consolidation mediastinal IFRT is still debated in view of the risk of secondary cancers and cardiac complications. We report the long-term results on a large series of PMBCL pts treated at our institution. Methods: 107 pts with PMBCL were treated between June 1991 and September 2006. The median age was 34yrs (15-61) and 71% were females; 80 pts had stage II and 27 stage IIE-IV; 75% had elevated LDH; bulky disease was present in 95 (88%) and 58 (55%) had clinical evidence of superior vena cava obstruction. The aaIPI score was 0-1 in 60 pts and 2-3 in 47. Ninety-two pts were treated with the standard MACOP-B regimen and 15 pts with Rituximab-MACOP-B regimen since March 2004. Overall, 101/107 pts (94%) received an IFRT at a dose of 30-36 Gy. The response was evaluated in all pts at the end of CHT and of IFRT. Results: At the end of CHT, a CR/CRu was obtained in 76 pts(71%), a PR in 22(21%), NR 3(4%), while 6 pts were not evaluable (5 received an early ASCT intensification and 1 died for toxicity). At the end of the IFRT: 14/22 PR became CR/CRu with an overall CR/CRu rate of 84%. Overall, 9 pts relapsed within 10 months and 4 of them died of progressive disease. After a median follow-up of 111 months (1-238) the 10-yrs OS, PFS and EFS were 88%, 85% and 83% respectively. No statistically significant difference in terms of PFS and OS was recorded for pts treated with or without Rituximab. Patients with an IPI 0-1 had a significantly better PFS (p=0.020) and OS (p= 0.015). In our cohort of pts, only 1/107 developed an acute myeloid leukemia (164 months after the end of therapy). No other secondary cancers, including breast cancer, occurred. Four of 107 pts presented late severe cardiac toxicity (3 congestive heart failures and 1 fatal arrhythmia). Conclusions: This is the largest reported series of pts with PMBCL treated with an uniform strategy at a single center. MACOP-B +/- Rituximab plus IFRT is highly effective and devoid of severe long-term toxicities. Future randomized trials should evaluate the real need of a consolidation IFRT in pts who obtain a CR after a Rituximab-CHT in attempt to reduce unexpected late toxicities.



P024

HEPATITIS B REACTIVATION IN PATIENTS WITH NON HODGKIN LYMPHOMA CD20+ UNDERGOING CHEMOTHERAPY WITH AND WITHOUT RITUXIMAB

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Background Reactivation of HBV infection is a well-recognized complication in infected patients who undergo cytotoxic chemotherapy for cancer. The highest incidence of reactivation was reported in patients with non-Hodgkin's lymphoma (NHL) and hematopoietic stem cell transplantation. Several case reports demonstrated that severe hepatitis due to HBV reactivation after rituximab administration occurred both in hepatitis B surface antigen(HBsAg)-positive and HBsAg-negative patients. However, systematic evaluation of the relationship between HBV reactivation and rituximab is still limited. Aims We conducted a study to investigate the relationship between rituximab-based therapy and HBV reactivation in 405 CD20-positive NHL patients at our institution. METHODS In our Unit, 405 CD20-positive NHL patients all newly diagnosed underwent measurement of HBsAg, anti-HBs,anti-HBc,HBeAg and anti-HBe. Patients were monitored by liver function tests during and after therapy as follows: on day 1and day 14 of each cycle, every month for a year. Results 154/405 (38%) patients were HBcAb-positive. 107 had an aggressive lymphoma, 42 had an indolent lymphoma. HBV reactivation was observed in 2 patient (1,2%) who had received chemotherapy including steroid and rituximab and in 3 patients(1,9%) who had received chemotherapy including regimen with only fludarabine without rituximab Immediate administration of lamivudine therapy after elevation of HBV DNA level was conducted, and this resulted in reduction of it and improvement of liver function test. Conclusions Rituximab plus steroid-containing regimens may increase the risk of HBV reactivation in HBsAg-negative and HBcAb-positive lymphoma patients but more attention should be paid on treatment with only fludarabine. More ambitious prospective studies are required to establish clinically useful or cost-effective follow-up methods for control of HBV reactivation in lymphoma patients with occult HBV infection.

P025

HIGH PREVALENCE OF HEPATITIS C (HCV) INFECTION AND FAVORABLE PROGNOSIS IN PRIMARY HEPATIC LYMPHOMA (PHL)

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Background. Primary Hepatic non-Hodgkin's lymphoma (PHL) is a rare disease, representing 0,4% of extranodal non-Hodgkin's lymphomas. To date, less than 150 cases have been published. We report 11 patients with PHL diagnosed in 1995 and 2009 at our center, with a study of the viral status and the result of cytotoxic treatment. Results. Eleven patients with PHL were identified. The disease occurred in middle-aged men (median age: 58 years). The main presenting complaint was right upper quadrant abdominal pain (4/11 patients). Tumour markers (-fetoprotein and CEA) were normal in 8 patients tested. Liver scans demonstrated either a solitary nodule or multiple lesions. Pathologic examination revealed diffuse large B cell lymphoma in six patients, one case of follicular lymphoma, one of small lymphocytic lymphoma and one case of T cell lymphoma. Eigth patients (72%) were HCV-positive. Seven patients received chemotherapy (6CHOP,1 R-CHOP), two patients received chemotherapy (R-FN), while a patient with a single focal lesion received surgical treatment. The complete remission rate was 100% (11/11); one patient, who had HCV-related cirrhosis, died of hepato-renal syndrome in complete remission from lymphoma, and another patient died of acute myeloid leukemia. Conclusions. The outcome of patients with PHL who are treated with combination chemotherapy seems excellent. The frequent association of PHL with HCV infection suggests a possible role of this virus in lymphomagenesis. HCV- infection does not appear to influence the outcome of therapy.

P026

SALVAGE TREATMENT WITH RITUXIMAB, IFOSFAMIDE AND ETOPOSIDE (R-IE REGIMEN) IN PATIENTS WITH PRIMARY CNS LYMPHOMA RELAPSED OR REFRACTORY TO HIGH-DOSE METHOTREXATE-BASED CHEMOTHERAPY (RRPCNSL)

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Background: High-dose methotrexate (HD-MTX)-based chemotherapy ± radiotherapy is associated with a high remission rate in patients (pts) with PCNSL; however, 35-60% of responsive pts experiences early relapse and an additional 10-15% is treatment refractory. A few studies focused on salvage treatment are available, often with disappointing results. We investigated feasibility and activity of a combination of rituximab, ifosfamide and etoposide (R-IE regimen) in pts with rrPCNSL. Herein, we report final results of this multicenter experience. METHODS: HIV-negative pts ≤75 years old, ECOG PS ≤3, with PCNSL relapsed or refractory to HD-MTX/HD-cytarabine-based chemotherapy ± radiotherapy treated with four courses of R-IE regimen (rituximab 375 mg/m2 day 0; ifosfamide 2 g/m2/d days 1-3; etoposide 250 mg/m2 day 1) were considered. Results: 22 pts (median age 60 ys, range 39-71; M/F ratio: 1.4) were analyzed. R-IE was the second-line treatment in 16 pts, the third-line in four and the fourth-line in two. Thirteen pts had refractory PCNSL (progressed during previous treatment) and nine had relapse. Twelve pts had been previously irradiated. Fifty-six (64%) of the 88 planned courses were actually delivered. Treatment is ongoing in one patient. R-IE was interrupted in 16 pts due to lymphoma progression (n=12), toxicity (n=3) and patient's refusal (n=1). G4 hematologic toxicity (neutropenia 50%; thrombocytopenia 25%; anemia 15%) was manageable; a single case of G4 non-hematologic toxicity (hepatic) was recorded. Response after R-IE was complete in 7 pts and partial in two (ORR=41%; 95%CI: 21-61%). Eight pts were successfully referred to autologous stem cell collection. Consolidation for responders was BCNU-thiotepa conditioning and ASCT in three pts and WBRT in one. At a median follow-up of 15 months, no responder experienced relapse, while 13 pts experienced lymphoma progression, with a 2-yr PFS of 31±11%. Eight pts are alive; eleven died of lymphoma, one of pulmonary aspergillosis and two of neurological impairment while in remission, with a 2-yr SAR of 23±10%. Type of failure was significantly associated with ORR, with 70% and 23% (p=0.04) respectively for relapsing and refractory pts. Conclusions: R-IE is a feasible and active combination for patients with rrPCNSL. This regimen made possible stem cell collection and consolidation with high-dose chemotherapy and autologous transplant. The use of this regimen in routine practice is suggested.

P027

THE ROLE OF CD200 EXPRESSION IN DIFFERENTIAL DOAGNOSIS AMONG NON HODGKIN LYMPHOMAS

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CD200 is a membrane glycoprotein, belonging to the immunoglobulin super family, expressed on a subset of T and all B lymphocytes (not on NK cells), dendritic cells and highly on central and peripheral nerve tissue. CD200 interacts with CD200R, expressed primarily on myeloid/monocytes lineage cells, and has a suppressive effect on T cell-mediated immune response. CD200 has been reported to be expressed in multiple myeloma, in acute myeloid leukemias and other non-hematological malignancies. CD200 expression was found to be up-regulated in some Non Hodgkin Lymphomas (NHL) when compared with normal B cells and so useful in their diagnosis and therapy. In particular chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL) express CD200 highly, allowing differential diagnosis between CLL and mantle cell lymphoma (MCL), HCL and splenic marginal zone lymphoma (MZL). We investigated CD200 expression on blood specimens (bone marrow and peripheral blood) of 46 normal controls, 105 patients with CLL, 22 with MZL, 7 with MCL, 9 with follicular lymphoma (FL), 7 with HCL, utilizing a BD FACSCanto II. CD200 expression was measured as mean fluorescence intensity (MFI) on B cell population for each control and patient specimen. Mean and standard deviation were then calculated on normal control group and on each group of NHL (Table 1). When compared with normal B cells, CD200 expression resulted high in CLL and HCL, almost normal on MZL and FL, and low in MCL. According to literature, we confirmed the utility of CD200 in differential diagnosis between HCL and MZL, especially in atypical HCL, and also between CLL and MCL. Comparing CD200 expression between NHL and normal B cells, we found that among MZL, FL and MCL, the latest has the lower expression. This finding may further help in differential diagnosis between MZL CD5+ and MCL. We need to validate our results on a bigger number of NHL, also in view of the proposed anti-CD200 targeted therapy in CD200 expressing cancers, in particular for CLL.

Type of samples	number of samples	CD200 expression (MFI)	
		Mean	Standard Deviation
Normal controls	46	286,33	142,86
CLL	105	851,92	509,20
HCL	7	769,14	582,62
MCL	7	184,43	163,61
MZL	22	299,59	239,68
FL	9	238,89	175,47

Table 1

MULTIPLE MYELOMA I

P028

SUPPRESSED OSTEOBLAST DIFFERENTIATION AND ACTIVITY IN MULTIPLE MYELOMA BONE DISEASE: ROLE OF SCLEROSTIN.

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Wnt signalling through the secretion of Wnt inhibitors DKK1, sFRP-2 and -3 plays a key role in the decreased osteoblast activity associated with multiple myeloma (MM) bone disease. We provide evidence that another Wnt antagonist, sclerostin, an osteocyte-expressed negative regulator of bone formation, is expressed by myeloma cells, i.e. human myeloma cell lines (HMCLs) and plasma cells (CD138+ cells) obtained from the bone marrow of a large number of MM patients with bone disease. We demonstrated that bone marrow stromal cells (BMSCs), differentiated into osteoblasts and co-cultured with HMCLs showed, compared with BMSCs alone, reduced expression of major osteoblastic specific proteins, decreased mineralized nodule formation, and attenuated expression of members of the AP-1 transcription factor family (Fra-1, Fra-2 and Jun-D). Moreover, in the same co-culture system the addition of neutralizing anti-sclerostin antibodies restored osteoblast functions by inducing nuclear accumulation of beta-catenin. We further demonstrated that the up-regulation of RANKL and the down-regulation of OPG in osteoblasts were also sclerostin mediated. Our data indicated that sclerostin secretion by myeloma cells contribute to the suppression of bone formation in the osteolytic bone disease associated to MM.

P029

THE ROLE OF FDG-PET/CT AND MRI IN THERAPEUTIC EVALUATION OF PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA (MM) AND SOLITARY PLASMACYTOMA (SP): WHAT IS IMAGING PROCEDURE MORE SENSIBLE FOR MONITORING RESPONSE TO THERAPY ?

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Introduction MM is a malignancy characterized by osteolityc lesions, bone marrow involvement and additional extramedullary lesions. The imaging techniques play an essential role in diagnosing and therapeutic evaluation. Several studies report that MRI presents difficulties to distinguish between post-treatment active MM and fibrotic lesions, while FDG-PET offers great opportunities because of its ability to visualise highly energy-consuming cells such as tumour cells. Methods Ten patients (7 male, 3 female) with symptomatic MM and 4 patients (2 male, 2 female) whit SP were subject to FDG-PET/CT and MRI with contrast material respectively after autologous stem cell transplantation (ASCT) and radiotherapy to monitor the response to treatment. Results In symptomatic MM group after ASCT, 8 patients out of 10 (5 with a VGPR and 5 with a CR) presented negative PET/CT scans, but only 3 demonstrated normal MRI. In SP group after radiotherapy, all patients presented negative PET/CT scans, but only 2 demonstrated normal MRI. In 1 patient with symptomatic MM, biopsy did not confirm the areas of increased uptake of some vertebral bodies (particularly D11), detected by MRI but not by PET/CT, and interpreted as positive for myeloma. In 2 patients with post-treatment abnormal MRI (1 case with symptomatic MM and 1 case with SP), the MRI, repeated after six months, did not confirm the areas of increased uptake documented above. Conclusions MRI may fail to demonstrate evidence of regression of the preceding bone marrow abnormalities; some lesions may remain unchanged in both responders and non-responders patients either due to treatment-induced necrosis/inflammation or because bone marrow lesions, seen at MRI, may persist a long time after treatment. Thus, it may be difficult to identify bone marrow involvements on the basis of imaging features alone, and biopsy may be necessary. PET/CT, due to the mechanism of FDG uptake that reflects the increased glycolysis occurring in tumour cells, has been shown to be useful in evaluating response to treatment, particularly when imaging techniques, such as MRI, remain abnormal. In this moment, it is recommendable that instrumental results should always be correlated with biological markers related to tumour burden and disease activity.

P030

WHOLE-BODY 64-SLICE MULTIDETECTOR COMPUTED TOMOGRAPHY (MDCT) VERSUS CONVENTIONAL RADIOGRAPHY (CR) AND MAGNETIC RESONANCE IMAGING (MRI) IN STAGING OF MULTIPLE MYELOMA (MM)

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Introduction MM is a malignancy characterized mainly by skeletal involvement. Based on the currently available evidence (IMWG), CR remains the standard method to detect osteolysis, although it shows osteolysis only when the cortical bone damage is more than 50%. It is universally accepted the superiority of MDCT in comparison with CR for detecting early osteolysis (< 5 mm), while MRI is an appropriate tool for detecting bone marrow involvement, but non osteolytic disease, which represents the major reason to treat patients with MM. The individuation of osteolysis reflects pivotal prognostic implications because the lytic bone lesions are indicator parameters of high-risk. Methods We underwent a MDCT without contrast material and MRI with contrast material 7 patients with MGUS, 11 with Symptomatic MM and 25 with Asymptomatic MM according to IMWG criteria. CR was the only imaging method employed for radiological screening at diagnosis. Results Patients with MGUS presented completely negative MRI and MDCT. MDCT was positive in all patients with Symptomatic MM, demonstrating in 6/11 patients a more extensive involvement in comparison with MRI; MRI was positive in 8/11 patients with Symptomatic MM. In Asymptomatic MM group, 13 patients with negative CR and 6 patients with negative MRI, showed multiple focal lesions on MDCT; in 7 patients with MDCT and MRI positive, MDCT demonstrated a more extensive involvement in comparison with MRI. Conclusions In patients without radiologically detectable osteolysis but with a reasonable suspicion of myeloma requiring therapy we recommend the use of MDCT. In our study, in some selected cases, MDCT was even superior to MRI. The false-negatives on MRI might be explained by the fact that very small lytic lesions can be visualized on MDCT before significant bone marrow replacement. The whole body information regarding both skeletal stability and extramedullary involvement in a single examination, the short acquisition time, the absence of contrast material and the 3D reconstruction algorithms permitting to explore difficult anatomic regions (clavicle, ribs) make MDCT an attractive diagnostic tool. A further advantage of MDCT is the ability to detect unsuspected pathological processes – additional malignancies -, especially those involving the lungs. The most important drawbacks in applying MDCT is the high-radiation dose. To overcome this problem, whole-body low-dose CT are being developed as very realistic alternative to CR.

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P031

LENALIDOMIDE FOR THE TREATMENT OF MULTIPLE MYELOMA ELDERLY PATIENTS: A SINGLE CENTRE EXPERIENCE

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Background. Lenalidomide is a new immunomodulatory drug with a dual mechanism of action: tumoricidal effect and immunomodulatory effect. It represents an important treatment option for multiple myeloma patient either as first line therapy, either in resistant/refractory disease or consolidation/maintenance therapy. In this way Lenalidomide increases the available treatment options. Aims. According to several studies which evaluated lenalidomide in the treatment of multiple myeloma, in our Department, lenalidomide was administered in resistant/relapsing myeloma patients and as consolidation/maintenance therapy in elderly myeloma patients with stable disease (partial remission) after induction therapy or more lines of chemotherapy. Methods. We treated 40 patients (21M and 19F) with median age of 70 years (range 66-81). We have evaluated 31 patients with a median follow up of 24

months. These patients were treated with lenalidomide at variable doses (5-25 mg/die p.o., according to tolerability of each patient, for 21 days every 28 days), in association of very low doses of dexametasone (10 mg/die p.o. days 1,2,3,4) or alone. We used Enoxaparin for prophylaxis of venous thromboembolisms.

Clinical restaging was performed after three, six and twelve months, in course of therapy. Results. At present we have not observed any progression of disease and in 18 cases we found a good impact on monoclonal component. In all patients the therapy was well tolerated and were not found significant adverse events.

Conclusions. There is an established role for lenalidomide with dexametasone or alone for consolidation/maintenance continuous therapy in previously treated elderly myeloma patients.

This therapy seems to lead an improvement in prognosis of these patients, without causing severe complications.

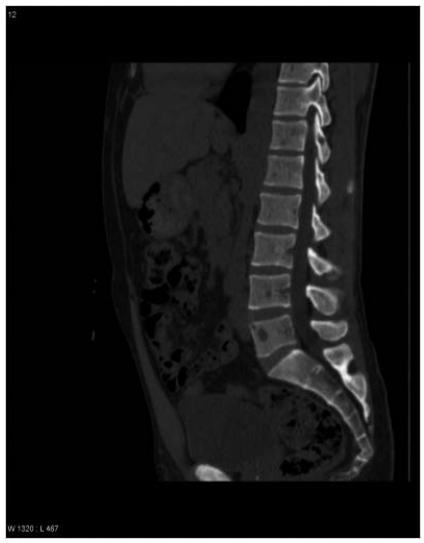

Fig. A = MDCT

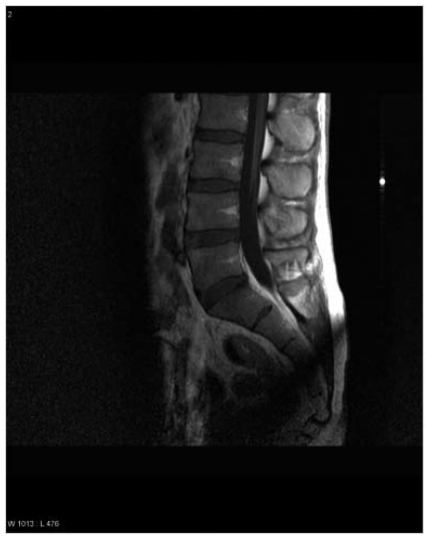

Fig. B = MRI

Comments: in Asymptomatic MM group MDCT showed focal lytic bone lesions that were not recognized on MRI.

P032

BORTEZOMIB IN COMBINATION WITH MELPHALAN AND PREDNISONE FOR THE TREATMENT OF FRAIL 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 frail patients affected by MM. In our institution we are following 20 elderly patients with stage II/III MM (12 F and 8 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 melphalan-prednisone-bortezomib combined administration a partial remission (reduction of M-component > 50-75%) was recorded in 15 out of 20 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 3 out of 20 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 +12) 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.

P033

CD117 (C-KIT) EXPRESSION AND CLINICAL OUTCOME IN PATIENTS AFFECTED BY MULTIPLE MYELOMA (MM)

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The survival of patients affected by MM is variable depending upon the tumour mass at the diagnosis and by the intrinsic biological characteristics of tumour cells. Flow cytometry and immunological methods have allowed the characterization of a series of surface antigenic molecules expressed on either MM or normal cells. With this technique several molecules differentially expressed on normal and MM cells and correlated with the prognosis of MM patients have been identified. In details B-associated antigens, growth factor receptors, myeloid antigens and adhesion molecules can be found on pathological plasma cells. At this regard some studies have demonstrated that in about 50% of MGUS patients and 33% of MM patients the plasma cells express CD117 (c-kit), while normal plasma cells are CD117 negative. We have analyzed the bone marrow blood of 57 patients affected by MM. 35 out of 57 presented a IgG component and the remaining 22 patients were IgA. On the basis of the staging criteria (Durie e Salmon), 29/57 pts. were in stage II and 28/57 in stage III; the clinical stage (remission, progression or stable disease) was defined with clinical re-evaluation after chemotherapy and/or re-staging at 6 months from diagnosis. The immunophenotype of bone marrow plasma cells demonstrated the expression of CD38 (very bright) and of CD138 while CD19 was absent; 50/57 were CD56+ and 15/57 CD117+(dim). 13 out of 15 CD117-positive patients were in stage II and showed a favorable clinical outcome, as demonstrated by a

higher DFS and OS than the remaining patients. The possible prognostic role of CD117 in MM warrants further clinical investigation on a larger series of patients even on the basis of new therapeutic strategies.

P034

TH1 AND TH2 CELLS IN MULTIPLE MYELOMA

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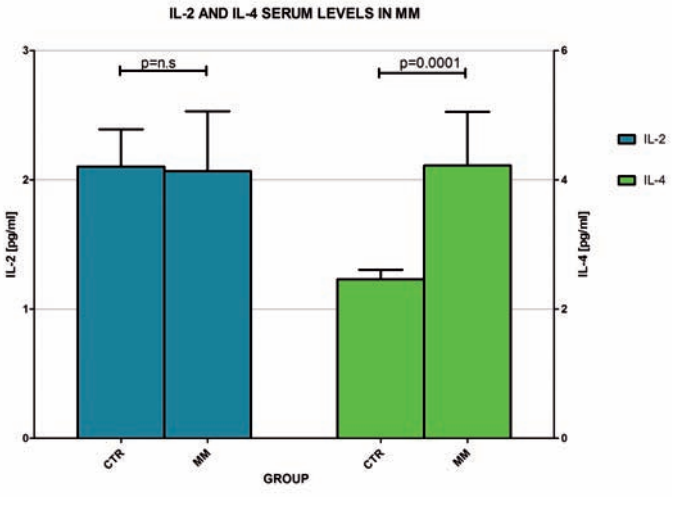
Background. Multiple myeloma (MM) is a B cell disorder characterized by the presence of malignant plasma cells. In bone-marrow the expansion of an idiotypic B and plasma cell clone causes a strong suppression of polyclonal B cells leading to reduced B cell activation and antibody synthesis. IL-2, IFN-gamma and Tumor Necrosis Factor-beta (TNF-B) are specific Th1 cytokines that induce macrophage activation with DTH stimulation and production of complement-fixing and opsonizing antibodies. IL-4, IL-5, IL-6 and IL-13 represent the cytokine pattern of Th2 cells that provide optimal help for antibody production. Beta2-microglobulin is widely distributed on leukocytes surface. Increased production or destruction of these cells causes Beta2-microglobulin plasmatic levels increase. IL-2 and IL-4 can be used respectively for Th1 or Th2 detection. Aims. The aim of this study was devoted to evaluate in MM at exordium the Th1 or Th2 role by IL-2, IL-4 and B2-microglobulin serum levels. Methods. Blood samples were collected from 12 patients with MM at exordium and from 10 healthy subjects as control. IL-2, IL-4 and B2-microglobulin serum levels were performed in both groups. Normality Test (Shapiro-Wilk) and Mann-Whitney test were used to analyze data. Results. IL-4 serum levels were significantly higher ($p = 0.02$) in MM patients (median 3.76; Q1-Q3 range 1.46) than in the control group (median 2.57; Q1-Q3 range 0.18). No significant difference in IL-2 serum level was observed between the two groups. B2-microglobulin serum values were significantly higher ($p = 0.0007$; median 3.12; Q1-Q3 range 3.88) in MM patients than in the control group (median 1.62; Q1-Q3 range 0.32). Conclusions. Our results showed that an abnormally high production of IL-4 and B2-microglobulin was found in MM. This seems to support a Th2 polarization in MM, but the role of Th2 cells is not defined. One could speculate that Th2 cells may promote the increased B lymphocytes immunoglobulin production, the increase of cellular turn-over, and B2-microglobulin serum levels. Further studies are necessary to confirm our data and thus define the Th2 in MM and its role as possible prognostic marker.



P035

LENALIDOMIDE (LEN)+DEXAMETHASONE (DEX) IN ELDERLY PATIENTS WITH ADVANCED, RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND RENAL FAILURE

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Salvage therapy of elderly patients with advanced, relapsed and refractory multiple myeloma (MM) is often limited by poor marrow reserve and multi-organ impairment. In particular, renal failure occurs in up to 50% of such patients, and this further limits the use of conventional chemotherapy. Recently, both thalidomide and bortezomib have proven effective in these patients, with an acceptable toxicity. LEN has not been considered so far a good option for MM patients with renal failure as its use can result into increased hematological toxicity unless dose reduction is applied. Aim of this study was a retrospective evaluation of the efficacy of the combination LEN +DEX in a population of elderly MM patients treated in 5 Italian Centers. The study included 18 consecutive MM patients (8 M, 10 F, median age 76.5 years) with relapsed (N= 6) or refractory (N=12) MM and renal failure, defined as creatinine clearance (Cr Cl) < 50ml/min. Three patient were undergoing hemodyalisis at study entry. Eight patients had been previously treated with thalidomide and 15 with Bortezomib-containing regimens. Two patients with moderate renal failure (Cr Cl 50 and 40ml/min) were treated with full dose (25mg/day) LEN, the remaining patients received a reduced drug dose according to renal function and clinical conditions (15mg every other day = 6 patients, 10mg/day = 7 patients). Three patients on hemodyalisis received 5mg/day. DEX was administered at 40mg/week. Median treatment duration was 5 months (1-22), therapy was interrupted after 1 21-day cycle in 2 patients, due to stroke (1) and diffuse tremors (1), and after 5 cycles in 1 patient due to recurrent severe infections. Hematological toxicity was observed in 8 patients (44%) 5 of whom developed grade III-IV neutropenia; grade II-III non hematological toxicity was recorded in 5 cases (28%). A > 50% decrease in serum or urine M component was observed in 7 patients (38%), 1 of whom obtained a VGPR, 8 additional patients achieved a stable disease. Median response duration was 9 months (range 2-24 months). Recovery of a normal renal function was observed in 4 patients, one additional patient interrupted hemodyalisis. According to our data, LEN+DEX has shown efficacy in this population of elderly patients with advanced MM and renal failure, although the incidence of hematological toxicity and other side effects should not be overlooked and careful monitoring is recommended. Work supported in part by RiminiAil

P036

BORTEZOMIB, MELPHALAN, PREDNISONE AND THALIDOMIDE FOLLOWED BY MAINTENANCE WITH BORTEZOMIB AND THALIDOMIDE (VMPT-VT) VS BORTEZOMIB, MELPHALAN, PREDNISONE (VMP) ALONE AS FIRST LINE TRATMENT OF MULTIPLE MYELOMA: UPDATED FOLLOW-UP AND IMPACT OF PROGNOSTIC FACTORS

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This phase 3 study compared bortezomib-melphalan-prednisone-

thalidomide followed by bortezomib-thalidomide maintenance (VMPT-VT) with VMP alone. 511 patients older than 65 years were randomized to receive nine 6-week cycles of VMPT-VT (N=254; induction: bortezomib 1.3 mg/m2, d 1, 4, 8, 11, 22, 25, 29, 32, cycles 1–4, d 1, 8, 22, 29, cycles 5–9; melphalan 9 mg/m2 d 1–4, prednisone 60 mg/m2, d 1–4, thalidomide 50 mg d 1-42; maintenance: bortezomib 1.3 mg/m2 every 14 days and thalidomide 50 mg/day) or VMP (N=257) alone. In March 2007, the protocol was amended: both VMPT-VT and VMP induction schedules were changed to nine 5-week cycles and bortezomib schedule was modified to weekly administration (1.3 mg/m2 d 1, 8, 15, 22, all cycles). Responses were superior in the VMPT-VT group, with a complete response (CR) rate of 42% vs 24% (p<0.0001). After a median follow-up of 32 months, 3-year PFS was 51% in VMPT-VT arm and 32% in VMP arm(P<0.0001). The overall survival at three years was 85 and 80% respectively (P= 0.35). Achievement of CR was a predictive factor of longer PFS in both groups (P=0.0001): in the VMPT-VT arm, 3-year PFS was 66% in patients who reached CR and 47% in those achieving PR; in the VMP arm, it was 70% and 30%, respectively. In patients older than 75 years (P=0.68) and in those at increased risk of disease progression, defined as presence of cytogenetic abnormalities [t(4;14) or t(14;16) or del17p] and ISS 3 (P=0.60), VMPT-VT seemed not to add any significant PFS advantage to VMP. Grade 3-4 neutropenia, cardiological events and thromboembolic events were more frequent among VMPT-VT patients. In both groups, the once-weekly infusion of bortezomib reduced the incidence of severe sensory peripheral neuropathy from 16% to 3% (p<0.0001). The 1-year landmark analysis of PFS in patients completing the 9 induction cycles, showed a 2-year PFS of 63% in the VMPT-VT group and 40% in the VMP group, demonstrating that maintenance with VT reduced the risk of disease progression of 51% (p=0.0003). This advantage was less evident in patients older than 75 years (P=0.91) and in those with high risk disease (P=0.77). In conclusion: VMPT-VT induced higher response rates resulting in prolonged PFS; once-weekly infusion of bortezomib improved safety without affecting outcome; maintenance therapy with VT further improved PFS with a good safety profile; VMPT-VT therapy seemed to be less effective in patients older than 75 years and in those with high-risk disease.

P037

CORRELATION BETWEEN B MEMORY COMPARTMENT AND CD38+ PLASMA CELLS IN MULTIPLE MYELOMA PATIENTS

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U.O. Ematologia con T.M.O. ARNAS Civico, Benfratelli E G. di Cristina

Introduction: Multiple myeloma (MM) is a neoplasm characterized by uncontrolled proliferation and accumulation in the bone marrow of a Plasma cells clone. Several groups have identified in the MM peripheral blood and bone marrow, a B cells population expressing an idiotype and Ig gene sequences identical to those of tumor Plasma cells, characterized by properties of stem cells and of memory B cells. We investigate the B memory compartment of patients with MM, using the Bm1-Bm5 (IgD/CD38) and CD27/IgD classifications. We have also compared the expression of CD19 and CD56 on the Plasma cells population. Materials and Methods: Fifteen MM bone marrow (BM) and peripheral blood (PB) samples of patients from Hematology Division of Civic Hospital of Palermo were studied. Phenotypic study was carried out using the following panels of monoclonal antibodies: 1) CD24FITC/IgD PE/CD45PercP-Cy5.5/CD38CD138APC/CD20APC-H7/CD19PacificBlue; 2)CD27FITC/CD56 PE/CD45PercP-Cy5.5/CD38CD138APC/CD20APC-H7/CD19PacificBlue.Almost one thousand events on CD19 and CD38+138 gates were acquired. Acquisition of these samples were performed with CyAN ADPTM and analyzed with sw Kaluza 1.1 (Beckman Coulter, Miami, FL, USA). Results: According to the expression of CD27 and IgD we have observed that: naïve B cells are the most representative population (55%) in samples of PB compared with late memory cells (37%), but in the BM there is a greater presence of the late memory (52,5%) than naïve B cells (40%). In PB and BM samples, according to the Bm1-Bm5 classification, B cells are distributed between Bm5 early (BM=17.5%;PB=3.18%) and Bm5 late (BM=35.3%;PB=34.9%) gates; as well as naïve B cells are included in Bm1 (BM=31.9%;PB=50.9%) and Bm2 (BM=8.52%;PB=6.29%) gates. In BM Plasma cells population it was noted that the CD19-CD56+ population when represented with a percentage <50% and between 10% and 50%, corresponds to the average attendance of 61 % and 62.3% respectively of late memory B cells, which decreases (47%) when the aforesaid Plasma cells population has a value >10%. Conclusion: In recent years several studies have focused on Multiple Myeloma cancer stem cells, giving them the phenotypic characteristics of memory B cells as well as stem cells properties. This has provided a starting point for the MFC analysis of B lymphocytes antigen-dependent maturation in BM and PB samples, and their relationship with the pathological counterpart of the Plasma cells population.

P038

LENALIDOMIDE LOW DOSE AND CONTINUED DEXAMETHASONE VERY LOW DOSE IN RELAPSED-REFRACTORY MULTIPLE MYELOMA

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Background: Lenalidomide in association with dexamethasone is an effective combination regimen for the treatment of either previously untreated or refractory-relapsed multiple myeloma (MM) patients. However, information dealing with efficacy of reduced doses of lenalidomide and dexamethasone in patients no suitable for a full-dose schedule are lacking. With this in mind we treated in the period November 2008 to November 2009 11 MM patients (4 refractory and 7 relapsed) all considered not eligible for therapy with full-dose lenalidomide : because of renal impairment (5 patients), heart failure (1 patient), ECOG > 2 (5 patients). There were 4 males and 7 females while median age was 80,5 years (range 62-86). Most patients had advanced disease (i.e., DS stage III 63 %). Median number of previous therapies was 2 (range,1-5). As far as creatinine clearance is concerned, it was less than 50 ml/min in 5 patients, between 50 and 80 ml/min in 2 patients and more than 80 ml/min in 4 patients. Treatment consisted of an association of lenalidomide 15 mg/day and oral dexamethasone 4 mg/day both given on a continuous daily schedule for 21 days. Cycles were repeated after a 7-day rest period. Results: After a median number of 5 cycles of therapy(range 1-6) the overall response rate was 63% . In detail, one patient achieved complete remission (CR) with negative immunofixation, a very good partial response(VGPR) was obtained in 2 patients, while 4 patients were considered in partial response (PR). Among not responder patients two had stable disease and two progressive disease. After a median follow up time of 18 months (range 1-28) 7 patients died and overall median survival was 20 months. At the time of present report all responder patients relapsed after a period ranging between 7 and 28 months. As far as toxicity is concerned : 2 patients had a DVT , 1 despite aspirin prophylaxis, two patients had neutropenic grade IV fever requiring hospitalization. Conclusion: This study although based on a relative small patient number points out that a schedule consisting of low dose desamethasone and lenalidomide can be successfully used in MM patients not eligible for a full-dose schedule. Further studies are needed in order to define whether this approach can be introduced in the current clinical practice especially when dealing with unfit older patients.

P039

CK2 AND HSP90 SIMULTANEOUS INHIBITION IN MULTIPLE MYELOMA: A POWERFUL TOOL TO KILL MYELOMA CELLS PERTURBING THE UNFOLDING PROTEIN RESPONSE SYSTEM

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Hsp90, a central chaperone molecule involved in the maturation and folding of several cellular client proteins, is essential for malignant plasma cell survival. Hsp90 inactivation in multiple myeloma (MM) cells causes perturbation of the ER stress/unfolded protein response (UPR) triggering the apoptotic cascades. Protein kinase CK2 is an important regulator of Hsp90 activity phosphorylating the Hsp90 co-chaperone Cdc37. CK2 is over-expressed in a fraction of MM patients and acts as a pro-survival molecule. We have here investigated the role of CK2 in the ER stress/UPR pathways and in Hsp90 inhibition-induced apoptosis in MM cells. We analyzed CK2 activity upon ER stress and the consequences of CK2 inhibition/silencing on ER stress induced-apoptosis triggered by the chemical Hsp90 inhibitor geldanamycin (GA). CK2 inac-

tivation together with Hsp90 inhibition was followed by apoptotic cell death to a much greater extent than that obtained with the single inhibition of the two molecules. Noteworthy, these effects were also reproduced upon modelling the MM bone marrow (BM) microenvironment by co-culturing MM cells with BM stromal cells and on plasma cells isolated from MM patients. Down-regulation of the catalytic CK2 subunit with chemical inhibitors or RNA interference resulted in modifications of the main UPR regulating signaling cascades, including a reduction of IRE1 and BiP/GRP78, an increase of phospho PERK and phospho eIF2 levels. CK2 partly localized to the ER and the ER-stressor Thapsigargin (TG) triggered its kinase activity. CK2 inactivation enhanced TG-induced apoptosis and opposed TG-driven CHOP/GADD153 and IRE1 rise. CK2 and Hsp90 double inhibition resulted in a more pronounced reduction of IRE1 protein levels, a marked inhibition of GA induced BiP/GRP78 rise and a more evident increase of eIF2 phosphorylation. CK2 inhibition led to a reduction of IRE1 /Hsp90/CDC37 complexes in MM cells, a phenomenon that could lead to a shortened IRE1 half-life. These data highlight the importance of CK2 in tuning Hsp90 function and the ER stress/UPR cascades in MM cells. In view of the very recent development of phase I clinical trials with both CK2 inhibitors and Hsp90 inhibitors as anti-MM agents, our results might provide useful insights to better set the groundwork in designing novel combination treatments for this disease.

P040

SIMULTANEOUS INHIBITION OF PROTEIN KINASE CK2 AND THE PROTEASOME INCREASES MULTIPLE MYELOMA CELL APOPTOSIS AND AFFECTS NF-KB AND STAT 3 SIGNALING PATHWAYS

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Multiple Myeloma (MM) cells are exquisitely sensitive to the cytotoxic effects of proteasome inhibitors (PI). Bortezomib (BZ) is a first-in class PI currently widely used in the therapy of MM patients. BZ causes MM cell apoptosis through different mechanisms. The protein kinase CK2 has been implicated in human cancer and we showed that it acts as a survival molecule for MM cells. Phase I clinical trials are currently ongoing with oral ATP-competitive CK2 inhibitors in MM and other tumors. In this study we have investigated the role of CK2 in the regulation of BZ-induced MM cell death and the pro-survival signalling pathways associated with MM cell resistance to BZ and chemotherapy. MM cell lines U-266, INA-6, human bone marrow stromal cells and freshly isolated plasma cells from patients were cultured and exposed to BZ and the CK2 inhibitors K27 and CX4945 for 6 and 18 hours. Cell growth and viability was assessed upon the different treatments by annexin V and propidium iodide staining, evaluation of mitochondrial potential depolarization and through analysis of PARP cleavage and SMAC/DIABLO expression by western blotting. BZ-induced apoptosis were significantly increased by the simultaneous inhibition of CK2 and the proteasome in all the MM models tested. Mitochondrial membrane potential measurements revealed that CK2 inhibition enhanced BZ-triggered intrinsic apoptotic cell death. Survival signalling pathways associated with STAT3 and NF-kB were studied with WB analysis and RT-PCR. We observed that unwanted side effects of BZ treatment were the activation of the NF-B and STAT3 signalling pathways and the rise in the levels of the unfolded protein response-associated kinase IRE1 . These changes could lend MM cells the ability to escape the cytotoxic effects of BZ. Oppositely, CK2 inhibition was associated with a strong reduction of phospho Ser 536 p65 NF- B, phosphor Ser 727 STAT3 and IRE1 levels in MM cells. Remarkably, the simultaneous treatment of BZ with CK2 inhibitors was accompanied by a significant reduction of BZ-triggered p65 NF- B and STAT3 activation and IRE1 protein level rise. These results suggest that protein kinase CK2 can antagonize BZ-induced apoptosis and regulates critical signaling pathways in MM cells, such as the NF- B and STAT3 cascades. Our findings indicate that CK2 inhibition could represent a rational therapeutic strategy to be tested in designing novel BZ-based anti-MM combination therapies.

P041

INTRAVENOUS MELPHALAN, BORTEZOMIB AND DEXAMETHASONE AS SALVAGE THERAPY IN MULTIPLE MYELOMA: FOLLOW UP AT 48 MONTHS

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Background In vitro studies have shown that bortezomib enhances sensitivity of myeloma cells to melphalan and does not significantly affect the growth of normal hemopoietic cells from normal donor. We explored the feasibility and efficacy of a three drug combination consisting of contemporary intravenous (i.v.) administration of standard doses of bortezomib, at days 1,4,8,11 or 1,8,15,22, intermediate doses of melphalan, and high doses of dexamethasone [BMD]. Methods Between May 2006 and December 2007 32 relapsed and 17 refractory patients have been included in this study, after a median of 2 previous lines (range 1-6). 19 patients (39%) had previously been treated with Bortezomib-containing regimens and 48/49 had been exposed to alkylant agents. At the beginning of the study, treatment consisted of monthly courses of intravenously Bortezomib at the dose of 1.3mg/m2 and Melphalan at the dose of 5 mg/m2 on day 1, 4, 8, 11. Dexamethasone 40 mg was administered intravenously on the day of bortezomib and melphalan and by mouth the day after each injection (1-2, 4-5, 8-9, 11-12, base). Since November 2006, after an interim evaluation indicated an excessive toxicity, the protocol was amended with administration Bortezomib and Melphalan on days 1, 8, 15, 22 and dexamethasone on days 1-2, 8-9.15-16, 22-23 (weekly) for 6 cycles. Results Base schedule was toxic and only 32% of patients completed the planned cycles, with a significant higher rate of ¬¬hematologic (anemia, thrombocytopenia, neutropenia) and gastro-intestinal side effects, compared to weekly schedule. Despite the different toxic profile between 1,4,8,11 and 1,8,15,22 schedules, no significant difference was noted in terms of efficacy. On intent-to treat basis, the overall response rate was 66%, with high rate (27.7%) of complete responses. However, in 6 cases after an initial response at 2nd cycle (>PR) evidence of progression disease occurred during treatment or in first month after the last dose. After median follow up of 24.5 months (range 2.7-50 months), the median OS was 33.8 months (respectively 29.82 and 38.2 in the base and weekly schedule, p=ns). The median PFS was 21.61 with no difference between the two schedules. Patients achieving at least PR had an improved OS (unreached median vs 18 months, p=0.0085) and PFS (35.25 vs 9 months, p=0.009) compared to other patients. Conclusions BMD is an effective regimen. However, the base schedule (1,4,8,11) is too toxic for the fragile subset of refractory/relapsed patients. Even better results may be achieved with a weekly schedule. In this study we confirm that achievement of at least a partial remission is more important than a rapid clearance of M-spike.

P042

EVALUATION OF HIF-1A MRNA INHIBITOR AS ANTI MULTIPLE MYELOMA AGENT

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Hypoxia-inducible factor-1 alpha (HIF-1a) is a transcription factor that plays a critical role in survival and angiogenesis. In solid tumors, elevated expression of HIF-1a, in response to hypoxia or activation of growth factor pathways, is associated with tumor proliferation, metastasis, and drug resistance and correlated with poor prognosis. In contrast to solid tumours, the role of HIF-1a in hematological malignancies is not completely known. In particular in multiple myeloma (MM) HIF-1a has been suggested to be constitutively expressed and HIF-1a knockdown cell lines have shown higher sensitivity to standard chemotherapy, suggesting a role in the pathophysiology of MM. In the present study, we explored the effect of EZN2968, an antisense oligonucleotide against HIF-1a, as a molecular target in MM. We showed that the expression of HIF-1a in several MM cell lines is detectable in either normoxia or hypoxia conditions and is increased in the presence of growth stimuli (IL-6 and stroma cells). In vitro, EZN2968 induced selective and stable down-modulation of HIF-1a mRNA and protein expression, either in normoxia or hypoxia conditions. Immunofluorescence analysis confirmed the

reduction of the protein after 24 hours of treatment, as well as a lower expression of VEGF. Clinically achievable doses of EZN2968 induced cell cycle arrest and cell death (10% to 60% of apoptotic cells compared to the controls after 24 to 96 hours, respectively) in MM cell lines. The activation of the apoptotic pathway was confirmed by western blot analysis at different time points. Moreover we observed an irreversible commitment to cell death after 48 hours of exposure. To evaluate the effect of microenvironment, MM cells treated with EZN2968 were exposed to IL-6 and stroma cells for additional 24 hours. EZN2968 overcame the proliferative effect induced by cytokines. EZN2968 showed moderate increase in efficacy in combination with conventional agents such as Melphalan, and immunomodulatory agents (Thalidomide and Lenalidomide). No significant effect on cell viability was observed on peripheral blood mononuclear cells and CD34+ cells from healthy donors treated with increasing doses of EZN2968. Our data suggest that HIF-1a inhibitor is an attractive therapeutic target for MM patients and provide the rationale for clinical evaluation of HIF inhibitor in combination with currently used MM agents.

P043

BENDAMUSTINE SALVAGE THERAPY IN REFRACTORY LAMBDA CHAIN MULTIPLE MYELOMA (MM) WITH EXTRAMEDULLARY MASS

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MM with extramedullary masses(EMM) at presentation is seen in about 15% of cases,but the incidence is increasing.It is an aggressive disease, poor responding to conventional chemotherapy .Lenalidomide and bortezomib have been used with no univocal results :the failures have been explained with the minor activity of these drugs on myeloma cells outside medullary microenvironment .Bendamustine (B) has proved efficacy in relapsed ,refractory MM but no date are about its activity on EMM .In the present report a rapid and complete disappearance of an extramedullary mass was obtained after B therapy in a patient refractory to 3 previous lines of chemotherapy .A 72-year-old man was referred to our hospital for refractory lambda chain MM, stage III B,presenting a paraskeletal mass that from the v^ rib was infiltrating the thoracic wall.In an other hospital he had received VAD(VCR-ADR-DEXA) therapy obtaining an initial response but resulting resistant at the end of the 6^ cycle.Then he was given 2 cycles of MP(MPH-PDN) therapy, without any response .When he was referred to us he was given 2 courses of PAD (bortezomib , liposomal doxorubicin,DEXA)therapy.At the end of the second one the patient showed a disease progression for a painful spreading of the mass ,as documented by thoracic CT scan and further impairment of renal function,as shown by creatinine level that rose from 2,62 to 5,4mg/dL and glomerular filtration rate(GFR)that reduced from 24 to 10.80 ml/min /1.73mq.The patient was treated with B at the dose of 90mg/mq dd 1-2 .Lenalidomide at reduced dose was started but discontinued after few days for diffuse skin rash.The volume of the breast mass rapidly reduced and disappeared into 15 days ,as shown by a thoracic CT scan .On day +16 from B therapy the patient developed fever, diarrhea that further impaired renal function ,the support of dialysis was necessary for one week .After the infection was resolved,a minimal but stable renal response was obtained.Hematologic toxicity occurred late(day + 39)lasted six days and was associated with CMV infection, that probably was partially responsible of it .On day + 45 the clinical and serological evaluation after one course of B therapy documented an improving of PS of the patient , disappearing of the extramedullary mass ,a renal minor response. Bendamustine is a particular bifunctional molecule with an alkylating group plus a purine-like benzimidazole ring ,it is only partial cross resistant with other alkylating agent.Ongoing trials are evaluating its association with lenalidomide and bortezomib.

P044

VALUE OF FDG PET IN THE ASSESSMENT OF PATIENTS WITH MULTIPLE MYELOMA: PRELIMINARY EXPERIENCE OF A SINGLE CENTRE

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Positron emission tomography (PET) is a tomographic nuclear imaging procedure that uses positrons as radiolabels and positron–electron annihilation reaction gamma-rays to locate the radiolabels. This technique has a high sensitivity (90–100%) and specificity (88–93%) for the detection of myeloma bone lesions. Materials and methods: 43 whole-body FDG PET scans were performed in 23 patients with multiple myeloma. The patients were referred for evaluation of extent of disease before therapy and were referred for assessment of therapy response (chemotherapy, radiation therapy, autologus transplant). FDG PET images were evaluated for distribution and uptake pattern. Results of other imaging examinations (MRI, CT, radiography), were used for verification of detected lesions. Results: FDG PET was able to detect medullary involvement of multiple myeloma and was helpful in differentiating between post-therapeutic changes and residual/recurrent tumor and in assessing response to therapy in six patients. FDG PET was positive at diagnosis in five patients and negative other ten patients. Sensitivity of FDG PET in detecting myelomatous involvement was 85% and specificity was 92%. Discussion: Myeloma has been enclosed into larger studies of PET/CT in the USA and PET/CT has been included as an option in the diagnosis and monitoring of myeloma patients within NCCN guidelines. Further targeted studies in myeloma are required to further clarify aspects of the specific utility in myeloma patients. In addition to demonstrating persistent or recurrent osseous disease, PET/CT studies are more sensitive than other imaging modalities for localizing extramedullary sites of disease, where they reveal additional lesions in almost 30% of the patients who had been diagnosed with solitary plasmacytoma by MRI. PET/CT was superior to plain radiographs to allowed detection of other unsuspected sites of bone involvement. The disadvantage of PET/CT is the false-positive. Conclusion: FDG PET is able to detect bone marrow involvement in patients with multiple myeloma and is useful in assessing extent of disease at time of initial diagnosis, contributing to staging that is more accurate. FDG PET is also useful for evaluating therapy response. The evidence for the sensitivity of PET scanning is most convincing in the setting of extramedullary disease when other imaging techniques have failed to clarify the situation.

P045

PANOBINOSTAT WITH MPT COMBINATION IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: RESULTS OF PHASE I/II TRIAL

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Panobinostat (Pan) is a potent inhibitor of histone deacetylase that induces apoptosis in multiple myeloma (MM) cell lines and it has been found to be effective in patients with relapsed / refractory MM with an acceptable toxicity profile. This study assessed the safety and efficacy of the combination of Pan-MPT in MM patients with relapsed/refractory. According to the Brian and Day design, the first stage of this phase I-II study of the optimal dose of Pan was considered the one with which we should have obtained at least 4 RP and less than 11 DLT out of 19 patients scheduled. The DLT was defined as any non-hematologic toxicity ≥ grade 3, grade 4 neutropenia lasting more than one week or any grade 4 hematologic toxicity except neutropenia. The initial dose of Pan was 15 mg orally on days 1, 3, 5, 8, 10, 12, 15, 17, 19 followed by 9 days of rest, thalidomide was administered at a dose of 50 mg/day continuously while melphalan and prednisone at doses of 0.18 mg/kg and 1.5 mg/kg, respectively, for 4 days every 28 days for 6 cycles. We enrolled 31 patients with a median age of 70 years (range 40-81), roughly 1/3 had been previously treated with ≥ 3 regimes and has proved to be refractory to last therapy. Thalidomide, bortezomib and lenalidomide had pre-

viously been administered to 73%, 77% and 47% of patients, respectively. The protocol was amended after enrollment of the first 12 patients since 8 developed severe hematologic toxicity that required modification or interruption of the protocol. Thus a new phase with Pan 10 mg with the same schedule was initiated. In this new cohort of 19 patients, 11 experienced DLT (two grade 3 gastrointestinal toxicity, 7 grade 4 neutropenia and 2 others grade 3 febrile neutropenia). Therefore, according to the study design, the optimal dose has not been defined either in this cohort. Two patients achieved a CR (6.5%), 2 a VGPR with a response ≥ PR of 38.5%. Even patients refractory to bortezomib or lenalidomide have had response of good quality. The median PFS and OS were 14 and 15 months, respectively. Although the combination of MPT-Pan show encouraging anti-myeloma activity with a manageable non-haematologic toxicity. However, different schedule of Pan should be explored to reduce hematologic toxicity since either Pan 15 mg and Pan 10 mg cause high rate of severe neutropenia although the latter dose, reducing the duration and the rate of thrombocytopenia, led to a significantly lower protocol interruption.

P046

BORTEZOMIB AND DEXAMETHASONE (DEX) AS SALVAGE THERAPY IN PATIENTS WITH MULTIPLE MYELOMA (MM): ANALYSIS OF LONG-TERM CLINICAL OUTCOMES

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Aim of the present analysis was to evaluate the long-term outcomes of a series of 85 MM patients who received bortezomib-dex as salvage treatment after conventional or high dose therapy. Bortezomib was administrated at the dose of 1.3 mg/sqm on days 1,4,8,11 every 3 week up to 12 cycles. Dex was given at the dose of 20 mg on the day and the day after of bortezomib administration. Median age of the patients was 62 years. Median lines of prior therapy were 2 (range: 1-5) (18% of the patients received more than 3 prior lines of therapy). Overall, median duration of bortezomib-dex therapy was 4.5 months. 21% of the patients received a maintenance treatment (one dose of bortezomib-dex every 2 weeks) after a median of 6 cycles, for a median time of 6.3 months. On ITT analysis, 55 % of the patients achieved at least a PR, including 19% of CR and 16% of VGPR, reaching the best response at a median time of 3 months from the start of bortezomib-dex treatment. The response rate was similar between patients who received 1 or 2 prior lines of therapy and more than 3. The median duration of CR and at least PR were 8 months. The median time to next therapy was 10.5 months and the median treatment free interval was 4 months. 28% of the patients received again bortezomib containing regimens at subsequent relapse; the response was as follows: PR 46%, VGPR 12%, CR 4%. With a median follow up of 17.6 months, median OS, TTP and PFS were 23, 8.9 and 10.2 months, respectively. Median TTP and PFS were significantly longer in the patients who received bortezomib after 1 or 2 lines of therapy with respect to those who received ≥ 3 lines (TTP: 9.1 vs 6.3 months, P=0.04, PFS: 11.9 vs 6.3 months, P=0.01). Median survival after relapse from bortezomib-dex therapy was 13 months. Patients achieving CR had a significantly longer PFS and OS (P= 0.03 and P= 0.01, respectively). The only dose-limiting toxicity was peripheral neuropathy (PN), which occurred in 55% pts (grade III: 18%), mainly at cycle 4. PN led to a dose reduction of bortezomib in 52% of the patients and to a discontinuation in 8% of the patients, after a median of 3 months. In conclusion, bortezomib in combination with dex demonstrated a high efficacy in relapsed/refractory MM, leading to a high rate of CR. The benefit for patients was long lasting, as demonstrated by the TTP and PFS, especially in those treated in earlier phases of the disease and in those achieving CR.

P047

VTD INDUCTION THERAPY DOES NOT IMPAIR STEM CELL COLLECTION IN MULTIPLE MYELOMA (MM)

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The introduction of novel agents as induction therapy in MM has raised concerns regarding their impact on periferal blood stem cell (PBSC) collection. To address this issue, we analyzed the effect on PBSC collection of a three drug induction therapy (VTD: bortezomib, thalidomide, dexamethasone) versus two drugs (TD). Analysis was based on the GIMEMA 2005 study, designed to assess the efficacy and safety of VTD versus TD in preparation for double autologous stem cell transplantation (ASCT) in newly diagnosed MM. Patients were mobilized with intermediate dose cyclophosphamide (CTX 4 g/m2) followed by G-CSF (10 mcg/Kg/die). The target threshold was 4 x 106 CD34+ cells/Kg. Patients evaluable for mobilization and PBSC collection were 435 (223 in VTD arm and 212 in TD arm). The median number of collected PBSC was 9.7 x 106 /Kg in VTD arm and 10.7 x 106/Kg in TD arm (p= n.s.). Only 3 VTD patients and 1 TD patient collected less than 2 x 106 CD34+ cells/Kg (p= n.s.). The planned yield of 4 x 106 CD34+ cells/Kg was achieved with one mobilization in 93% and 91% of patients in VTD and TD respectively (p= n.s.). A yield of more than 10 x 106 CD34+ cells/Kg was achieved in 50% and 59% of VTD and TD patients, respectively (p= n.s.). A second mobilization was needed in 30 patients (18 VTD and 12 TD, p= n.s.). The majority of patients (86% VTD and 82% TD, p=n.s.) received CTX as an in-patient procedure (median time of hospitalization of 4 days). Less than 5% of patients developed grade 3-4 infective complications (2% VTD vs 3% TD, p=n.s.). Following ASCT, no significant difference was observed in term of haematological and non haematological toxicities. In a multivariate analysis, variables independently associated with a longer TTP and EFS were a yield of CD34+ greater than 4 x 106 CD34+/Kg, VTD treatment and absence of del(17) and/or t(4;14); longer OS was associated with a yield of CD34+ greater than 4 x 106 CD34+/Kg and absence of del(17) and/or t(4;14). Kaplan-Meier curves projected at 5 years showed that OS, TTP and EFS were significantly shorter for TD patients who collected less than 4 x 106 CD34+/Kg (p=0.0001). Our results demonstrate that: 1) a short induction regimen containing bortezomib and thalidomide added to dexamethasone does not affect SC yield; 2) a poor yield of CD34+ is independently associated with a worse outcome.

P048

PEGYLATED LIPOSOMAL DOXORUBICIN, CYCLOPHOSPHAMIDE AND DEXAMETHASONE IN ADVANCED AND REFRACTORY MYELOMA

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Patients affected by multiple myeloma almost invariably become chemo-resistant: hence, one of the main topics in clinical research is the quest for therapeutic alternatives to overcome refractory disease. We are treating patients relapsed and refractory to most of the therapeutic options available using a combination of pegylated liposomal doxorubicin (Caelix®), cyclophosphamide and dexamethasone (CED), at monthly doses respectively of 35 mg/m2 on day 1, 800 mg/m2 on day 1 and 20 mg/d on days 1-4, with interesting results. Fifteen patients (10 f, 5 m; mean age 60 mos.; r.: 43-75) affected by advanced, resistant and progressive multiple myeloma (mean number of previous treatment lines: 3, r.: 2-6) were treated with monthly CED courses (mean courses: 3; r.: 1-17). In one patient the monoclonal component disappeared and in eight there was a partial response; the disease remained stable in two patients and four did not benefit from the treatment. Mean response duration was 6 mos. (r.: 2-20). The toxicity profile of CED was satisfactory: hematological toxicity was frequently observed (WHO grade 2 in 40% of the patients); we observed an episode of acute renal failure in one patient and bradycardia in another. We believe that these results warrant further studies as they have been obtained in patients with severely advanced disease stage, previously not exposed to anthracyclines, who did not benefit anymore from the combinations received.

CHRONIC LYMPHOCYTIC LEUKEMIA I

P049

PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) HAVING DEVELOPED MALIGNANT LYMPHOMAS. CONTINUING COMPLETE REMISSION OF LYMPHOMA FOLLOWING HIGH-DOSE CHEMOTHERAPY, BUT NOT OF SLE.

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The development of malignant lymphomas, generally of the non-Hodgkin type (NHL), and with a preference to diffuse large cell B lymphomas (DLCBL), in systemic lupus erythematosus (SLE), has been proven and analyzed in an exhaustive recent literature. The combination of germline and somatic mutations, persistent immune overstimulation and the impairment of immune surveillance facilitated by immunosuppressive drugs, is thought to be at the origin of the increased lymphoma genesis. However the treatment and course of such affected patients is less known, and prognosis is generally estimated as poor. Out of 258 patients with complete/incomplete lupus and secondary antiphospholipid syndrome (APS) seen and treated at the institutional Day Hospital between 1982 and 2009, 6 developed lymphomas (4 DLCBL, 1 Hodgkin's and 1 indolent lymphocytic lymphoma). The first 5 patients were treated with high dose chemotherapy (HDCT) and achieved continuous complete remissions (CCR) with a follow-up comprised between 18 and 172 months. One patient achieved complete remission (CR) of both diseases. In the other 4 lupus serology (ANA, APA) persisted, with occasional lupus flares and vascular complications. While eradication of the last cancer stem cell is tantamount to cure in neoplastic disease, persistent autoantigenic overstimulation may contribute to the refractoriness of autoimmunity. The implications of these results for the increasing utilization of hematopoietic stem cell transplantation for severe autoimmune diseases (SADS), with lupus as a paradigm, are discussed.

P050

RITUXIMAB CONSOLIDATION AND MAINTENANCE IMPROVE OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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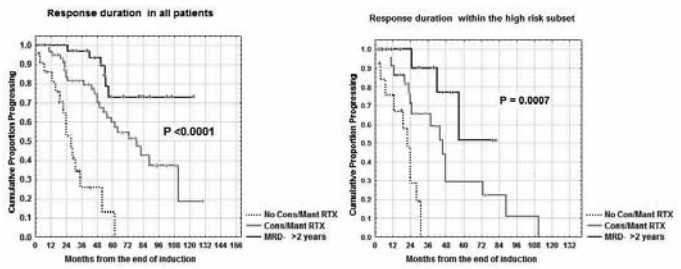
When added to chemotherapy, rituximab (rtx) has resulted in improved complete remission (CR) rate, longer response duration (RD) and overall survival (OS) in CLL. In addition, rituximab consolidation and maintenance therapy may provide a further RD and OS benefit in CLL patients (pts). We treated in first line 140 CLL symptomatic pts, median age 63 years, with six monthly courses of intravenous (25 mg/m2) or oral fludarabine (30-40 mg/m2) and then with four weekly doses (375 mg/m2) of rtx. Fourteen pts had a modified low Rai stage, 123 an intermediate 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/poor cytogenetics (trisomy 12 or del11q or del17p). Sixty-one pts belonged to the high risk subset. Based on NCI criteria, 107/140 pts (76%) achieved CR, 26/140 (19%) a partial remission (PR) and 7/140 (5%) no response or progression. Phenotypic CR (CD19+CD5+CD79b- BM cells <1%) was achieved in 82/140 (58%) CLL pts and MRD+ pts showed a significant shorter OS in comparison with MRD- pts (24% vs 72% at 16 years, P=0.0001). Hematologic toxicity was mild including neutropenia (grade 3 and/or 4 in 60 pts) and thrombocytopenia (grade 3 and/or 4 in 8 pts). Fifty-seven pts either in CR with B-CLL BM cells >1% (MRD+, n=15 pts) or in CR MRD negative, but developing MRD positivity within 2 years after induction (n=24 pts) or in PR (n=18 pts), underwent consolidation and maintenance therapy with four monthly cycles of rtx at 375 mg/m2 followed by twelve monthly doses of rtx at 150 mg/m2. Noteworthy, both persistently MRD negative (>2 years) pts (n=54) and pts undergoing consolidation/maintenance therapy (n=57) showed a longer RD vs MRD+ not consolidated pts (n=22) [73% vs 57% vs 0% at 5 years, P<0.0001]. Equally, OS was shorter in MRD+ not consolidated pts in comparison with the other two subsets (0% vs 61% vs 97% at 15 years; P=0.03). Importantly, also within the high risk subset (n=61), pts in persistent phenotypic CR (n=19) and consolidated pts (n=22) showed a longer RD (90% vs 66% vs 0% at 2.6 years, P=0.0007) vs MRD+ not consolidated pts (n=13). In multivariate analysis, consolidation/maintenance (P<0.0001) and biologic risk classes (P=0.001 and P<0.0001) were confirmed as independent prognosticators with regard to RD and OS. In conclusion, both persistent MRD negativity and/or rituximab consolidation/maintenance therapy improve RD and OS in CLL, also within the high risk subset.



P051

CARBONYL GROUPS SERUM LEVELS ARE ASSOCIATED WITH POOR PROGNOSTIC MARKERS IN PATIENTS WITH B CHRONIC LYMPHOCYTIC LEUKEMIA.

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Oxidative stress is responsible for deoxyribonucleic acid, lipid and protein damage, and play an important role in carcinogenesis. Accumulation of oxidation products leads to genomic instability, and increasing evidence indicates that oxidative stress play an important role in cell proliferation and participates in cell signaling regulation. Mitogen-activated protein kinase family members extracellular-regulated kinase, Jun N-terminal kinase, and p38 respond to oxidative stress for their activation. Transcription factors nuclear factor-kappa B and activating protein 1 are also activated by oxidative stress Aim of our study was to analyze the serum levels of Carbonyl Groups (CG) as parameters of oxidation, in order to quantify the oxidative stress in 24 patients with B-chronic lymphocytic leukemia. In the same subjects we also evaluated IgVH gene analysis, CD38 positivity, Zap-70 expression, and two Multidrug resistance-1 gene (MDR-1) polymorphisms, G2677T polymorphism in exon 21 and C3435T polymorphism in exon 26, to evaluate a possible correlation between biomarkers for oxidative stress and gene polymorphisms or biological risk. The serum content of protein CG was evaluated with use of the Levine method; briefly, 100 ul of serum was incubated with 100 ul of a 20 mM 2,4-dinitro-phenylhydrazine solution for 60 min. Then the proteins were precipitated from the solution with the use of 20% trichloroacetate; the protein pellet was washed three times with ethanol and ethyl acetate and resuspended in 1 ml of 6 M guanidine at 37°C for 15 min. The carbonyl content was determined from the absorbance at 366 nm (molar absorption coefficient, 22,000 M-1/cm). Serum levels of CG were significantly increased in B-CLL patients in comparison to controls (1.690 ± 1.030 nmol/mg proteins vs 0.648 ± 0.568; p< 0.0001). We found a strong correlation between CG with IgVH (r= 0.515; p< 0.02) and CD38 expression (r= 0.568; p< 0.02). Moreover a significant correlation was found between CG and LDH serum levels (r = 0.515 ; p< 0.02), a known marker of tumour burden. In conclusion B-CLL patients displayed an unbalance of the oxidative stress. Carbonyl groups serum levels could be considered as a prognostic factor in patients with B-CLL.

P052

CHEMOIMMUNOTHERAPY REGIMENS FOR THE TREATMENT OF YOUNG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: LONG-TERM FOLLOW-UP

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B-cell chronic lymphocytic leukemia (CLL) has traditionally been considered indolent, with a prolonged clinical course. However, a large proportion of patients with CLL often require more immediate treatment of their leukaemia. The addition of rituximab to fludarabine-based regimens in CLL has been shown to produce high response rates with extended remissions. Moreover, monotherapy with alemtuzumab has also been shown to achieve a complete response with undetectable MRD in several patients with relapsed/refractory disease. We investigated, in a small cohort of young untreated CLL patients, the feasibility and effectiveness of a combination therapy using alemtuzumab consolidation to improve the quality of response to FC-R (Fludarabine-Cyclophosphamide-Rituximab) induction. In our institution we treated, in the last 6 years, 30 patients (11 F and 19 M; median age: 45 years, r.: 35-52 years; Rai stage III-IV) with 6 cycles of FC-R (fludarabine at a dose of 25 mg/m2 i.v. on days 1-3, cyclophosphamide at a dose of 250 mg/m2 i.v. on days 1-3, and rituximab at a dose of 375 mg/m2 on day 0). One month after the last cycle all patients were subjected to a disease re-staging that showed a clinical CR, but 25 out of 30 patients showed the presence of MRD in the bone marrow. Thereafter all patients received, after an initial dose escalation over 3 days, alemtuzumab 10 mg subcutaneously three times per week for 12 weeks. Cytomegalovirus reactivation occurred in 15 patients, all of whom were successfully treated with oral valganciclovir. At a clinical re-staging performed after one, three, six and twelve months from the end of therapy 26 out of 30 patients showed a CR with undetectable MRD (molecular CR). 18 out of 30 patients have a median follow up of 63 months (range: 60-64 months). In this cohort of patients the median PFS was 38 months, and 30% were progression free at 5 years; the median OS has not yet been achieved, and 95% of patients were alive at 5 years. FC-R is highly active as initial therapy also in young CLL patients. However, a consolidation therapy with alemtuzumab seems to be required for achieving a stable molecular CR. Our results show acceptable toxicity profile of this therapeutic approach. Moreover, preliminary data on PFS and OS are very promising.

P053

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH DEL13Q14 AS SOLE ABNORMALITY: PROGNOSTIC SIGNIFICANCE OF INTERPHASE FISH PATTERN AT DIAGNOSIS AND CLONAL EVOLUTION DURING LONG-TERM FOLLOW UP

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Interphase FISH, a technique applicable in clinical practice, was employed to analyse and monitor untreated CLL pts with del13q as sole abnormality, to establish the incidence and prognostic impact of clonal evolution within this favourable group. Among 352 CLL pts followed at our Institution (2001-2009), 137 (39%;) carried del13q as sole abnormality (FISH probes: LSI-D13S319 and LSI-Rb1, LSI-ATM, LSI-p53, CEP12;). Characteristics: Binet stage A: 92%, ZAP70 positive: 28 pts, CD38 positive: 5 pts; IGHV mutated (IMGT): 69%. D13S319 deletion only was detected in 91 pts (66%), deletion encompassing Rb1 locus in 46 (34%), monoallelic deletion (del13qx1) in 121 (88%), and biallelic or concomitant biallelic/monoallelic deletion (del13qx2/x1) in 16 (12%). Ten pts had two different subclones. The median percentage of abnormal nuclei was 50% (range: 11-96%) and was higher in pts with a biallelic or mixed pattern (73% vs 49%; p<0.0001, Mann-Whitney). 60 pts (44%) required treatment; at 5 yrs, treatment free survival (TFS) was 53%, not significantly different from 118 pts with no FISH abnormalities (p=0.142). As a continuous variable, the baseline percentage of del13q14 cells was associated to progression risk (HR: 1.02; 95%CI 1.01-1.04, p=0.0002). Deletion of Rb1 did not influence TFS, while presence of a nullisomic clone at diagnosis was associated with a shorter TFS (p=0.004, Wilcoxon). After categorising by % of deleted cells, median TFS was 12mos for the >75% group, 43mos for group 50-75%, and not reached for pts with <50% (p<0.001). In multivariate Cox analysis, the % category and IGHV mutational status were independent risk factors for treatment (HR 2.01 p=0.02, 5.15 p<0.001 and 2.46 p=0.001 for categories 50-75% vs. <50%, >75% vs. <50% and IGHV unmutated vs. mutated respectively). In 97 pts re-evaluated for FISH (median interval from baseline to re-evaluation: 40mos, range: 9-97) a significant increase in the proportion of del13q cells was detected (p<0.0001, Wilcoxon matched pairs). Change in % of deleted cells per unit time (1mo) affected the risk of treatment requirement (HR 2.03, p=0.016 95%CI 2.03-3.6). Among re-evaluated cases, 11.3% acquired a new clone on FISH: 6 pts acquired a nullisomic clone, 1 del13qx2 pt acquired a del13qx1 clone; 4 pts acquired Rb1 deletion, and 1 pt acquired del17p13. Clonal evolution, however, was not associated with clinical progression requiring treatment (OR=1.07), and only in one case determined risk category upgrading.

P054

IMMUNOPHENOTYPIC PROFILE AND CYTOGENETIC ABERRATIONS DETECTED BY INTERPHASE FISH IN B-CLL PATIENTS

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Chronic Lymphocytic Leukaemia of B-lineage (B-CLL) is the most common lymphoid malignancy in adults, with a variable clinical course, ranging from indolent to aggressive. Flow-cytometry allows the evaluation of malignant B-cell immunophenotypical characteristics and affords the study of important prognostic factors, such as either CD38 and ZAP-70 expression. The immunophenotipic profile of neoplastic B lymphocytes, from peripheral blood or bone marrow samples, was evaluated in 792 patients using a 6-colour panel of monoclonal antibodies: CD19, CD23, CD20, CD22, CD5, CD43, FMC7, CD38, ZAP-70 and surface Ig Kappa/Lambda. Recently, we introduced the analysis of CD200 which was evaluated in 140 patients. FISH on interphase nuclei, from magnetical purified B-cells was assessed in 500 patients in order to detect the most common chromosomal abnormalities with prognostic impact in B-CLL: 11q22.3, 13q14.3 and 17p13.1 deletions and trisomy of chromosome 12; 6q23.3 deletion, IgH rearrangement, t(11;14) and t(14;18) were also analyzed on 250 patients. At least one chromosomal abnormality was found in 97% of B-CLL patients. Del13q14.3 was the most common aberration (found in 45% of patients) and was significantly most frequent in CD38 negative (p<0.01), ZAP-70 negative (p=0.03) and CD43 positive (p<0.01) patients. Del11q22.3 was identified in 9% of patients and was significantly most frequent in CD38 positive (p<0.01) and ZAP-70 positive (p=0.05) patients. Trisomy of chromosome 12 was identified in 21% of patients and was significantly more frequent in CD38 positive (p<0.01), ZAP-70 positive (p<0.01) and CD43 negative (p<0.01) patients. Del17p13.1 was observed in 13% of all patients and no significant correlation was found with any phenotypic parameter. Del6q23.3 and t(14;18) were respectively detected in 3% and 1.7% of patients. Atypical B-CLL with CD23 positivity and CD43 negativity was identified in 61 patients and t(11;14) evaluation was performed in 33 patients. Among this group, t(11;14) was positive in 10 patients allowing an appropriate diagnosis of Mantle Cell Lymphoma. More recently, CD200 expression has been added to better discriminate between Mantle Cell Lymphoma (CD200 negative) and B-CLL tumor cells (CD200 positive).

P055

B-CELL RECEPTOR STEREOTYPY, BCL3 TRANSLOCATION, AND RICHTER'S SYNDROME: A CONTROVERSIAL ISSUE

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In Chronic Lymphocytic Leukaemia (CLL) the relationship among BCR-stereotypy, BCL3 rearrangement and Richter's transformation (RS) is controversial. We describe our experience in CLL pts carrying IGHV4-39 gene with a stereotyped HCDR3-subset 8 and BCL3 translocation. Diagnosis was based on clonal B-cells > $5.00x10^9$/L (phenotype score >3). Among 408 unselected CLL pts, 15 (3.7%) carried the IGHV4-39 gene; 3/15 showed the IGH4-39/IGHD6-13/IGHJ5 rearrangement with stereotyped HCDR3-subset 8. No other pts displayed this stereotyped

HCDR3. Presenting biologic characteristics were similar: IGHV unmutated status (according to IMGT), ZAP70 and CD38 expression (flow cytometry), surface IgG, and isolated trisomy 12 on FISH (probes: LSI13, LSID13S319, CEP12, LSIp53, LSIATM). Pts 1 and 3 carried a BCL3 translocation (BCL3 split signal probe [Dako]), occurring as a consequence of t(14;19)(q32.3;q13.2). Treatment was required at 20, 23 and 8 mos from diagnosis: pt 1 and 2 (age >70 yrs and comorbidities) received mono-chemotherapy, while pt 3 received a fludarabine-cyclophosphamide combination achieving a complete response. Transformation occurred at 65, 43, and 45 mos from diagnosis and was characterised by a sudden increase in lymphocyte count to > 150.00x10^9/L. Morphologic evaluation revealed the predominance of large lymphoma-like cells; enlarged retroperitoneal lymph-nodes were detected in pt 2, but poor performance status prevented a diagnostic biopsy. LDH value was extremely high in all cases (from 6,300mU/ml to 42,900mU/ml). Bone marrow was extensively infiltrated by large B-cells. Cytogenetic evaluation detected del17p in pt 1, no clonal evolution in pt 2 and a complex karyotype in pt 3. In all cases, analysis of IGHV-D-J rearrangement confirmed clonal relationship to the CLL phase. Pt 1 died two mos after transformation; pt 2 is receiving supportive care; pt 3 experienced an incredibly poor clinical course and died of respiratory failure caused by multiple pulmonary infiltrates shortly hospitalisation. In our experience, HCD3-subset 8 stereotypy was associated with an aggressive outcome characterised by a predominant involvement of peripheral blood and bone marrow. Independently of a pathologically-proven shift to lymphoma, progression in pts with this peculiar HCD3 stereotypy might be considered as a Richter's transformation. In CLL the t(14;19) may cooperate with the stereotyped IGHV4-39/IGHD6-13/IGHJ5 rearrangement in causing aggressive evolution.

P056

SPLEEN INVOLVEMENT IN GASTRIC, B-CELL, MALT-LYMPHOMA PATIENTS: REPORT OF 3 CASES.

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Background: Spleen involvement in patients with gastric lymphoma has been rarely reported, and all reported cases underwent both gastrectomy and splenectomy. No data on medical management in these patients are available. Methods: We described therapeutic approaches, and long-term follow-up in 3 patients with gastric, MALT-lymphoma and spleen involvement. Results: Case 1. A 71-year old male was diagnosed with a H. pylori-associated, low grade B-cell, gastric MALT-lymphoma, with both spleen and bone marrow involvement (stage IV). Following bacterial eradication, remission of gastric localization of disease was achieved, whilst spleen involvement persisted despite a therapy with chlorambucil (splenomegaly 15 cm, PET negative; immunophenotype CD5, CD23, and FMC7 positive). At 6-years follow-up a stable disease is still present. Case 2. A 77-year old male was diagnosed with gastric, B-cell gastric lymphoma H. pylori negative. At bone marrow biopsy, a 30% infiltration by CD 20+, BCl6+, CD19/FMC 7+, CD 5- e CD 23- lymphocytes was detected, and peripheral lymphocytosis (40%) was present. A therapy with chlorambucil and prednisone was administered. Gastric localization of disease disappeared at 1-year follow-up, whilst both splenomegaly and peripheral lymphocytosis (58%; CD19+) persisted. Case 3. A 75-year old female, was diagnosed with DLBCL, gastric lymphoma which regressed following CHOP chemotherapy (Stage II). At 4-year follow-up she presented with H. pylori active gastritis without lymphoma recurrence, marked splenomegaly (22 cm) with a positivity of PET signal, abdominal lymph node megaly, and bone marrow infiltration of by a low-grade, MALT-lymphoma (CD 20+, CD3-, CD5-,CD10-). Chemotherapy with R-COMP q 21 was administered, with a remission of disease in both bone marrow and lymph nodes, and persistence of splenomegaly (20 cm). At 1 year, a stable disease is present. Conclusions: Spleen involvement in patients with gastric MALT-lymphoma seems to persist at long-term following chemotherapy, despite regression of both gastric and bone marrow localization of disease.

P057

A CASE OF RICHTER SYNDROME: CYTOMETRIC AND MOLECULAR INVESTIGATION

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Background. Richter syndrome (RS) is the transformation of B-chronic lymphocytic leukemia (B-CLL) to a diffuse large B cell lymphoma (DLBCL). A number of studies have recently focused on identifying clonally related/unrelated diseases and on the molecular mechanisms leading to a high grade lymphoma that is biologically different from de novo DLBCL. We report the cytometric and molecular characterization of such a case of RS. Materials and methods. A 62 year old man was referred to our department for leucocytosis (WBC 51.000/mmc) and low platelet count (PLT 28.000/mmc), lymph nodes and spleen enlargement, systemic symptoms. Lactate dehidrogenase was 2757 U/L. The diagnosis of concomitant B-CLL and DLBCL was made by peripheral blood (PB) multicolour flow cytometric analysis and bone marrow (BM) biopsy. Molecular investigation of immunoglobulin heavy chain variable region (IgVH) gene rearrangements was performed using a multiplex fluorescent PCR with family specific primers. The IgVH mutational status was investigated using IMGT/V-QUEST software. Cytogenetic conventional analysis was performed. Results. Flow cytometry showed two distinct B cell populations, both CD19 and CD5 positive: one represented by CD10-/CD20+/CD23+/lambda restricted small lymphocytes and the other by CD10+/CD20++/CD23-/kappa restricted large cells. BM biopsy showed large blastic B cells CD20+, CD5+, CD10+, CD23-, MUM1+, Bcl2+ (DLBCL) and a few clusters of small lymphocytes CD5+ and CD23+ (residual B-CLL). Molecular analysis identified two different clonal rearrangements involving IGHV3-30 and IGHV4-59 genes, respectively. Both rearrangements resulted productive and mutated. Cytogenetic analysis showed a normal karyotype. Conclusions. RS is the clinical definition for two different conditions: a DLBCL clonally related to CLL, which accounts for the majority of cases, and a DLBCL developed in the contest of CLL, but clonally unrelated. A few reports suggest a relationship between the mutational status of CLL-VH genes and the pathogenesis of RS. Whereas unmutated CLL can give rise to both clonally related and unrelated aggressive lymphomas, RS in mutated CLL appears to develop as a clonally unrelated secondary neoplasm. Our case is in keeping with this hypothesis as we identified two unrelated clones both showing mutated IgVH genes status.

P058

MALDI-TOF MS PROFILING IDENTIFIES N-ACETYLATED THYMOSIN BETA4 (T 4) AS DOWN-REGULATED IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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MALDI-TOF MS profiling was applied to analyze low-molecular weight protein profiles of highly purified B-cells from 100 untreated Binet stage A CLL patients and 17 healthy controls. We detected 17 statistically significant differentially expressed ion signals in CLL compared to controls, 10 were considered technically identifiable. Applying the Olex data-mining method, it appeared that 2 ion signals (4963.7 m/z, and 7345.3 m/z) corresponding to 5.0 and 7.3 kDa proteins, respectively could correctly differentiate normal (F-measure=0.79)from CLL cases (F-measure=0.96). The 5.0 kDa protein was characterized as T 4 while the 7.3-kDa protein remains to be identified. In particular, the down-regulation of the 4963.7 ion signal (T 4) was shown to be more strongly associated with CLL [% Relative peak area (%RPA) normal vs CLL, 49.36±14.46 vs 34.50±12.52; mean±SD, p<0.00001, by Student-T test]. Moreover, T 4 expression resulted unchanged in CLL cases when classified according to CD38 and ZAP-70 expression, and IGVH mutational status. The down- regulation of T 4 protein in CLL was also con-

firmed by a ROC analysis which established %RPA=44 as the cut-off value which could effectively differentiate CLL patients from controls. To validate the results of the MALDI-TOF analysis, we conducted an independent GEP analysis comparing CLL cases (n=80) and controls (n=6), which also confirmed T 4 mRNA down-regulation in CLL (3604±1244 vs 5715±1004, respectively; mean±SD; p=0.001). T 4 is a ubiquitously expressed G- actin binding and sequestering protein involved in tissue repair processes, such as wound healing, in response to different forms of trauma (e.g. ischemia). Coherent with its role in tissue normalization, extracellular T 4 exerts anti-inflammatory activity due to its ability to down-regulate prototypical inflammatory cytokines and chemokines, such as TNF, found to be elevated in the serum of CLL patients. Recent data has shown that ectopic T 4 suppresses TNF-mediated NF B activation, a prognostic marker in CLL, by direct targeting of the RelA/p65 subunit. On these bases, a role for T 4 down-modulation in CLL cells may be envisaged in sustaining TNF levels and the consequent NF B phosphorylation via a TNFRII-mediated activation pathway triggering pro-inflammatory and pro-survival signals. Further studies are required to confirm the potential relevance of T 4 modulation in CLL pathobiology.

P059

PROGNOSTIC SIGNIFICANCE OF TELOMERE LENGTH IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS IN EARLY STAGE DISEASE

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Chronic lymphocytic leukemia (CLL) is a genetically heterogeneous disease with a variable outcome. The identification of factors that could predict the clinical course of early-stage CLL represents a crucial objective. Although previous studies indicated that telomere length may be a useful independent prognostic factor in the risk stratification of CLL, limited information has been reported in asymptomatic early stage patients (Binet stage A). We investigate the association of telomere length with the major biological and cytogenetic markers known to predict clinical outcome in CLL. The global DNA methylation levels of Alu and LINE sequences, was also investigated. Correlation with disease progression, measured as the time elapsed from diagnosis to first treatment, was evaluated. We measured relative telomere length (RTL) by real-time PCR in a panel of highly purified (>90%) peripheral mononuclear CD19+ cells from 7 healthy donors and 77 untreated CLLs. All cases were characterized by FISH for the most frequent chromosomal aberrations (Fabris et al. GCC, 2008). Molecular markers including mutation status of the heavy chain variable regions of immunoglobulin genes (IGVH), the expression of the 70-kd zeta-chain T-cell receptor-associated protein kinase (ZAP-70) and CD38 cell surface antigen protocols were previously reported (Cutrona et al. Haematologica, 2008). A quantitative bisulfite-PCR Pyrosequencing method was used to evaluate methylation of Alu and LINE-1. We found a significantly lower RTL values in CLLs (median RTL=0.4 IQR 0.3-0.6) as compared with controls (median RTL=1.0 IQR 0.9-1.3) ($P < 0.001$). A progressive and significant RTL decrease in low (13q- and normal karyotype), intermediate (+12) and high (11q- and 17p-) cytogenetic risk categories (P for trend =0.008) was observed. Patients with IGVH mutated genes had longer telomeres than patients with unmutated genes ($P<0.001$). No significant association between telomere length and either CD38 or ZAP-70 expression was found. Telomere shortening was significantly correlated with hypomethylation of Alu (P=0.048) and LINE-1 (P=0.001), indicating a contribution to chromosome instability. Finally, follow-up analysis, showed a significantly higher risk of starting treatment for patients with shorter telomeres (P=0.0037). Our results extended previous evidence that telomere length could be used as marker for the identification of CLLs with a different prognostic risk.

P060

MULTIPLE LIGATION-DEPENDENT PROBE AMPLIFICATION AND FLUORESCENCE IN SITU HYBRIDIZATION TO DETECT CHROMOSOMAL ABNORMALITIES IN CHRONIC LYMPHOCYTIC LEUKEMIA: A COMPARATIVE STUDY

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Chronic Lymphocytic Leukemia (CLL) is a clinically heterogeneous disease characterized by recurrent chromosomal aberrations of prognostic significance. Although Fluorescence in situ Hybridization (FISH) is the most common technique used to detect these abnormalities, it still remains a quite expensive time-consuming method. We aimed to evaluate the potential of the novel Multiplex Ligation-dependent Probe Amplification (MLPA) technique, to detect genomic alterations in CLL. Highly purified (>90%) peripheral mononuclear CD19+ cell populations from 100 untreated CLLs in early stage disease (Binet stage A) were included in this study. All samples were investigated by fluorescence in situ hybridization (FISH) for the presence of trisomy 12 and 17p13.1, 11q22.3 and 13q14.3 deletions. For MPLA analysis, DNA was amplified by means of 2 commercially available probes sets allowing the simultaneous screening of 56 genomic sequences. Overall, a high degree of concordance (95%) between MPLA and FISH results was found provided that abnormal clone was present in more than 30% of leukemic cell population. The use of multiple MPLA probes allowed the fine the mapping of the 13q14 deletion and the identification of intragenic or small alterations undetected by FISH. Moreover, additional alterations in 2p24 (MYCN) (3pts), 8q24 (C-MYC) (1pt), 9p21 (CDKN2A-2B) (1pt), 1q21 (LMNA) (1pt), and 6q25-26 (1pt) regions not covered by a standard FISH assay were detected and all confirmed by FISH. Our data extend previous limited evidence that MLPA may represent a useful technique to characterize well-known lesions as well as to investigate additional genomic changes in CLL.

P061

EXPRESSION AND FUNCTIONAL ACTIVITY OF DEATH RECEPTOR 3 IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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In B-cell chronic lymphocytic leukemia (B-CLL) microenvironments, interplaying between tumor necrosis factor (TNF) superfamily members and their cognate receptors has been shown to play a relevant role in controlling B-CLL growth and survival. Death Receptor 3 (DR3), a TNF receptor superfamily member, has been recently associated with modulation of immune and inflammatory functions. The ligand of DR3, TNF-like protein 1A (TL1A), is expressed by various cell types, including bystander cells in B-CLL microenvironments, i.e. macrophages and activated T cells. The aim of this study was to test the hypothesis that DR3 may be involved in the molecular network that modulates leukemic growth in B-CLL microenvironments. The expression of DR3 was measured in peripheral blood B cells and in lymph node specimens from 35 B-CLL patients by flow cytometry and immunofluorescence, respectively. PBMCs and lymph nodes from healthy donors were used as control. Although B cells from either B-CLL patients or healthy donors did not express DR3 in basal conditions, stimulation of B cell receptor (BCR) with anti-IgM antibodies induced de novo expression of DR3 in B cells from both B-CLL patients and healthy donors. However, DR3 expression was significantly higher in B cells from B-CLL patients than that in healthy donors (mean fold-induction ± SD = 2.51±1.1 in B-CLL and 1.91±0.5 in healthy donors; p 0.05). DR3 expression in B-CLL cells was confirmed at the mRNA level by quantitative RT-PCR (4-fold induction with respect to basal conditions). Immunofluorescence analysis showed DR3 expression both in scattered CD20 positive cells in B-CLL lymph

node and in CD20 positive cells in germinal centers of reactive lymph nodes. DR3 functional activity was analyzed in anti-IgM-stimulated B-CLL cells by engaging DR3 with agonistic antibodies. DR3 engagement induced signaling activity, as shown by the downstream phosphorylation of Extracellular Signal-Regulated Kinase 1 and 2 (Erk1/2) measured by phospho-specific flow cytometry. In conclusion, we described for the first time the expression of functional DR3 molecules in B-CLL cells. The finding that anti-IgM-induced DR3 expression was higher in B-CLL cells if compared with healthy B cells suggests that DR3 may have significance in B-CLL physiopathology.

P062

PROSPECTIVE ENROLLMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA IN A REGIONAL REGISTRY (CLL VENETO): HOW UNSELECTED PATIENTS PRESENT TO OUR CENTERS.

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The Chronic Lymphocytic Leukemia regional registry (CLL-Veneto) was established in 2008 with the aims of exploring the history and real world management of patients diagnosed with CLL in our region, standardizing the techniques for diagnosis and prognostication among different labs, and providing insights into the management and therapeutic strategies employed in both the community and academic settings. Among hematologists of our region, five sub-committees were established to explore epidemiological, clinical, phenotypical, cytogenetical, and biological aspects of the disease at patient diagnosis. The registry was approved at each center institutional review board, and all patients were required to sign an informed consent before being subject to study procedures. Since september 2008, 317 patients have been prospectively enrolled in the 7 Veneto provinces. Patients were uniformly interviewed at presentation, and peripheral blood specimens were collected for phenotypic, molecular, and cytogenetic studies. Median age was 66 (31-86), and 185 (58%) were males. Overall, 11% of the patients declared a job related exposure to chemical or toxic materials or substances, 4% had a degree level of scholar education, 14% had a history of second malignancy requiring treatment, and 13% reported a family history of CLL or leukemia. RAI and Binet stage at diagnosis were 0: 50%, 1: 26%, 2: 15%, 3: 3%, 4: 6%, and A: 77%, B: 16%, C: 7%, respectively. At least one constitutional symptom related to CLL was present in 26% of patients. Median lymphocyte count was 24.7x103/mmc (range 0.9-824), with CLL-phenotype B-cell lymphocytes <5x103/mmc (consistent with the diagnosis of monoclonal B-cell lymphocytosis) in 32% of patients. Coombs' test was positive in 7% of patients, while ZAP-70 was positive in 46%, CD38 in 24%, IgVH status was mutated in 55%, del(11q23) and del(17p13) were present in 7% and 6% of patients respectively. After a median follow-up of 14 months (1-32), 60 patients (19%) started treatment, and two patients died. Median time to first treatment was 4.6 months, with 20 patients (6%) necessitating treatment within 2 months from diagnosis. Eight patients (2.5%) had autoimmune hemolytic anemia and 5 (1.5%) had immune thrombocytopenia. The clinical and biological characteristics of patients enrolled so far in the CLL Veneto registry represent an overall picture of how unselected patients with CLL present to hematological centers. Supported by: Regione Veneto "Ricerca Sanitaria Finalizzata"; "Fondazione G. Berlucchi per la Ricerca sul Cancro"; AIRC - Associazione Italiana Ricerca sul Cancro; Fondazione CARIVERONA.

P063

ALTERED COMPOSITION OF CIRCULATING MONOCYTIC POPULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA B CELL PATIENTS

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Bidirectional interactions between chronic lymphocytic leukemia (CLL) B cells and non-transformed cells of stromal and immune compartments may contribute to CLL pathogenesis and progression. Monocytes are recruited from the bloodstream into tumor sites and, as they extravasate across the tumor vasculature, begin to differentiate into macrophages. The aim of this study was to characterize circulating monocytic populations, including Tie2-expressing cells, in CLL patients and normal controls. CD14+monocytes can be divided into two subset defined resident and inflammatory according to the expression of CD16, a Fc gamma receptor III. Here, we found that inflammatory monocytes (CD14+CD16-) accounted for 71.1% and 74.5% of total monocytes in normal controls and CLL patients respectively as assessed by flow cytometry. Increased CD4+CD16++ monocytic population was seen in CLL compared to normal controls (mean+/-SEM, 10.4%+/- 1.1% vs. 6.9%+/-0.99%) (p=0.029). CLL patients with high risk cytogenetic abnormalities were characterized by increased percentage of resident monocytes (CD14+CD16+) compared to low risk subset (31.7% vs. 25.3%) (p=0.038). A subset of monocytes called TEM is known to express Tie2 receptor of Angiopoietins. In cancer patients, TEM were observed within tumors, but hardly detected in non-neoplastic tissues. Angiopoietin-2 (Ang2) was reported to determine the recruitment and M2 polarization of TEM. In this study, peripheral blood mononuclear cells (PBMCs) from CLL patients and normal controls were stained with anti-human Tie2 mAb and analysed by flow cytometry. Increased number of Tie2+ PBMCs were measured in CLL (138.3 +/- 24.0 cells/ul) compared to controls (67.9 +/- 18.5/ul) (p <0.05). The number of Tie2-expressing monocytes was 84.5 cell count/ul in CLL and 52.3 cell count/ul in controls. TEM were particularly increased in CLL patients with adverse cytogenetics compared to cases with low risk cytogenetics (157.9+/-50.2/ul vs. 57.1 +/- 17.9/ul) (p=0.04). Moreover, we found a positive correlation between Ang2 plasma levels and number of circulating TEM (r=0.51, p=0.002). In conclusion, aberrant composition of circulating monocytic subpopulations was detected in CLL, with particular increase of CD16++ monocytes and TEM subset. The capacity of producing Ang2 in BM and LN compartments and the correlation between plasmatic Ang2 and TEM in CLL patients suggest a possible role of TEM in CLL pathogenesis that has to be elucidated by further studies.

P064

EFFICACY, TOLERABILITY AND COST-SAVING OF FRONTLINE ORAL FLUDARABINE AND CICLOFOSFAMIDE THERAPY FOR CHRONIC B-CELL LEUKAEMIA AND LOW GRADE NON HODGKIN LYMPHOMA IN ELDERLY PATIENTS.

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Background: Fludarabine has an oral formulation that offers equivalent efficacy and an improved tolerability profile compared to the IV formulation. IV fludarabine requires several administrations that will expose patients to the risk relating to IV injections and the cost of trip to the hospital. Methods: Between April 2005 and January 2011, 10 elderly untreated patients (pts) (mean age 75, range 68-86) with treatment requiring B-cell lymphatic leukaemia and 11 elderly indolent stage≥3 lymphoma non Hodgkin untreated pts (mean age 74, range 59-80) received therapy with low dose of oral fludarabine (25mg/mq/die) and cyclophosphamide (150mg/mq/die)(FC) both days 1-3 once a day. Study design consisted of 6 cycles repeated every 4weeks in outpatient regimen. Pts received antibiotic prophylaxis with trimethoprim/sulphamethoxazole (160/800 mg twice a day, 3 times a week) and allopurinole (300 mg once a day, days 0-4). Performance status was WHO 2 in all pts. Comorbidities, as diabetes, hypertension and chronic heart dis-

ease, were present in 15pts. No pts reduced dose and number of cycle because of haematologic and extra-haematologic toxicities, only 2 pts experienced grade III neutropenia treated with GCSF. Results: Response was assessed according to the updated IWCLL-NCI 2008 criteria.18 of 21 obtained a response (9RC and 9 RP) with an overall response 86%. All responder pts are alive and maintained response after mean follow-up of 27 months (range3-53). We used Genzyme sponsored Excel program to compare direct hospital cost of oral to IV FC (both 3 days regimen):18 day-hospital accesses for IV therapy with total cost of €7.527, 6 ambulatory accesses for oral regimen with total cost of €1.642 (including: pharmacy, nurses and physicians resources). In this analysis we didn't consider social and psychological cost: transports, relatives' lost of working hours, disease consciousness, trauma of repeated venipunctures. Conclusions: Our results suggest that oral FC is effective and well tolerated for pts unfit for more aggressive treatments. Moreover this therapy compared to chlorambucil, the most used agent in these pts, is more effective and better tolerated. No pts suffered of major toxicity obtaining an effective control of the disease. Ambulatory regimen is preferred by our pts, who are treated in an friendly environment, with fewer complications and reduced use of hospital resources.

P065

IDENTIFICATION BY SEROLOGICAL PROTEOME ANALYSIS (SERPA) OF TUMOR-ASSOCIATED ANTIGENS ELICITING ANTIBODY-RESPONSES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Serological proteome analysis (SERPA) has been demonstrated to be a useful method to detect tumor antigens (Ag) eliciting antibody responses in human malignancies. Ag identified in tumor patients can provide new biomarkers and may be useful for the development of immune-based therapeutic strategies. In this study SERPA was applied for the first time to identify tumor Ag in chronic lymphocytic leukemia (CLL). Twenty-one untreated CLL patients were included in the study. Proteins extracted from purified CLL cells were separated by 2-D electrophoresis and transferred onto membranes by electroblotting to obtain 21 2-D proteomic maps. Each map was probed with the corresponding autologous serum by Western Blot (WB). To verify the CLL-specificity of antibodies recognition, 7 out of 21 CLL patients' maps were also probed with sera collected from 7 healthy donors (HD). For identification, Ag spots were analyzed by peptide mass fingerprint by MALDI-TOF Mass Spectroscopy (MS). Sixteen out of 21 CLL sera (76%) showed at least one immunoreactivity and produced an overall number of 45 Ag spots. By contrast, sera from HD were significantly less reactive ($p<.03$) and produced only a total of 3 Ag spots. Eleven out of 16 (69%) reactive CLL sera recognized from 2 to 6 different Ag. All the 45 spots were characterized and consisted of 16 different Ag. Sera from 48% CLL patients exhibited reactivity against a protein which was identified by MS as -Enolase (ENOA). ENOA recognition was CLL specific since none of the sera from HD showed reactivity against this protein. The IGHV mutational status was available in 20 CLL patients. Interestingly, ENOA was recognized from sera of 7 out of 12 mutated patients (58%), but only from sera of 2 out of 8 unmutated patients (25%). The ability of ENOA to induce Ag-specific T cell responses was also evaluated. T cells isolated from the peripheral blood of 3 CLL patients with anti-ENOA antibodies were stimulated with autologous ENOA-pulsed dendritic cells (DC), and evaluated by IFN ELISPOT assay. ENOA-pulsed DC stimulated autologous T cells to secrete IFN . This response was ENOA-specific because it was not induced by unpulsed DC or DC pulsed with an irrelevant protein, and also CLL-specific because IFN release was not induced when T cells from a HD were stimulated with autologous ENOA-pulsed DC. Our results indicate that ENOA can be considered a promising biomarker and a potential target for immune-based approaches in CLL

CHRONIC MYELOID LEUKEMIA I

P066

TRANSIENT EFFECT BUT GOOD TOLERANCE OF NILOTINIB IN A CASE OF CML WITH F317L MUTATION INTOLERANT AND RESISTANT TO IMATINIB AND DASATINIB

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Case report: female,70 years,with CML from 1997 treated with alphaIFN, hydroxyurea, then imatinib. After a CCyR during treatment with imatinib (+4 years), the patient, because of several episodes of grade IV pancytopenia and repeated IMA discontinuations, in 2007 developed hematologic and cytogenetic progression(Ph+100%,ACA). From August 2007 to December 2007 the patient was treated with dasatinib. However, the drug was poorly tolerated because of grade IV anemia and thrombocytopenia (many interruptions and dose reductions) and, also, ineffective (Ph 100% but ACA eliminated,bcr/abl 34.7 IS). Since February 2010 the patient was treated with nilotinib 600. The treatment was very well tolerated with only asymptomatic moderate pancytopenia, asymptomatic bradycardia (QTc lengthening until 480 msec, after normalized); never oedema, serous effusions, dermatitis, dyspepsia, diarrhea, headache, fatigue; it has never been necessary,even temporarily, to stop nilotinib. Leukocytosis and splenomegaly rapidly regressed. After the 7 months of treatment the patient had a satisfactory cytogenetic debulking quantifiable as a MCyR(Ph+30%). However, the molecular monitoring has shown, after an initial transcript reduction, a gradual increase of bcr/abl. Parameters of hamatologic, cytogenetic and molecular follow up are summarized in the table. F317L mutation is described as sensitive to nilotinib, but in the long-term patients may become unresponsive. In this case of advanced phase CML at high genetic instability, resistant and intolerant to imatinib and dasatinib, nilotinib has been shown active in controlling the hematological and cytogenetic parameters of the disease and very well tolerated.

	feb/2010 (basal)	apr/2010	jul/2010	sept/2010	feb/2011 (+12 months)
wbc	42.300	3.500	2.900	2.500	3.300
hgb	10.5	9.5	9.8	9.4	9.7
plts	596.000	88.000	61.000	41.000	53.000
spleen, cm	18	14	12	12	12
karyotype	Ph+ 100%			Ph+ 30%	
bcr/abl	34,7		20,7	13	22
nilotinib	600 mg	>	>	800 mg	>
QTc, msec	480	420	400	400	400

P067

DELAYING THE INITIATION OF DASATINIB AFTER IMATINIB FAILURE HAS A NEGATIVE IMPACT ON OUTCOME FOR PATIENTS WITH CHRONIC-PHASE MYELOID LEUKEMIA (CP-CML): RESULTS FROM A EUROPEAN OBSERVATIONAL STUDY (FORTE; CA180-211)

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Background: Approximately 50% of patients (pts) with CP-CML discontinue 1st-line imatinib (IM), mostly due to inadequate response and/or intolerance. Interventional studies in IM-resistant pts suggest that earlier and deeper responses to dasatinib correlate with better outcomes, and time from IM failure to starting 2nd-line BCR-ABL inhibitor therapy significantly predicts response. However, limited real-life observational data have been gathered. Aims: To estimate the relationship between time from IM failure to initiation of dasatinib and best response to dasatinib. Methods: In this real-life study, data were collected retrospectively and prospectively for up to 6 months from adult CP-CML pts who had failed IM and received dasatinib for ≥2 months. A Proportional Odds model was used to identify covariates potentially influencing best response to dasatinib over the entire observation period. Results: Of 457 eligible pts, 176 (39%) were IM intolerant and 352 (77%) IM resistant; 71 pts were both resistant and intolerant. Median age at dasatinib initiation was 57.2 years and median times from diagnosis to IM and dasatinib initiation were 2.2 and 45.1 months, respectively. 52% of pts had a complete cytogenetic response (CCyR) or major molecular response (MMR) on prior IM. Median time from IM failure to dasatinib initiation was 8.8 months and 68% of pts received a starting dasatinib dose of 100 mg/day. 74% pts achieved CCyR or MMR on dasatinib. A statistically significant effect of time (months) from IM failure to dasatinib initiation on the achievement of a better response to dasatinib was observed ($p<0.023$), with an estimated odds ratio [95% CI] of 0.987 [0.976–0.998], suggesting that a 6- or 12-month delay in starting dasatinib would decrease the probability of achieving a better response to dasatinib by 7.5% or 14.4%, respectively. Conclusions: Delaying dasatinib initiation negatively impacts response to dasatinib in CP-CML pts who have failed IM. Results are consistent with data from interventional trials in resistant pts and underscore the importance of early intervention after imatinib failure.

P068

PHARMACOGENETICS FOR THE OPTIMIZATION OF CHRONIC MYELOID LEUKEMIA (CML) THERAPY IN THE ERA OF SECOND-GENERATION TYROSINE KINASE INHIBITORS (TKIS): SPECIFIC DRUG TRANSPORTER GENOTYPES ARE ASSOCIATED WITH DECREASED RATES OF MAJOR MOLECULAR RESPONSE (MMR) AND COMPLETE MOLECULAR RESPONSE (CMR) IN PATIENTS RECEIVING IMATINIB (IM)

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MMR and CMR are the current goals of CML therapy and 2nd generation TKIs might allow to achieve these endpoints in pts who fail to do so on IM. The availability of multiple options for CML treatment should be paralleled by the availability of biological predictors of outcome allowing to identify, at the time of diagnosis, those pts who are more likely to benefit from dasatinib or nilotinib rather than from IM. Pharmacogenetics has proven to be a potential source of biomarkers given the known influence of polymorphisms in key genes encoding drug transporters and metabolizing enzymes on drug delivery and effectiveness. We thus investigated a panel of 20 single nucleotide polymorphisms (SNPs) in ABCB1 (MDR1), ABCG2, SLC22A1 (hOCT1), OCTN1, OATP1A2, CYP3A4 and CYP3A5 genes in 189 newly diagnosed CML pts receiving IM in the framework of the TOPS phase III trial; 156 (83%) pts were Caucasian and 23 (12%) were Asian; low, intermediate and high Sokal risk pts were 84 (44.4%), 65 (34.4%) and 40 (21.2%), respectively. MMR and CMR rates were compared according to i) each candidate genotype ii) summary measures based on combinations of SNPs in the same gene and iii) summary measures based on combinations of SNPs in functionally related genes (uptake; efflux). Statistically significant associations observed with the Cox regression analyses were further evaluated through cumulative incidence plots based on the Kaplan-Meier method. The homozygous wild-type CC and the heterozygous CT genotype at OCTN1 rs1050152 correlated with MMR ($P = 0.03$). With respect to the summary measures, the number of major alleles at 4 SNPs in the SLC22A1 (hOCT1) gene (rs72552763, rs12208357, rs683369, rs2282143) correlated with MMR ($P = 0.03$). When considering summary measures of uptake and efflux, the former (number of major alleles at: OCT1 rs72552763, rs12208357, rs683369, rs2282143; OATP1A2 rs11568563; OCTN1 rs1050152) was found to be associated with CMR ($P = 0.01$). In addition, analysis of the Caucasian subgroup evidenced a significant association between the CC genotype in MDR1 rs60023214 and CMR ($P = 0.005$). Genotyping of CML patients might be taken into account in an attempt to further individualize treatment, with the aim of enhancing efficacy in terms of MMR and CMR achievement. Stratification of pts according to genotypes may be proposed for selection between IM and 2nd generation TKIs, and represents an attractive opportunity for new clinical trials. Supported by Novartis Oncology.

P069

THE ALTERATION OF CPK IN CML PATINTS TREATED WITH IMATINIB, HAS IMPACT ON RESPONSE AND TOXICITY?

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The observation of increased CPK in some CML patients treated with imatinib, has raised questions how to affect this change in patient management. The aim of this study was to evaluate whether there are differences in treatment response and toxicity between the 2 groups of patients. From 2003 to 2010 we followed 47 consecutive new patients with CML, 44 are evaluable; 57% showed increase of CPK (CPK+) and 43% did not show this increase (CPK-). We observed that there is no difference between the 2 groups in terms of OS, PFS and EFS. In CPK+ group 11/25 (44%) are in CMR, 10/25(36%)in MMR and 4/25(16%) in suboptimal response, whereas in CPK- group 7/19 (37%)were in CMR , 6/19 (32%)in MMR and 6/19 (31%) in suboptimal response. The median months for the major molecular response in these cohorts of patients was 12 and 17 months in CPK+ and CPK- groups respectively. In CPK+ 76% patients suffered of muscle pain and fatigue of grade I-II (sec. CTC) correlated with the values of CPK. In CPK- group only 5% suffered of muscle pain. No patient discontinued treatment for toxicity in both groups. 5/44 (11%) patients changed TKI, 2 to progression (1 CPK+ and 1 CPK-), 3 for suboptimal response (0 CPK+ and 3 CPK-). In conclusion, although the number of patients is low, the alteration of CPK in patients with CML treated with imatinib seems to correlate with increased muscle toxicity. More rapid and intense achievement of major molecular response with significant difference (80% vs 69%). Such observations may be considered CPK as a prognostic factor for response and compliance treatment and may affect the management of early shift therapy in these patients to other TKI.

P070

FREQUENCY OF COMPLETE MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS: EXPERIENCE OF A SINGLE INSTITUTION

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Imatinib mesylate, at a daily dose of 400 mg, represents the gold standard of care for patients with chronic myeloid leukemia (CML) in chronic phase. The aim of this study is to assess the frequency of Complete Molecular Response (CMR) in CML patients treated with imatinib at standard dose at our institution. All CML patients included in this study had been followed by molecular monitoring for their bcr-abl transcript. Clinical and biological responses were defined according to the ELN 2009 recommendations. 44 CML patients in chronic phase were included in this study. Median age at diagnosis was 57 years (range: 16-81 years) and 48 % was males. 30% of patients had low risk, 50% intermediate risk and 20% hjgh risk according to Sokal. Imatinib alone was administrated as a first line treatment in 71% of patients; 29% of patients started interferon or hydroxyurea treatment before 2000 and then was switched to imatinib. Major molecular response (MMR) with a ratio of BCR-ABL to ABL < 0.1% was observed in 15% of samples available at 6 months, in 37% of samples at 12 months and in 50% of samples at 18 months. Complete molecular response (CMR) with an undetectable bcr-abl was observed in none patients after 6 months of treatment, in 5% of samples at 12 months and in 20% of samples at 18 months. The rate of MMR and CMR at our institution is 76% and 62%, respectively. Patients achieved MMR and CMR at a median of 13 months (range: 3-73) and 35 months (range: 9-62), respectively. In our experience patients who obtained MMR or CMR did not lose CCR during follow-up. Only one patient, who achieved MMR at 36th month, lost MMR at 65th month. In conclusion, imatinib is an highly effective drug as first line therapy for CML patients. In our experience patients who obtained major or complete molecular response have an excellent long-term follow-up.

P071

OUTCOME OF MYELOID LEUKEMIA PATIENTS WITH SUBOPTIMAL RESPONSE AT 18 MONTHS

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Tyrosine kinase inhibitors have changed the approach to the management of chronic myeloid leukaemia. Imatinib mesylate is today the first line therapy for patients with Chronic Myeloid Leukaemia (CML) in chronic phase. In 2009 the European LeukemiaNet published recommendations for the management of patients with chronic myeloid leukaemia, including monitoring and response definition. Patients with a suboptimal response to imatinib may continue on imatinib, at the same or higher dose, or may be eligible for therapy with second-generation TKIs. In this study we analysed the outcome of 14 patients with CML in chronic phase treated with imatinib at standard dose at a single institution who not achieved major molecular response and were considered as a suboptimal responders at 18 months. The molecular response was defined as a ratio of BCR-ABL to ABL. BCR-ABL/ABL >0.1% identified patients with a suboptimal response. Three patients had a BCR-ABL/ABL ratio >1% and were shifted to a second generation TKIs. 11 patients (78%) had a median of BCR-ABL/ABL ratio of 0.17% (range: 0.11-0.84%). The median of follow up of this patients was 90 months (range: 45-109). The 91% of them had achieved RMM after a median of 33 months (range: 19-73) and 55% of patients achieved RMC after a median of 51 months (range 35-62). Only one patient lost MMR at 65th month. Suboptimal response to imatinib represents a "gray zone" and frequently a transitory state in which the best treatment option is under investigation. However in our experience suboptimal responders with a BCR-ABL/ABL ratio <1% may continue on imatinib at the same dose and may obtain a late major or complete molecular response.

P072

REVERSIBLE PULMONARY ARTERIAL HYPERTENSION (PAH) LIKELY RELATED TO LONG-TERM, LOW-DOSE DASATINIB TREATMENT FOR CHRONIC MYELOID LEUKEMIA (CML)

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Under dasatinib treatment for imatinib resistant/intolerant CML, cardiac and pulmonary side effects have been reported, the most common being pleural effusion (PE). We describe a case of severe PAH that resolved after dasatinib discontinuation. In February 2008 a 53 year-old female CML patient switched to dasatinib at the dose of 100mg once daily because of suboptimal response to imatinib 600mg daily. She was a non-smoker and was receiving a calcium-channel blocker and a beta-blocker for systemic hypertension. Baseline ECG was normal and echocardiography showed only minimal mitral valve regurgitation. After 1 mo, the patient complained of fever. After extensive evaluation fever was attributed to a hypersensitivity reaction and dasatinib dose was reduced to 70mg daily. A major molecular response (BCR-ABL/ABL 0.09% IS) was achieved and the patient remained on low-dose dasatinib. In September 2010 (31 mos after starting dasatinib), she complained of breathlessness and progressive exertional dyspnoea. Moderate liver enlargement was detected. Chest-X ray was negative for PE, but showed enlargement of right cardiac image. The ECG showed right bundle branch block and negative T-waves in leads V1-V4. Transthoracic Doppler echocardiography documented normal systolic and diastolic left ventricular function, no intracardiac shunts, an estimated pulmonary arterial pressure (PAP) of 65-70mmHg, and reduced right ventricular function. Contrast-enhanced spiral CT ruled out pulmonary thromboembolism, pulmonary veno-occlusive syndrome and lung parenchymal involvement. Dasatinib was permanently discontinued and furosemide therapy was started. One month later, right heart catheterisation was performed and results were as follows: PAPs/d/m: 53/23/33 mmHg; pulmonary vascular resistance: 6WU; right atrial pressure: 5mmHg; cardiac index: 2.8l/min/mq; the vasodilator test with nitric oxide test was negative. PAH possibly related to dasatinib therapy was diagnosed. Sildenafil therapy was initiated at the dose of 20mg TID. Two months later, right heart catheterisation was repeated and hemodynamic parameters were normal. Improvement in symptomatic PAH after dasatinib discontinuation and before starting Sildenafil is suggestive of an ethiopatological role of the TKI. The occurrence of symptomatic PAH may reflect a chronic off-target mechanism that after an insidious onset might become clinically relevant in the long-term, independently of the dose of the drug and occurrence of PE.

P073

THE GROWTH OF A PH-NEGATIVE, MONOSOMY 7 CLONE ARISEN UNDER SECOND-GENERATION TYROSINE-KINASE INHIBITOR THERAPY IS UNEXPECTEDLY SLOW DOWN BY BCR-ABL IN A CHRONIC MYELOID LEUKEMIA (CML) PATIENT.

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The appearance of clonal abnormalities in the Ph-negative metaphases during tyrosine-kinase inhibitor (TKI) therapy has been recognized in CML pts, but clinical implications of this event have not been assessed. We describe the unusual indolent course of a –7, BCR-ABL negative acute leukemia occurring in a Ph+ CML under second-generation TKI. A 58-year-old CML patient switched from imatinb to dasatinib because of cytogenetic failure at 18 mos. Under dasatinib, he achieved a partial response and a near-major molecular response (BCR-ABL mRNA transcript: 0.5%-0.9% according to International Scale, IS). After 48 mos, he developed grade II cytopenia and monocytosis (2.66x109/L), with 10% peripheral blasts (CD34+ CD117+, CD33+, Cd13+, DR+). Bone marrow biopsy documented 40%-50% of CD34+, CD117+, KP1/CD68+, PGM/CD68R+, MPO-, CD56- blasts. Bone marrow aspiration was consistent with acute leukemia with 50% of atypical promonocytes and blasts. On immunophenotyping 2 blast populations were observed: 26% CD34+, CD117-, CD33+, DR+ blasts and 40% CD34+, CD117+, CD33+, CD14+, DR+, Cd11c+ blasts. BCR-ABL mRNA transcript was 0.5% IS. Conventional karyotype was 46,XY, t(9;22)(q34;q11)[9]/45,XY, -7[11], and FISH on peripheral blood revealed monosomy 7 in 240/300 (80%) of the interphase nuclei (LSI D7S486 (7q31) SO/CEP7 SG probes,

Abbott Mol.) and BCR-ABL rearrangement in 8% of the interphase nuclei (LSI BCR/ABL ES dual color translocation probe, Abbott Mol). Interphase FISH revealed that the abnormal clone was undetectable at CML diagnosis, but was present in 6% of interphase nuclei at the time of switching to dasatinib and progressively increased over time. After dasatinib discontinuation, cytopenia improved and bone marrow blast percentage slightly decreased. At the latest evaluation (8 mos after documentation of overt leukemia), BCR-ABL transcript load was 3,4% and marrow karyotype was 46,XY, t(9;22)(q34;q11)[8]/45, XY, -7[11]/46,XY, del(7)(q31;q35)[1]. Interphase FISH showed that monosomy 7 was present in 199/300 (66%) nuclei and BCR-ABL rearrangement in 18% nuclei. This case shows an unusual discordance between the unfavorable cytogenetic and morphologic data and the indolent clinical course, as the –7, Ph-negative leukemic clone expanded slowly over 4 years under the selective pressure of the second TKI. This patient has refused allotransplant from a full-matched unrelated donor and maintains normal Hb level and neutrophil and platelet counts without any treatment.

P074

NILOTINIB DOES NOT RESULT TO INDUCE MODIFICATIONS OF CHOLESTEROL METABOLISM OR FASTING GLUCOSE LEVEL OF CLINICAL SIGNIFICANCE IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS

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Nilotinib is a second-generation tyrosine kinase inhibitor 30-fold more potent than imatinib, with high affinity and selectivity on BCR/ABL1. Both in resistant patients to imatinib and in newly diagnosed patients, the spectrum of adverse events are similar with few grade 3/4 non-haematological side effects (most commonly headache, skin rash and diarrhoea) and more frequently the occurrence of laboratory abnormalities, such as increased pancreatic enzymes, hyperbilirubinemia and hyperglycaemia. All safety data described were retrieved from sponsorized clinical trial and only few data are known in real life clinical practice. We described for the first time hyperglycaemic metabolic off-target events during treatment with nilotinib as second line treatment in patients resistant/intolerant to imatinib [6]: more prone to observe metabolic side effects we tested in all our CML patients treated with nilotinib total cholesterol, with HDL and LDL fraction, triglycerides, fasting glucose level and lipase at baseline, after 3, 6 and 12 months of treatment. Sixty-two patients receiving nilotinib in our Institution as second line treatment (30 patients, of whom 12 enrolled in phase II and IIIB sponsorized Novartis trial, all treated with 400 mg BID) and 32 as first-line treatment (27 patients enrolled in GIMEMA phase II trial and 5 patients enrolled in phase III ENESTnd trial treated with 400 mg BID, except 2 patients who received 300 mg BID). Median age was 45.9 years (range 22-77.7), 38 were males and 24 females. Median level of total cholesterol at baseline was 187 mg/dl (range 129-306) with only 3 patients having value higher than normal and was not modified during the first year of treatment: median level at 3 months was 195 mg/dl (p=ns), at 6 months 194 mg/dl (p=ns) and at 12 months 197 mg/dl (p=ns). Before nilotinib, case history revealed that 11 patients were affected by hypertension, 12 patients had nicotine consumption and 2 patients had asymptomatic coronary heart disease: although these comorbidities, we did not reveal cerebrovascular accidents or progressive arterial occlusive disease (PAOD) during treatment. At baseline again, only 3 patients had high triglycerides value: median concentration in the overall cohort was 156 mg/dl at baseline (range 52-337), 56 mg/dl at 3 months, 66 mg/dl at 6 months and 74 mg/dl at 12 months (p=ns). Median fasting glucose level at baseline was 98 mg/dl and after a median follow-up of 1.5 years (1-4 years) it was changed to 125 mg/dl (p=0.05). We detected grade 3/4 hyperglycaemia in only 7 patients (11%), but none of these developed severe diabetic consequences. In conclusion, we proved that the drug does not modify prospective cholesterol and triglycerides levels. In conclusion, based on our experience we do not recommend particular attention to screen and negatively select patients with increased risk of metabolic and off-target effects on nilotinib treatment.

P075

DETECTION OF BCR-ABL FUSION PROTEINS BY FLOW CYTOMETRIC BEAD (FC) ASSAY: PREPARATORY RESULTS FOR A PROSPECTIVE MULTICENTER SCREEN STUDY IN CHRONIC MYELOID LEUKEMIA (CML)

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The effectiveness of a simple FC assay (BCR-ABL Protein Kit, BD Biosciences) for detecting BCR-ABL fusion proteins was evaluated in order to organize a prospective multicentre study in CML within the SCREEN cooperative group involving Hematology Centers from Sicily and Calabria. BCR-ABLPOS and BCR-ABLNEG cell lines, peripheral blood (PB) or bone marrow (BM) samples from 18 CML patients (14 on therapy and 4 newly diagnosed cases), samples from 2 pediatric ALL and 5 other disorders were analysed. PB from 10 healthy volunteers were used as controls. FC assay results were compared with those obtained by RT-PCR normalized to BCR-ABLIS. FC assay results using BCR-ABLNEG (HEL, HL60) and BCR-ABLPOS (p210: K562, BV173; p190: SD1) cell lines were concordant with RT-PCR independently of the breakpoint position in the BCR gene. Using serial dilution of K562 cells in HEL cells, the detection limit of the FC assay was determined as >0.1%, also confirmed using parallel frozen lysates thawed after 6 days. We observed protein instability in frozen lysates and DMSO cryopreserved patient samples, with FC signals reduced by 0.05 to 46.9% compared to identical fresh samples, and false negatives seen in some cases. Using fresh CML samples, fusion protein was detectable up to 1% dilution. However, time-course experiments using PB or BM stored at room temperature up to 108h showed progressive decline of FC signal within 48h. Finally, the presence of the BCR-ABL protein was investigated on fresh BM and/or PB from 18 cases of CML. The BCR-ABL protein was detected in 9/18 (all 4 onset cases and 5 cases after 1-12 months of therapy). In these CML follow-up patients with detectable fusion protein, BCR-ABLIS was between 1-10%. For the remaining 9/18 with undetectable fusion protein, BCR-ABLIS was <1%. The remaining patients with other hematological disorders (ET, IMF, CLL, MM, AML) tested negative both in the FC immunoassay and RT-PCR. Of the 2 pediatric ALL cases, 1 was positive in both FC and RT-PCR. We conclude that the BCR-ABL FC assay is an easy technique which detects all types of BCR-ABL proteins with high specificity and sensitivity. FC can be used for routine monitoring of CML patients with BCR-ABLIS above 1%, thus circumventing more elaborate techniques for the initial follow-up phase of the disease. SCREEN multicenter study will establish the validity of this assumption and the relationship, if any, with cytogenetic and molecular analysis.

P076

SYNERGISTIC EFFECT OF PI3K AND TYROSINE KINASE INHIBITORS ON APOPTOSIS AND STRESS ENDOPLASMIC RETICULUM IN K-562 CELLS

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Background: The tyrosine kinase protein (PTK) product of the BCR-ABL fusion gene that results from the t(9;22) translocation of chronic myelogenous leukemia (CML) is an attractive therapeutic target. Our research has shown that lymphomonocytes of CML patients displayed decreased [Ca2+]i fluxes after Myo-inositol (M-IP3) and Adenosine triphosphate (ATP) administration. Aims: The purpose of this study was to verify in K562 cells the activities of Imatinib (IM) and Nilotinib (NIL), selective inhibitors of Bcr-Abl protein or Phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002 (LY) to verify the ability to modulate M-IP3, Thapsigargin or ATP-induced reticulum endoplasmic (ER) stress, apoptosis and cell viability. Methods: [Ca2+]i was measured by using FURA-2/AM. The levels were evaluated on K-562 cells balanced in medium containing 1mM calcium after in vitro incubation with 2 μM Imatinib

or 5 µM NIL or LY or Myo-inositol (M-IP3) or Thapsigargin (TG). Apoptosis was evaluated on K-562 cells after incubation for 18 hours with various drugs assaying the levels of caspase-3. Cell viability was determined by MTT test assay. Results: Our results showed that IM, NIL and LY alone at the used concentrations did not induced any significant change in the levels of [Ca2+]i but significantly reduced the levels of [Ca2+]i M-IP3 or TG or ATP-induced, from 395±48.3 to 210±23.9 (IM+M-IP3) or 279±30.7 nM (NIL+M-IP3), from 317±24.8 to 197±20.1 (IM+TG) or 224±26.5 nM (NIL+TG) and from 239±18.4 to 188±16.6 (IM+ATP) or 208±20.8 nM (NIL+ATP). IM and NIL, in our experiments, were able to increase M-IP3 and TG-induced apoptosis respectively of 77.1% and 76.5% for IM and of 60.9% and 52.1% for NIL while LY did not modify this parameter. Finally, LY increases apoptosis induced from IM and NIL of 82.7% and 82.3% respectively. M-IP3, TG and LY significantly decreased cell viability in IM and NIL treated cells from 57.9% (M-IP3 alone) to 43.4% (IM) and 46.4% (NIL), from 61.8% (TG alone) to 37.3% (IM) and 48.9% (NIL), and from 77.9% (LY alone) to 55.3% (IM) and 64.2% (NIL) respectively. ATP had no effect on cell viability. Conclusion: Our studies have shown that association between inhibitors of Bcr-Abl and of PI3k seems to significantly enhance the activity of IM and NIL on apoptosis and cells viability by inhibition of M-IP3 or TG-induced ER stress. Work supported by grant from: Associazione Italiana contro le Leucemie e i Linfomi (A.I.L.)-Caserta-ONLUS "Valentina Picazio".

P077

IMATINIB INHIBITS AUTOPHAGY M-IP3 AND THAPSIGARGIN-INDUCED IN LYMPHOCYTES OF CML PATIENTS OF FIRST DIAGNOSIS IN CHRONIC PHASE

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Background: Autophagy is a degradative process that in certain contexts can serve as an alternative cell death mechanism named type II cell death, but it is becoming increasingly clear that this process can also act as a cell survival mechanism. Our studies have shown that lymphocytes of CML patients displayed decreased [Ca2+]i. Aims: In present study we evaluated in lymphocytes of patients CML in chronic phase the activity of Imatinib (IM) on the mobilization of [Ca2+]i, autophagy, apoptosis and cell viability induced from M-1,4,5-inositol-triphosphate (M-IP3), thapsigargin (TG), lithium (Li) and rapamycin (RP). Methods: [Ca2+]i, autophagy, cell viability and apoptosis were evaluated on lymphocytes isolated from blood of CML patients of first diagnosis and after in vitro incubation for 18h with 5µM IM or M-IP3 or TG or RP or 25mM Li. [Ca2+]i was measured by using FURA-2/AM. Autophagy was evaluated by immunoblotting by assaying light chain 3 (LC3-I and LC3-II) protein. Cell viability was determined by MTT test assay. Apoptosis was evaluated assaying levels of caspase-3. Results: Our results showed that IM alone did not induce any significant change in the levels of [Ca2+]i but significantly reduces the levels M-IP3 or TG-induced [Ca2+]i whose values decreased from 465±39.8 (M-IP3) to 221±39.5nM (IM+M-IP3) and from 622±43.8 (TG) to 293±55.1 nM (IM+TG) respectively. The basal levels of LC3-II were low in untreated lymphocytes or after treatment with IM but were ~3-fold increased in lymphocytes treated with RP or M-IP3 or Li. Imatinib significantly inhibited TG or M-IP3 or Li-induced autophagy but not rapamicin-induced autophagy. M-IP3, TG and Li induced a significantly reduction in cell viability, respectively of 41.3%, 52.1% and 44.8% while IM and RP did not produce any effect. IM associated to M-IP3, TG and Li, significantly decrease cell viability from 58.7% to 41.7%, from 55.2% to 34.6% and from 55.2% to 42.8% respectively. All substances used alone induced apoptosis and IM increased this effect. Conclusion: Our results show that the induction of endoplasmic reticulum stress by TG and M-IP3 relies on ability of intracellular Ca2+ to induce autophagy and that IM treatment rapidly inhibit this autophagic process. Also inhibition of autophagy induced by IM potentiates cell death IM-induced in lymphocytes of CML patients. Work supported by grant from: Associazione Italiana contro le Leucemie e i Linfomi (A.I.L.)-Caserta-ONLUS "Valentina Picazio".

P078

A SINGLE-CENTRE OUTCOME RESEARCH STUDY ON IMATINIB TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML) LARGELY CONFIRMS THE MAIN RESULTS OF THE IRIS STUDY

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Background. In the last 10 years the management of CML has dramatically been improved by tyrosine kinase inhibitors (TKIs), as indicated by the updated analysis of the seminal IRIS trial (Hochhaus et al, Leukemia 2009). Imatinib (IM) represents the standard 1st line treatment while 2nd generation TKIs are usually reserved to resistant/intolerant patients. Aim. To evaluate single centre, long-term results in a large cohort of CML patients treated with IM. Methods. Data from CML patients treated with 1st line IM were analyzed. Response was evaluated by means of standard cytogenetics every 6 months until complete cytogenetic response (CCyR) and quantitative PCR analysis on blood samples every 3 months until major molecular response (MMolR), and 6-monthly thereafter. Failure, optimal and suboptimal response were defined using European LeukemiaNet (ELN) criteria. Events were failure to achieve hematologic/cytogenetic response or their loss (ELN criteria), IM discontinuation, death for any cause. Patients with suboptimal response at 12 months or later received higher dose IM or 2nd generation TKI. Results. From 2003 to 2010, 89 patients were registered: median age 52 years (range 27-83); 70% male; chronic phase 84 (94%), accelerated/blastic 5; Sokal index low 39 (44%), intermediate 30 (34%), high 19 (21%). After a median follow-up of 4.02 years (range 0.25-8.3), 80 patients (90%) are alive while 9 patients died due to CML progression (3, 3.3%), other cancer (4), cardiac (1) and bone marrow transplantation (1). The 5-year OS is 89%, 97% when considering only CML-related deaths. Nine patients (10%) failed IM, 3 discontinued and 5 died in optimal response for other causes. The 5-year EFS is 77% with no significant difference by Sokal index. The 5-year PFS is 96%, and 97%, 100%, 94% for low, intermediate, high risk Sokal, respectively (p=0.07), with a cumulative incidence of accelerated/blastic phase at 5 years of 4%. Patients with optimal response at 6 months had a significantly better EFS and PFS than patients with suboptimal response although OS was not different (Table). Conclusions. These results confirm the reproducibility of IRIS data in the clinical practice and emphasize the need of an accurate clinical, cytogenetic and molecular follow up to ensure an optimal treatment quality. Patients with suboptimal response at 6 months had a worse outcome than responsive patients and could benefit from an early shift to a 2nd generation of TKI.

	Response at 6 months		
	Optimal (n=69)	Suboptimal (n=11)	
EFS at 5 years (%)	86	51	P=0.0082
PFS at 5 years (%)	100	89	P=0.01
OS at 5 years (%)	92	83	P=0.7
OS at 5 years (%) (only CML-related deaths)	100	100	P=ns

Table. Clinical outcome according to cytogenetic response at six months

P079

RARE BCR-ABL ISOFORMS: MOLECULAR APPROACH TO INVESTIGATE B2A3 REARRANGEMENT IN CML PATIENTS

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Backgroung: Chronic Myeloid Leukemia (CML) can be characterized by the presence of BCR-ABL fusion gene with abnormal onco-protein expression. Most cases of BCR-ABL rearrangement involve the b3a2 (BCR exon 14-ABL exon 2) and the b2a2 (BCR exon 13-ABL exon 2) isoforms with a frequency of 55% and 40% respectively. A remaining 5% of CML cases are due to the uncommon b3a3 and b2a3 forms. A GeneXpert system is routinely used in our laboratory that automates the

processes for the detection and quantification of p210 (b3a2 and b2a2 isoforms). Aim: Our aim was to demonstrate the value of an integrating approach based on rapid and sensible automated analysis together with the qualitative wider range molecular assays, in order to identify also this rare BCR-ABL isoforms that the automated system not recognize. Materials and methods: A single case we would discuss in the present study enters in our transplant centre of the Oncology Department of Businco Hospital, in Cagliari (Italy) with CML clinical feature. Peripheral and bone marrow blood samples were collected and BCR-ABL automated diagnostic analysis was carried out. Results and Conclusions: GeneXpert analysis of this patient gave a negative result for the detection of BCR-ABL RNA transcript. Nevertheless, the result of the successive cytogenetic response demonstrated a t (9;22) chromosomal rearrangement, indicating the presenceone of the most uncommon b3a3 or b2a3 isoforms. Qualitative PCR-nested molecular assay has been done confirming the positivity for the uncommon b2a3 BCR-ABL isoform the automated system is not able to recognize. The present report reviews the need of integration of automated systems with manual diagnostics procedures to avoid false negative results in detecting BCR-ABL positive CML patients. Moreover, we would point-out the commercial lack of standardized and certified diagnostic system to detect and quantify also the rare BCR-ABL isoforms. This integration of diagnostic methods is essential to complete the molecular approach in monitoring all CML patients at onset and during the follow-up.

P080

EFFECTS OF AURORA KINASE INHIBITOR MK-0457 ON H3 POST-TRANSLATIONAL MODIFICATIONS IN CHRONIC MYELOID LEUKEMIA

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The mutated phenotype is one major feature of chronic myeloid leukemia (CML) and has a key role in the disease progression. It is driven by the costitutive tyrosine kinase (TK) activity of Bcr-Abl fusion protein and concurrently mediated by an excess of endogenous DNA damage (due to reactive oxygen species [ROS] increased production) and reduced efficiency of DNA repair (Kim JH et al, Blood 2005; Penserga ETP et al, Oncogene 2007; Naughton R et al, Leukemia 2009). Genomic instability associated with Bcr-Abl is further integrated by impaired cell cycle checkpoint induction (Perrotti D et al, J Clin Invest 2010). Growth arrest DNA damage (Gadd)-inducible 45 proteins (a, b and g) function as stress sensors in myeloid progenitors and are involved in base- and nucleotide-excision repair (Hoffmann B and Liebermann DA. J Cell Physiol 2009). Accordingly, they have a critical role in cell cycle arrest and genomic integrity. In preliminary experiments we found that Bcr-Abl prevents Gadd45a induction in response to stress. Our purpose was to investigate whether epigenetic chromatin modifications associated with p210 Bcr-Abl TK play a role in Gadd45a transcriptional regulation. Aurora kinase (AK) inhibitor MK-0457 (formerly reffered to as VX-680) has been proposed for CML therapy due to its inhibitory activity on either wild type or mutated Bcr-Abl protein (Young MA et al, Cancer Res 2006) and for its effects on AK activity. Here we show that serine 10 at the N-terminal tail of histone H3 (H3S10) is de-phosphorylated and lysine 9 (H3K9) is tri-methylated following AK A and B inactivating de-phosphorylation in response to MK-0457 (100 nM for 24 h) in K562 cell line and BaF3 cell clones stably transduced with Bcr-Abl constructs coding for the wild-type and T315I-mutated proteins. Such nucleosomal histone H3 post-translational modifications promoted the recruitment of Oct1/PUO2F1 transcription factor (the major player of p53-independent Gadd45 induction) at the Gadd45a promoter (Jin S et al, Oncogene 2001). This event drives Gadd45a transcript increase and a consequent restoration of cell cycle control. In conclusion, our results show that MK-0457 triggers an histone H3 methylation/phosphorylation switch relevant for transcription of genes involved in genomic instability of CML.

P081

TET2 "LOSS OF FUNCTION" IS AN INTEGRAL COMPONENT OF CHROMATIN HYPERMETHYLATION AT PROMOTERS OF GENES INVOLVED IN APOPTOSIS RESISTANCE OF CHRONIC MYELOID LEUKEMIA.

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DNA hypermethylation at promoter-associated CpG islands is a common mechanism for the inactivation of tumor suppressor genes in hematopoietic malignancies. It is mainly driven by the recruitment of DNA methyltransferase (DNMT) in complex with other chromatin remodelling factors by oncogenic transcription factors. An integral component of DNMT-mediated DNA methylation is the conversion of 5-methylcitosine (5mC) to 5-hydroxymethylcitosine (5hmC) by the family of Ten-Eleven-Translocation (TET) oxydases ((Dahl C et al, Clin Chim Acta 2011). In chronic myeloid leukemia (CML) TET2 mutations leading to the loss of its catalytic activity are late events associated with the disease progression towards accelerated phase (AP) or blast crisis (BC) (Makishima H et al, Blood 2011). However, the low levels of 5hmC observed in patients with wild-type TET2 suggest the participation of alternative events in the "loss of function" of TET2 associated with Bcr-Abl. Informational spectrum method (ISM), a virtual spectroscopy method for functional analysis of protein interactions based on their sequence, depicted multiple interactions between p210 Bcr-Abl fusion protein and TET2 product possibly involved in TET2 cytoplasmatic compatimentalization and loss of nuclear function (Mancini M et al, Traffic 2008). Such interactions were predicted at residues 544-576 and 1077-1109 in TET2 and 305-329, 428-459, 1175-1192, 1240-1257,1611-1628 and 1644-1661 in p210 Bcr-Abl. They were contingent upon the fusion protein tyrosine kinase (TK) activity, since 24 hr exposure to Imatinib (leading to p210 Bcr-Abl TK inhibition) let the two protein dissociation within the cytoplasmatic compartment and TET2 nuclear import. TET2 re-activation following its release from p210 Bcr-Abl TK was associated with the induction of BCL2-interacting mediator (BIM), a pro-apoptotic protein mediating IM cytotoxicity in Bcr-Abl+ cells (Kuroda et al, Proc Natl Acad Sci USA 2006). BIM downregulation in CML cells depends on promoter hypermethylation and has a role in IM resistance (San José-Eneriz et al, Eur J Cancer 2009). In conclusion, our results expand the knowledge on DNA epigenetic marks of CML as putative therapeutic targets.

P082

ANEMIA IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH IMATINIB AND ON COMPLETE CYTOGENETIC RESPONSE (CCYR) AND MAJOR MOLECULAR RESPONSE (MMOR)

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Introduction Mild anemia (≤ 1 degree WHO) was rarely reported in CML patients treated with imatinib on CCyR and MMoR; Song et al (Leukemia Research, 2009) identified the cause of a mild macrocytic anemia in an imatinib- induced inhibition of c-kit and Larson et al (Blood, 2008) showed that the incidence of anemia (grade 1 - 4) at 5 years of treatment was related to the blood levels of the drug. The aim of this study was the evaluation of the incidence of anemia in a large group of CML patients on CCyR and MMoR. Material and Methods The study population included 104 CML patients, diagnosed, treated with imatinib and followed in our Departments. In each patient, when on CCyR and MMoR, the hemoglobin (Hb) level was evaluated; anemia was defined as an Hb level <13g/dl in male and <12g/dl in female patients; other causes of anemias were excluded with appriopriate tests. In the

anemic patients the Hb levels was related to the Hb levels at diagnosis, gender, age, Sokal score and MCV. Results Mean value of Hb level was 12.5g/dl at diagnosis (13,0 range 9,2-18,8 in male and 11,9 range 6,8-14,8 in female patients); 12.8g/dl on CCyR (13.6 range 11,6-15,6 in male and 11,9 range 9,4-14,0 in female patients); 12.8g/dl on MMoR (13.6 range 11,0-15,3 in male and 12,0 range 9,4-14,2 in female patients). 33% of patients (34/104) were anemic on CCyR (27% in males and 42% in females: Hb range 11.6-12.8 in males and 9.4-11.8 in females); 31% of patients (32/104) were anemic on MMoR (22% in males and 44% in females: Hb range 11.0-12.9 in males and 11,1-11.9 in females); 82% (28/34) of the patients were anemic both on CCyR and on MMoR. There was no significant relationship between anemia and Hb at diagnosis (p 0,201), Sokal score (p 0,52) and MCV (p 0,239). The only correlation was found between anemia and age of male patients (p 0,0001). Mean value of MCV was 95,2 fl (range 82-109) at CCyR and 95,1 fl (range 82-109) at MMoR. 17 patients (9 male and 8 female) showed MCV values > 98 fl. Conclusion Mild anemia was found in a large number of our patients; Hb levels were stable in the period of time from CCyR to MMoR. Our data clearly support the hypothesis that anemia is linked and secondary to imatinib treatment. Further studies are necessary to clarify the exact mechanisms involved in the generation of anemia and the relationship with the blood levels of imatinib.

P082

PROGNOSTIC EVALUATION ACCORDING TO THE HAMMERSMITH AND MDACC SCORES OF CML PATIENTS RESISTANT TO IMATINIB BEFORE TREATMENT WITH SECOND-GENERATION TKIS

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The Hammersmith group proposed a prognostic score aimed at the early identification of CML patient sensitivity to second generation TKIs. The score, created on a cohort of 80 patients and validated on a small series of 28 patients, was based on 3 factors: previous cytogenetic response to imatinib, Sokal risk and recurrent neutropenia during imatinib. Recently, another prognostic score was proposed by MDACC group, which was based on performance status > 1 at the time of start of second-line treatment and lack of any previous cytogenetic response to imatinib. Aim of our study was to confirm the validity of these scores and to establish their strength on a large group of CML patients resistant to imatinib and treated with second generation TKIs. One hundred twenty-seven patients were collected from 6 different Italian hematologic centers. There were 66 males and 61 females, median age 54 years (range 25-80). Twenty-seven patients had received interferon before imatinib. Thirty patients had primary resistance to imatinib, whereas 97 patients had secondary resistance (39 patients were treated with nilotinib and 88 with dasatinib). Data availability allowed the application of the Hammersmith score was possible in 118 patients who had available data: 52 patients were identified as good risk, 27 patients as intermediate risk and 38 patients as poor risk. The 1-year cumulative incidence of complete cytogenetic response (CCR) was 73% in good risk patients, 40% in intermediate risk patients and 22% in poor risk patients (p=0.0001). Similarly, the cumulative incidence of major molecular response (MMR) was 52% in good risk, 28% in intermediate risk and 13% in poor risk category (p=0.001). Estimated 2-year event-free survival (EFS) - considered as loss of hematologic or cytogenetic response, disease progression, death for any cause, toxicity - was 89% in good risk subjects, 70% in intermediate risk and 54% in poor risk patients (p=0.0001). Two-year estimates for progression-free survival (PFS) - defined as survival without evidence of accelerated or blastic phase - were 95% in good risk individuals, 93% in intermediate risk and 87% in poor risk patients (p=0.05). Kaplan Meier estimated 2-year overall survival (OS) was 100% in the good risk, 93% in the intermediate risk and 82% in the poor risk category (p=0.001). With MDACC score we identified 28 patients as low risk, 55 as intermediate and 41 as high risk. The score applied to our series of patients was able to identify different 1-year probability of achieving MCyR (90%, 72%, 53%, respectively p=0.001) and 3-year EFS (97%, 80% and 61%, p=0.001). However, we did not find substantial differences in 3-year OS within the stratified patients (100%, 96%, 85%) or in 3-year PFS (100%, 91% and 88%). In conclusion, some prognostic factors before starting second generation TKIs might predict cytogenetic response and outcome. Our results indicate that the proposed models can identify patients with low probabilities to achieve optimal responses to dasatinib and nilotinib and with poor EFS, but MDACC score has limitations in predicting OS and PFS in the cohort of high-risk patients.

P084

PROTEIN SIGNALLING TRIGGERED BY IMATINIB AND DASATINIB IN THE MYELOID BLASTIC CRISIS OF CML: AN IN VITRO PROTEOMIC AND PHOSPHOPROTEOMIC STUDY

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Background: Imatinib resistance (IM-R) developing in approximately 30% of Chronic Myeloid Leukemia (CML) patients (pts) is due to point mutations in the BCR-ABL kinase domain and amplification of the BCR-ABL gene locus or to improper activation/inactivation of BCR- ABL-independent signaling pathways. Aims: We describe preliminary results of a study employing immortalized CML cells - either sensitive or resistant to IM - to perform Reverse Phase Protein Micro-Arrays (RPMAs) aimed at characterizing the proteomic profiles and at identifying BCR-ABL-independent pathways that contribute to the development of IM-resistant CML. Methods: RPMA is a reproducible, high-throughput system for protein signaling pathway profiling. The phosphorylation state of kinase- associated therapeutic targets provides direct information regarding the target and off-target effects of different drug treatments. Human CML cell lines, sensitive (K562S, LAMA84S) and resistant (K562R, LAMA84R) to Imatinib, were incubated with IM 1uM, Dasatinib (DAS) 1uM or LY-294002 10uM (LY) used as a control. After 2 or 12 hours, cells were placed in a preservative that suppresses fluctuations in kinase pathway proteins. RPMAs were used to quantitatively map 45 cell signaling pathway endpoints, including autophagy, DNA repair systems, DNA damage and transcriptional factors crucial for CML. Results: Previous evidence has demonstrated that K562R are unresponsive to IM because of unknown BCR-ABL-independent mechanisms, while LAMA84R display BCR-ABL genomic amplification. Compared to their sensitive counterpart, K562R exhibited a paradoxical reduction in BCR-ABL signaling at BCR(Y177), CRKL(Y207), ERK(T202/Y204) and Cofilin(D59), associated with over-expression of autophagy markers (ATG5 and LC3B). Conversely, LAMA84R presented increased BCR-ABL signaling at BCR(Y177), CRKL(Y207) and ERK(T202/Y204). In drug treatment endpoints, DAS was confirmed stronger than IM in abrogating BCR-ABL auto-phosphorylation on BCR(Y177). We found HDAC3 induction in both K562R and LAMA84R and this event was positively associated with increased expression of c-MYC and NUMB and higher phosphorylation on mTOR(S2445), pRb(S608), CHK1(S345), FOXO1(T24), FOXO3a(T32), but not on BCR(Y177) or c-ABL(Y245). Conclusions: Taken together, our data confirm the value of the RPMA assay to investigate improperly activated pathways that could identify one or more potential targets for future treatment strategies of IM- resistant pts.

CELL THERAPY AND ALLOGENEIC TRANSPLANTATION I

P085

CD133+ STEM CELLS FOR THE TREATMENT OF END-STAGE LIVER DISEASE

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Previous studies have shown that bone marrow (BM) cells contribute to liver regeneration after tissue injury. The main objective of the present study was to evaluate the feasibility and the safety of the purification and intrahepatic reinfusion of increasing numbers of autologous BM-derived G-CSF-mobilized CD133+ stem cells (SCs) in patients with end-stage liver disease. For this purpose, G-CSF at 7.5µg/Kg/b.i.d. is administered subcutaneosly (sc) from day 1 until the completion of peripheral blood stem cells (PBSC) collection. Collection of PBSC begin on day + 5 only if the concentration of CD133+ cells is 8/µL. CliniMacs device is used for the positive selection of CD133+ SCs (under GMP conditions) from PB of mobilized standard-volume leukapheresis. At least 4 weeks after SC mobilization, collection and cryopreservation, highly purified autologous G-CSF-mobilized CD133+ cells are re-infused through the hepatic artery by transfemoral or transbranchial arteriography. CD133+ cells are administered to patients starting from 5x104/Kg patient's body weight and increased every 3 patients up to 1x106/kg. G-CSF at 5µg/Kg/day is administered sc for 3 days after the reinfusion of SCs for their expansion and to induce a selective proliferative advantage of reinfused cells in vivo. Biological assays (phenotype of circulating SCs, clonogenic assays, serum cytokines) were done during the mobilization and re-infusion phases together with the phenotypic characterization of the isolated CD133+ SCs. The clinical trial is ongoing. Up to date, six patients have been successfully mobilized with G-CSF and highly purified autologous CD133+ SCs have been re-infused in 5 cases. Based on preliminary data, we suggest the feasibility and safety of intrahepatic reinfusion of highly purified CD133+ stem cells in patients with end-stage liver disease. Biological studies show that: 1) circulating hematopoietic and endothelial progenitors are increased after G-CSF treatment; 2) highly purified CD133+ cells express hematopoietic and endothelial markers; 3) serum concentration of HGF, SDF-1, VEGF and MMP9 and clonogenic capability of hematopoietic progenitors is increased during the mobilization and re-infusion phases; 4) clonogenic potential of endothelial progenitors show variable expression.

P086

RETARGETING OF CITOKINE-INDUCED KILLER (CIK) CELLS WITH MOLECULARLY ENGRAFTED T-CELL RECEPTORS (TCR): A PRECLINICAL STUDY

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Cytokine Induced Killer (CIK) cells are a heterogeneous population of T lymphocytes sharing NK phenotype and functional properties: they are CD3+/CD56+ and have a potent MHC-unrestricted antitumor activity. We hypothesized that the therapeutic potential of CIK cells might be increased if they acquired the ability to recognize MHC-restricted tumor associated antigens. To this end, we transduced CIK cells with an HLA-A2 restricted T-Cell Receptor (TCR) directed against the melanocyte associated antigen Mart-1. PBMC were incubated with IFN-on day 0 and supplemented with anti-CD3 and IL-2 on day +1 to generate CIK cells. Cultures were transduced at day 4 with concentrated lentiviral particles and successfully expanded over a 4 week period. This allowed to generate CIK cells that contained 13±9% Mart-1 TCR positive cells, as detected by staining with a Mart-1 specific tetramer. Transduced CIK cultures contained 61±19% CD3+/CD56+ cells. Tetramer positive cells were both CD3+/CD56- and CD3+/CD56+ (31±8% and 60±9%, respectively), suggesting that both MHC-restricted T-cells and MHC-unrestricted CIK cells could be targeted by lentiviral transduction. TCR-transduced CIK cells specifically recognized tumor cells presenting the relevant peptide and maintained their MHC-unrestricted tumor activity at the same time. The cytotoxic activity of Mart-1 redirected CIK against HLA-A2+ melanoma cell lines was 2.8 fold higher than the untransduced counterparts (62%±9 vs 22±6% lysis), while the cytotoxic activity against a Mart-1+/HLA-A2- melanoma cell line was similar in transduced and untransduced CIK cells (24%±8 vs 22±6% lysis), indicating that the increased activity was due to HLA-restricted recognition. This was confirmed by blocking experiments with an HLA-Class I antibody. At the end of the culture, the majority of both unmodified and transduced CIK cells expressed an effector memory phenotype, with few residual central memory cells. In TCR redirected cells there was a slight but significant increase of cells with a naive phenotype compared to unmodified cells (34±7% vs 21±5%). These data suggest that the naive and central memory pool of redirected CIK cells might efficiently expand in vivo and support a long lived memory response, whereas the terminally differentiated pool might mediate short lived but potent MHC-restricted and unrestricted activity. We are now validating these in vitro data in a humanized mouse model and generating a new TCR directed also against hematologic tumors.

P087

HOW TO IMPROVE ADOPTIVE T CELL IMMUNE-GENE THERAPY: THE IMPORTANCE OF BE(GINN)ING NAÏVE

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Immunotherapy with engineered T cells is attractive for its promise to provide high- avidity tumor specific lymphocytes to cancer patients. Unfortunately, current protocols of ex vivo T cell manipulation often induce terminal differentiation, resulting in poor persistence and limited activity of the transferred cells. We previously showed that costimulation and culture with g-chain cytokines generate gene-modified T cells with a functional central memory (TCM) phenotype superior to effector/effector memory (TEM) counterparts for expansion potential and antitumor activity. Here we investigated the consequence of initial targeting of selected T cell subpopulations on the ultimate function of engineered cells. We activated and efficiently transduced FACS-sorted T Naive (TN), TCM and TEM cells by both RV and LV. Manipulated TN showed the highest expansion potential (mean fold increase: TN 40, TCM 10, TEM 5). Strikingly, activation of TN resulted in a predominant population (80%) of CD45RA+CD62L+CCR7+ cells. This population did not produce IFNg nor cytotoxic molecules, and required IL7 and IL15 for its generation and maintenance. In contrast to naturally occurring unmanipulated counterparts, CD45RA+CD62L+CCR7+ manipulated-TN were post-mitotic, expressed CD45RO and memory markers (IL2-Rb, CXCR3, CD95). Compared to manipulated TCM and TEM, naïve-derived T cells expressed higher levels of IL7-Ra, c-Kit and CXCR4 and lower levels of CCR5, HLA-DR, PD-1. To verify the differentiation potential upon antigen stimulation, TN, TCM and TEM were transduced to express a WT1-specific TCR and stimulated with the cognate peptide in presence of IL2. In this context, TN greatly expanded, differentiated into CD45RA+CD62L- and CD45RA-CD62L- cells and were unique in the ability to generate a mixed population of CD127± lymphocytes. When infused in immunodeficient mice, manipulated TN showed higher engraftment and persistence than memory counterparts, reconstituted a mixed CD45RA±CD62L± phenotype and displayed a degree of xenoreactivity comparable to that of unmanipulated lymphocytes. To test the self-renewal ability, we performed a serial transplantation assay. We found that only manipulated TN cells engrafted in secondary recipients and expressed IL7-Ra. Our results indicate that gene transfer into TN cells, when manipulated according to our protocol, might increase the therapeutic effect of immune-gene therapy by combining target specificity with the most extensive self renewal.

P088

HIGH-AVIDITY CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR A NEW PEPTIDE DERIVED FROM PREFERENTIALLY EXPRESSED ANTIGEN OF MELANOMA (PRAME) CAN TARGET LEUKEMIC PRECURSOR CELLS.

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The cancer testis antigen (CTA) PRAME is overexpressed by many hematological malignancies, but is absent on normal tissues, including hematopoietic progenitor cells, and may therefore be an appropriate candidate for T-cell mediated immunotherapy. The sensitivity of CML to donor lymphocyte infusion after allogeneic stem cell transplantation suggests this leukemia can be highly susceptible to cellular immunotherapy targeted to tumor associated antigens. We therefore tested whether functional PRAME-specific cytotoxic T lymphocytes (PRAME CTLs) could be generated and expanded from healthy donors and leukemia patients, or whether the limited immunogenicity of this CTA coupled with tumor-associated anergy would preclude this approach. Using optimized culture conditions and HLA-A*02-restricted PRAME-peptides, we have consistently generated PRAME CTLs from 8/9 healthy donors and 5/6 CML patients. These CTLs released IFN in response to PRAME peptides (between 113±8 and 795±23 spot forming cells/10^5 T cells) and lysed PRAME peptide-loaded cells (45±19% at an effector:target [E:T] ratio of 20:1) in a MHC-restricted fashion. Importantly, these CTLs recognized and had cytotoxic activity against HLA-A*02(+)/PRAME(+) tumor cell lines, and could recognize and respond to primary CML cells. Although clonal analysis showed these cells could have high TCR-peptide avidity, the majority of expanded PRAME CTLs were characterized by low peptide avidity. Thus, we attempted to generate high avidity PRAME-specific CTL by using professional and artificial antigen presenting cells (APCs) loaded with a peptide-library spanning the entire PRAME protein and consisting of 125 synthetic pentadecapeptides, overlapping by 11 amino acids. We successfully generated polyclonal PRAME-specific CTL lines, and elicited high avidity CTLs, with a high proportion of cells recognizing a previously uninvestigated HLA-A*02-restricted epitope P435-9mer (NLTHVLYPV). These PRAME-CTLs could be generated both from normal donors and from subjects with PRAME+ hematologic malignancies. The cytotoxic activity of our PRAME-specific CTLs was directed not only against leukemic blasts but also against leukemic progenitor cells as assessed by colony forming inhibition assays, which have been implicated in leukemia relapse. Of note, these PRAME-directed CTLs do not affect normal hematopoietic progenitors indicating that this approach may be of value for immunotherapy of PRAME+ hematologic malignancies.

P089

RISK-ADAPTED TRANSPLANT SELECTION DRAMATICALLY IMPROVES OUTCOME OF YOUNG ADULT PATIENTS WITH HIGH RISK AML: RESULTS OF A PILOT STUDY

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Treatment of adult with AML still remains controversial for at least two reasons: 1) risk-category allocation based on cytogenetic/genetic findings may fail to predict relapse in individual patients; 2) carrying out allogeneic SCT (ASCT) in all high-risk patients is hampered by the paucity of candidates (25-30%) with a full matched family donor. Minimal residual disease (MRD) promises to be a strong predictive factor since it may contribute to refine genetic/cytogenetic risk assignment. We designed a risk-adapted therapeutic strategy for patients <60 years where risk stratification was determined on the basis of the genetics/cytogenetics and post-consolidation flow cytometric MRD status. High-risk patients [unfavorable karyotype (K) or FLT3-ITD positive or MRD positive] were addressed to ASCT whatever the source of stem cells: matched sibling (MSD), matched unrelated (MUD), haploidentical related donor (HRD) or umbilical cord blood (UBC). For comparison, we analyzed the outcome of a matched historical cohort of high-risk patients whose transplantation choice was oriented according to family donor availability: ASCT for those with MSD and autologous stem cell transplant (AuSCT) for those without. The prospective cohort included 23 high-risk patients (4 unfavorable K, 6 FLT3-ITD, 4 MRD+ favorable K and 9 MRD+ intermediate K); 19 received ASCT after consolidation (4 relapsed early). The historical cohort accounted for 59 high-risk patients (1 unfavorable K, 10 FLT3-ITD, 8 MRD+ favorable K, 33 MRD+ intermediate K and 7 MRD+/unknown cytogenetic) of whom 14 received ASCT, 28 AuSCT, 12 relapsed early and 5 were not transplanted for medical reasons. After a median follow-up of 20 months, survival estimates of the prospective cohort were dramatically superior as compared to the historical one (DFS 73% vs 15%, p=0.011; OS 69% vs 20%, p=0.020; CIR 21% vs 76%, p<0.001). We believe that the improvement in survival estimates of the prospective cohort is determined by the higher percentage of patients who were submitted to ASCT [19/23 (83%) vs 14/59 (24%), p<0.001]. Notably, in the prospective cohort, only 8/19 patients had MSD whereas in 5 and 6 a HRD or MUD/UCB was found. We conclude that for young patients with AML, decision of giving or not ASCT should rely on a "transplant vs no transplant" rather than "donor vs no donor" strategy and, once high-risk patients are identified, transplant should be accomplished in due time evaluating all possible stem cell sources.

P090

INCIDENCE, OUTCOME AND RISK FACTORS OF LATE-ONSET NON INFECTIOUS PULMONARY COMPLICATIONS AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION.

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Object: We retrospectively evaluated incidence, risk factors and outcome of late-onset non infectious pulmonary complications (LONIPCs) among adult patients with haematological malignancies who underwent reduced intensity conditioning (RIC) allogeneic SCT at our center from 2002 to 2009. Patients and methods: Of the 177 consecutive RIC transplants, we evaluated 124 patients who survived at least 3 months after transplantation. The diagnosis of LONIPCs was based on clinical evaluation, high-resolution tomography scan, pulmonary function tests and, if possible, histological findings. Results: At a median time of 14 months after transplant (range 5-43), 11/124 patients (9%) fulfilled the diagnostic criteria of LONIPCs and were subclassified as having bronchiolitis obliterans (6), bronchiolitis obliterans with organizing pneumonia (2) and interstitial pneumonia (3). Overall, 4/11 patients (36%) obtained a partial response after first-line therapy with steroids alone or combined with cyclosporine or mycophenolate mofetil (2) and after salvage treatment (2). The remaining 7 patients were unresponsive to salvage treatments (imatinib, rituximab, mycophenolate mofetil, photophoresis); six of them died after a median of 6,5 months mainly due to respiratory failure. Relapse rate of primary disease was significantly lower in LONIPC than in non-LONIPC patients (0% vs 43%, p=0.002) and TRM rate was significantly higher (64% vs 16%, p=0.001); the 5-year OS was similar in LONIPC and non-LONIPC patients (43% vs 48%, p=0,82). Univariate analysis showed that in vivo T-cell depletion (used in unrelated transplants) was a protective factor (OR 0.17, 95% CI 0.03-0.85; p=0.03) while moderate-severe chronic GvHD was a risk factor for the occurrence of LONIPCs (OR 16.9, 95% CI 2.0-136.7; p=0.008). Conclusions: We observed an incidence of 9% of LONIPCs after RIC transplants, which is not inferior to that reported after myeloablative transplants. In spite of a higher TRM rate, the overall survival of LONIPC patients was similar to that of non-LONIPC patients, because of a low relapse rate. Our study confirms the strong association of LONIPCs with moderate-severe chronic GvHD and indicates that in vivo T-cell depletion can prevent the occurrence of these lung complications.

P091

INTRABONE CORD BLOOD TRANSPLANT: PRELIMINARY RESULTS FROM A PROSPECTIVE PHASE II STUDY.

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Intrabone transplantation has been described as an efficient way to infuse cord blood. Here we report on the first 10 patients undergoing to intrabone transplantation for advanced haematological malignancies. The study was a phase II prospective monocenter study approved by local ethical Committee and registered at http://clinicaltrials.gov/ ; the primary endpoint was engraftment rate and 17 patients are planned. All patients signed a written informed consent. End of recruitment is planned within april 2011. Conditioning regimen was myeloablative (Bu- Cy or unfractioned TBI-Cy); prophylaxis of GVHD was CsA, micophenolate 30 mg/kg/die and ATG-F 30 mg/kg, total dose. G-CSF was routinely given from Day +7 until recovery. CB processing was as described by Frassoni et al. Briefly, cord blood units were thawed, washed with the Rubinstein solution to remove DMSO and reduced the final volume up to 30 ml. Infusion was performed in operating room using a monitored anaesthesia care sedation with propofol and remifentanil. Median age was 36 years (29-54), median weight of recipient was 60 kg (51-93); diagnosis were AML (7) ALL (1) MM (1) CML (1). Phase at transplant was mainly advanced (for AML 5 with resistant/relapsed disease, 2 II CR; for ALL 1 II CR; MM: 1 progressive disease and CB for CML). 3 patients had a previous allotransplant and 3 a autotransplant. All CB units were 4/6 except for one (5/6). Median total cell infused was 1.91 x 10e7/kg and median CD34 pos cells 0.52 x 10e5/kg. Median time to 0.5 x 10e9/L ANC was 21 days and median time to 20x 10e9/L and 50 x10e9/L platelets were 46 and 60.5 days, respectively. At day +100 the evaluable patients had 121x 10e9/L plt (range 104-192). Two patient died before engraftment for CNS bleeding (+6) or for myocarditis (+16). One patient didn't graft and a second unit, again via intrabone, was given, after 2 months and with a RIC regimen, with successful engraftment. All the evaluable patient achieve a complete response (CR), except for the MM pt, who obtain a nCR); two patients relapse at 4 and 7 months from transplant. GVHD occurrence was very low: no severe acute GVHD and only one case of extensive GVHD were recorded. Preliminary results of this study with advanced disease suggest that intrabone injection of CB resulted in short term good engraftment, expecially for platelets, low GVHD and good outcome. Longer follow up is needed to estimate the actual antileukemic effect.

P092

A SALVAGE TREATMENT WITH NEW DRUGS FOLLOWED BY REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA RELAPSED AFTER AUTOGRAFTING

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Background and objectives. The use of allogeneic stem cell transplantation (allo-SCT) with reduced intensity conditioning (RIC) in relapsed multiple myeloma (MM) is controversial due to the mortality and the morbidity related to the procedure and the availability of novel antimyeloma drugs. We investigated prognostic factors and outcome of RIC allo-SCT in MM patients who relapsed after autologous SCT and were then treated with a salvage therapy based on novel agents. Patients and methods: Sixty-eight patients, median age 53 years, were retrospectively evaluated in a multicenter study. Median time between auto-SCT and relapse was 15 months (2-87). Salvage treatment was thalidomide-based (38 patients), bortezomib-based (24) , lenalidomide-based (6), and achieved 23 complete remissions (CR) + very good partial remission (VGPR) (33%) and 32 partial remissions (PR) (47%). Twenty-four (35%) had an HLA identical sibling donor and 44 (65%) had an unrelated donor. Fifty-seven patients (84%) received peripheral stem cells. Most used preparative regimens consisted of fludarabine, melphalan ± thiotepa (28 patients) and fludarabine + 2 Gy TBI (24 cases). Results: Two-year non-relapse-mortality (NMR) was 22%. Two-year progression-free-survival (PFS) and 2-year overall survival (OS) were 38%, and 55%, respectively . Grade II-IV acute GVHD and chronic GVHD occurred in 28 (41%) and 21 (39%) evaluable patients, respectively. Thirty patients (44%) showed relapse or progression and received one or more new drugs (21 patients) ± donor lymphocyte infusions (DLI) (9 patients). The observed median OS after relapse post-allo-SCT was 4,5 months. At a median follow-up of 29 months after allo-SCT , 27 patients (40%) maintained a clinical objective response , 9 of them achieved again a response to new drugs ± DLI after they had progressed post allo-SCT. In multivariate analysis chronic GVHD significantly prolonged OS (HR 0.11; 95% CI, 0.17-0.68, p=0.02), whereas a longer time between diagnosis and allo-SCT was significantly associated with poor OS (HR 1.07; 95% CI, 1.01-1.13, p=0.02). Conclusions: These results suggest that an earlier timing of allo-SCT and new transplant strategies that will incorporate novel agents before and after allo-SCT could improve clinical results of RIC allo-SCT in relapsed MM.

P093

GENERATION OF HUMAN THIRD-PARTY UMBILICAL CORD MESENCHYMAL STEM CELLS LINES ENGINEERED TO ALLOW NONINVASIVE IN VIVO TRACKING BY PET AND INCREASE IMMUNOSUPPRESSIVE CAPACITY IN THE TREATMENT OF GRAFT-VERSUS-HOST DISEASE

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La graft-versus host disease (GvHD) causa circa il 20% della mortalità correlata all'allotrapianto di cellule staminali ematopoietiche. Nella GvHD acuta grave resistente agli steroidi, studi clinici di fase II hanno mostrato l'efficacia delle cellule staminali mesenchimali umane (hMSC) da sangue midollare di terze parti[1]. Tuttavia in non tutti i casi descritti le hMSC sono state in grado di trattare la GvHD, ed il monitoraggio della loro biodistribuzione richiede ad oggi biopsie tissutali. Una fonte alternativa di hMSC (immediatamente disponibile all'uso e che non richiede il coinvolgimento del donatore di cellule staminali ematopoietiche) è rappresentata dalle hMSC dalla gelatina di Wharton del cordone ombelicale (hUCMSC)[2]. Viene qui descritta la metodica di validazione cGMP di linee di hUCMSC di terze parti ad aumentata potenza immunosoppressiva e monitorabili in vivo in modo non-invasivo. Linee di hUCMSC verranno trasdotte col vettore lentivirale LAW34 recante il gene della IL-10 del virus EBV (che a differenza della IL-10 umana non ha alcuna attività immunostimolante residua[3]) e dal gene della timidina chinasi del virus herpes simplex. Ques'ultimo permette, dopo somministrazione sistemica di analoghi dell'aciclovir marcati con 18F, di monitorare in vivo vitalità e biodistribuzione delle hUCMSC mediante PET[4]. Tali linee transgeniche mantengono identica capacità differenziative e di inibizione delle reazione linfocitaria mista, garantendo latrasferibilità in una sperimentazione clinica di fase I. BIBLIOGRAFIA : 1. Le Blanc, K., et al., Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet, 2008 371(9624): p. 1579-86. 2. Petsa, A., et al., Effectiveness of protocol for the isolation of Wharton's Jelly stem cells in large-scale applications. In Vitro Cell Dev Biol Anim, 2009 p. Jul 16. 3. Min, C., et al., IL-10-transduced bone marrow mesenchymal stem cells can attenuate the severity of acute graft-versus-host disease after experimental allogeneic stem cell transplantation. Bone Marrow Transplant, 2007 39(10): p. 637-45. 4. Buursma, A., et al., 18F-FEAU as a radiotracer for herpes simplex virus thymidine kinase gene expression: in-vitro comparison with other PET tracers. Nucl Med Commun, 2006 27(1): p. 25-30.

P094

ALLOGENEIC BONE MARROW TRANSPLANTATION FROM UNRELATED DONORS IN MULTIPLE MYELOMA: AN ITALIAN BONE MARROW TRANSPLANTATION DONOR REGISTRY (IBMDR) STUDY.

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To evaluate the role of allografting from unrelated donors in the treatment of myeloma we conducted a retrospective study through the IBMDR. Overall, from 2000 through 2009, 196 myeloma patients, median age 51 years (32-67), for a total of 199 allografts, were transplanted from an unrelated donor at 34 Centers in Italy. Fifty-two (28.1%%), 69 (37.3%), and 64 (34.6%) patients were prepared for transplant with a myeloablative, a reduced-intensity and a non-myeloablative conditioning respectively. In 14 transplants the conditioning was not reported. Patient characteristics of the 3 cohorts are reported in Table 1. Overall, the cumulative incidence of transplant related mortality (TRM) was 29.6% at 1 year and 32.4% at 5 years post-transplant. Incidence of acute grade II-IV graft-versus-host-disease (GVHD) was 46.4% whereas chronic GVHD was 45.1%. There was no difference in GVHD incidence among the 3 cohorts defined by type of conditioning. Complete and partial remissions in patients who survived at least 3 months post-transplant were 27.1% and 28.1%. At a median follow up of 32 (0-118) months post-transplant, median OS from diagnosis was 70.6 months while OS and EFS from the allograft were 18.9 and 14.9 months, in the entire study population. OS from diagnosis and EFS from transplant were 70.6 and 28.2 months; 66.8 and 9.1 months; and 111.9 and 22.4 months in patients who respectively underwent a myeloablative, a reduced-intensity and a non-myeloablative transplant. One-year and 5-year TRM was 33.3% and 35.7%, 32.2% and 34.4%, and 22.1% and 26.5% respectively. Overall, by multivariate analysis, higher doses of CD34-pos cells in the graft (HR 0.52 and 0.60), limited (HR 0.42 and 0.44) or extensive (HR 0.35 and 0.45) chronic graft-vs.-host disease (GVHD) were significantly associated with both longer OS and EFS. Acute GVHD was associated with both poorer OS (HR 2.89) and EFS (HR 2.96) whereas age > 50 years (HR 1.61) was correlated with worse EFS. In conclusion, with a degree of caution given the retrospective nature of this study, there appears to be a strong association between both limited and extensive chronic GVHD and graft-vs.-myeloma effects. However, long term disease control remains an issue independent of the conditioning employed. Prospective trials may allow to define which patient category may most benefit from an unrelated donor allograft.

Conditioning	Myeloablative	Reduced-intensity	Non-myeloablative
Patient number (%)	52/185 (28)	69/185 (37)	64/185 (35)
Median Age	45	53	55
Previous therapy lines < 2 (%)	23 (27)	33 (38)	30 (35)
Previous therapy lines > 2 (%)	29 (29)	36 (37)	34 (34)
Stem Cell Source BM (%)	24 (57)	18 (43)	0 (0)
Stem Cell Source PBSC (%)	28 (19)	51 (36)	64 (45)

Table 1

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MICRORNA-OME OF CELL PRODUCTS DERIVED FROM DIFFERENT DONOR SOURCES SUCH AS APHERESIS AND BONE MARROW.

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Rationale. A large body of publications has provided compelling evidence that microRNAs are involved in immune homeostasis including innate and adaptive responses. Aim. In this study we wondered if exist differences among microRNA-ome of product cells derived from different donor sources such as apheresis (HPC-A) and bone marrow (HPC-BM). Material. Five samples HPC-BM was collected from 5 donors. Five HPC-A samples were obtained from healthy volunteers mobilized with G-CSF. MirNAs were extracted from unfractionated units. Equal amounts of miRNA from each sample were mixed to generate two different miRNA pools: HPC-BM and HPC-A. TaqMan Low Density Arrays (Applied Biosystem) were used for expression profiling of 384 miRNAs. A microRNA of Universal Sample (Stratagene) was considered as calibrator. Results. We observed that there were 3 different miRNA expression patterns between HPC-A and HPC-M: 1° subset with identical number of copy, 2° with expression level different for +1 log, and 3° with opposite expression. From the immunological point of view, our data showed interesting interpretations. For example, mir-21, -25, -27b, -100, -140, -194, -155 were all expressed as in second subset. All these were involved in innate immune responses during recognition of the "non self" from "self". However, the interpretation in immune control is not easy because other miRNAs (mir-146a and mir-125b) involved negatively in innate immune regulation showed opposite behaviours in our samples. In fact, both were not expressed in HPC-BM indeed they were high in HPC-A. The miRNAs -181 and-223 showed the same number copies in HPC-A and HPC-BM. They are very important actors in immune network because they regulate central elements of adaptive immune response such as T-cell receptor signalling. As previous reported, miR17-92 cluster, mir-150, mir-155, mir-181, mir-223 are highly expressed in immune cells and have permissive function in maturation of myeloid and lymphoid cells. In our experimental setting, all these showed high expression values suggesting that both donor products exhibit similar potential properties to obtain a complete engraftment. Conclusions. It's likely that this study is just the tip of the iceberg in terms of the involvement of regulatory RNAs in orchestrating immune response. However, it's may suggest that apheresis and bone marrow have different immune properties. In future it could image therapeutic interventions based on microRNA manipulation.

miRNAs ID	miRNA pools/Apheresis	miRNA pools/Bone Marrow
hsa-let-7c	4,30E+07	5,26E-02
hsa-miR-107	3,14E+07	2,85E-02
hsa-miR-125b	2,54E+07	1,25E-01
hsa-miR-128	6,20E+06	6,01E-01
hsa-miR-130a	3,16E+07	7,08E-03
hsa-miR-135a	1,14E+07	5,24E-02
hsa-miR-135b	1,32E+07	7,53E-03
hsa-miR-142-3p	1,02E+07	6,75E-01
hsa-miR-146a	1,92E+07	7,13E-01
hsa-miR-195	1,00E+06	7,03E-03
hsa-miR-193b	2,39E+06	3,02E-01
hsa-miR-19b	1,30E+07	8,43E-01
hsa-miR-212	2,08E+07	4,46E-01
hsa-miR-22	1,95E+07	2,69E-03
hsa-miR-296-5p	5,39E+07	5,17E-01
hsa-miR-29b	2,02E+07	3,99E-01
hsa-miR-330-3p	1,03E-01	4,81E+07
hsa-miR-335	9,59E+06	2,85E-02
hsa-miR-32	2,08E+07	1,94E-02
hsa-miR-339-5p	5,70E+07	4,25E-03
hsa-miR-34a	2,28E+07	1,17E-01
hsa-miR-34c-5p	2,06E+07	4,61E-01
hsa-miR-370	3,75E-02	6,87E+06
hsa-miR-374a	2,05E-03	3,97E+06

hsa-miR-410	1,29E+07	3,60E-01
hsa-miR-423-5p	5,23E+07	7,51E-01
hsa-miR-425	1,47E-03	4,51E+07
hsa-miR-486-5p	1,16E+07	9,52E-01
hsa-miR-99a	4,80E-03	1,79E+07
hsa-miR-502-5p	2,39E+06	4,37E-03
hsa-miR-517a	1,25E+07	9,36E-01
hsa-miR-519d	6,67E-01	5,29E+06
hsa-miR-548b-5p	2,50E-01	1,47E+07
hsa-miR-548d-3p	5,65E-01	3,13E+07
hsa-miR-574-3p	2,40E-01	8,21E+06
hsa-miR-636	9,83E-01	1,74E+06
hsa-miR-660	1,66E-03	8,14E+06
hsa-miR-671-3p	8,22E-03	7,98E+07
hsa-miR-885-5p	6,89E+00	2,84E-02
hsa-miR-886-3p	1,28E-01	4,28E+07
hsa-miR-539	2,08E-02	7,69E+06

Table 1. Espression level of representative microRNAs with opposite values between apheresis and bone marrow samples

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INTERMEDIATE DOSE OF ANTI HUMAN THYMOCYTE IMMUNOGLOBULIN (ATG) REDUCE TRANSPLANT RELATED MORTALITY WITH NO IMPACT ON THE RELAPSE RATE OF ACUTE LEUKAEMIA PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A SINGLE CENTRE REPORT.

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Background The addition of anti human thymocyte immunoglobulin (ATG) to GVHD prophylaxis results in decreased incidence of acute and chronic GVHD after allogeneic haematopoietic stem cell transplantation (AlloHSCT). However, its use may delay T- cell reconstitution, prolong susceptibility to opportunistic infections and increase leukaemia relapse. The main purpose of this study was to evaluate retrospectively the impact of different doses of ATG on overall survival (OS), cumulative incidence of relapse (CIR) and transplant related mortality (TRM) of acute leukaemia patients undergoing AlloHSCT from both sibling and unrelated donors following a myeloablative conditioning regimen. Patients and methods We analysed 156 consecutive patients (median age 37,5) with acute lymphoblastic (n= 62) or myeloblastic (n= 94) leukaemia who underwent alloHSCT between May 1994 and August 2010. For GVHD prophylaxis, a conventional Cyclosporin A and Methotrexate program (with no ATG) was given to the first 72 patients, while ATG (Genzyme, Italy) at >5 mg/kg was added in 40 patients and at the dose of 5 mg/kg in 44. The donor was a related sibling in 78 or unrelated in 78 patients. The disease status at transplant was complete remission (CR) in the majority of cases (CR1= 81, CR > 2 = 29) and active disease in 46 cases. All patients (pts) underwent myeloablative conditioning (71% TBI based). The stem cell source was bone marrow in 38 pts and peripheral blood in 118 pts. Results and conclusions With a median follow-up of 17 months (4-186), for the whole cohort of pts the 5 years OS is 60% for whom receiving ATG at a dose of 5 mg/kg compared to 48% and 44% for pts receiving ATG >5 mg/kg or no ATG. In the subgroup of MUD transplant (n=78) the 5 years OS is 76% , 44% and 43% respectively in the three groups (ATG=5 mg/Kg, ATG > 5mg/Kg and no ATG) (p=0.046). In this analysis, the use of ATG does not increase the risk of leukaemia. A comparable higher risk of TRM is seen in patients who either received no ATG or ATG at more than 5mg/kg. For acute leukaemia pts an appropriate dose of ATG may reduce TRM with no detrimental effect on the relapse rate and thus with an overall benefit on survival, that in MUD setting is statistically significant.

	CIR 5y	p	TRM 5y	p	OS 5y	p
ATG						
None	35	NS	30	NS	44	NS
> 5 mg/kg	37		26		48	
= 5 mg/kg	36		14		60	

Table .

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ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT IN RELAPSE/REFRACTORY HODGKIN LYMPHOMA: A SINGLE CENTER EXPERIENCE.

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Hodgkin lymphoma (HL) can be cured in more than 90% of early-stage and about 75% of advanced-stage patients with conventional chemotherapy. Thus, only a minority of patients became candidate for allogeneic stem cell transplant (allo-SCT), however it remains the only curative option for refractory HL. We report a retrospective analysis of 33 relapsed or refractory HL patients, median age 33 (range 18 - 55) years, treated with reduced intensity (n 26/33, 79%) or myeloablative (n 7/33, 21%) allo-SCT between May 2000 and December 2010. Donors were HLA identical siblings (n 13/33, 39%) or unrelated (n 20/33, 61%). Twentynine/33 (88%) patients received more than 2 lines of treatment before alloSCT, and 25/33(76%) patients had chemorefractory disease at the time of transplant. Thirtyone/33 (94%) patients received at least one autologous-SCT, and 23/33 (70%) were treated with radiotherapy before allo-SCT. The cumulative incidence of non-relapse mortality (NRM) was 12,3% at 12 months from transplant. All NRM events occurred in patients treated with reduced intensity conditioning. The cumulative incidence of any grades acute GVHD was 43% at day 100, whereas that of overall chronic GvHD was 52% at 400 days. After a median follow-up of 48 months (range 1 – 106), median OS and EFS were respectively not reached and 23 months. Among patients with OS longer than 46 months (n=12, 36%), 8/12 (67%) were disease-free. With the limitation of a small sample size no difference in terms of OS and EFS were detected between the conventional and reduced intensity conditionings. Allo-SCT is a feasible option in heavily pre-treated relapsed or refractory HL patients with low NRM, and can induce durable clinical remissions. Furthermore, this study suggests graft-vs-HL effect exists and gives rise to long term disease control.

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UNMANIPULATED HAPLOIDENTICAL BONE MARROW TRANSPLANTATION FOR ADVANCED HEMATOLOGICAL MALIGNANCIES WITH HIGH DOESE CYCLOPHOSPHAMIDE (CY) AS GVHD PROPHYLAXIS

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High dose, post- transplantation CY, promotes tolerance of alloreactive host - donor T cells and is effective as prophylaxis of GVHD. The safety and efficacy of post-transplant CY has been shown in the haploidentical related donor setting , following non myeloablative (NMA) conditioning. We now report our experience on 31 patients with advanced disease who underwent related haploidentical T replete bone marrow transplantation, following both NMA or myeloabaltive (MA) conditioning. The diagnoses were AML (n=7), ALL (n=7), CML BC (n=3), MPD (n=1), MDS (n=1), HD (n = 12). The first nineteen patients were prepared with a MA regimen including thiotepa , fludarabine and busulfan (TFB) . The 12 patients with Hodgkin Lymphoma received a NMA regimen (CY, fludarabine and TBI 200 rads). GVHD prophylaxis consisted of CY (50mg/kg intravenously) on days + 3 and + 5, MMF 2 gr /day and Cyclosporine. At transplantation, median age was 38 years (range 18 –66). Most patients (20/31: 64%) had refractory or active disease at BMT, 14 had failed autologous BMT and 5 patients were receiving a second allogeneic BMT. Two patients died of hemorrhage before day 10. Of the 29 evaluable patients all except one engrafted with 100% donor chimerism by day +30.Hematological recovery was complete and stable in all patients. The median time to neutrophils recovery (>500/mcl) and platelets recovery (> 20.000/mcl) was respectively +19 and +25 days from BMT. GVHD was scored as grade I in 7 patients and grade II in one patient. Grade III – IV GVHD was absent. Chronic GvHD was absent. Non relapse mortality was 16%: 2 patients died for cerebral hemorrhage, 1 for interstitial pneumonia, 2 for sepsis. Three patients died in disease relapse. The median follow up was 127 days from

BMT(range 58 – 589). The OS and DFS was respectively 68% and 58%. In conclusion our experience confirms that , following NMA or MA conditioning, high dose post transplant CY is effective 1) as prophylaxis of GVHD after haploidentical BMT 2) can prevent rejection with complete hematologic recovery and 3) non relapse mortality is acceptable for a group of advanced patients.

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NK AND NK-T CELL RECONSTITUTION FOLLOWING NON T CELL DEPLETED, G-CSF PRIMED, HAPLOIDENTICAL BONE MARROW TRANSPLANTATION

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NK cells favorably influence the outcome of myeloid leukemia patients (pts) undergoing allogeneic stem cell transplantation (SCT).The molecular mechanism underlying the GVL activity of NK cells is due to KIRs/HLA class I disparity between donor/recipient pairs. T cell depleted haploidentical SCT is the setting where NK cells are more likely to mediate GVL effects while the role of NK cells in the outcome of non T cell depleted haploidentical bone marrow transplantation (BMT) is unclear. Here, we evaluated the immunological reconstitution of 18 pts undergoing non T cell depleted, G-CSF primed, haploidentical-BMT focusing on NK and NK-T cell populations. In the peripheral blood (PB) of healthy donors (HD), 2 classical NK cell types (CD16+CD56+CD3- and CD16-CD56+CD3-) and 3 distinct populations of NK-T cells including CD16-CD56+CD3+, CD16+CD56-CD3+ and CD16+CD56+CD3+ were identified. To evaluate the phenotypic reconstitution of the indicated cells, their absolute number (per micro liter) was taken before, 30, 60, 90,180,≥360 days post-BMT and compared with that obtained in the PB of 9 HD. Classical T cells were examined as control. The number of CD16+CD56+CD3- NK cells was not significantly lower than that detected in the PB of HD (182±58) at 30 (123±85), 60 (145±195), and 90 (173±6) days post-BMT while returned to pre-BMT levels (55±14) at 180 (85±50:P=0.001) and 360 days (94.5±76:P=0.02) post-BMT.CD16-CD56+CD3- NK cells quickly recovered after BMT, reaching a significantly higher pick than that observed in HD (39.1±31) at 30 (183±156:P=0.02) and 60(159±140:P=0.01) days while returned to normal levels at 90(92±85), 180(54±34) and 360(47±53) days post BMT. In contrast, the number of NK-T and T cells was deeply depressed even after 360 days post-BMT. Further studies showed that IL-2 stimulation induced in early and normal CD16+CD56+CD3- NK cells an equivalent level of CD16 upregulation and perforin content. Allo-ML-2 efficiently triggered CD107A translocation in IL-2 stimulated early and normal NK cells either in CD16+CD56+CD3- or in CD16-CD56+CD3- cells suggesting that early NK cells may have efficient killing machinery. In conclusion, after non T cell depleted, G-CSF primed, haplo-BMT, potentially competent NK cells rapidly repopulated PB while NK-T and T cell regeneration was deeply impaired. These results may help understand a possible GVL phenomenon in non T cell depleted,G-CSF primed, haplo-BMT.

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HAPLOIDENTICAL HEMOPOIETIC STEM CELL TRANSPLANTATION: THE HIGHER MODULATING EFFECT OF GRANULOCYTE COLONY-STIMULATING FACTOR ON BONE MARROW IMMUNE CELL POPULATIONS

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Background. Unmanipulated G-CSF-primed bone marrow transplantation from haploidentical donor represents an innovative strategy for patients lacking an HLA identical sibling and the preliminary results in 80 patients transplanted for hematological malignancy are particularly encouraging. Associated to the clinical protocol, this in vitro study has been addressed to evaluate the effects induced by 7-days G-CSF treatment (4mcg/Kg/day) on hematopoietic and immune cell populations in either bone marrow (G-BM) or peripheral blood (G-PB). Results. The results are reported in the table. The effects of G-CSF on hematopoietic compartment were more impressive in G-PB than in G-BM with an increase of CD34+ cells over the baseline levels respectively of 4.5 and 3.2 fold and of the hematopoietic progenitors (CFU) of 10.4 fold in G-PB vs 4.7 fold in G-BM. Conversely, the immune cell fractions resulted highly up-regulated in BM than in PB. G-CSF treatment significantly enhances the CD4+CD25+ (p=0.014) and CD4+CD25+ 127- (p=0.035) T cell subsets in BM, whereas, in PB, the absolute number of T-reg cells remained unchanged. The monocytes were significantly increased either at BM or PB levels. As to the dendritic cells, G-CSF induced an increase of both myeloid (DC1) and plasmocytoid (DC2) cell fractions in BM, whereas in PB a significant rise was detected only for DC1 cells. A striking rise (15 fold, p<0.001) of mesenchymal progenitors (CFU-F) was detected in BM after G-CSF priming. Finally, IL-10 sera levels were significantly increased after G-CSF priming (1.6±0.3 vs 2.7±0.3, p=0.001). Conclusions. The increase of T-regulatory cells, DC2 cells and mesenchymal progenitors, which was higher in BM than in PB after 7-day G-CSF, together with the increasing level of IL-10, allows to speculate about the specific mechamisms of immunotolerance contributing to hematopoietic engraftment and GVHD modulation in the setting of haploidentical BM transplant. These biological data are consistent with our clinical results showing high engraftment rate and low incidence of acute and chronic GVHD in unmanipulated G-CSF-primed bone marrow transplant from haploidentical donor.

Cell Subpopulations	BM vs G-BM		PB vs G-PB	
	fold increase	p	fold increase	p
CD34+	3.2	<0.001	4.5	<0.001
CFU	4.7	<0.001	10.4	<0.001
CFU-F	15.2	<0.001	NT	
CD4+25+	2.3	0.014	1.8	NS
CD4+25+127-	2.8	0.035	1.2	NS
CD14+	4.9	<0.001	3.3	<0.001
DC1	5.6	0.004	5.1	0.021
DC2	3.1	0.018	1.6	NS

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UNRELATED ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THALASSEMIA: 15 YEARS OF EXPERIENCE OF THE ITALIAN GROUP "GITMO"

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Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for patients with thalassemia major. However, most candidates for HSCT do not have a suitable donor within the family and therefore need to consider transplantation from an unrelated donor. In this study, we report the outcome of patients with thalassemia who underwent allogeneic HSCT from an HLA-matched unrelated donor in one of the four major Italian Centers performing unrelated HSCT for hemoglobinopathies. Patients and Methods: 124 patients (72 males and 52 females) were transplanted in 4 different Italian Centers between 1996 and 2010. The median age of the patients at the time of HSCT was 10.5 years (range 1-35). Donors were selected using DNA high-resolution typing of both HLA Class I and Class II loci. Forty patients (32%) were classified in risk class 1; 39 (31%) in risk class 2 and 43 (35%) in risk class 3 of the Pesaro classification system; 26 of the patients in risk class 3 (21%) were adults (>18 years). The conditioning regimens were BU/CY in 14 pts, BU/FLU/TT in 33 pts, TREO/FLU/TT in 35 pts and BU/CY/TT in 41 pts. Results: Sixteen patients (13%) experienced either primary or secondary graft failure. Nineteen patients (15%) died of transplantation-related causes. Grade II–IV acute graft-versus-host disease (GVHD) developed in 37 cases (35%), and chronic GVHD in 16 cases (16%). Overall survival (OS) in the cohort of 124 patients was 85%, whereas the Kaplan–Meier estimates of disease-free survival (DFS) with transfusion independence was 74%. In the group of

79 thalassemia patients in risk classes 1 and 2, the probabilities of OS and DFS were 93% and 83%, respectively. Conclusions: The results of unrelated allogeneic HSCT observed in our cohort of thalassemia patients were comparable to those of patients transplanted from HLA-matched sibling donors. These date confirm that unrelated HSCT for thalassemia is an acceptable procedure provided that high resolution DNA typing methods are used for donor selection.

P102

FECAL CALPROTECTIN AS BIOMARKER OF GVHD AFTER ALLOGENEIC STEM CELL TRANSPLANTATION.

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Graft-versus-host disease (GHVD) is one of the main complications after allogeneic stem cell transplantation (SCT). Since there are no validated laboratory test for GvHD, confirmed clinical suspicion requires tissue biopsies. Recently, some authors focused their attention on the proteomic pattern of biologic samples obtained by patients (pts) with GHVD with the aim to identify a sensitive and specific marker of GVHD activity, with a predictive and prognostic value. For this purpose, we focused our attention on fecal calprotectin (FC), a dimeric S100 (A8-A9) protein, widely studied in inflammatory bowel diseases. We studied 39 pts (M/F 20/19, median age 49 years, 17-56) receiving SCT. Underlying diseases were: 1 plasma cell leukemia, 1 HL, 5 ALL, 2 CLL, 18 AML, 5 NHL, 1 MF and 6 MDS. Stem cells source were peripheral blood 37 pts, bone marrow in one case and cord blood in another one. Graft was performed by a sibling donor in 22 cases and by a matched unrelated donor in 17 cases. A reduced intensity conditioning was performed in 27 cases and myeloablative regimen was used in 12 cases. GvHD prophylaxis was performed with Ciclosporine A (CSA)+micophenolic acid in 18 cases, only CSA in 3 cases, CSA+ short course of methotrexate in 18 cases. Anti-thymocyte globulin and Campath-1H were added in 6 and 3 cases respectively. Stools samples were collected montly after SCT and FC was measured by a quantitative enzyme immunoassay. We considered the threshold of 100 mg/Kg to subdivide pts in two groups: pts with high or low fecal calprotectin levels. Seventeen pts developed aGvHD at a median time of 25 days (range 10-97 days) after SCT (43.6%) (grade I in 1 case, grade II in 4 cases, grade III in 6 cases and grade IV in other 6 cases), and 7 pts (41%) presented a gastro-intestinal involvement (GI). The onset of cGvHD was detected in 15 pts (51.7%): classic chronic GvHD in 6 pts and an overlap syndrome in 9 pts. Two pts (13.3%) presented a mild form, 3 pts (20%) a moderate form and the other 10 pts (66.7%) a severe form. GI involvement was seen in 8 pts (53%). Our data suggest that FC level at day +30 after SCT was a useful marker of aGvHD (p=0.049) distinguishing GI involvement vs other organ involvement (p=0.02), and correlated with the aGvHD onset time after SCT (p=0.027, Figure 1). Later after SCT, FC median value within the first 100 days after SCT was able to predict cGvHD development (p=0.03).

Figure 1 Fecal calprotectin levels at day +30 vs time of aGVHD onset

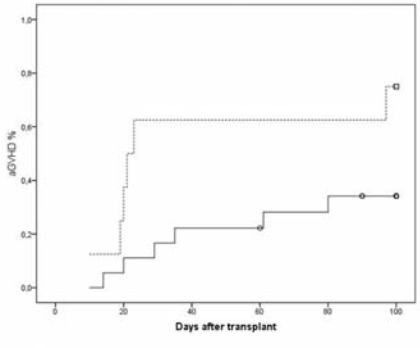

Figure 1. Legend:
□ patients with fecal calprotectin at day +30 >100mg/kg
○ patients with fecal calprotectin at day +30 <100mg/kg

HEMOSTASIS AND PLATELETS

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SUCCESSFUL TREATMENT OF SEVERE GASTROINTESTINAL BLEEDING AFTER CHEMOTHERAPY IN ACUTE MYELOBLASTIC LEUKEMIA WITH RECOMBINANT ACTIVATED FACTOR VII

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Haemorrhage is a frequent complication in patients with acute leukemias. Gastrointestinal (GI) bleeding frequently occurs due to the chemotherapy-induced mucosal damage and represents a life-threatening condition with a high rate of mortality. Patients are generally managed with red blood cell transfusion, platelet suspensions, fresh frozen plasma; and sometimes with pharmacologic and endoscopic interventions. If these therapeutic measures fail, patients might be treated with haemostatic drugs. Recombinant activated factor VII (rFVIIa) is an effective haemostatic agent originally developed for the treatment of bleeding episodes in patients with haemophilia and inhibitors. Recently, unlicensed use of rFVIIa has provided benefit in many other bleeding situations unresponsive to conventional therapy. These include the management of critical bleeding in patients with haematological malignancies. We present our experience on the management of 9 patients (age range 46-55) who had GI bleeding during induction therapy for acute myeloid leukaemia (AML) (5 FAB-M5, 3 FAB-M2, 1 FAB-M1) using low doses of rFVIIa. At the time of GI haemorrhage, neither patient had evidence of disseminated intravascular coagulation (DIC) or prior bleeding diathesis (by history or by pre-chemotherapy measurements of PT and APTT). Patients received a median dose of 30μg/Kg of rFVIIa (range 20-50μg/Kg) and a median number of 4 injections (range 2-6). The mean total dose of rFVIIa administered was 150μg/Kg. Bleeding stopped in all patients. In most patients, the response was achieved within 4-6 h from the administration of a single dose of rFVIIa. All patients demonstrated clinical improvement with stabilization of haemoglobin and a marked reduction in blood product support requirement. There were no evidences of DIC, thrombotic complications and other drug-related adverse events. Our experience indicates that rFVIIa might be a useful treatment alternative in the management of severe GI bleeding in patients with acute leukaemia. Even if its high cost may be a concern, it should be considered that low doses can be administered and further doses might be escalated if needed. Of course, further experiences should evaluate the efficacy and safety of rFVIIa as a single haemostatic agent in the setting of refractory GI haemorrhage and as part of standard first line therapy for GI bleeding in patients with leukaemia.

P104

MOTOR DISABILITY IN THE SETTING OF ORAL ANTICOAGULANT THERAPY: A CROSS-SECTIONAL STUDY

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Introduction. Oral Anticoagulant Therapy (OAT) is a widely applied measure to prevent thrombotic events in a broad group of diseases. OAT is chronically administered and its management requires a close monitoring of prothrombin time (PT) and of the international normalized ratio (INR), given its narrow therapeutic range, in order to balance the risk of hemorrhage and thrombosis. Although PT/INR can be tested by a healthcare professional or by the patients themselves, the majority of individuals on OAT are usually followed by an hematological specialized center for both analysis and OAT management. Therefore, the need of hospital visits may be concerned by the complex burden of frailty and disability afflicting many OAT patients, although the the incidence of these findings in this setting is unknown. Aims and Methods. In order to address this issue, we performed a cross-sectional evaluation of the motor disability (MD) in a group of consecutive outpatients on OAT, by handling in a MD assessment questionnaire at the moment of therapy schedule delivering. MD was assessed using Barthel Index (BI), which was used as basic ADL ability scale, and was classified as mild (BI>66%),

moderate (BI: 33-66%) and severe (BI<33%). Results. There were 132 patients with median age was of 65 (20-76) years. Disability was present in 51/132 (39%) patients. Out of the 51 disabled patients, the motor impairment was mild in 30 (59%), moderate in 16 (31%) and severe in 5 (10%) of them. The frequency of BI items reduction revealed that complex activities, such as ascending and descending stairs, moving on level surface, dressing and transferring from chair to bed, are the most frequently impaired basic ADL in disabled OAT patients. Conclusions. Although preliminary and related to a limited series of patients, our data provide new insights on a neglected issue, such as disability, regarding the management of OAT. Indeed, MD is a frequent feature, but extended data analysis is required to achieve a better understanding about the disablement process, onset, causes, related risk factors and progression. A targeted rehabilitative approach to prevent and treat disability may lead to positive effects on both patient's quality of life and caregiver work-load. Moreover, home care management for physically impaired patients on OAT may be a suitable option, although requiring a trained healthcare professionals team and adequate resources.

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RECOMBINANT ACTIVATED FACTOR VII (RFVIIA) TREATMENT FOR SEVERE BLEEDING IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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AML is frequently complicated by severe bleeding episodes, that can be unresponsive to standard therapy and life threatening. Unlicensed use of rFVIIa (Novoseven,Novo Nordisk) for severe and resistant bleeding in hemato-oncological diseases proved to be beneficial suggesting a potential role of this product in the management of life threatening haemorrhages in non haemophilic pts. From January 2008 to May 2010, 5 AML pts(1F/4M, median age 53, range 20-72) experienced life threatening haemorrhages during(2 pts)and after(3pts)intensive induction chemotherapy. Median PLTs count was 10.000/mm3 (range 4000-16.000,median INR was 1.44 (range 1.20-1.65) and all pts were resistant to standard antihaemorrhagic therapy. 4 pts had gastrointestinal haemorrhages(2 melena,2 rectal bleeding)confirmed by endoscopy; 1 pt had hemothorax confirmed by CT scan and treated successively by surgery (pleural drainage. The dose of rFVIIa administered was 90 microg/Kg every 6-8 hours for a median number of 9 doses (range 8-15),associated to PLTs transfusions and tranexamic acid infusion. In 3 pts INR was >1.5 and FPP (20ml/Kg/die) was transfused. The criteria to stop the rFVIIa administration were: end of haemorrhages; reduction in RBC transfusion requirements; clinical recovery. Three pts (2 gastrointestinal haemorrhages, 1 hemothorax) had a complete response fulfilling all the above criteria; 2 pts failed to respond to rFVIIa treatment: 1 underwent gastrectomy and died of fatal ischemic stroke and one die for uncontrolled haemorrhagic shock. Of the 3 responding pts 2 experienced thromboembolic episodes: 1 succlavian vein thrombosis and 1 non fatal pulmonary embolism after 21 days from rFVIIa treatment and concomitant with a rapid increase in PLTs count(PLTs >1.000.000/mm3). In our experience, rFVIIa administration was beneficial,with rapid clinical recovery and bleeding stop in 3/5 AML pts. As for the 3 thrombosis related complications,only the succlavian vein thrombosis,occurred during rFVIIa administration, can be considered treatment related, even if it was associated with a indwelling line; fatal stroke and non fatal polmonary embolism occurred 7 and 20 days after the stop of rFVIIa respectively. To date the use of rFVIIa in non haemophilic pts is unlicensed and the administration schedule is not yet well defined, therefore, to better evaluate the beneficial role of rFVIIa treatment in AML pts with resistant life threatening haemorrhages, and the role of additional pro-thrombotic risk factors in the development of thromboembolic complications, a larger study is needed.

P106

PREVALENCE OF ACQUIRED VON WILLEBRAND SYNDROME (AVWS) IN A SMALL COHORT OF PATIENTS AFFECTED BY MONOCLONAL GAMMOPATIES (MG)

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Background Acquired von Willebrand syndrome (AVWS) is a rare bleeding disease similar to the congenital von Willebrand disease (VWD) in terms of clinical and laboratory findings, being characterized by bleeding episodes and low plasma levels of FVIII-von Willebrand factor (VWF) activities. Lymphoproliferative and myeloproliferative disorders appear to be the most frequently associated with AVWS in both literature and the registry, accounting for 48-63% of cases: among these monoclonal gammopathies (MG) are highly present . AIM Prevalence evaluation of AVWS in patients with monoclonal gammopaties (MGUS, MM, WD) and examination of the effective correlation between Bleeding Severity Score and AVWS diagnosis. PATIENTS and METHODS From September 2010 to March 2011, a cohort of 60 consecutive patients with monoclonal gammopaties (31 M and 29 F, age 31-87) followed at day-hospital and outpatient care of our Division was enrolled. 39/60 were affected by MGUS (25 IgG, 3 IgA, 7 IgM, 4 with >1 component); 20 patients were affected by multiple myeloma (16 IgG, 2 IgA and 2 micromolecolar). 1 patients was affected by light chains disease. The questionnaire for Bleeding Severity Score evaluation (validated for congenital VWD1) was administered to the cohort of patients. The patients were evaluated for APTT and FVIII/VWF activities. Results 59/60 of the patients showed normal or increased values of VWF activities: VWF:Ag median 153 (range 49-366) U/dL. Only one patient with IgGk-MGUS showed reduced levels of FVIII/VWF activities: FVIII: 22, VWF RCo 4, VWF:Ag 19 U/dL. Such laboratory abnormalities was clearly associated with bleeding events as shown by the increased bleeding severity score calculated after the analysis of the questionnaire (BSS: 6). Bleeding episodes were non-mucocutaneous (surgical ematoma and post-traumatic hemarthrosis) and occurred before diagnosis requiring PRBC transfusions. Conclusions We confirm that AVWS is a relatively rare bleeding disorder but can be diagnosed sometimes in patients with MG only when an accurate bleeding history and lab test for hemostasis are investigated. BSS is useful to identify patients at high risk of bleeding. Based on this small cohort study, AVWS should be suspected and searched for with appropriate clinical and laboratory parameters in MG patients showing minor bleedings and before surgery or invasive procedures.

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PLASMA-DERIVED PROTEIN C FOR TREATING DISSEMINATED INTRAVASCULAR COAGULATION IN ADULT PATIENTS WITH ACTIVE CANCER

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Introduction. Cancer-related disseminated intravascular coagulation (DIC) is a life-treating condition without effective treatment, mainly based on fresh-frozen plasma. Protein C (PC), a modulator of the coagulation as well as the inflammatory system, has been successfully tested (in its activated recombinant [a-rPC] form) in sepsis-related coagulopathy but with increase for major bleeding. Plasma-derived PC (pd-PC) is more suitable than a-rPC in patients at high risk for bleeding, such as cancer patients, since its self-limiting process in activating anticoagulation. Aim. To evaluate the role of pd-PC in adult cancer patients with DIC. Materials and Methods. Adult cancer patients affected by DIC and PC plasma concentration < 50%, were treated with PC concentrate (Ceprotin®, Baxter) in adjusted doses to restore normal PC values (70-120%). Clinical outcomes (bleeding, thrombosis and mortality) were recorded up to a follow-up of 28 days. Blood coagulation and haematological tests as well as DIC score were recorded after 12, 24, 48 hours, 7 and 10 days. Results. Over a period of 3 years, 22 patients with advanced cancer (16 with solid and 6 with haematological neoplasm) were evaluated. Within 48h from the beginning of pd-PC therapy, all laboratory tests normalized as well as the DIC score. No bleeding or thrombosis was observed; baseline PC levels were lower in non-survivors than in survivors although this difference was non-significant. Overall mortality at 28 days was 22.7%. Conclusions. Our investigation shows that pd-PC therapy is safe and normalizes laboratory variables in cancer patients with DIC.

Table 2. Changes in laboratory findings obtained from all patients during the study period (mean ± SD)

	Baseline	24 h	48 h	72 h	7th day	14th day
PC (%)	27.3 ± 7.1	71 ± 15.6*	85.9 ± 12.5*	91.2 ± 11.6 *	92.2 ± 13.4 *	99.1 ± 13.5 *
WBC (×10⁹/L)	8.2 ± 3.1	7.8 ± 2.2	6.5 ± 1.9	6.7 ± 1.5	7.3 ± 1.5	8.1 ± 0.6
Platelet (×10⁹/L)	49.3 ± 20.4	51.2 ± 19.4	71.2 ± 33.4	91.7 ± 41.1	113.4 ± 65.1	154.8 ± 109.2*
d-Dimer (µg/L)	2.133.6 ± 1.643	2.366 ± 1.561	1.230 ± 1.045 *	800.2 ± 686 *	350 ± 225*	541 ± 246*
Fibrinogen (g/L)	2.1 ± 1.4	2.8 ± 1.1	3.6 ± 1.5	4.4 ± 1.4*	4.5 ± 1.2*	4.2 ± 1.3
PT (%)	46.4 ± 11.5	46.2 ± 12.1	51.8 ± 13.8	63.3 ± 15.2	65.4 ± 0.9*	69.7 ± 14.3 *
aPTT (s)	40.1 ± 13.4	34.8 ± 7.6	35.4 ± 6.1	33.4 ± 6.1	32.9 ± 7.5	31.2 ± 3.6*
AT (%)	54.2 ± 12.2	61.6 ± 23.3	73.4 ± 21.4	77.7 ± 22.2 *	80.6 ± 16.5 *	87.1 ± 18.5 *
DIC score	6.26 ± 1.12	5.38 ± 1.42	4.26 ± 0.96	3.16 ± 0.98 *	2.97 ± 0.87 *	2.21 ± 1.43 *

*$P < 0.05$ versus baseline

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THE IMPACT OF VENOUS THROMBOEMBOLISM IN CRITICALLY ILL PATIENTS: A META-ANALYSIS OF MAJOR CLINICAL OUTCOMES

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Background: Critically ill patients appear to be at high risk of developing deep vein thrombosis (DVT) and pulmonary embolism during their stay in the intensive care unit (ICU). Although frequently unrecognized, the clinical consequences of DVT have the potential to be serious in the ICU. However, little is known about the clinical course of this disease in the ICU setting. Purpose: To evaluate whether a diagnosis of DVT affects length of hospital and ICU stay, duration of mechanical ventilation and mortality in critically ill patients. Material and Methods: MEDLINE and EMBASE databases were searched up to June 2010. Two reviewers, independently, performed study selection and extracted data. Association between DVT and hospital and ICU mortality, and duration difference of hospital and ICU stay, and of mechanical ventilation in patients with and without DVT were calculated using a random-effects model. Pooled results were reported as relative risk (RR) and weighted mean difference (WMD) and were presented with 95% confidence interval (CI). Results: Seven studies for a total of 1518 patients were included. Patients with DVT had increased ICU and hospital stay compared to those without DVT (WMD 7.3 days, 95% CI 1.4, 13.2; P= 0.02 and 16.5 days, 95% CI 1.51, 30.59; P= 0.03 respectively). Duration of mechanical ventilation was not significantly increased in DVT patients (3.41 days 95% CI 1.12, 7.94; P=0.14). DVT patients had a marginally significant increase risk in hospital mortality (RR 1.31 95% CI 0.99, 1.74,P=0.06), and a non statistically significant increase risk in ICU mortality (RR 1.96; 95% CI 0.74, 5.19; P = 0.17). Conclusions: A diagnosis of DVT upon ICU admission appears to affect clinically important outcomes including length of ICU and hospital stay and hospital mortality. Larger prospective studies are warranted to verify our preliminary results.

Outcomes

Study	Duration of mechanical ventilation in days (DVT vs No DVT)	Hospitalization length in days (DVT vs No DVT)	ICU Stay in days (DVT vs No DVT)	Hospital mortality rate (DVT vs No DVT) [95% Confidence Intervals]	ICU mortality rate (DVT vs No DVT, n.) [95% Confidence Intervals]
Ibrahim 2002	18.9±19.7 vs 14.6±12.9 p=0.310	31.4±21.7 vs 27.5±18.2 p=0.375	18.6±14.6 vs 15.9±1.04 p=0.388	8.9 (34.6%) vs 26.8 (32.1) p=0.815	n/a
Velmahos 1998	Not given^	49±32 vs 31±24 p=< 0.05	34±31 vs 19±18 p=<0.05	n/a	31% (8) vs 18%,(31) P= 0.04
Major 2003	n/a	n/a	n/a	n/a	17% (2) vs 2% (15) p=0.03
Cook 2005	9** (4,25)* vs 6 (3,13)* p=0.03	51** (24,73)* vs 23 ** (12,47)* p=<0.001	17.5** (8.5, 30.5)* vs 9** (5,17)*	17 (53.1%) vs 85 (37.4%) p=0.04	-, 8 ** vs -, 62** p=0.78
Joynt (2006)	4 (0-14) vs 2(0-46), p=0.81	0 (0-24) vs 0 (0-57), p=0.73	4 (5-21) vs 3(2-61), p=0.89	5 (33%) vs 18 (28%), p=0.75	n/a
Khouli (2009)	5.5 (2.20) vs 6 (1.36), p=0.90	n/a	8 (3-23) vs 8 (3-36), p=0.52	17%;95% CI,5.51)vs (21%;95%CI,10-23; p=0.70)	n/a
Boddi (2009)	14.5 (6.5-19.75) vs 3 (1-9) p=0.001	n/a	[14 (9-26) vs 10 (7-29), p=0.05], [19(13.5-30) vs 7 (4-15), p=0.001]"	n/a	n/a

Legend

DVT: Deep Vein Thrombosis
Vs: versus
ICU: Intensive Care Unit
* IQR (Interquartile range)
** median
^Necessity for ventilation measured by PEEP (positive end-expiratory pressure)
- means missing value
/- means missing control data
" retrospective phase and prospective phase of the study

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IN FAMILIES WITH INHERITED THROMBOPHILIA THE RISK OF FETAL LOSS IN THE CARRIER WOMEN IS DEPENDENT ON THE CLINICAL PHENOTYPE OF THE PROBAND.

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Several studies have demonstrated an association between inherited thrombophilia and obstetric complications (OC), in particular recurrent fetal loss (> 2) and unexplained intrauterine fetal death after the 20th gestational week. However, the strength of association is weak and it is uncertain whether women with inherited thrombophilia are more prone to OC than non-carriers. To assess the risk of OC among the carriers, we analyzed the clinical history of 415 women recruited from a large family cohort of 1,720 relatives of 563 probands with inherited thrombophilia. The inclusion criteria were a) to be relative of a proband diagnosed as carrier of inherited thrombophilia because of a history of venous thromboembolism (VTE) or OC; b) to have been genotyped for inherited thrombophilia; c) to have been pregnant at least once. The presence of antiphospholipids was a criterion of exclusion. The two study groups consisted of 93 relatives (54 carriers) of a proband with OC, and 322 relatives (187 carriers) of a proband with VTE. Out of 241 carriers, 13 had antithrombin, protein C or S deficiency, and the remaining ones had factor V Leiden and/or prothrombin G20210A. Overall, we recorded 1,038 pregnancies. If the proband had OC, the rate of fetal loss was 21.4% among carriers (32 of 149 pregnancies) and 15.3% among non-carriers (15 of 98 pregnancies); if the proband had VTE, the rate of fetal loss was 14.2% among carriers (64 of 450 pregnancies) and 12.6% among non-carriers (43 of 341 pregnancies). The carriers with a history of fetal loss were 22 among the relatives of a proband with OC (40.7% of the carriers), and 47 among the relatives of a proband with VTE (25.1%); the non-carriers with a history of fetal loss were 9 and 34, respectively. In women with thrombophilia in comparison with non-carrier women, the odds ratio (OR) of having at least one fetal loss was 2.29 (95%CI 0.91-5.76) among the relatives of a proband with OC and 0.99 (95%CI 0.59-1.66) among the relatives of a proband with VTE. The risk for fetal loss was double in the carriers identified because a history of OC in the proband in respect to the carriers identified because a history of VTE in the proband (OR 2.04, 95%CI 1.08-3.86). In conclusion, in women with inherited thrombophilia and family history of OC the risk of fetal loss is increased. Whether this information could be applied in tailoring pharmacological prophylaxis in their pregnacies should be investigated in proper studies.

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SELECTION CRITERIA OF PATIENTS WITH VENOUS THROMBOEMBOLISM FOR LABORATORY INVESTIGATION OF INHERITED THROMBOPHILIA.

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Laboratory investigation for inherited thrombophilia is warranted in

young patients, especially those with severe venous thromboembolism (VTE) occurred spontaneously or recurrently. Investigation of older patients is discouraged, especially when events are mild or provoked. Such policy could miss a number of carriers, leaving undiagnosed their families. To investigate whether clinical parameters are predictive of the presence of inherited thrombophilia in VTE patients, we analyzed the files of 1,835 patients referred to our Thrombosis Center. The median age at the first VTE was 37 years (range 0-89); 736 were males (40.1%). Patients were stratified according to family history of VTE, age of first VTE (younger or older than 45 years), type of first VTE (defined severe for proximal DVT and/or pulmonary embolism and mild for distal DVT or superficial vein thrombosis), circumstances of first VTE (unprovoked or provoked), history of recurrent VTE. Multiple regression was carried out labelling as dependent variable diagnosis of overall thrombophilia or severe thrombophilia (antithrombin or protein C or protein S deficiency, homozygous or multiple defects, n=211) or mild thrombophilia (heterozygous factor V Leiden or prothrombin G20210A, n=415). Diagnosis of overall thrombophilia was associated with family history (p=0.005), severity of VTE (p=0.008) and recurrent events (p less than 0.0001); the aforementioned criteria were all absent only in 8% of patients with thrombophilia. Among patients with thrombophilia 30% had clinical onset after 45 years of age, 62% had a first provoked VTE, and 11% had both. Severe thrombophilia was associated with family history (p=0.02), first unprovoked VTE (p=0.015) and recurrent events (p=0.04). Mild thrombophilia was associated with family history (p=0.05), severity of VTE (p=0.03) and recurrent events (p less than 0.0001). In conclusion, family history, clinical severity and recurrence of VTE are strong predictors of inherited thrombophilia, and at least one of these parameters is present in more than 90% of cases. Selection of the patients to be investigated according only to age and/or circumstances of the first VTE could miss diagnosis of thrombophilia in a relevant number of cases.

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DIFFERENT PROPHYLAXIS REGIMENS IN YOUNG SEVERE HEMOPHILIA A PATIENTS: EFFICACY, FVIII CONSUMPTION, TROUGH FVIII LEVELS AND THERAPY COMPLIANCE.

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Background. Long-term prophylaxis is the gold standard treatment for severe hemophilia A patients (pts); it is effective in the prevention of hemophilic arthropathy in children and in young pts. The standard prophylaxis regimen consists of the administration of FVIII ~30 IU kg-1, every other day or three times a week, with the aim of maintaining a level of FVIII >1%. Venous access, especially in children, can be a barrier to prophylaxis; thus, different regimens which involve a lower number of venipunctures are under evaluation. Aims. To evaluate different prophylaxis regimens in young severe hemophilia A pts, and to compare efficacy, FVIII consumption, trough FVIII levels and patient/family's compliance. Methods. Twenty severe hemophilia A pts (≤18 years) started prophylaxis because of either increasing hemorrhages or presence of a target joint. Three prophylaxis regimens were planned: FVIII, 50 IU kg-1 once a week in 2 pts, 50 IU kg-1 twice a week in 12, 30 IU kg-1 thrice a week in 6. The median age of pts at the start of prophylaxis was 6.9 years (1.1–13.5). Results. All pts were treated with rFVIII. Actual rFVIII doses were: once a week, 50 IU kg-1; twice a week, mean dose 46.5 IU kg-1; thrice a week, mean dose 37 IU kg-1. The median annual number of hemarthroses/other bleedings pre-prophylaxis was 4 (1-12) and 5 (1-20), respectively. During the last 12 months of prophylaxis, we recorded: hemarthroses, median 0 (0-2), other bleedings, median 1 (0-2). Mean/median values of trough FVIII levels were 1.1% and 0.7% (0.22%-8%), respectively. Values of trough FVIII >1% were recorded in 5 pts (4 under twice, 1 under thrice a week regimen). No significant differences in concentrate consumption were recorded between twice and thrice a week schedules. There was no difference in the orthopedic score before (median 0; 0-2) and during prophylaxis (median 0.5; 0-2). Median follow-up was 12.7 years (2.6-17.3). During prophylaxis, no inhibitor development was recorded; moreover, 4 low-responding inhibitors (titer <5 BU mL-1) which were present before the start of prophylaxis start disappeared. Conclusions. Twice a week prophylaxis can be an alternative regimen to the standard one in young severe hemophilia A pts. Indeed, we found no significant differences both in trough FVIII levels and efficacy between twice/week and thrice/week regimens. Moreover, reduction of venipunctures, especially in small children, improves the compliance of pts and their families.

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CHRONIC REFRACTORY THOMBOCYTOPENIA WITH DEL (20Q) IDENTIFIED BY FISH TREATED WITH HIGH DOSES ROMIPLOSTIN

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Case report: female/71y, with chronic refractory thrombocytopenia treated at another institution with PDN,ivIg,azathioprine(only partial and not sustained responses);after influenza vaccination recurrence of severe thrombocytopenia (severe mucocutaneous bleeding). 2009,November: normal spleen,no enlarged lymphnodes,wbc 6600 hgb 12.3 plts 2000, normal coagulation,PaIgG,ANA,HCV,HBV,HIV negative;LDH,B12 normal; B.M. hypercellular, mild trilinear dysplasia, MKC hyperplasia, normal blasts. Karyotype normal. After failure with PDN,danazole,ivIg.,refractoriness to plts concentrates and repeated hospitalizations for mucocutaneous bleeding because of the persistence of severe thrombocytopenia, the patient was evaluated for romiplostin. Splenectomy contraindicated because of surgical risk for severe refractory thrombocytopenia, comorbidities (diabetes mellitus,hypertensive cardiomyopathy),a previous suspected thromboembolism post-cholecystectomy and the patient's refusal. In March 2010, treatment with romiplostin (Nplate,Amgen) 1 mcg/kg subcutaneously began,followed by weekly dose increases because of persistent severe thrombocytopenia. At a new evaluation, marrow was hypercellular, with massive MKC hyperplasia, trilinear dysplasia, normal blasts, mild diffuse increase of lymphocytes. Karyotype was still normal,but FISH analysis(LSI D20S10 probe) was positive for del 20(q12) in 12% of nuclei. After 10 doses romiplostin at the maximum dose expected (10 mcg/kg) the plts recovery was still incomplete (wide fluctuations from 3000 to 51000) but consisting of a clinical benefit because of the complete and permanent disappearance of bleeding. A further increase to 11 mcg/kg was followed,after an initial stabilization of plts without significant fluctuations(40000-58000), by a progressive and stable increase up to 100000. The patient is currently on home treatment with romiplostin at 14 months without clinical toxicity or abnormal laboratory tests. In pts with chronic refractory thrombocytopenia FISH analysis for del(20q)identifies a subset of thrombocytopenic patients with myelodisplastic features unlikely to respond to standard ITP therapies including splenectomy. Nplate has a dose dependent activity in many patients with chronic ITP;high doses may provide clinical benefits in MDS patients with thrombocytopenia. In this case of refractory thrombocytopenia a prolonged home treatment with high doses of romiplostin achieved a stable clinical benefit without adverse events.

P113

ACUTE ARTERIAL THROMBOSIS WITH GANGRENE AS COMPLICATION OF ROMIPLOSTIM TREATMENT IN A PATIENT WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Introduction: Suboptimal platelet production in ITP is a direct effect of autoantibodies on the megakaryocyte, which may be affected further by failure to substantially increase circulating thrombopoietin (TPO) levels despite often marked thrombocytopenia. The treatments of ITP with steroids, intravenous immunoglobulin or anti-D, rituximab, danazol or azathioprine in some cases induced short-term response. Currently romiplostim is a thrombopoietic agents stimulating platelet production and stimulates platelet production by a mechanism similar to endogenous TPO . It produce increased of platelet counts with reduction of risk of bleeding but with and a potential risk of thrombosis. Case report: A 75 years old man with ITP was treated with cortisone, intravenous Ig HD and rituximab without benefit. A cerebral haemorrhage occurred for the persistence of thrombocytopenia which was successfully overcame by the patient. After failures of therapy and refusal of splenectomy, the

patient began therapy with ramiplostim at the initial dose of 1 ug / kg and did not get any results until they practiced the dosage of 10 ug / kg: with this dose there was an increase platelet levels which exceed the 1000000/mmc. The value of platelet decreased gradually after sospension of ramiplostim ; the dose was adjusted weekly for obtainer a stable response. During treatment at 10 ug / kg, the patient had pain on left foot that was badly controlled with drugs and gradually established a left lower limb acute ischemia. It wasn't possible to avoid the surgery of amputation of the left foot, following the gangrene, although treatment implemented. During the ischemic episode ramiplostim was stopped and transfusion of platelets were infused for low values of Platelets (<5000/mmc) . After surgery the patient resumed ramiplostim with heparin because the platelets were less 10.000/mmc and for absence of other treatment. The response to romiplostim occurred again and the number of PLT is more high with a dose of 8 ug / kg. Conclusion: romiplostim is an effective and well-tolerated maintenance treatment in patients with chronic ITP. During the treatment with romiplostim the thrombotic event are associated with the excessive elevation of platelet count: for this reason it should be used with precaution in patients with vascular problems because the risk for thrombotic complications may increase significantly.

P114

FRONT LINE THERAPY WITH DEXAMETHASONE PLUS RITUXIMAB LEADS TO INCREASED DURABLE RESPONSE RATE THAN DEXAMETHASONE MONOTHERAPY IN ADULTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA: LONG TERM FOLLOW UP ANALYSIS OF ML18542 TRIAL

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Background. In adults with primary immune thrombocytopenia (ITP) the addition of rituximab to a single course of dexamethasone improves the rate of sustained response (SR, i.e. platelet count 50 x 109/L or more at month 6 from the beginning of treatment) (63% vs 36%, P= 0.004, Blood 2010;115:2755). Purpose. In order to better address the long term impact of therapy, a subsequent observational follow-up study up which was planned. Patients and Methods. Participating centers were asked to continue to monitor the patients from month 6 up to month 36 documenting the occurrence of delayed side effects and the response status. Results. Eighty-one patients were systematically followed-up beyond month 6 for a median overall period of 34 months (range: 4-54 months) and were valuable for safety. Fifty-four out of 65 patients who achieved SR could be evaluated for long term efficacy. This group included 13 out of 19 patients of the dexamethasone monotherapy arm (group A), 27 out of 31 patients of the dexamethasone plus rituximab arm (group B), and 14 out of 15 patients of the dexamethasone plus rituximab salvage therapy arm (group C). As far as the safety profile, we documented: 1) Group A (15 patients): no delayed toxic events; 2) group B (42 patients): 1 osteoporotic vertebral collapse; 3) group C (24 patients): 1 Herpes Zoster reactivation, 1 deep venous thrombosis. As far as efficacy, the relapse rate in the three groups was 23% (3/13), 26% (7/27), and 14% (2/14) respectively (P= 0.837). The 40-month estimated probability of response duration for each of the above treatment groups was 77%, 73%, and 86% (P= 0.700), respectively. Arbitrarily considering as relapse events those 11 patients who achieved SR but were subsequently lost to follow up, the percentage of patients in the three groups who achieved and maintained long term response was 19%, 41% and 44%, respectively. Conclusions. The results of this study indicate: i) very low pattern of long term toxicity between ITP patients treated either with single course of dexamethasone or dexamethasone plus rituximab; ii) nearly 40% vs. 20% probability to achieve and maintain long term response after dexamethasone plus rituximab and dexamethasone monotherapy, respectively; iii) no significant differences in the relapse rate and RD among patients who achieved SR in the three groups of patients, suggesting that favouable long term outcome may be a possible surrogate of SR, rather than of the initial therapeutic choice.

P115

CLONALITY AND PHENOTYPE IN SPLEENS FROM PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA

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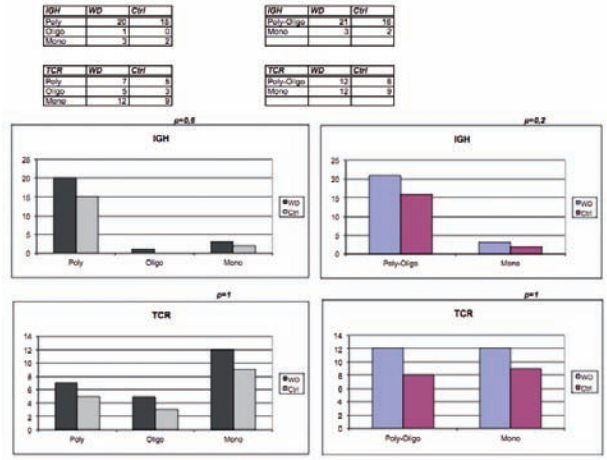
We analysed the phenotype and IgH and TCR gene rearrangements of the splenic lymphocytes in primary immune thrombocytopenia (ITP) patients to assess possible impact on diagnosis and course, comparing the same data in post trauma spleens. MATERIAL & METHODS 31 and 19 spleen samples respectively from ITP and control patients were studied by histology, immunohistochemistry for CD3, CD20, CD4, CD8, CD56, CD57, PD1, Tia1, Granzyme B, FoxP3, CD72, and molecular analysis for IgH and TCRgamma gene rearrangements. Age ranged from 15 to 68 (mean 41.61y) with M/F of 9/22 in ITP and 18 to 89 (mean: 66y) with M/F 11/8 in controls. Results All cases but 2, that underwent Rituximab before surgery, showed well developed white pulp and regular sinusoid-rich red pulp with moderate lymphocytic and modest granulocytic infiltrates. Large haemorrages were seen in post trauma spleens. Immunohistochemistry showed similar stains in the 2 groups (white pulp: CD20+ B cells, CD72-+, CD3+/CD4+ T cells and rare FoxP3+; red pulp: regular CD20/CD3 ratio, <20% CD3+ T cells also expressed PD1/Tia1/Granzyme B; scattered CD57+/CD56- T cells). At PCR, 24/31 ITPs and 17/19 control spleens were evaluable for IgH and TCRg. Discussion The results didn't show statistically significant differences between ITP and controls as for morphology, phenotype and T/B cell clonality. Since a decrease of regulatory T cells (T regs) is reported in ITP we tested T reg-related nuclear molecule Foxp3 in immunohistochemnistry. In control cases few cells in the white and red pulp were observed, while in ITP spleens fewer Foxp3 positive cells could only be seen in the red pulp: although slightly different, the low amount of positive cells in both groups decreases the reliable reproducibility of such observation. Overlapping molecular results were also obtained in the two groups (table 1).



Our data agree with previous reports, although with slightly different rates. The attempt to translate the molecular findings into possible immunomorphologic differences between monoclonal and non monoclonal cases in both case series failed since neither amount or distribution of B and T cells nor T cell subtypes showed evident differences. On

the whole, our results show that neither lymphoid phenotype nor IgH or TCR clonality can be used as specific features of refractory ITP or guide treatment choice.

P116

LONG-TERM RESULTS OF SPLENECTOMY IN IMMUNE THROMBOCYTOPENIA: RESULTS OF 91 CASES WITH A MEDIAN FOLLOW-UP OF 24 YEARS

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Background. The recent introduction of new medical therapies (anti-CD20 and thrombopoietin mimetics) for the management of chronic immune thrombocytopenia (ITP) has encouraged the tendency to delay splenectomy. The definition of the efficacy and safety of splenectomy in the long-term is therefore substantial. Patients and Methods. We retrospectively analyzed the data of 91 ITP patients followed at our Institution who underwent laparotomic splenectomy between 1970 and 2000. Results. All patients have a minimum follow-up of 10 years after splenectomy (median, 24; range, 10-39). Sixty-seven percent were women; median time from diagnosis to splenectomy was 13 months (range, 0-254) and median age at splenectomy was 33 years (range, 6-74). Three patients (3%) underwent splenectomy front-line; the other patients were splenectomized after failure of at least one course of conventional medical therapy. Overall, 80 patients (88%) achieved a response (platelet >30x109/L), which was complete (platelet >100x109/L) in 72 cases (79%), while 11 patients (12%) were refractory. Nineteen out of 80 responding patients (24%) relapsed during the follow-up, after a median time of 12 months (range, 3-256), for a relapse-free survival of 75% at 20 years. In 11 cases (58%), relapse occurred within the first year from splenectomy. Overall, 27 patients (29.6%) needed further treatment after surgery. At last contact, 72 patients (79%) were in complete response, 9 patients were in response and 10 patients had a platelet count <30x109/L. Six patients (6.5%) remained in on-demand steroid therapy after a median time of 19 years (range, 11.2-38). Forty-two hemorrhagic events (6 of which grade 3-4 WHO) were observed in 19 patients (21%), after a median time of 83 months. Nineteen patients (21%) experienced one or more infectious complications after surgery, for a total of 23 events which occurred after a median time of 74 months. Infections were pulmonary (9 cases), gastrointestinal (3), perioperative (2) or minor (8). One patient developed dilatative cardiomiopathy after viral miocardiditis. Fourteen patients (15%) died, 13 of whom for causes unrelated to ITP. One patient, with refractory thrombocytopenia died for bleeding complications. Conclusions. After a median follow-up of 24 years, 67% of the patients maintained a stable response to splenectomy, removed from any treatment. The incidence of severe infectious complications was not negligible, but no cases of OPSI were recorded.

P117

RITUXIMAB TREATMENT BEFORE SPLENECTOMY FOR CHRONIC IMMUNE THROMBOCYTOPENIA (ITP): IMPACT ON RESPONSE AND COMPLICATIONS

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Background. Rituximab is increasingly becoming a treatment of choice in patients with chronic immune thrombocytopenia (ITP) refractory to other therapies and is often administered before submitting patients to splenectomy, in order to avoid a surgical procedure. However, the potential impact of Rituximab on the outcome of the patients who finally undergo splenectomy is unknown. Patients and Methods. We retrospectively analyzed the data of 15 ITP patients who underwent lapascopic splenectomy between 2000 and 2009, after failure of Rituximab treatment. Patients were followed at the Divisions of Hematology of Bologna or Udine, Italy, for a median time of 3.5 years (range, 1.1- 11) from splenectomy. Results. Median age at ITP diagnosis was 48 years (range, 4-76); 80% were women. Fourteen patients received Rituximab after failure of one or more previous medical therapies and all but two patients were resistant to steroids; one patient received Rituximab front-line. Average time from diagnosis to Rituximab and to splenectomy was 29 months (SD 54) and 37 months (SD 57). Consequently, average interval between Rituximab and splenectomy was 8 months (SD 6). All patients received prophylactic vaccination before Rituximab therapy. Overall, all patients but one achieved a response (platelet >30x109/L), which was complete (platelet >100x109/L) in 12 cases (80%). Five out of 14 responding patients (36%) relapsed, all within 6 months from splenectomy. Overall, 6 patients (40%) needed further treatment after surgery. At last contact, 13 patients (87%) were in response (complete in 11 cases), while 2 patients had a platelet count <30x109/L. Four patients (27%) remained on continuative treatment (TPO mimetics or dapsone) at last contact. Six patients experienced a WHO grade 1-2 hemorrhagic event during the follow-up, mostly in the peri-operative period. Five patients (33%) presented an infectious complication immediately before or after surgery. Infections were pulmonary (3), gastrointestinal (1) or related to the surgical wound (1). No patient was lost to follow-up and all were alive at last contact. Conclusions. After a median follow-up of 3.5 years, 60% of the patients maintained a stable response to splenectomy, removed from any treatment. Although one third of the patients experienced an infectious complication, all events happened earlier in the follow-up, with no cases of fatal or late complications.

P118

AN EMBLEMATIC CASE OF REFRACTORY THROMBOCYTOPENIA

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We report the case of a 32 years old men, affected since early childhood by PTI (Idiopathic thrombocytopenic purpura). He failed previous therapy with Prednisone (1 mg/Kg), high dose of Dexamethasone, Immunoglobulin and anti CD20-Rituximab. Finally the patient was send to splenectomy obtaining a good response (median platelet count of 243 x 10*9/L) for about 11 months, when he suffered another symptomatic thrombocytopenia relapse (62.000 x 10*9/L) with marked epistaxis and strong headache. So we decided to initiate azathioprine therapy (50 mg for 3 time day) performing a good platelet count (median of 310 x 10*9/L) for about 24 months when the patient, by himself opinion, suspended azathioprine resulting in severe thrombocytopenia (platelets count 4 x 10*9/L). A new Azathioprine treatment failed, so we began Cyclophosphamide (1500 mg for days) associated with Dexamethasone (40 mg for 2 days) treatment but the platelet count remained very low (8 x 10*9/L) with hemorrhagic symptoms: epistaxis, petechiaes and gingival hemorrhages. Therefore we decided to treat the patient with Romiplostim, a thrombopoietic recombinant protein defined as a "peptibody", given as a weekly subcutaneous injection. For the first nine administrations the platelets count remained very low (7 x 10*9/L) but the hemorrhagic signs disappeared. After the tenth injection (10 µg/Kg) the platelets count was extraordinary elevated (> 1300 x 10*9/L), so we suspended Romiplostim and in the following three weeks platelets raised to 2400 x 10*9/L. Later the platelets count settled down about 350 x 10*9/L. Now the patient has been free from any treatment for the last 4 months, and his latest platelet count was 358 x 10*9/L. We performed a bone marrow biopsy that excluded fibrosis tracks. Actually we do not know whether this response will be lasting or not, nor if in the case of a new thrombocytopenia relapse there will be another good result starting again Romiplostim. This case of a late response to Romiplostim, in our opinion, is paradigmatic because it empathies the importance to reach the higher dosages before giving up Romiplostim.

P119

TREATMENT WITH ROMIPLOSTIM IN ACCORDING TO THERAPEUTIC INDICATIONS IN CHRONIC ITP PATIENTS: A SINGLE-CENTER EXPERIENCE

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Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by immunologic destruction of otherwise normal platelets. ITP was believed to be caused by increased platelet destruction at a rate that exceeded production by a compensating bone marrow. New knowledge has questioned this model, providing evidence that platelet production is also decreased in many patients with ITP. Romiplostim (Nplate™) is thrombopoietic agent stimulating platelet production that have recently been shown to induce increases in platelet count in ITP patients by a mechanism similar to that of endogenous TPO. We evaluated 6 patients

(4 female; 2 men) with severe refractory ITP, all patients had no response to conventional treatments, including splenectomy (4/6). The median age was 45 years. The median platelet count at baseline was 12.000/mmc; all patients were receiving corticosteroids and 4/6 patients had undergone a splenectomy, 2/6 patient had contraindication to splenectomy. All patients were to receive Romiplostim once weekly by subcutaneous injection and the treatment was initiated at 1 g/kg per week.The dose of Romiplostim was adjusted on the basis of the patient's platelet count. The targeted platelet range (platelet count >50,000/mmc) was reached in 5/6 patients and the median time from the first dose to targeted platelet range was three weeks. Durable response was achieved receiving the 2 g/kg dose in 2/6 patients, 3 g/kg dose in 1/6, 6 g/kg dose in 1/6 and only for one patient it was necessary the max dose of 10 g/kg. One patient, nonsplenectomized, had not achieved the targeted platelet response but he had increased in platelet count and his median peak platelet count, after 10 weeks of treatment, was 43.000/mmc (range 260.000/mmc – 6.000/mmc; baseline count 3.000/mmc) with a dose of 6 g/kg and he has no more clinical manifestation like petechiae and ecchymoses. 5/6 patients had been changes in the corticosteroid dose when count platelet begin stable and after a median time of treatment of ten weeks and 4/6 patients stopped this drug after about nine months of treatment with Romiplostim. No one reported adverse effects. On the basis of our observation Romiplostim appears a well-tolerated and effective treatment for patient with ITP. It was proven to increase platelet counts, reduce the need for other ITP therapies and emergency treatments, and demonstrate an acceptable safety profile.In addition, romiplostim improves patient-quality of life.

P120

FLUCTUATIONS OF WEEKLY PLATELET COUNT DURING TREATMENT WITH ROMIPLOSTIM IN PATIENTS AFFECTED BY CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Romiplostim is a thrombopoietin mimetic agent recorded for treatment of immune thrombocytopenic purpura (ITP) refractory or relapsed after splenectomy or in patients having contraindications to spleen surgery. Many patients achieve a stable dosage (weekly platelet count between 50-400 x 109/l without changing dose neither using rescue therapies for 4 consecutive weeks), but with important fluctuations in weekly platelet counts. Stable dosage allows patients to get self injection of romiplostim and monitoring platelet count monthly. Therefore, it's important to understand the kinetics of platelet response, especially considering that risk of bleeding is higher during the first 24 weeks. Particularly, we have focused on weekly platelet counts out of range 50-400 x 109/l, which require a dose adjustment, on counts < 30 x 109/l, because of a higher risk of bleeding, and on the median duration of first stable dosage. From November 2008 until August 2010, 10 chronic ITP patients aged 56-85 years (median 68) were treated with weekly subcutaneous injections of romiplostim, starting from 1 mcg/kg and adjusting dose as standard on the basis of weekly counts. Data are available for 10 patients at week 12, 8 patients at week 18 and 5 patients at week 24; splenectomised patients were 30%, 25% and 20% of the total respectively. Results: platelets counts > 400 x 109/l were 2,5% of the total, but their incidence was higher in splenectomised patients (8,3% of their total counts at week 12 and 18, 7,3% at week 24) than in not ones (0,01% during all 24 weeks). There was less difference in counts < 50 x 109/l between splenectomised (incidence 25% during all 24 weeks) and not splenectomised patients (18% at week 12, 16% at week 18 and 24). Percentage of counts < 30 x 109/l on all counts < 50 x 109/l was also higher in splenectomised patients, but reduced during the treatment (77% at week 12, 57% at week 24), while increased in not splenectomised (33% at week 12, 48% at week 24). Median time to achieve first stable dosage among all the patients was 7 weeks (range 5-15), but it's median duration was only 0,5 weeks (range 0-14). Therefore, though more data are needed, we believe that weekly check of platelet count during the first months of treatment should be performed even in patients that achieve a stable dosage, particularly for avoiding important thrombocytopenia both in splenectomised and not splenectomised patients and thrombocytosis in splenectomised ones.

P121

PLATELET KINETIC STUDY (PKS) MAY IDENTIFY A SUBSET OF PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) PROBABLY CURED BY ROMIPLOSTIM .

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Background. A platelet kinetic study (PKS) is not indicated in the evaluation of adult patients with idiopathic thrombocytopenic purpura (ITP) at presentation not having a proven role in the differential diagnosis of ITP from other thrombocytopenias and not being helpful in patient management.Moreover, in ITP patients refractory to or relapsing after corticosteroid therapy, its appropriateness is considered uncertain. Striking,PKS, in our experience,was able, to identify a subset of patients probably cured by therapy with romiplostim Methods. A total of 18 adult patients, female (63%),median age 55 (range 31 - 78) years, median baseline platelet count 19 (range 3 – 32) x 109/L.,median of 4 (range 1 – 7) prior ITP therapies,received romiplostim administered once weekly sc, with dose adjustments to maintain platelet counts in the target range of 50-150x109/L..The median time since ITP diagnosis was 6,8 years (range, 0.6-12.8 years) and 10% had undergone a splenectomy . Patients received romiplostim for a median of 87 weeks (range, 18-90); taking the average weekly dose of all patients, the median was 4 mcg/kg . Home administration was started by 16% of patients (3/18) but 2/3 patients discontinued home administration and resumed weekly outpatient injection .All patients achieved a platelet count ≥50x109/L. 3 out of 18 experienced thrombocytosis and rebound thrombocytopenia.A PKS with (111)In oxine-labeled autologous platelets was performed in all patients failing steroid treatment , before romiplostim was started. A gamma function was used for the calculation of platelet mean life span (MLS) that was greatly reduced in 100% of patients. Results. 3 patients, despite the discontinuation of romiplostim maintain normal platelet count after 10 months of follow-up.Sticking a second PKS in these subset shows a normal platelet half-life with normal uptake of the spleen and liver. In our opinion PKS in romiplostim responding patients who discontinued treatment for the stability of response, may identify a subset of patients probably cured.

P122

TREATMENT WITH ROMIPLOSTIM IN ADULT PATIENTS WITH PERSISTENT/CHRONIC IMMUNE THROMBOCYTOPENIA: A SINGLE CENTER EXPERIENCE.

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Background. Romiplostim, a thrombopoietin mimetic, is capable of increasing the platelet count in patients (pts) with persistent/chronic immune thrombocitopenia (ITP). Aim. To report our experience with Romiplostim in persistent/chronic ITP pts Patients and Methods. Twenty adult ITP pts (10M, 10F; median age, 62.4 years 33.8-82) were treated with Romiplostim (initial dose 1 µg Kg-1week-1). The dosage was adjusted in order to maintain platelet counts of 50-250 x 109/L. Median time between diagnosis and Romiplostim start: 14.4 years (0.08-37.9). All pts had already received ≥2 lines of treatment (median 2.5 2-6): prednisone, pulsed high-dose dexamethasone, immunoglobulins, rituximab, interferon, azathioprine and splenectomy (8 pts). At the start of Romiplostim, the median platelet count was 11 x 109/L (2-32 x 109/L). Results. Sixteen/20 (80%) pts were persistent responders and 4/20 (20%) were non-responders. Median follow-up from the start of therapy: 5.5 months (1-27). Median platelet count during therapy: maximum, 385.5 x 109/L-1 (17-1649 x 109L-1); lowest, 8 x 109/L-1(1-79 x 109/L-1). Eighteen pts were receiving prednisone at the start of Romiplostim treatment: 12 pts discontinued it at a median time of 13 weeks (5-37), while 6 pts never stopped it. In the 16 responding pts, the median Romiplostim dose to achieve a response was 2.5 µg Kg-1/week-1 (1-9), the median maintenance dose 3 µg Kg-1/week-1 (0-10) and the median time to response 3 weeks (1-10). Two pts discontinued Romiplostim for 7 and 11 months, respectively, maintaining a persistent response at the last follow-up. Among non-responding pts, 2 obtained a transient response (2 and 5 weeks, respectively), 2 showed no response (1 suspended treatment at

a dose of 4 µg Kg-1/week-1 because of no compliance). Fifteen pts reported transitory adverse events: bone pain, headache, legs edema, itching, flu-like syndrome. Two serious adverse events (SAEs) occurred: one arterial thrombosis (a pt with diffuse arteriopathy); one death of non Hodgkin lymphoma (NHL) (the diagnosis was made after recovery) which was considered non-related with Romiplostim. Conclusions. At the last follow-up, 16/20 pts (80%) showed a persistent response. Two/16 (12.5%) responding pts discontinued Romiplostim. Three/4 non-responding pts were splenectomized. Two SAEs occurred: death due to NHL was considered non-related to Romiplostim; arterial thrombosis (resolved without sequelae) arose in an elderly pt with arteriopathy. Romiplostim is effective and safe as 2nd or 3rd line therapy in pts with ITP either refractory or with contraindication to splenectomy.

P123

PREGNANCY OUTCOME AND DISEASE COURSE IN A WOMAN WITH ACQUIRED AMEGACARIOCYTIC THROMBOCYTOPENIA.

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Amegakaryocytic thrombocytopenia is a rare disease presenting with thrombocytopenia and megakaryocytic reduction. Pathogenesis of this disorder is not entirely clear but the immune system seems to play a crucial role as shows by the effectiveness of immunosuppresive drugs as antithymocyte globuline (ATG), cyclosporine (CsA), mycophenolate mofetil and steroids. In case of refractoriness of these therapies bone marrow transplant is indicated. There are very few cases of pregnancies recorded in patients with amegakacaryocytic thrombocytopenia Here we report the case of a female diagnosed to have the disease when she was 19 years and presented platelets 1x10^9/L .Diagnosis was confirmed by bone marrow biopsy. Initially she was treated with CsA (250 mg/die) continous associated with metilprednisolon (100 mg/die for 5 days followed by slow decrease) and achieved a complete response (CR). In May 2003 she relapsed (PLTs 12x10^9/L) and received ATG (40 ml for 5 days) followed by CR. In February 2005 she started metilprednisolon (60 mg/die for 15 days followed by oral prednisolon at low dose continous) because of new decrease of PLTs (9x10^9/L). In 2007 CsA 150 mg daily was added with partial response. This combination therapy is still ongoing. When she was 31 years she started an unplanned pregnancy and we know that when she was at 4th week of pregnancy. She was extensively informed about the risk of relapse or flare-up of the disease during pregnancy and about the safety of CsA, being very low the incidence of theratogenic effect as recorded in literature regarding pregnancies in patients following renal transplant and affected by aplastic anemia receiving long term CsA. Patient decided to complete pregnancy. At that moment she was in good clinical conditions, blood count was Hb 11 g/dl, WBC 8x10^9/L and platelets 70x10^9/L, vital signs, renal and liver function were normal. She was on CYA 150 mg/daily and metilprednisolon 8 mg/daily. The CYA dose was decreased to 100 mg/daily in order to reduce possible effect on the newborn body weight and immune system and to avoid side effects on the pregnant woman. Patient was strictly followed-up from the hematological and obstetrician point of view. During pregnancy median PLT value was 70x10^9/L during the first, second and the main part of third trimester, the Hb and WBC values has been always normal. Embryonal and fetal developments were regular as the placental one; there were not complications associated to pregnancy and the serum parameters were normal. At the 37thweek PLTs decreased to 50x10^9/L and to avoid worsening of this condition a caesarian delivery was planned at the 38th week (see figure 1). Intravenous polyspecific immunoglobuline at the dose of 400/mg daily for 3 consecutive days and a platelet apheresis transfusion in the 3th day were administered ; in the same day a Caesarian delivery was performed without complications (PLTs: 90x10^9/L). The newborn was a healthy female weighted 2,7 Kg (normal range: 2,8-3,3 Kg) with a normal morphological development and Apgar index. Blood counts were: Hb 17.5 gr/dl, WBC 22x10^9/L then dropped to 10x10^9/L, Plt 115x10^9/L then increased to 398x10^9/L (in 15 days). The baby immunological parameters and the lymphocytes subpopulations were normal. Conclusions A normal pregnancy is possible in acquired amegacaryocytic thrombocytopenia in partial response to therapy. Low doses CsA are sufficient for disease control and to allow a safe pregnancy and a normal fetal development. In particular, in our case, the newborn weight, even if in the lower range, was normal as the immunological parameters. A close hematological and obstetrician follow-up is crucial to discover early signs of worsening of hematological parameters and to proceed in order to allow a regular and safe pregnancy.

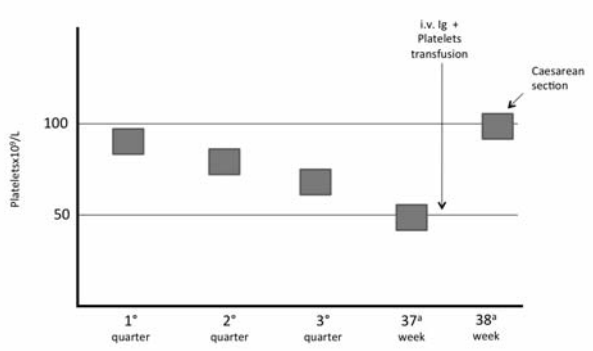

Figure 1- Platelets values during pregnancy and after supportive therapy preceding caesarian section

CYTOGENETICS AND LABORATORY

P124

IN B-CLL CPG OLIGONUCLEOTIDE+INTERLEUKIN-2 SHOULD COMPLEMENT IFISH RESULTS

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The principal aim of the present study, which includes ninety-one B-CLL patients diagnosed at our Institution between January 2007 and July 2010, was to compare the chromosomal pattern revealed by pokeweed (PKW) mitogen and by the ODN+IL2 combination with interphase FISH (i-FISH) results. An additional aim was to correlate the chromosomal pattern with clinical parameters and prognostic markers. There were thirty-eight females and fifty-three males with a median age of 64 years (range 42-83). According to Binet, sixty-three patients (69.2%) were considered as stage A, seventeen (18.7%) as stage B and eleven (12.1%) as stage C. PKW stimulation was unsuccessful/detected clonal defects in 14.2%/17.5% of patients, whereas the ODN+IL-2 combination was unsuccessful/revealed clonal abnormalities in 3.3%/61.5% of patients. The ODN+IL-2 combination revealed a complex karyotype (≥ three defects) in twelve patients (13.2%), two chromosomal defects in fourteen patients (15.4%) and a single chromosomal defect in thirty patients (32.9%). Fifteen (16.4%) harboured various chromosomal rearrangements. iFISH with the B-CLL panel (Vysis, Downers Grove, IL, USA) revealed clonal abnormalities in sixty-two patients (68.1%); 13q-, +12, 11q- and 17p- were observed in 46.1%, 14.2%, 7.7% and 6.5% of patients. The ODN+IL2 combination discovered a +12 in three patients with a normal FISH pattern and a structural 17p13 defect in five patients who showed the loss of one p53 signal on iFISH. In addition, the ODN+IL2 combination showed that five of the forty-two patients, who harboured a 13q- on iFISH analyses, presented this defect as part of a complex karyotype. From a clinical point of view, nine of the fifteen patients with various chromosomal rearrangements were classified as stage A, four as stage B and two as stage C and three of the twelve patients with a complex karyotype were considered as stage B and nine as stage C. In conclusion, the ODN+IL2 combination i) allows a precise definition of the chromosomal pattern as it is the only method that reveals complex karyotypes, ii) detects clonal defects at a rate similar to that of iFISH, iii) reveals minor +12 clonal cell populations; iv) does not always discover 13q- because of the sub-microscopic nature of this defect; v) shows that the loss of one p53 signal is frequently due to an unbalanced rearrangement. Thus, the ODN+IL2 combination should be used to complement iFISH results.

P125

NEXT GENERATION TRANSCRIPTOME SEQUENCING UNVEIL A CRYPTIC ETV6/ABL FUSION IN A PH NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASM WITH NORMAL KARYOTYPE

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Background In recent years, the discovery of new genetic lesions such as mutations of JAK2 and TET2 genes has provided crucial insight to understand the molecular pathogenesis of chronic myeloproliferative neoplasms (MPNs); nonetheless, the underlying genetic lesion remains unknown in many cases. High throughput transcriptome sequencing may represent a rapid and widely applicable tool to identify genetic lesions and to offer the possibility to apply new molecular targeted therapies. Patients and methods In 1997 a diagnosis of atypical Ph-, chronic MPN was posed to a 63 years old woman with leukocytosis and splenomegaly. Cytogenetic analysis performed at diagnosis and repeated in year 2009, showed a normal karyotype and molecular analysis (FISH and RT-PCR) repeatedly proved negative for BCR-ABL p210, p190 and p230. Whole transcriptome sequencing (RNAseq) was performed on RNA derived from peripheral blood granulocytes (Illumina platform) and SNP analysis (Affymetrix) was applied to granulocytes as well as CD3 positive T-lymphocytes derived DNA to discriminate individual variations from neoplasm-associated alterations. The identified chimeric fusion gene was validated with FISH, RT-PCR and conventional sequencing. Imatinib was administered at 400mg/die and the fusion transcript monitored with RQ-PCR during treatment. Results RNAseq unveiled the presence of an ETV6/ABL chimeric fusion transcript as the only fusion gene emerging among a huge amount of potentially interesting results. The concomitant SNP analysis allowed to demonstrate the presence of a deletion on the long arm of chromosome 9 and a duplicated region on the short arm of chromosome 12. FISH analysis showed the co localization of the ETV6 and ABL probes on chromosome 9 thus confirming the presence of a cryptic translocation; RT-PCR and direct sequencing of PCR product confirmed the presence of a fusion between ETV6 exon 5 and ABL exon 2. Due to the reported sensitivity of ETV6-ABL positive diseases to TKI, Imatinib was given to the patient at 400 mg/die. A rapid and complete normalization of blood counts and splenomegaly was documented in less than 2 weeks and molecular monitoring by sequential RQ-PCR showed a transcript reduction during the 3 month treatment. Conclusion Our results underline the clinical utility of high throughput whole transcriptome sequencing to identify cryptic genetic lesions in patients with a normal karyotype. This approach can lead to tailored, effective treatments.

P126

15 YEAR EXPERIENCE IN MOLECULAR DIAGNOSIS AT CASTELFRANCO VENETO: PRENATAL DIAGNOSIS FOR HAEMOPHILIA IN A SINGLE CENTRE

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Since 1996 our centre have been involved in carrier status investigation for 483 and 241 females for HA and HB respectively. among these females, 263 and 163 have been defined as carriers for HA and HB respectively. As stated by the Italian Association of Haemophilia Centres (AICE), PND would be offered only in severe cases. In total 88 PNDs were required. We have performed 78 prenatal diagnosis (PND), 53 for HA and 25 for HB, on 48 severe HA and HB carriers. 10 further cases would have required PND, but DNA analysis was not performed because of spontaneous miscarriage. female foetal gender determination was obtained from cariotypic or maternal peripheral blood analysis in 12 cases and no further investigation was needed. Genomic DNA was used and molecular investigation were performed in all within the 12th weeks of pregnancy. For sex determination we used specific primers for single copy amelogenin-encoding gene (AMD) mapping both on X and Y chromosomes. In male foetus the mutation detection was performed by Long Distance PCR for F8 gene inversion involving intron 22 and 1. for other defects, PCR was followed by conformation sensitive gel electrophoresis (CSGE) or BY direct sequencing OF F8 or F9 fragments containing the mutation which was previously identified in the carrier mother. For severe cases of haemophilia A, F8 gene inversions represent the most important causative mutations: invint22 accounts for 23 causative mutation and invint1 for 1 in our cohort. 3 large deletions and 51 diverse point mutations accounted for the remaining cases. Gender determination was done for 78 foetal samples and we found 36 females (46%) and 42 males (54%). Among the males, 22 were affected and 20 were not (28% and 26% respectively). PNDs were done within four days after CVS sampling, leading to complete results as soon as possible. many carriers' requests came from other Italian centres and we do not have detailed data: anyway, we have the strong suspicion that in the majority of affected males' diagnosis the carrier women decided for voluntary interruption of the pregnancy.

P127

ITALIAN HAEMOPHILIA B (HB) MUTATION DATABASE: AN UPDATE AND SUGGESTION FOR GENETIC COUNSELLING BOTH IN PATIENTS AND IN FEMALES

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Causative defects identification for HB can be obtained through molecular investigation: mutations are extremely diverse and span whole Fac-

tor IX gene (F9). The HB mutations database created for Italian Association of Haemophilia Centre (AICE) should be a powerful tool for the management of the bulk of recorded data. The database includes so far 432 diverse patients (269 severe, 86 moderate and 77 mild): 354 were unrelated (215 severe, 73 moderate and 66 mild). In 3 unrelated patients (1 severe and 2 mild) the mutation was not found. 183 unique mutations encompass 10 large and 11 small deletions, 1 deletion/insertion, 2 insertions, 118 missense, 21 nonsense, 15 splicing mutations, 4 in the promoter and 1 silent variant. Most patients (70%) have missense mutations (247 out of 351), showing different distribution in severe (57%) and in moderate/mild (91%) patients. In severe patients deletions and nonsense mutations are more frequent, the first accounting for 34 cases in severe and 1 in moderate/mild class; the second account for 41 cases in severe and 2 in moderate/mild patients. Nine of severe patients (3%) developed inhibitors: 4 showed a complete gene deletion while the other 5 have nonsense mutations. The HB database provide opportunity to perform carrier testing and Prenatal Diagnosis (PND) by mutation analysis in females. So far, 233 women at risk of transmitting the disease have been investigated, resulting in 127 carriers, 36 obliged carriers, 70 not carriers. So far, 22 HB carriers asked for PND in 29 instances. In 19 out of 29 requests, foetal DNA analysis was performed in our lab. Foetal gender was also determined by kariotypic or maternal peripheral blood test elsewhere, for a total of 25 foetal sex determinations: the foetus was female in 14 cases (56%) and male in 11 cases (44%). Of the 11 male foetuses, 4 were affected and 7 were not (16% and 28% respectively). The HB database could give useful information both for risk of inhibitor or anaphilaxis development in patients and for counselling in carrier status determination and PND.

P128

MOLECULAR ANALYSIS AND GENETIC COUNSELLING FOR HAEMOPHILIA A AT CASTELFRANCO VENETO

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Starting from 1996 our centre has been working in genetic counselling for patients and families with haemophilia A (HA) and B (HB). In our centre 539 HA patients' DNA have been collected and most of them have been investigated for the causative mutation. According to their severity, the HA patients are 480 severe, 37 moderate and 22 mild. F8 gene inversions involving intron 22 (invint22) and intron 1 (invint1) were investigated in 480 severe patients and were found in 223 (46%) and 10 (2.1%) respectively. All the mild/moderate patients and inversions negative severe patients were further investigated for the causative mutation. Among severe HA characterized patients, 223 (51%) have Invint22, 10 (2.3%) have Invint1, 133 (30.4%) have point mutations (46 nonsense, 71 missense, 14 splicing site defects and 2 missense in splicing site), 18 (4.2%) have large deletions, 29 (6.6%) have small deletions, 22 (5%) have small insertions and 2 (0.5%) have a combined deletion/insertion. Among non severe HA characterized patients we found 3 (5.1%) small deletion, 1 (1.7%) small insertion, 54 (91.5%) point mutations (47 missense and 7 splicing site defects) and 1 (1.7%) duplication of exon 13. Most patients (45%) have invint22 (223 out of 496) and missense mutations (118 out of 496) show a different distribution in severe (16%) and in moderate/mild (79.7%) patients. Large deletions and nonsense mutations are present only in severe patients. Genetic counselling led to investigation of 468 related females. Among them, 263 females (56%) were found to be carrier. To date, we have performed 47 prenatal diagnosis (PND). In 12 further cases the DNA analysis was not performed here because of miscarriage (6 cases) or female foetal gender determination obtained elsewhere by PCR on foetal DNA, by cariotype analysis or maternal peripheral blood analysis (6 cases). In this cohort, the gender sex determination was done for 53 foetal samples and we found 22 female foetus (41,5%) and 31 male foetus (58,5%). Among the male foetus for which we carried on with molecular analysis, 18 (34%) were affected and 13 (24.5%) were not affected.

P129

ROLE OF GATA-1 ISOFORMS IN TRANSIENT MIELOPROLIFERATIVE DISEASE ASSOCIATED WITH DOWN SYNDROME

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Transient myeloproliferative disorder (TMD) is a leukemoid reaction occurring occasionally in Down syndrome (DS) newborn infants. Acute megakaryocytic leukemia (AMKL) develops in approximately 20% to 30% of the cases with TMD. Blast cells in most patients with TMD have mutations in exon 2 of the gene coding the transcription factor GATA-1. Recently, we found GATA-1 mutations in the pheripheral blood of two newborns with TMD-DS: a nonsense mutation occurring at position c189 (Tyr63Stop) (TAC/TAA), already described (1), and an insertion of 22 nucleotides at position c153, not yet reported in literature, which creates a premature stop at codon 74. Both these mutations occur in exon 2 and lead to a truncated protein missing the N-terminal transactivation domain (GATA-1 short or GATA-1s) (2). Moreover, according to literature data, in these patients WT1 expression levels in pheripheral blood were found elevated at birth and then normalized within the first month of life, following TMD remission (3). In order to evaluate effects of GATA-1 isoforms on WT1 expression, we performed over-expression experiments of GATA-1 and GATA-1s in K562 cells. Results showed that GATA-1s was able to induce a more dramatic increase of WT1 expression respect to GATA-1. GATA-1 binding sites have been described in WT1 gene regulatory elements, particularly in the proximal promoter and in two enhancer regions, located in the third intron and the 3' UTR, respectively (4). On the basis of all these data we have now performed reporter gene assays using luciferase vectors containing one or more of the GATA-1 binding sites reported in the WT1 gene. Transient co-transfection experiments with GATA-1 and GATA-1s isoforms in K562 cells have shown that higher 3'UTR enhancer activity is associated with the full-lenght isoform of GATA-1, whereas GATA-1s plays a major transacting role on the proximal promoter and intron 3 enhancer elements. These results suggest that GATA-1 and GATA-1s could transactive WT1 gene expression through different mechanisms, probably mediated by different protein complexes. Understanding these mechanisms could contribute to clarify the molecular basis of leukemic transformation in this disease. 1. Kanezaki R. et al. Blood (2010); 116: 4631-8. 2. Crispino JD. Pediatric Blood & Cancer (2005); 44:40-4 3. Hasle H. et al. Leukemia (2006); 30: 543-6. 4. Furuhata A. et al. Leukemia (2009); 23: 1270-7.

P130

CRYPTIC ASPECTS IN ACUTE PROMYELOCYTIC LEUKEMIA(APL): PITFALL IN MORPHOLOGICAL DIAGNOSIS

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Two main morphological subtypes of APL are recognised: hypergranular or classic and hypogranular or variant subtypes. Recently a few cases of atypical APL were described, which were characterized by hyperbasophilic microgranular blasts with cytoplasmic blebs resembling micromegakaryoblasts. In these cryptic forms, cytogenetic and molecular biology are required to confirm the APL diagnosis. We report three female cases (aged 54, 59 and 80 years respectively) of cryptic APL, observed between February 2005 and May 2010. At light microscopy, both bone marrow and peripheral blood showed in the 3 cases hyperbasophilic agranular/ microgranular blasts with cytoplasmic budding mimicking M7 blast cells. As to immunophenotypic features, blast cells stained positive for HLA-DR,,CD34,CD13, CD33, CD2, and CD9. Cytogenetic and molecular biology analyses demonstrated in all cases the presence of t (15;17) (q2;q21) and PML/RARa rearrangement thereby confirming APL diagnosis. Patients 1 and 2 were enrolled in the APL-specific GIMEMA protocol AIDA Morphological Complete Remission(CR) was achieved in both cases after induction therapy. Interestingly, patient 3 was firstly diagnosed by morphology as AML without maturation (WHO 2008). Subsequently, the results of molecular studies indicated a diagnosis of APL, but patient died of cardiac failure soon thereafter. To date patients 1 and 2 are

alive: case 1 is in 2nd CR (+31m) after Arsenic Trioxide therapy and case 2 is in CCR (+59 m). These three cases prove how heterogeneous the morphology of APL blasts can be, and confirm that cytogenetic and molecular biology are mandatory for correct diagnosis of APL..As to the morphological evaluation of CR or relapse, we recommend that the presence of either typical or atypical APL blasts should be sought in order to avoid a misdiagnosis.

P131

QUANTITATIVE PCR ASSAY FOR THE DETECTION OF JAK2 WILD TYPE AND JAK2 V617F GENE EXSPRESSION: VALIDATION ON PATIENTS WITH PH-NEGATIVE MYLOPROLIFERATIVE NEOPLASMS

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The JAK2V617F mutation is associated with Ph-negative myeloproliferative neoplasms (MPN) and the quantity of JAK2V617F allele burden may be associated with the myeloproliferative phenotype. Currently, the JAK2V617F allele quantification is performed on genomic DNA, but Zhao et al (2005) demonstrated that the percentage of JAK2V617F is usually higher in cDNA than in genomic DNA when cell samples from patients with polycythemia vera (PV) are analyzed. We developed an absolute allelic-specific RQ-PCR method to quantify JAK2 wild type (wt), JAK2V617F and ABL (housekeeping gene) transcript levels. In order to construct reference curves, two plasmid standards were manufactured to contain 150 bp of JAK2 cDNA wt and JAK2V617F sequence, respectively. Each plasmid vector includes 10-fold serial dilutions. cDNA was obtained from RNA extracted from K562 (JAK2wt) and HEL (JAK2V617F) human erythroleukemic cell lines. We modified the primers described by Merker et al (2010) to perform an allelic discrimination: reverse primers are complementary to the wild type or to the mutant cDNA sequence. Sensitivity was determined by a serial 10-fold dilutions of the K562 and HEL cell lines which were included in each experiment as negative and positive controls. We tested cDNA from peripheral blood buffy coat specimens of 18 patients with myeloproliferative neoplasms (5 PV; 12 essential thrombocythaemia-TE; 1 primary myelofibrosis-PMF), 8 patients with secondary polycythemia (PS) and 12 healthy donors. For all patients, JAK2V617F mutation was previously identified by qualitative PCR method (Baxter el al, 2005); the results of our RQ-PCR assay show a correlation of 100% with the qualitative method. Our assay was specifically designed and evaluated for the detection of the JAK2wt and JAK2V617F transcript levels. The mean expression of JAK2wt and JAK2V617F allele, indicated as absolute measure of averaged copies x102 + SEM x102, respectively, was: 157+38 and 0 for healthy donors; 144±32 and 0 for PS; 60±38 and 242±110 for PV; 27±13 and 0 for JAK2wt TE; 26±9 and 50±14 for JAK2V617F TE; 40 and 201 for PMF patient. For each patient we calculated the JAK2V617F/JAK2wt allele ratio and the ratio mean was 10.8±4 for PV; 0.73±0.18 for JAK2V617F TE and 4.95 for PMF patient. In summary, our initial data demonstrated that this method is sensitive, specific and reproducible. Its aim is to detect the absolute quantity of JAK2 transcripts and subsequently, to monitor minimal residual disease.

P132

EVIDENCE FOR REDUCED ANGIOGENESIS IN BONE MARROW (BM) IN SYSTEMIC SCLEROSIS (SSC)

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Background: Dysfunctional angiogenesis is a pathogenetic marker of SSc. Microvascular endothelial cells have a reduced capacity to form capillaries, reduced circulating levels of endothelial progenitor cells have been found and mesenchymal stromal cells differentiated in endothelial cells have shown a defective capacity to form capillaries .The hypothesis is that some modifications are in act already in the BM of SSc patients. Objectives: To study in SSc BM the angiogenetic process, the cellular immune system and fibrosis Methods: 8 SSc patients affected by a severe diffuse SSc and screened for autologous hemopoietic stem cells transplantation, underwent a BM biopsy to assess cellularity and morphology.BM biopsies were compared with 5 healty controls. To evaluate angiogenesis, immune system and fibrosis the following antibodies were used: VEGF, KDR/flk-1, MMP-9, CD34/QBEND10, vWF, CD20, CD3, CD4, CD8, CD38, K, lambda, CD68/PGM-1, CD61. To evaluate fibrosis silver impegnation for reticulum was used. The number of vessels, the mean area of vascularisation, the perimeter and microvessel density (MVD) were measured with a multiparametric computerized imagine analysis. Results: Morphology of BM was similar in SSc and controls. Also B cells population was similar but only a reduction of CD4/CD8 ratio was observed in SSc. A significant reduction in BM vascularity was found: both microvessel density and number of vessels were lower while VEGF expression was much higher than in controls. In seven patients a weak expression of KDR/flk-1 was observed and MMP-9 expression was low in all cases.Out of 8 patients 2 had a maximal while other 2 had a moderate grade of BM fibrosis. Conclusion: In SSc,BM is characterised by a reduction of angiogenesis that may induce the increase of VEGF.

P133

CELL POPULATION DATA PROVIDED BY UNICEL DXH 800 ALLOW IDENTIFICATION AND CLASSIFICATION OF LYMPHOPROLIFERATIVE DISORDERS.

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Objectives: Laboratory diagnosis of lymphoproliferative disorders (LD) is based on automated WBC count, microscopy and immunophenotyping assessed by flow cytometry according the consensus panels. Our previous experience with VCS technology of Beckman Coulter showed important correlation between VCS Cell Population Data (CPD) and other data coming from microscopy, flow-cytometry, cytogenetics and molecular biology. For this purpose, we decided to explore if UniCel DxH800 CPD could provide useful information to laboratory and clinical hematology. Methods: More than 50 new diagnosed untreated patients and 90 healthy donors were analyzed in this study with UniCel DxH800 that performs leukocytes differential with the Flow Cytometric Digital Morphology (FCDM) technology, based on the measurements of Volume (V), Conductivity (C) and 5-angle Scatter light laser (UMALS, MALS, LMALS, LALS, AL2). Mean and standard deviation of FCDM measurements are collected in 56 CPD. Results: We present some of the most important findings on CPD usefulness in comparison between normal values and lymphocyte pathologies according morphology and immunophenotype. Reference interval lymphocyte CPD were calculated from 90 healthy donor samples. In CLL samples, we confirmed our previous data showing that mean volume LY (MV-LY) is significantly lower than in normal samples (83 au vs 89 au) and we discovered that lymphocyte axial-light- loss (AL2) is also lower (41 vs 68). These 2 CPD can describe the morphological findings of both lymphocyte populations with low homogeneous volume and CLL with heterogeneous features. Even in leukemic lymphomas we found correlation between MV-LY (95), AL2-LY (74) and morphological abnormalities related to lymphoma cells. One of most important findings was the discovering of 3-years old ALL sample with lymphocytosis and without leukocytosis; the higher SD-LY-AL2 (14) vs normal (10), induced us to follow-up in the diagnosis. Conclusions: We presented the first consideration on how UniCel DxH800 CPD can provide useful information to clinical hematology. Different patterns of scatterplot with different CPD values can help Coulter users in the validating process both in large laboratories and in clinical hematology lab. The first ones need screening tools while the second ones need classification tools useful also in the follow-up of the patients' therapy and prognosis. These preliminary observations are now under investigation in a multicentric evaluation.

P134

CORRELATION BETWEEN IMMUNOPHENOTYPE, CYTOGENETIC ABERRATIONS AND IMMUNOGLOBULIN VARIABLE REGION MUTATION FOR IDENTIFICATION OF CHRONIC LYMPHOCYTIC LEUKEMIA HIGH RISK SUBGROUP

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Background: The presence of unmutated Ig VH genes and ZAP-70 or CD 38 immunophenotype positivity has been shown to identify a subgroup of B-CLL (chronic lymphocytic leukemia) patients with progressive

disease and a poor outcome, but their concordance is variable and not completely confirmed. AIM: To Identify biological variables to correctly stratify B-CLL patients for accurate prediction of clinical course. METHODS: In this study, 53 CLL patients (26 female and 27 male) observed at our institution, were analyzed for CLL antigen panel expression by flow cytometry , VH status by DNA sequencing and genomic aberrations by fluorescence in situ hybridization (FISH) on peripheral blood samples. Results: In our cohort of patients most frequently expressed VH gene family was found to be VH3 (60.9%) followed by VH2 (7.8%) , VH5 (7.8%), VH4 (6.3%) and VH6 (6.3%), VH1 (3.1%) ,no expression of VH7 gene families or VH1-69 and VH3-21 and the 7.8% of blood sample were not available. Most of the patients were VH mutated (46/53:86.8%) and the minority (7/53:13.2%) were VH unmutated with cut off 98-100%. VH unmutation status and CD79b expression were strongly associated in our CLL patients (86%). In 71,5% of unmutated IgVH patients was observed high risk cytogenetic aberrations as del(17p13) or del(11q22.3),these were all CLL patients with B and C Binet stages(advanced or symptomatic disease), while in 28.5% of unmutated IgVH presented del(13q14) with Binet stages A (early stage) but associated aberrant high CD79b expression and rapid disease progression (lymphocyte doubling time <6 months). By multivariate regression analysis that include high-risk genomic aberrations and, alternatively or contestual, variables as VH mutation status and CD79b expression, the correlation reach a significantly result ($p<0.001$)as independent outcome predictors. SUMMARY/Conclusions: Further extensive studies may be required to confirm the multivariate correlation between the mutational status of IgVH genes, CD79b expression, Binet stage and cytogenetic abnormalities.useful to define an CLL patients score system for routine diagnosis and prognostic impact.

P135

EFFICACY OF A PANEL OF SHORT TANDEM REPEATS IN CHIMERIS STATUS ANALYSIS: INFORMATIVITY OF THE SINGLE MARKERS EVALUATED ON A LARGE NUMBER OF DONOR/RECIPIENT COUPLE

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Short Tandem Repeats (STR) polymorphysm analisys is actually the most employed method for the study of Chimerism Status (CS) after Hematopoietic Stem Cell Transplantation (HSCT). Quantitative or semiquantitative assays based on PCR can do a good assessment of the Donor/Recipient (D/R) ratio in a cellular subset. Despite these considerations, actually there is not a standardized STR panel for the CS study only and many laboratories employ panels used in forensic studies. Here we present our experience in CS analysis employing a forensic kit (Powerplex 16, Promega USA) in 233 D/R couples: 120 derived from sibling transplants (ST) and 113 derived from unrelated donor transplant(MUD) Aim of the study: primary end point was to evaluate the efficacy of the kit in providing informative markers. Secondary end points was to define the more and the less useful markers in CS analysis considering two different kinds of D/R couples: familiar in ST and not familiar in MUD. Result: The panel provide at least one informative marker for the CS analysis in 99% of MUD and 98% of SB. The majority of D/R couples had 2 informative markers (42% in ST and 40% in MUD), 19% and 13% had only 1 marker and 39% and 48% had from 3 to 6 markers in ST and MUD respectively. Considering the single markers, we have seen that 10 of them have an high frequency of informativeness either in ST or in MUD, despite there are 3 markers that are useless because rarely significant and never single. The single STR markers in order of significance are: Amelogenin, Penta D, TH01, Penta E, D18S51, TPOX, D13S317, D8S1179, D7S820, D21S11, D16S539, D3S1358, FGA, D5S818, Vwa, CSF1PO. These data have been confirmed also with a statistical analysis based on the linear regression test which compares the level of significance of each marker in our population with the level of variability of each STR allele in the general population. We have seen a direct correlation of these two variables (R2: 0,20). We have also seen that in our population 12 STR alleles are sufficient to provide at least 1 markers for CS in 99% of D/R couples, but considering the general population and the level of variability of each markers we estimate that only the last 3 ones could be eliminated. Conclusion: these data confirm the high efficacy of this panel in providing informative markers for CS study. We retain that some STR alleles which have a low variability level in the population could be replaced with other more informative.

ANEMIAS AND THALASSEMIAS

P136

A FATAL CASE OF LEGIONNAIRE'S DISEASE ASSOCIATED WITH COLD AGGLUTININ DISEASE

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Cold agglutinin disease usually develops as a result of the production of a specific immunoglobulin M auto-antibody directed against red blood cells. Autoimmune and lymphoproliferative disorders, or infections can be associated with the production of cold agglutinins. We report a case of fatal cold agglutinin disease associated at Legionnaire's disease. A young man was admitted to our Hospital in August 2010 for anemia, jaundice and fever. The bone marrow aspirate showed erythroid hyperplasia of the marrow, while the direct Coombs test showed the presence of cold auto-antibodies. Viral and tumoral markers were absent, not evidence of lymphoproliferative disease and mycoplasma infection. He was diagnosed with autoimmune cold agglutinin disease, and he was treated with steroids at doses of 1.5 mg/ kg. On day +7 the patient was in haematological recovery and fever had disappeared. Two weeks later, the patient was hospitalized again for another episode of haemolysis. He had high fever and dyspnoea, and needed for blood transfusions daily. A chest CT showed diffuse pulmonary consolidations. The Legionella pneumophila antigen in urine proved positive and he was diagnosed with Legionnaire's disease. The patient was then treated with macrolides and fluorochinolones and steroid therapy was slowly suspended, the patient underwent therapy with immunoglobulins 30g/die for five days . On days +10 from second hospitalization he was severely anaemic and needed daily blood transfusions. The fever had disappeared and dyspnoea was improved. On days + 15 fever reappeared and it showed an increase of direct bilirubin and transaminase. Legionella pneumophila antigen in urine was still positive. On days + 16 it was decided to refer the patient to plasmapheresis. On days + 17 he died in intensive care for multi-organ failure. The legionnaire's disease is responsible for 1-8% of all community- acquired pneumonia requiring hospitalization, and about 4% of fatal nosocomial pneumonia. In this case , the patient has been infected before the first hospitalization, and,subsequently,because of immunosuppression caused by steroid, he died of Legionnaire's disease. Cases of Legionnaire's disease and cold agglutinin disease are very rare in the literature. This clinical case shows that Legionella pneumophila should be considered a potential causative agent in patients with pneumonia and cold agglutinin disease, to avoid, if possible, immunosuppressive therapy.

P137

RITUXIMAB AS LIFE SAVING DRUG IN AUTOIMMUNE HEMOLITYC ANEMIA REFRACTORY TO SPLENECTOMY:A CASE REPORT.

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Autoimmune haemolytic anemia (AIHA) is an immune disorder, potentially lethal ,caused by antibodies directed against autologous red cells. Rituximab-induced B-cell depletion has been proven to be a useful therapy for AIHA. In literature is debated to use rituximab after splenectomy for high incidence of fatal infections. We describe a case of AIHA refractory to medical and surgical therapy but responsive to therapy with rituximab. A young woman with a medical history positive for Hodgkin lymphoma in complete remission for five years, was admitted to our Hospital in July 2010, for anemia, jaundice and fever. She was diagnosed with autoimmune hemolytic anemia with Coombs direct and indirect positive. She was treated with steroids at doses of 1.5 mg/ kg which responded not satisfactorily, thus this treatment was enhanced with the infusion of high-dose immunoglobulin to which the patient responded slowly. Day +21 after diagnosis, she was discharged. In august 2010 she was hospitalized again for new episode of haemolysis and fever. She began antibiotic and antiviral therapy, and she associated with high-dose steroid therapy, treatment with cyclophosphamide. At days +14 after second hospitalization, the patient was apiretic ;she not responded to therapy for AIHA; clinical condition was poor and she was

constantly anemic (Hb 4.5 g / dl), and needed daily support for urgent blood transfusion with blood bags no tested. For refractory to medical therapy and critical status, she underwent emergency splenectomy. At day +18 the condition of the patient did not vary. The patient made, between August and September 2010, Rrituximab treatment at dose of 375 mg/m2 weekly for four weeks . At days +40, transfusion support wasn't reduced, Coombs tests was still positive, she also reactivated cytomegalovirus and she started specific therapy with ganciclovir. At days +60, transfusion support was reduced considerably and the haemoglobin was increasing; Coombs tests and molecular CMV was negative and she stopped ganciclovir treatment. At + 6 months after Rituximab treatment she is in good condition, no needs transfusion support and Coombs tests is negative. This clinical case indicate that rituximab is a saving life drug in patients with AIHA in this setting, should not be a limit to such therapy; a good prophylaxis and a constant monitoring of opportunistic infections are appropriate.

P138

A CASE OF CONGENITAL RED CELL PYRUVATE KINASE DEFICIENCY ASSOCIATED WITH HEREDITARY SPHEROCYTOSIS

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Pyruvate kinase (PK) deficiency, transmitted as an autosomal recessive trait, is the most common erythroenzymopathy of glycolytic pathway (prevalence of 1:20,000) associated with chronic non spherocytic haemolytic anaemia from mild to severe. More than 180 mutations in the PK-LR gene have been so far reported, and genotype-phenotype correlation has been established for some of them. Hereditary Spherocytosis (HS) is the most common congenital haemolytic anaemia in Caucasians, with an estimated prevalence ranging from 1:2000 to 1:5000. The main clinical features are haemolytic anaemia from compensated to severe, variable jaundice, splenomegaly and cholelythiasis. The molecular defect is highly heterogeneous, caused by proteins involved in the attachment of cytoskeleton to the membrane integral domain (spectrin, ankyrin, band 3 and protein 4.2). We describe a case of PK deficiency associated with HS. The propositus was a 13 years-old Italian male with neonatal jaundice and need of blood transfusion (Hb 5.8 g/dL) during an infectious episode. The study of the most important red cell enzymes revealed reduced PK activity (59% of normal). Direct sequencing of PK-LR gene showed compound heterozygosity for 994A mutation (Gly332Ser) and -148T localized the erythroid specific promoter region. The presence of spherocytes in peripheral blood smear prompted us to investigate for the coexistence of HS. SDS–PAGE analysis of red cell membrane proteins reveals a 30% spectrin reduction. Family study demonstrated an heterozygous condition for the 994A mutation in the father who also displayed comparable enzyme deficiency. Mutation -148T was detected in the brother and mother. No red cell membrane abnormalities were present in the family members, although positive EMA binding test and increased osmotic fragility were found in the father and brother. The co-existence of HS and PK deficiency is very rare event, only few cases are described to date. Clinical, family and molecular studies allowed the determination of the interrelationship between the two RBC abnormalities in the patient and his relatives. The reduced PK activity in the propositus and his father is justified by heterozugous 994A. More severe clinical picture in the propositus could be due to the coexistence HS and presence of -148T mutation, that although it seems not to have effects on mRNA expression is, in literature often detected in PK deficient patients with heterozygous PK mutations.

P139

MOLECULAR ANALYSIS OF THE SEC23B GENE IN PATIENTS AFFECTED BY CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II (CDAII)

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CDAII, the most frequent type of congenital dyserythropoietic anemia, is an autosomal recessive disease characterized by ineffective erythropoiesis, peripheral hemolysis, erythroblast morphological abnormalities and hypoglycosylation of some RBC membrane proteins. In 2009 we and others identified SEC23B as the gene responsible for CDAII (Schwarz et al, 2009, Bianchi et al, 2009). SEC23B is a member of the SEC23/SEC24 family, a component of COPII coat protein complex which is involved in protein trafficking through membrane vesicles from the endoplasmic reticulum to the Golgi apparatus. The gene, localized on chromosome 20p11, is split in 20 exons and codifies for a 767 aa protein. The aim of the study was to characterize the molecular defect in a large series of CDAII patients of Caucasian origin. 25 CDAII patients from 23 unrelated families (17 Italians, 4 Dutch) were analyzed by direct exon sequencing. We identified 15 different mutations, 6 of which were not described before (c. 1798 G>A, Asp600Asn; c.1129_1130del, p. Asp377Phefs*17; c.733_735del, p. Leu 245del; c. 28C>T, p. Gln10X; c.52 C>T, p.Arg 18Cys; c.640 c>t, p. Gln214X). Eight mutations were disruptive and 7 were missense mutations. All the missense mutations affected highly conserved aminoacids and were not found in 200 normal alleles examined. The c.325G>A mutation was identified in 8 homozygous patients and in three cases in combination with other mutations. The change c.40C>T was detected in 10 unrelated patients as heterozygous mutation, never at homozygote level. Considering the entire series of patients (including previously published cases) characterized by our group (40 CDAII patients from 35 unrelated families), c.325G>A and c.40C>T mutations account for 52% (37/70) of the unrelated mutated alleles and therefore should be firstly screened during molecular diagnosis of CDAII (SEC23B gene exons 2 and 4). A c.325G>A mutation usually results in a mild to moderate clinical picture at homozygous level, whereas it may cause a very severe clinical pictures when combined with other mutations (Fermo, et al 2010, and this study). Despite the sequencing of all exons and flanking intronic regions, 1 patient displayed only one mutation, suggesting the possibility that mutations could be located in regulatory regions or that a second gene could be involved in the pathogenesis of CDAII.

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LOW-DOSE RITUXIMAB IN IDIOPATHIC AUTOIMMUNE HEMOLYTIC ANEMIA

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Conventional therapy of warm autoimmune hemolytic anemia (WAIHA) includes corticosteroids and immunosuppressive agents, or splenectomy, whereas no effective treatment exists for cold hemagglutinin disease (CHD). A substantial proportion of patients with WAIHA does not respond to or relapse after corticosteroid therapy. Favourable responses to rituximab at standard doses have been reported in both WAIHA and CHD, and in other autoimmune diseases. 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. Aims: 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. days 1-30, 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. Results: 23 patients (16 F, 7 M; median age 56 yrs, range 27-75) were enrolled, with a median Hb value at enrolment of 9.1 g/dL (range 4.4-12.3). An iCR and iPR were observed in 15/23 (65.2%) and 4/23 (34.8%) patients, respectively; a SR at month 6 was observed in 20/22 (91%) and at month 12 in 16/19

(89%) evaluable patients. The 6 and 12 months cumulative relapse-free survival(RFS) estimated by Kaplan-Mayer analysis was 95.5% and 80.0%, respectively. Univariate analysis showed that response at month 2, 6, and 12 was significantly associated with warm AIHA (P=0.023, P=0.047, and P=0.013 respectively). General estimating equation analysis showed that a lower cumulative dose (roughly 50%) of steroid was administered to relapsed patients during the study compared with previous therapy. These results indicate that LD-rituximab associated with standard steroid therapy is a safe and effective treatment, particularly in WAIHA; re-treatment with LD-rituximab is also effective, leaving patients free of therapy for long periods and resulting in a steroid-sparing effect.

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NEW EPIDEMIOLOGY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN ITALY

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Background. Glucose 6-phosphate dehydrogenase (G6PD) deficiency is one of the most common red cell abnormalities characterized by a wide clinical, biochemical, and molecular heterogeneity. So far more than 200 G6PD mutations have been described. The distribution and frequency of genetic variants differs among various populations depending on ethnicity and geographical areas. Because of new migration, some variants, previously never detected in Europe, are now present. Aims. This is a retrospective study aimed to identify the G6PD variants among all the G6PD deficient subjects referred since 2007 to the Hereditary Anemia Centre at Policlinico "Ca' Granda" Foundation in Milan. The studied subjects were sent because of known G6PD deficiency, or for preconceptional genetic counselling or to define genotype. METHODS. Haematological routine parameters, G6PD activity and molecular analysis of G6PD gene were performed for each subject. The molecular analysis has been carried out by restriction fragments length and direct DNA sequencing. Results. Since 2007, 138 G6PD deficient subjects have been identified, 11.6 % of whom were immigrants, mainly from Central Africa and South East Asia. 94 (68.1%) subjects had the Mediterranean variant and 19 (13.8%) had the Seattle variant. Seven (5.1%) had Chatam variant, 5 (3.6%) had Cassano variant and 3 (2.2%) had Portici variant, that is responsible for chronic non-spherocytic haemolytic anaemia. We also registered in 4 subjects the mutation 376A/G present alone in A variant or coupled with 202 G/A mutation in A- variant and with the 542 A/T in Santamaria variant. Other variants identified in less than 1% of subjects studied were: Cairo, Mahidol, Ludhiana, Rignano, Santamaria, Sant'Antioco and Union. Conclusions. These data suggest that, due to new migration during the last 5 years, the G6PD deficiency and the identification of G6PD variant is a diagnostic issue in Italy and particularly in the north, where G6PD deficiency is not so common. It is important to suspect and soon recognize G6PD deficiency to avoid the administration of drugs or other xenobiotics which could be the triggers of hemolytic crisis.

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SCREENING OF ITALIAN BLOOD DONORS FOR ERYTHROCYTE MEMBRANE DEFECTS

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Introduction - Hereditary Spherocytosis (HS) is the most common hereditary hemolytic anemia due to a defect/deficiency in RBC membrane proteins. Clinical manifestations vary widely, ranging from asymptomatic to anemic transfusion-dependent patients and the diagnosis of HS may be missed. Severity has been related to inheritance pattern and protein defects. The aim of this study was to perform a screening of blood donors to reveal the occurrence of HS asymptomatic carriers in healthy population. Methods – We have screened for RBC osmotic fragility 2953 blood donors from Policlinico Umberto I Blood Bank (Rome) using a modified acidified glycerol lysis test (AGLT50). All positive donors were further investigated for membrane protein defects according to our diagnostic protocol for HS. It includes the evaluation of i) red cell morphology; ii) SDS-PAGE of membrane proteins and spectrin extracts; iii) non-denaturing electrophoresis of spectrin extracts. Rheological characterization of RBCs was also performed on some positive donors by measuring blood viscosity, RBCs viscoelasticity and aggregation index (Rheo-Microscope, Anton Paar). Results - 35 donors resulted positive to the screening (AGLT50 < 30 min): 9/35 donors with pathologic values (AGLT50 < 5min), as found in HS, 26/35 donors with possibly pathological values (AGLT50: 5-30 min). Up today, we have investigated 23/35 positive donors to confirm the presence of RBC membrane defects. Abnormal RBC morphology, mainly spherocytes and elliptocytes, has been observed in 18/23 cases. SDS-PAGE analyses pointed out membrane protein defects in 20/23 cases: spectrin, protein 4.1 and ankyrin deficiencies were the most frequent. Moreover in 8/23 cases an increase in spectrin dimers confirmed alteration of membrane-skeleton organization. Rheological characterization on 14/23 positive donors showed differences between positive donors with pathological and intermediate AGLT50 values; altered RBC viscoelastic profiles, that highlight RBCs with structural anomalies, have been found in donors with AGLT50 < 5 min. Conclusions – This study reveals the presence of membrane protein defects in healthy Italian blood donors suggesting that the frequency of HS trait carriers in general population could be high and most likely underestimated. The screening of blood donors for RBC osmotic fragility could be proposed in order to identify HS trait carriers and avoid consequent post-transfusion adverse events.

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A CASE OF CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II AND CONTEMPORARY ACANTHOCYTOSIS IN FAMILIAR BENIGN HYPOBETALIPOPROTEINEMIA DIAGNOSED IN ELDERLY PATIENT

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An 87 years old male was admitted in Emergency Room of our hospital for angina by discrepancy in mild anemia. In the medical history of the patient was reported splenectomy about 1960 following the diagnosis of costitutional hemolytic jaundice. Despite this surgical, subsequentely persisted mild anemia and jaundice. About 20 years ago the patient was reevaluated in other hematological institution, where was diagnosed Gilbert's syndrome with unconjugated hyperbilirubinemia in the absence of signs of hemolysis. His hemoglobin level was 9.5 g/dl, MCV was 104, WBC and platelet count were in range. Signs of hemolysis were absent (reticulocyte count, haptoglobin, LDH all in range), but was confirmed about 4 mg/dl of unconjugated bilirubina. The examination of peripheral blood smear showed a marked acanthocytosis (30%) of red cells with 3% of NRBC. The remaining blood tests showed very low values of total cholesterol, HDL, LDL and triglycerides; was also performed assay of apolipoptoteina B well below the normal range. The abdominal ultrasonography noted intense hepatic steatosis and cholelithiasis. Was therefore given a diagnosis of familiar benign hypobetalipoproteinemia, disease often completely asymptomatic and associated with a good longevity of patients. But that diagnosis did not explain the anemia and jaundice. The patient was subjected to bone marrow biopsy that showed the presence of a marked hyperplasia of erythroid precursors, with 20% of bi- or tri- or plurinucleated erythroblasts, highly suggestive of a congenital dyserythropoietic anemia type II (CDA II). For further confirmation, molecular analysis was performed with a finding of a homozygous missense mutation in exon 4 (c.325 G>A cod. GAA 109 AAA) determining an amino acid substitution (Glu109Lys) causative of CDA II. CDA II is the most common congenital dyserythropoietic anemia. Once it was frequently confused with constitutional hemolytic jaundice and in effect splenectomy can improve the anemia but does not eliminate the jaundice. Many patients die early for hemochromatosis and its complications, but there are reports of survivors more than 70 years. We did not found other report of CDA II associated with familiar benign hypobetalipoproteinemia: it may be the simultaneous presence of the latter to explain the unusual longevity of our patient.

P144

STEM CELL TRANSPLANTATION TO CURE SICKLE CELL ANEMIA

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Background. Sickle Cell anemia (SCA) remain a disease with high risk of morbidity and early death. Although medical treatments are life-extending, end-organ damage could not be avoided in most patients over time. Allogeneic haematopoietic stem cell transplantation (HSCT) is the only curative treatment for SCA. We report our experience concerning 11 geno-identical HSCT for SCA-patients prepared with the same myeloablative conditioning regiment consisting of Busulfan, Cyclophosphamide and rabbit ATG. Patients and Methods. Eleven patients with a median age of 12 years (range, 2-16), affected by sickle cell anemia (SCA), received hematopoietic stem cell transplantations from HLA-identical, related donors following a myeloablative conditioning regimen. Indications for transplantation were vaso-occlusive crisis, acute chest syndrome, avascular bone necrosis, chronic red blood cell transfusions, or hemorrhagic stroke. The cell souce was bone marrow. GVHD prophylaxis consisted of the association of cyclosporine(CSA)-short MTX. Results. All patients had sustained engraftment. One patient became a stable mixed chimera with 25% of donor cells four years after transplantation. One patient died one year after transplantation. The probability of survival, SCA-free survival, and transplant related mortality at five years after transplant were 90%, 90%, and 10%, respectively. All ten surviving patients remained free of any SCA-related events after transplantation. Conclusion. Allogeneic stem cell gene therapy is the only treatment option for patients with SCA. These results confirm that is possible to offer more than 90% chances of cure to SCA children. There is an excellent survival rate and a return to normal life, free of SCA- related events. HSCT should be considered as standard of care for SCA children.

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A USEFUL RELATIONSHIP BETWEEN THE PRESENCE OF EXTRAMEDULLARY ERYTHROPOEISIS AND THE LEVEL OF THE SOLUBLE FORM OF THE TRANSFERRIN RECEPTOR IN A LARGE COHORT OF ADULT PATIENTS AFFECTED BY THALASSEMIA INTERMEDIA: A PROSPECTIVE STUDY.

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Introduction: In thalassemia intermedia (TI) the increase in bone marrow haemopoietic activity frequently leads to extramedullary erythropoeisis (EE). No predictive factors for the presence of EE have yet been found; similarly, its relationship with the level of the soluble form of the transferrin receptor (soluble transferrin receptor, sTfR) which fully reflects the marrow erythropoietic activity, has not yet been explored. Material and methods: From January 2009 to December 2010 all TI patients attending at our center underwent sTfR assay and MRI or CT (if claustrophobic) scan evaluation for the presence of paraspinal EE. Results: a total of 55 patients with TI were studied, 52(95 %)with MRI and 5(10%) with CT, respectively. The age range was 15 to 75 (median=39) years and the haemoglobin (Hb) value range was 7.5 to 12.1 (mean=9.5) g/dl. Thirty-one (56 %) were splenectomized; thirty-two (58%) were on chelation therapy because of the presence of pathologic ferritin levels and/or pathologic liver T2* value. EE involved 21 (42%) patients; overall, the concentration of sTfR varied from 2.6 to 20.6 (mean=8.7) mg/L, but in splenectomized group and in unsplenectomized group it varied from 4.2 to 17.8 (mean ± SD= 9.9 ± 3.41) mg/L and from 2.66 to 20.6 (mean ± SD=7.1 ± 3.9) mg/L, respectively with a statistically significant intergroup difference ($p<0.05$). The T student's test revealed also that sTfR level, but not Hb level, was statistically significant different ($p<0.01$) between groups of patients with EE and in chelation therapy as compared with those without EE and not in chelation therapy, respectively. The cut-off point at 8.6 mg/L of sTfR using the ROC curve (Medcalc) showed a sensitivity of 76.2% and a specificity of 72.7%, in predicting EE but, considering the unsplenectomized subgroup, the sensitivity and the specificity raised to 100% and 95%, respectively. On the other hand, The Fisher's exact test showed that EE more frequently affected splenectomized patients with respect to unsplenectomized ones (31% vs 7,2 % , $p<0,01$). Conclusion: These data showed that in TI sTfR but not Hb level, could represent a good predictive factor of EE particularly in patients with spleen. Splenectomy, by increasing the level of sTfR level reduced its sensitivity and specifity in predicting EE but per se represented a risk factor for the presence of EE. High concentration of sTfR strongly correlated with the need of chelation therapy.

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PERSISTENT MIXED CHIMERISM AFTER BONE MARROW TRANSPLANTATION IN A CHILD WITH SICKLE CELL ANEMIA : PERIPHERAL RED BLOOD CELL SPLIT CHIMERISM AS A CONSEQUENCE OF INTRAMEDULLARY SELECTIVE APOPTOSIS OF RECIPIENT RED BLOOD CELLS

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Introduction. Allogeneic cellular gene therapy through the hemopoietic stem cell transplantation is the only radical cure for the congenital hemoglobinopathies as the thalassemia and the sickle cell anemia. Persistent mixed hemopoietic chimerism has been described after bone marrow transplant. Here we report a case of PMC four years after the transplant with 30% donor hemopoietic stem cells in the bone marrow. In the peripheral blood there is evidence of 30% of the nucleated cells of the donor while the split chimerism of circulating red blood cells (RBC) shows 80% /donor type and 20 %/host type. Materials and Methods. We performed analysis of engraftment in nucleated cells and in RBC in bone marrow and in peripheral blood after 4,5 years post transplantation. Haematological and immunological reconstitution, CD95 expression in erythroblast and in recipient and donor RBC in bone marrow and in peripheral blood are also evaluated. Results. Data of engraftment shows the presence of 80% of donor RBC at peripheral blood level and 40 % of donor RBC in bone marrow whereas no different engraftment of donor nucleated cells was observed between bone marrow and peripheral blood (40% vs 39%). At bone marrow level we found an increase of CD95% in recipient RBC respect donor RBC whereas no difference was observed in peripheral blood. An increase of caspases 3 was observed in peripheral blood vs. bone marrow. An increase of T reg cell % was observed in patient respect control. Conclusion. We found that Fas was expressed by a significantly higher proportion of bone marrow erythroblasts. This observation may suggest that Fas, expressed by early erytroblasts in vivo, might contribute to the cell death of erythroid precursors in bone marrow in mixed chimerism condition. Moreover we observed an increase of CD95% in recipient RBC respect to donor RBC in bone marrow whereas no differences were observed in the peripheral blood.

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UNIVERSAL NEWBORN SCREENING FOR SICKLE CELL DISEASE AND OTHER HEMOGLOBINOPATHIES IN FERRARA

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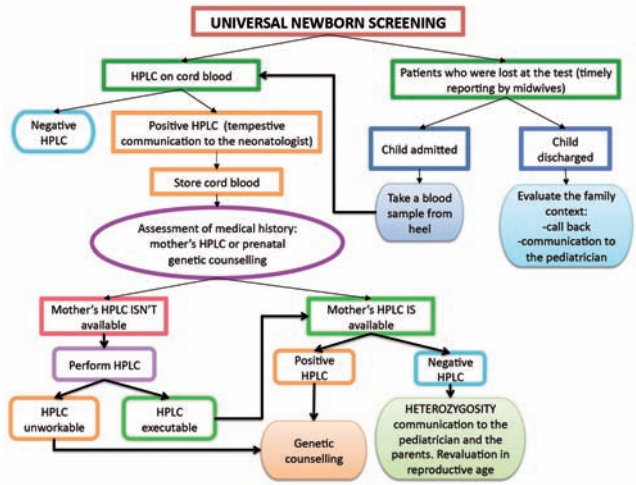
Sickle cell anaemia (SCA) is one of the commonest hemoglobinopathies in Africa, Middle East and India. Recently its incidence has increased dramatically also in Europe due to the high migration rate from endemic areas. In the province of Ferrara, from Jan 2009 to Jan 2010 the number of foreign residents increased by 14,6% most of them coming from countries where the frequency of the sickle gene is high. Neonatal screening and the introduction of prophylactic penicillin in early childhood have been proven to reduce the mortality in infancy. Also the knowledge of carrier status is important to ensure correct counselling for the family and later for the patient. Currently in Italy genetic counselling for hemoglobinophaties is provided only during the preconception period when free HPLC (high performance liquid chromatography) is offered, but only to patients with abnormal count blood. Because Ferrara is the Regional hub for the diagnosis and treatment of the hemoglobinopathies, and it has an historical record for genetic counselling of thalassemia, we decided to evaluate here the feasibility of a universal neonatal screening for SCA. The first stage of this study was

meant to assess how many pregnant women underwent HPLC and how many of them were carriers of hemoglobinopathies. The results showed that 59% of patients who delivered at the University Hospital of Ferrara, from 2007 to 2009, (4648 women) underwent the test. HbS was evidenced in 0,7% of them while HbC was present in 0,15%. However 41% of patients, many coming from endemic areas, were not tested during pregnancy. The second stage of the study included neonatal screening on cord blood, analysed by HPLC for the diagnosis of hemoglobinopathies. Finally, we aimed to create a complete database to monitor the frequency of SCA in our territory. From Sept 2010, 752 tests were performed and 10 carriers of hemoglobinopathies (1,3%) were identified: 6 HbS, 2 HbC, 1 HbDPunjab; one patient had an abnormal peak in the preHbA0 window. When a test is positive, a protocol (tab1) is applied in collaboration with other groups (Medical Genetics, Medical Laboratory and Obstetrics) to diagnose heterozygosity or homozygosity of the newborn in order to implement all the appropriate prophylactic and therapeutic measures. The parents and family pediatrician of every positive patient are given detailed information on the case. The protocol has been submitted to the Ethics Committee of the University Hospital in Ferrara.



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ABNORMAL VASCULAR ENDOTHELIAL FUNCTION AND ELEVATED FACTOR VIII-VON WILLEBRAND FACTOR RELEASE MIGHT CONTRIBUTE TO PROGRESSIVE PULMONARY ARTERIAL HYPERTENSION IN SICKLE CELL DISEASE:AN EMERGING LIFE-THREATENING ADVERSE COMPLICATION.

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During painful crisis the well known interactions between sickled erythrocytes (S-RBC),platelets,plasma adhesive proteins,abnormal cytokine release, hypercoagulation,hypofibrinolysis,endothelium suffering promote in adult/elder SCD patients a progressive pulmonary arterial hypertension(PAH)and continuous vessel remodeling as well as chronic inflammation directed at the vessel wall.Thus,PAH leads to progressive devastating clinical course of SCD patients.In this scenario, the S-RBC, platelets and plasma adhesive proteins together with chronic endothelium suffering might cause micro-and macro-vascular occlusions culminating in multiorgan damage/failure.In this context, the factor VIII/von Willebrand factor (FVIII-vWF)might play a pivotal role into microcirculatory district pulmonary reperfusion.Therefore,because of the continuous endothelial cells repair and vascular intima trophism/remodelling and repeated 'no-reflow' phenomena the PAH inevitably occurs and represents the major complication in adult/elder SCD subjects even if they are well treated .15 SCD patients(aged 44-67,females n=8,males=7)without iron overload and regularly transfused were considered.Echocardiograms were performed prospectively in SCD pts(with hypertension,diabetes and coronary artery disease n=3, hypertension alone n=3)and 11 controls.PAH was defined as a tricuspid regurgitant jet≥2.5m/s and "more severe PAH" as≥3.0m/s by a single reader according to recommendations from ASE.Pulmonary artery systolic pressure (PAP) and estimated right atrial pressure(RAP)were calculated.PAH was defined by peak tricuspid regurgitant(TR)jet velocity only.The cardiologist defined PAH as a TR jet≥2.5m/s(approximately equivalent to PAP≥35mmHg assuming a RAP of 10 mmHg);"more severePAH"as TR jet≥3.0m/s.9 SCD pts met definition of PH and3 had severe PH with larger right atrial(RA) and right ventricular(RV) sizes,higher RAP and decreased RV function. As expected, we found a significant ($p<0.001$) increase of FVIII-vWF in conjunction with significantly ($p<0.001$)elevated plasma levels of indexes of thrombin activation as Thrombin/antiThrombin (TAT)complex,Fibrinogen fragment F1+2(F1+2)and D-dimer both in steady state and during painful crisis respect to control ones.From our data we here confirm that a chronic endothelial damage with decreased nitric oxide and increased cytokines formation/release is present in SCD with continuous plasma thrombin generation and several coagulation abnormalities which contribute to impaired microcirculation and thromboembolism, as we previously reported(Musso R. et al.Blood 1993;82 (1):472).The impact of PAH on clinical outcomes of SCD pts carries a poor long-term prognosis.Emerging therapies targeting PAH and diastolic dysfunction are confirming beneficial pulmonary hemodynamic effects in SCD patients with PAH.However,what are the implications of these findings?.First, the PAH represents the major cause of mortality in SCD patients.Secondly, in the absence of clinical trials and any evidence-based guidelines an optimal care may be required to provide a better cardiovascular risk reduction.

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RARE MOLECULAR ASSOCIATION (BETA°COD 39/HB MISSISSIPPI) IN A PATIENT WHO REJECTED A BONE MARROW TRANSPLANTATION (BMT), BUT BECOME TRANSFUSION INDEPENDENT

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In the last years, owing to the considerable increase of immigrants in our Country, thalassemia (thal) and hemoglobinopathy genetic variety raised. Recently, we observed two sisters of Bulgarian origin, PMO, 27 and PEO, 26 years old: the first defined as a previous thal-major and the other diagnosed as a simple healthy carrier. The proband, splenectomised in 1993, underwent BMT from her sister in the same year. In 1995 the BMT has been rejected. In spite of transplantation rejection, the patient became transfusion therapy-free, except in 2006 in the occasion of a left hepatectomy, owing to hepatic adenoma. At the time of presentation to our Centre, the proband presented rather critical values: RBC=2.72 (mil/ul);Hb=7,4(g%);MCH=27,2(pg);MCV=85(mc);abundant and heterogeneous morphological red cell abnormalities, increased globular osmotic resistance (70% of hemolysis), HbA2=4,8% and HbF=27,2%.Total bilirubin was 3,34(mg/dL) and serum ferritin was 2.183(ng/ml). Molecular study revealed an association between the known Mediterranean defect Beta°39 (CAG—TAG) and the rare Hb Mississippi at cod 44 (TCC—TGC, Ser—Cys), only once described, but in association with a Beta+ thalassemia [Hemoglobin,11(5),435-452(1987)–JG Adams III,WT Morrison,RL Barlow,MH Steinberg], in a 6-year-old Chinese girl with chronic anemia and thal-intermedia. The simple heterozygotes for this variant Hb are clinically and hematologically normal. It is undetectable using electrophoretical procedure and cation exchange HPLC but, afterwards functional and structural studies, it has been defined mildly unstable and has been demonstrated that polymerized with itself as well as with other globin chains. Molecular analyses, extended to Alpha and Gamma globin genes, didn't reveal any other defects to justify the variation in the phenotype, resulting in the abolishing the transfusion therapy requirement. The sister of the proband presented the typical phenotype of the Beta°-thal trait, characterized by reduced MCV, MCH and increased HbA2 (Beta°39). It is very difficult to speculate on the causes involved in the independence of the proband from transfusion therapy, even if we observed similar situations yet in

the past. Between the theories it is possible to put forward, there is a derepression in the Gamma-globin genes activity, performing in an adequate Hb F production (27.2%). But, the need of a more exhaustive answer persists unresolved: it would be useful to explain similar clinical cases and to devise new therapeutic strategies.

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THE HYPERCOAGULABILITY AND VASCULAR ENDOTHELIUM ABNORMAL CYTOKINES' RELEASE MAY INFLUENCE THE PROGRESSION OF THE OSTEOPATHY IN HOMOZYGOUS -THALASSEMIA PATIENTS

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Blood transfusions are necessary for survival in patients with homozygous -thalassaemia (-Th) but a severe myocardial and hepatic iron deposition, despite their relatively low ferritin levels and apparently adequate chelation therapy,are widely documented by magnetic resonance imaging and hepatic sonography pattern together with multi-organ damage.Of course,the iron overload from the chronic hemolysis determines a vascular endothelium dysfunction with abnormal cytokines' release,activation of plasma coagulation, platelet hyperfunction, leucocytes and erythrocytes membrane changes/interactions.In this context,the chronic hypercoagulation along with reduced fibrinolysis and endothelial cells suffering would be responsible for thromboembolic events in homozygous -Th patients.In this scenario,an other severe complication such as the osteopathy,which becomes certain in adult -Th subjects,has mainly been referred to the several endocrine deficiencies,while the contribution of the hypercoagulation and abnormal vascular endothelium cytokines' release has not been considered.11 Sicilian homozygous -Th patients(6 females and 5 males),aging 26-69 yrs, were studied.15 Sicilian heterozygous -Th subjects and 10 healthy individuals of comparable age served as controls.Bone density scans showed severely low bone mass in 7/11pts and low bone mass in 4/11 respect to the control groups.The osteoblastic cytokines' net-work as the Platelet-derived growth factor(PDGF),Transforming growth factor- (TGF-)and Interferon- (IFN-)together with the osteoclastic cytokines such as the Interleukin-1(IL-1),Interleukin-6(IL-6)and Tumor necrosis factor- (TNF-)were determined by ELISA.Our results showed a significant($p<0.001$)increase of the osteoclast cytokines in homozygous -Th respect to those observed in the controls groups.The osteoblastic cytokines were in normal range in all groups.From these observations we suggest that the chronic red blood cells haemolysis,blood transfusions and iron chelation 'quoad vitam' would determine continuously biochemical changes in -Th leading also to an abnormal release of several cytokines from injured vascular endothelium of the bone microenvironment.The osteoclastic cytokines' formation is dominant over the osteoblastic one and by reducing bone mineral density so potentiating the osteoporosis in adult homozygous -Th patients.In this context,the chronic vascular endothelium suffering of the bone microenvironment together with the hypercoagulability,leukocytes and platelet hyper-activation could enhance further the osteoclast cells functions in -Th.

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GENE THERAPY OF BETA-THALASSEMIA: A PILOT STUDY TO EVALUATE THE FEASIBILITY AND SAFETY OF G-CSF MOBILIZATION OF HEMATOPOIETIC PROGENITOR CELLS (CD34 +)

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Introduction.. This preliminary and functional study (ID No.: NTC000658385 at clinicaltrials.gov), prior further clinical trials of gene therapy for the treatment of beta-thalassemia, was carried out with the primary objective of the feasibility and safety of PBSC (peripheral bood stem cells) mobilization with G-CSF in patients affected by -thalassemia major, given the lack of such data in the literature, and the secondary objective to determine the ability of these cells to be transfected with lentiviral vector Thalagen® MATERIALS AND METHODS. Mobilization with G-CSF and PBSC apheresis was performed on five patients affected by -thalassemia major. The PBSC collected after CD34 + selection, were transduced with lentiviral vector Thalagen® Results. The data obtained show the possibility to mobilize with this protocol a good number of PBSC in patients with -Thalassemia Major (8-12 x106/Kg CD34 + cells). Side effects that occurred were mild: bone pain in all patients, headache and fatigue in the first three patients in the last 2 days of treatment and pain at the insertion of central venous catheter in the third patient, who was the only one need a central street for collection. The cells were transduced with an average of 0.8-1 copies of vector per cell. Conclusion. The protocol was found to be safe and effective, and the average number of copies of vector transduced per cell falls within the range suggested by FDA for approval of gene therapy protocol.

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IMPACT OF IL28B GENOTYPE IN THE NATURAL CLEARANCE OF HEPATITIS C VIRUS IN PATIENTS WITH HAEMOGLOBINOPATHIES

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Thalassemia major and sickle/ -thalassemia patients have an high risk of hepatitis C virus chronic infection. Genome-wide association studies detected a single nucleotide polymorphism on chromosome 19q13, 3 kilobases upstream of the IL28B gene (-3kbC>T, IL-28B rs12979860) associated to HCV-RNA spontaneous clearance and in linkage disequilibrium with a non-synonymous variant in the IL28B exon2 (K70RA>G). In a cohort of 42 not consecutive thalassemia major and sickle/ -thalassemia patients evaluated for antiviral treatment we analyzed the 2 variants to evaluate their allele frequencies and to correlate them to the hepatitis C virus natural clearance (defined as the lack of HCV-RNA detection in the serum in absence of antiviral therapy). Forty-two patients who had not been treated with PEG-IFN- /RBV were included in this study. According to the literature the genotype IL28B IL-28B rs12979860CC/K70RAA was considered to be most frequently associated with spontaneous HCV-RNA clearance whereas the genotypes IL-28B rs12979860TT/K70RGG and IL-28B rs12979860CT/K70RAG were considered to be most frequently associated with a persistent infection. Twenty out of 42 patients (47.6%) showed a spontaneous HCV-RNA clearance -15 out of 20 (75%) had a IL28B genotype IL-28B rs12979860 CC/K70RAA, 5 out of 20 (25%) had an IL28B genotype IL-28B rs12979860CT/K70RAG - showing a discrepancy with the natural clearance rate in the general population. However the natural clearance rate reported on literature is referred mainly to the no-thalassemia population therefore the different rate of HCV-RNA clearance could be due to the difference in the kind of the disease in which infection occurred. It may be possible also that chelation treatment determining binding of iron, essential even for the function of polymerase enzymes, could facilitate HCV-RNA clearance. In conclusion IL28B genotype seems to play a role in natural clearance of HCV-RNA: 15 out of 20 (75%) untreated patients who showed a spontaneous HCV-RNA clearance had a genotype favoring viral clearance (IL-28B rs12979860CC/K70RAA). These findings suggest that a "waiting and watch" approach could be maintained in patients with haemoglobinopathies until liver damage, defined as an increase of serum ALT levels >2 times for six months not related to iron overloading, will not be detected.

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THALASSEMIA SYNDROMES AND HEPATOCELLULAR CARCINOMA: CLINICAL CHARACTERISTICS, OUTCOME AND LONG TERM FOLLOW-UP IN A SINGLE CENTRE EXPERIENCE.

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Thalassemia major and thalassemia intermedia adult patients are frequently cronically infected with hepatitis C (HCV) or B (HBV) virus – or both - related to blood transfusion. Most of them present also secondary iron overload. In the last years hepatocellular carcinoma (HCC) on cirrhosis is a common reported complication and cause of mortality in these patients. The aim of our study is to describe in a homogeneous cohort of thalassemia patients the clinical characteristics, outcome and long term follow-up of HCC cases in a single center experience. During surveillance 9 new cases of HCC, since January 2000 until April 2011, were detected - 3 cases in TM and 6 cases in TI (2 men and a woman in TM and 3 men and 3 women in TI). The mean age was 54.8 years, 59,25 years in women and 51.4 years in men. Mean value of serum ferritin levels, in the last 2 years before diagnosis, was 1049±721 ng/ml. All patients except one were anti-HCV positive. Seven of them were HCV-RNA positive. All the nine HCC cases were HBsAg negative, but three of them had previous HBV infection. Only one patient had severe stage C cirrhosis. None of the patients had significant high levels of alpha fetoprotein (AFP) at diagnosis; however, a slightly increase of AFP levels was shown, during the 6 months before diagnosis, in 50% of cases, but AFP value in these cases remained in all of them <75 ng/ml.. Five patients had multifocal HCC at the diagnosis. Four showed an unifocal HCC, developing two of them multifocal HCC during the follow up. Four patients died during the follow-up for decompensated cirrhosis. Five patients are alive after treatment. The overall mean survival was 9.9±30.4 months (range 3-87). Our data indicate that HCC in Thalassemia patients is not a rare complication in Thalassemia syndromes and significantly affect prognosis. Both TM and TI patients can develop it in presence of risks factor; in our experience, but further data are needed, the prevalence of this complication is double in TI respect of TM. In seven cases the patients were anti-HCV positive and in six of these cases the virus was in active replication – HCV-RNA positive. The absence of cronic infection with HCV doesn't preserve from the risk of developing HCC because hepatic iron overload plays also an important role. In only was case HCC was non suitable for treatment for the presence of decompensated cirrhosis. Two patients went to orthotopic liver transplantation (OLT) and are alive.

INFECTIONS

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PROGRESSIVE LEUKOENCEPHALOPATHY IN A PATIENT WITH WALDENSTROM'S MACROGLOBULINEMIA TREATED WITH RITUXIMAB

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Progressive multifocal leukoencephalopathy (PML) is a rare demjelinating brain desease, caused by reactivation of a latent poliomavirus (JCV), recognized as a rare complication of hematological malignancies. PML has also been described with the use some antineoplastic therapy as Fludarabine. A marked increase in the incidence of PML has been reported with the use of monoclonal antibodies as Rituximab. We describe a case of PML in a patient treated 3 years before with a Rituximab containing regimen. In May 2007, a 74 years old man presenting progressive Waldenstrom's macroglobulinemia, received 6 cycles FCR (Fludarabine, Cyclophsphamide, Rituximab) achieving CR. In August 2010, in persistent CR, the patient complained a slowly progressive poorly defined visual defect. CD4 lymphocyte were 360/Ul. Brain MRI showed a large area of signal alteration in the left occipital lobe, suspected as vascular ischaemic lesion. Owing to the worsening of the visual defect he was admitted in a neurological department in November. A right hemianopsia and left inferior quadrantic visual field defect were detected. A new brain MRI showed better defined signal abnormality located mainly in the left but also in the right occipital lobe, sparing the cortex and without contrast enhancement. A left occipital-temporal lobe hypoperfusion was seen at cerebral perfusion SPECT. Neuropsycological examination showed a moderate cognitive impairment. Cerebrospinal fluid (CSF) routine analyses were normal and cytology was negative for malignat cells. CSF detection of JCV-DNA by PCR amplification was positive at high titre (83,000 copies/ml); DNA for other virus and pathogens, 14-3-3 protein and anti Hu,Yo,Ri antibodies were negative. The patient complained progressive deterioration of visual functions and developed right hemiparesis and aphasia. In December brain MRI showed an anterior spreading of the white matter lesions involving both bilateral temporal, left frontal lobes and the posterior part of the corpus callosum. No specific treatment for PML post Rituximab therapy was established. Patient is presently at home, presenting severe cognitive impairment, global aphasia, right hemiplegia. This case is interesting because of the delay between Rituximab dose and PML diagnosis (36 months). In other reported cases median time was 3 months if CD4<500/ul vs 17 months in CD4>500/ul. Awareness is therefore needed of the potential of PML among Rituximab treated patients.

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THERAPEUTIC DRUG MONITORING (TDM) OF POSACONAZOLE IN ACUTE LEUKEMIA PATIENTS: PRELIMINARY RESULTS OF A MONOCENTRIC EXPERIENCE

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Posaconazole is an orally broad-spectrum triazole antifungal which has recently been approved for prophylaxis and treatment of invasive fungal infections (IFI) in immunocompromised patients (pts). Posaconazole has to be taken with fatty food for optimal intestinal resorption, which is likely to be influenced by mucositis and administration of proton-pump inhibitors. Consequently therapeutic drug monitoring (TDM) of antifungal plasma concentrations is increasingly recommended. Adequate levels are ≥ 0.5 µg/ml for prophylaxis and ≥ 1,25 µg/ml for therapy. We collected preliminary data on the posaconazole TDM in acute myeloid leukemia (AML) pts, undergoing first remission-induction chemotherapy and treated with posaconazole as primary prophylaxis at 200 mg three times / day. From January 2010 to December 2010, 16 posaconazole plasma samples were processed in 13 consecutive pts (mean-age 63 years). All plasma samples were obtained after 5-7 days of primary prophylaxis and in 3 pts also after 12-14 days. Mean duration of prophylaxis was 23 days. Posaconazole plasma levels were determined by HPLC with UV set at 255 nm. Median trough levels obtained after 5-7 days were <0.5 µg/ml in 46% of pts; 0.51 to 0.99 µg/ml in 8% of pts and ≥ 1 µg/ml in 46%.of pts. Empirical antifungal therapy was

started in 23% of pts, preemptive therapy in 8%, while in 69% no antifungal therapy was needed. Patients with levels consistently > 0.5/ µg/ml were more likely to have successful outcome. No breakthrough aspergillosis was documented. Absence of proton-pump inhibitors were associated with higher levels of posaconazole; compliance was good in 77% of pts. In a previous TDM analysis, posaconazole was employed as treatment for suspected IFI in 9 AML pts refractory or intolerant to conventional antifungal therapy, at 800 mg/day . All pts received proton-pump inhibitors. In this experience 6 pts had levels < 1 µg/ml and 3 pts ≥ 1,25 µg/ml; outcome was favourable in 33% of pts. Our preliminary data on posaconazole prophylaxis are encouraged, but must be integrated with future multicenter experience. The role of posaconazole in refractory IFI (suspected/proven) remains controversial and must be confirmed in several clinical setting. In all cases the presence of mucositis and the use of proton-pump inhibitors are important conditioning factors and TDM essential to optimize safety and efficacy of antifungal in an attempt to improve patient outcomes

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VORICONAZOLE TREATMENT IN NEUTROPENIC PATIENTS POSITIVE FOR CANDIDA AFTER ANTIFUNGAL PROPHYLAXIS

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Background. The frequency of invasive fungal infections in neutropenia patients has increased in the last few decades. These infections are associated with high mortality depending on patients characteristics and localization of infection. Several therapeutic strategies have been proposed. Methods. In the last four years, we used Voriconazole (6 mg/kg i.v., twice daily immediately followed by 4mg/kg twice daily i.v.) in neutropenic patients with at least one positive blood culture for Candida who have received prophylaxis with Itraconazole. Results. 70 neutropenic patients affected by Acute Leukemias and Non-Hodgkin Lymphomas were treated with Itraconazole as antifungal prophylaxis. In 25 cases we administered Voriconazole after positive blood culture for Candida. Voriconazole therapy started within 24 hours after Candidemia even in absence of clinic signs or symptoms. Positive response with negativization of blood culture are obtained in all patients. Voriconazole was administered until resolution of neutropenia. No significative adverse events were observed. Conclusions. Because of the substantial morbidity and mortality associated with invasive fungal infections (IFI), there is a need to accurately define patient groups at greatest risk of IFI and, when appropriate, to initiate effective antifungal therapy. Our experience in a subset of neutropenic patients showed that Voriconazole is safe and effective.

P157

VALUE OF CHEST X-RAY IN THE DIAGNOSIS OF LUNG INFECTIONS IN THE EARLY PERIOD AFTER REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION

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Lung infection is the most frequent complication of any neutropenic phase and also of the early period after allogeneic transplantation (alloSCT). The first exam usually performed to detect lung infections is chest X-ray (CXR), but its diagnostic value is still a matter of debate. The gold standard imaging for diagnosis of lung infections is high resolution CT (HRCT). This study aimed at understanding the value of CXR to detect lung infections during the early post-transplant phase after reduced intensity (RIC) alloSCT. We reviewed the CXR and HRCT performed from day 0 to +30 by 260 consecutive patients receiving RIC alloSCT at our center between 2002 and 2010. Median age was 49 years (range, 15-68), diagnoses were: lymphoma (154), multiple myeloma (55), leukemia (36 patients), and solid tumors (15). Patients were allografted from HLA identical siblings (117), matched unrelated (99) or haploidentical donors (44). CXR was performed in 192 patients at a median of 13 days (range, 0-27) after alloSCT. Fifty patients were evaluated with both CXR and HRCT. The indications for HRCT were: fever of unknown origin (FUO, 27 patients), respiratory symptoms +/- fever (6), chest pain +/- fever (6), high C-reactive protein (11). HRCT after CXR was considered the reference standard to assess sensibility, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CXR. Of 50 patients performing CXR and HRCT, 20 had evidence of lung infection at CXR. The sites of infection were: low inferior lobes (right, 10; left, 8), median right lobe (2), upper right lobe (1), perihilar region (2); 6 patients had pleural effusion, 3 had interstitial infiltrates. Of 20 patients with positive CXR, 18 had diagnosis of lung infection confirmed by HRCT, whereas 2 patients had negative HRCT and no clinical signs of pneumonia. Lung infection was diagnosed by HRCT in 4 of 30 patients with a negative CXR. Sensibility of CXR was 82% (IC95% 66-98%), specificity was 93% (IC95% 83-100%), PPV was 90% (IC95% 77-100%), NPV was 87% (IC95% 75-99%). In conclusion, CXR has high specificity and PPV to assess lung infections early after RIC alloSCT. Confirmatory HRCT could be avoided in patients with positive CXR, obviating the radiation exposure and the costs of HRCT. Instead, the reduced sensibility and NPV of CXR suggest that patients with a negative CXR but with respiratory symptoms, chest pain, FUO or high C-reactive protein should be investigated with HRCT.

P158

SURVEY ON ANTIFUNGAL COMBINATION THERAPY FOR TREATMENT OF PROVEN OR PROBABLE INVASIVE FUNGAL DISEASES IN ITALIAN HEMATOLOGICAL CENTERS. THE SEIFEM-COMBO STUDY (NCT 00906633).

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Background: This prospective observational Clinical Trial (NCT 00906633) evaluated the feasibility, efficacy and toxicity of Antifungal Combination Therapy (Combo) as treatment of proven or probable Invasive Fungal Diseases (IFDs) in Hematological patients (pts). Materials and Methods: Between Jan 2005 and Dec 2009, 84 cases of Combo were reported from 20 Hematological Centers in Italy. Median age of pts was 34 yrs (range 1-73) and 37% had less than 18 yrs. Acute Leukemia was the most common underlying hematologic disease (68/84; 81%). The status of hematologic disease was: onset 21/84 (25%), remission 18/84 (21%), refractory/relapse 45/84 (54%). The main site of fungal infection was lung with or without other sites. The etiological agents were: Aspergillus sp 68 cases (81%), Candida sp 6 cases (8%), Zygomycetes 4 cases (5%), Fusarium sp 4 cases (5%) and other (2 cases). Results: The most used Combo were: Caspofungin + Voriconazole 35/84 (42%), Caspofungin + Liposomal Amphotericin B (L-AmB) 20/84 (24%), and L-AmB+Voriconazole 15/84 (18%). The median duration of Combo was 19 days (range 3-180). The Overall Response Rate (ORR) was 73% (61/84 responders) without significant differences between the Combo regimens. The most important factor that significantly influenced the response rate (in univariate and multivariate analysis) was PMN recovery during Combo (P < 0,0001). Only one patient discontinued therapy (voriconazole related neurotoxicity) and 22% experienced mild and reversible adverse events (hypokalemia, ALT/AST increase, creatinine increase) without differences between pediatric and adult pts. The IFD attributable mortality rate was only 17%. Conclusion. This is the first multicenter, prospective, observational study exploring feasibility, efficacy and toxicity of Antifungal Combination Therapy (Combo) as treatment of proven or probable Invasive Fungal Diseases (IFDs) in Hematological patients (pts). The results of this study indicate that: 1) Combo was well tolerated in both children and adults hematologic pts. 2) The Overall Response Rate was 73% and the mortality IFDs related was only 17%. 3) The most used Combo regimens were caspofungin+voriconazole (ORR 80%) and Caspofungin+L-AmB (ORR 70%). 4) In univariate and multivariate analysis PMN recovery during Combo predicts a better outcome.

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PROPHYLAXIS OF INVASIVE FUNGAL DISEASES WITH POSACONAZOLE IN ACUTE MYELOID LEUKEMIA. A REAL LIFE EXPERIENCE.

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Background. Acute Myeloid Leukemia (AML) patients are at high risk of Invasive Fungal Diseases (IFDs). We report our real-life experience with POS prophylaxis in AML. We also compare the performance of POS prophylaxis with an historical, well matched, control group of AML pts who received prophylaxis with Fluconazole (FLUCO) or Itraconazole (ITRA). Patients and Results. Fifty-five unselected and consecutive AML pts received POS prophylaxis (600 mg daily) between Jan 2009 and Oct 2010. Median age of this population was 47 yrs (range 18-69). All cases were given chemotherapy with anthracyclines and cytarabine. The POS was started when neutrophil (PMN) count was less than 1000 mL and was stopped at PMN recovery. The median duration of severe neutropenia (PMN lower than 500 mL) was 15 days (range 7-41); 10/55(18%) of cases had an oral mucositis grade II-III CTC (common toxicity criteria) and 73%(40/55) of these pts received a proton pump inhibitor. An active diagnostic work up was made in all cases with Galactomannan assay, standard chest X-ray and thoracic CT scan in case of fever (FUO) lasting over 48 hours. The median duration of POS prophylaxis was 15 days (range 7 to 41). Only 4/55(7%) of pts required parenteral empiric or preemptive antimycotic therapy and only 2/55(4%) experienced a proven IFDs (Fusarium solanii fungemia and Aspergillus sp pneumonia). Mortality IFDs related was 0%. POS was well tolerated and only 9%(5/55) of pts experienced mild drug related side effects. No cases of POS discontinuation, due to the side effects or intolerance, were reported. When we compare the 55 pts who received POS with an historical control group of 55 AML pts who received FLUCO (45/55) or ITRA (10/55)prophylaxis, between Jan 2008 and Jun 2009, no significant differences were observed for underling disease status, age, IFDs risk factors, days of severe neutropenia and days of prophylaxis.Instead, there were significant differences in breakthrough IFDs (4% in POS group vs 16% in control group; P=0,02), and in days of parenteral antymicotic therapyn (37 vs 163). Conclusions. This real-life experience confirms that POS prophylaxis is feasible, safe, well tolerated and effective (prevention of IFDs) in unselected AML patients. Only 7% of these high risk pts required parenteral antimycotic therapy and only 4% experienced breakthrough IFDs. We also confirm that POS is more effective than FLUCO or ITRA as antifungal prophylaxis in AML pts.

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CAN BE LIGTH CYCLER DETECTION LIMIT (LOD) A PREDICTOR OF VIRAL CMV REPLICATION USING REAL-TIME QUANTIFICATION?

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Introduction: Quantitative detection of cytomegalovirus (CMV) by real-time PCR is currently the primary choice for the surveillance of active CMV infection in allogenic stem cell transplant (Allo-SCT) recipients. Different assays are planned for detection of CMV in plasma samples, but sensibility and detection limits are different among them. Aims: The aim of the study is to investigate the utility of the Limit of Detection (LOD) in CMV monitoring by quantitative PCR assay as early predictor of viral replications. Materials and methods. Plasma samples were collected at transplant centre of Oncology Department of Businco Hospital, Cagliari (Italy). DNA was extracted from plasma with specific Roche kit. Viral CMV copies/ml were quantified using the Roche Light Cycler CMV Quant PCR Kit using by Light Cycler 2.0 instrument. Seventeen Allo-SCT patients were enrolled in this evaluation. Patients included in the study had an history of negativity for CMV. The kit LOD was estimated following procedure, using 235 copies/ml in 200 ul of plasma with a range of confidence between (153-500 copies/ml). During follow- up were performed about 360 determinations for all cases. All detections were performed each two days, in the first month after Allo-SCT, and each week after the first month. In the positive cases of determination, CMV copies analysis was performed until test negativity. Results. Eight cases (37.7%) show as first indicator, after all days post Allo-SCT, measurable quantities of CMV viral load even if under detection limits. In the same cases, after 2 or 4 days without antiviral treatment, it has been an increase of 20-30 fold respect to the initial copies number. Calibrator and internal standard (IC) were inside the acceptability limits. Conclusions: The manual DNA plasma extraction systems plus real time Light Cycler Quant can be considered excellent commercial real-time PCR assays for measurements in quantitative analysis of CMV DNA. These complete system guarantees optimal test sensitivity and a low detection limit (detection of 95% of all copies of the sample containing CMV). In our experience we have seen that every time any value of CMV number copies/ml was below the detection limit (in the absence of CMV antiviral therapy) we successively registered an increased by 20-30 times in the number of CMV copies. For this reason, we suggest that this parameter can be considered an early viral replication predictor.

P161

GALATTOMANNAN TESTING IN BRONCHOALVEOLAR LAVAGE FLUID CONTRIBUTES TO THE DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS (IPA) IN BONE MARROW TRANSPLANT (BMT) RECIPIENTS.

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Background: Invasive pulmonary aspergillosis (IPA) is an important cause of mortality in patients undergoing hematopoietic stem cell transplant. Timely diagnosis of IPA improves survival but is difficult to make. Standardized typical (EORTC/MSG) radiological criteria are helpful but not universally present in patients with IPA. Aspergillus galattomannan (GM) antigen detection in serum by Platelia enzyme immunoassay (EIA) has been approved for diagnosing invasive aspergillosis (IA). However, serum GM levels may be lower in patients with less angioinvasive aspergillosis, thus detection of GM in broncoalveolar lavage fluid (BAL) might be more sensitive. The aim of our study was to evaluate the diagnostic utility of GM in BAL in BMT recipients with different types of pulmonary CT lesions. Methods: We reviewed retrospectively clinical and radiological records of 73 consecutive patients, with hematological malignancies and underwent BMT, in whom 84 BAL were performed. Both BAL fluid and serum samples were evaluated for GM and an optical density index (ODI) ≥0.5 was considered positive. Extensive search for other pathogens was performed on BAL fluid, including direct examination and culture for fungi. Results: Twenty two patients (26%) had a positive BAL GM test, but IPA was diagnosed as probable according to EORTC/MSG consensus group criteria only in 12 (14%) patients. Of these 12 IPA, only five (41%) had a positive serum GM test, while in 7 (59%) BAL GM was necessary for diagnosing IPA. The other 10 patients, despite the positive GM in BAL, were not diagnosed with IPA because CT scan signs were aspecific. The median value of GM ODI in BAL was not different for patients with IPA vs patients without IPA (1.5 vs 1.2). All 22 patients received antifungal treatment. The 12-week overall survival (OS) did not differ between the two groups (IPA OS=66% vs no IPA OS=60%). Conclusions: 1) BAL GM added sensitivity to serum GM: positive GM BAL was the sole microbiologic criterion in more than half of the patient with IPA. 2) The interpretation of the diagnosis in patients with GM BAL positive but with atypical pulmonary lesions remain controversial.

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CENTRAL VENOUS CATHETER INFECTIONS IN PATIENTS WITH MALIGNANT HEMOPATHIES: REPORT FROM A SINGLE INSTITUTION

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Introduction: central venous catheters (CVCs) are currently used in malignant hemopathies for intravenous therapy, stem cell transplant (SCT), blood products and fluids infusions. Aims: The study aimed at retrospectively analyzing CVC infectious complications in the setting of malignant hemopathies. Patients and methods: One hundred fifty-five CVCs (100 Port-a-cath, 57 external CVC), inserted from March 2001 to June 2010, have been analyzed. Patients with external CVC received chemotherapy for acute leukemia or autologous SCT for myeloma or lymphoma. Patients with port-a-cath systems were treated with standard chemotherapies for lymphoma or myeloma. Results: Out of 57

external CVCs, 6 experienced infections: 4 sepsis (P.aeruginosa), 1 skin tunnel infection (P.aeruginosa and S.epidermidis), 1 endocarditis (Streptococcus oralis). Of these 6 patients, 3 leukemic patients treated with intensive chemotherapy died because of infections. Infections did not affect catheter survival. Performance status did not influence catheter infections, neither fever nor infections at the time of procedure. Percutaneous technique was associated to catheter-related bloodstream infections (CRBSIs) with statistical significance (p=0.023). CVC infections were more frequently related to left side insertion and to percutaneous technique (p=0.023). CRBSI was not statistically related to the type of immunosoppressive therapy (p= 0.72). The underlying disease (p=0.03) and the type of therapy (p=0.029) were significantly associated with development of P.aeruginosa infections. Patients with a neutrophil counts <500/mmc for more than 10 days more frequently developed Pseudomonas infections (p=0.002). Out of 100 port-a-cath, sepsis occurred in 3 port-a-cath, 2 by S.epidermidis, and one by fungal infection. Overall, the infection rate per 1000cvc days was 0.71 in external CVCs, and 0.65 in port-a-cath systems. Cumulative survival was mainly influenced by infectious complications (Log Rank and Breslow p<0.0001). Although not statistically different, patients who developed a CRBSI had lower neutrophil counts at the time of insertion. Less intensive chemotherapy in patients with port-a-cath systems did not influence CRBSIs. Summary/conclusions: CRBSIs are an important cause of morbidity and mortality in patients with malignant hemopaties. There are few studies reported that CRBSIs are more frequent in patients with infections and unfavorable performance status. However, the presence of infections and unfavorable performance status did not influence CRBSIs in our patients with external CVC and port-a-cath. There are conflicting data from the literature upon the importance of neutrophil counts at the time of insertion: according to some authors, neutropenia is a risk factor for CVC-related infections. Our study did not report a correlation between neutrophil counts and infection development neither in external CVCs or in ports. Diagnosis and therapy did not influence CRBSIs rate probably because of heterogeneity and/or low number of patients. In this retrospective study we confirmed the data reported by others that the side (left) and type of venous access (external) represent risk factors for CRBSIs. Materials and type of device may promote colonization and biofilm formation, with higher infection risk, but we were not able to find this association in 157 CVCs. The present study contributes to underline the complex management of patients with malignant hemopathies

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POSACONAZOLE PROPHYLAXIS DURING FRONT-LINE CHEMOTHERAPY OF ACUTE MYELOID LEUKEMIA: A SINGLE CENTER REAL LIFE EXPERIENCE

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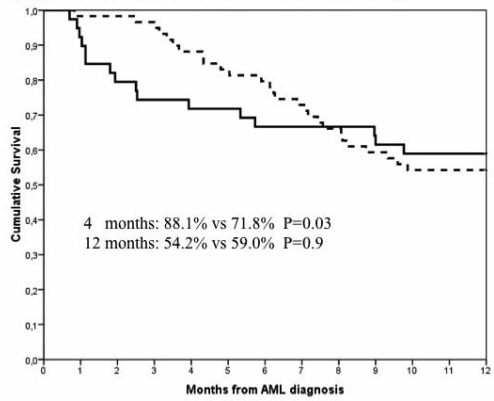
Posaconazole is effective as primary antifungal prophylaxis (PAP) of invasive fungal diseases (IFDs) in acute myeloid leukemia (AML) patients undergoing induction chemotherapy. We evaluated the impact of posaconazole PAP administered during the whole front-line chemotherapy in a "real life" scenario of consecutive AML patients. We retrospectively compared 58 patients who received oral non-absorbable amphotericin B (control group) to 99 patients who received oral posaconazole (posaconazole group) as PAP during induction, reinduction and consolidation chemotheray. The primary endpoint was the incidence of proven/probable IFDs during front-line chemotherapy. Secondary endpoints included: the overall incidence of proven/probable and possible IFDs and the use of empiric antifungal therapy during front-line chemotherapy; overall survival at 4 and 12 months from the AML diagnosis; mortality attributable to IFD; difference in the costs of antifungal drugs administered either in primary or secondary prophylaxis or in therapy and in the days of hospitalization during the first 12 months from the AML diagnosis ; occurrence of severe toxicity or side effects related to PAP. Proven/probable IFDs were documented in 51.7% of patients in the control group and in 23.2% in the posaconazole group (absolute risk reduction, 28.5%; 95% CI, –12.9 to –42.8; P=0.0002). IA was the most common IFD and there were fewer cases of IA in the posaconazole group (15.1% vs 43.1%, P=0.0002). 58% of patients in the control group and 52.5 % in the posaconazole group died within 12 months from the AML diagnosis. Mortality attributable to IFD occurred in 5 of 58 (8.6%) and in 5 of 99 (5.0%) patients of the control group and posaconazole group, respectively (p=0.17). Cumulative survival did not differ considering the overall population, but in patients aged <60 years a significant survival advantage was observed at 4 months in the posaconazole group (p=0.03) (Figure). A mean reduction of more than 10,000 Euros per patient in cost of antifungal drugs was calculated in the posaconazole group. The mean hospitalization time during the first 12 months after AML diagnosis was not significantly different. Three percent of patients who received posaconazole early discontinued treatment due to poor oral compliance. PAP with posaconazole was effective in preventing IFDs in a "real life" scenario of AML patients and resulted in an early but transitory survival advantage in younger patients.

Figure: Kaplan-Meier curves for survival in in younger patients aged ≤60 years . Differences between the posaconazole group (dotted line) and the control group (solid line) were calculated at 4 and 12 months from AML diagnosis.



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POSACONAZOLE AS ANTIFUNGAL PROPHYLAXIS IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS: SAFETY AND EFFICACY

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Invasive fungal infections (IFI) are a growing cause of morbidity and mortality in patients undergoing allogeneic haemopoietic stem cells transplantation (alloHSCT). Posaconazole is a new generation oral-azole with a wide spectrum activity and a safety profile. We compared posaconazole with fluconazole/itraconazole for prevention of IFI in alloHSCT recipients. 26 consecutive patients undergoing alloHSCT from February 2008 through March 2011 received posaconazole and were compared with 20alloHSCT recipients transplanted before February 2008 treated with fluconazole/itraconazole. Patient characteristics are showed in table1.No significant differences were found between the two cohorts of patients.The median CD34+cells infused was $5{,}03x10^6$/Kg (range 2,01-20).The median time to platelet and neutrophil engraftment higher than $20x10^9$/L and $0.5x10^9$/L were 14 (range10-33) and12 days (range10-23), respectively. 32patients (71%) experienced a febrile neutropenia (Table 2), with11episodes of FUO (34%). Four pneumonitis(12%)and seventeen bloodstream infections (54%)occurred. Three IFI were reported (9%). One *C.Albicans* sepsis was documented in the posaconazole group.Two GM positive plus imaging findings suggestive of invasive aspergillosis (IA) occurred (1 in posaconazole and 1 in other group; these patients died for IA). All patients received a systemic antimicrobial therapy. 10 patients(33%) had a favorable response to primary antimicrobial treatment. The empiric/pre-emptive antifungal therapy was administered in 13 patients(41%).4patients(31%)in the posaconazole group and 9 patients (69%) in the other group underwent antifungal empiric/pre-emptive therapy (p<0.05). The median days of antifungal treatment were 10, range1-30,without statistical difference between the two groups. No differences were observed regarding the febrile neutropenia episodes, the proven fungal infections and duration

of antifungal treatment between the two group of patients. TRM at +100 days was 28%, similar in the two cohorts. No adverse serious events occurred during the antifungal prophylaxis. 3 patients(13%)treated with posaconazole withdrawn the drug because of gastrointestinal symptoms. Despite the need of a prospective and definitive study,our data indicate that posaconazole is effective and safe in patients undergoing allogeneic transplant. A lower rate of empiric or pre-emptive systemic antifungal treatment has been documented in recipients treated with posaconazole as antifungal prophylaxis.

Table 1: Patient's characteristics

	All	Posaconazole		p
	46	YES 26 (56%)	NO 20 (44%)	
Median age, y (range)	47(13-69)			
>55 years	13(28%)	9 (69%)	4 (31%)	0,27
Primary underlying diagnosis,no(%)				
Lymphomas	25(54%)	10(40%)	15(60%)	
Multiple Myeloma	3 (7%)	3		0,1
Acute Leukemia	12 (26%)	9 (75%)	3(25%)	
Myelodysplastic disorder	5 (11%)	4 (80%)	1 (20%)	
Other	1 (2%)		1	
Median N prior chemotherapy,(range)	2 (1-4)			
> 2	22 (48%)	12 (55%)	10 (45%)	0,79
Type of allograft donor				
Matched, related	40 (87%)	26 (60%)	16 (40 %)	0,5
Mismatched, related	4 (9%)	1 (25%)	43(75%)	
Matched, unrelated	2 (4%)	1	1	
Status at transplant				
CR	15 (33%)	11 (73%)	4 (27%)	0,23
PR	12 (26%)	5 (42%)	7 (58%)	
Progression/NR	19 (41%)	10 (53%)	9 (47%)	
Median time to HST, months (range)	12(4-117)			0,42
Antimicrobial prophylaxis				
Chinolonic drugs	34 (79%)	18 (53%)	16 (47%)	
Other	9 (21%)	6 (67%)	3 (33%)	
Median days of prophylaxis(range)	14 (6-32)			0,7
Median days of antifungal proph(range)	21 (7-39)	20 (7-33)	22(10-39)	0,7

Table 2: Results

	All patients	POSACONAZOLE		P
		YES (26)	NO (20)	
FEVER	**32 (71%)**	17 (53%)	15 (47%)	0,55
FUO	11 (34%)			
Microbiological	17 (54%)			
Pneumonitis	2 (6%)			
Microbiological/radiological	2 (6%)			
Median day of onset	4 (-3; +13)			
Median duration of fever	7 (1-26)			
IFI	3 (9%)	2	1	0,7
Use of systemic antifungal therapy (AFT)	**13 (41%)**	**4/13 (31%)**	**9/13 (69%)**	**0,03**
Amphotericin B colloidal dispersion	5			
Liposomal amphotericin B	4			
Voriconazole	2			
Caspofungin	2			
Median days of AFT*, range	10 (1-30)	13(4-30)	10(1-29)	
>10 days	6 (46%)	3	3	0,16
Median time spent in Hospital, range	28 (11-59)			0,4
> 28 days	21(61%)	11(42%)	10(50%)	
TRM at + 100	13 (28%)	7(26%)	6(30%)	0,82

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INFECTIOUS COMPLICATIONS IN HOME-CARE-MANAGED HEMATOLOGICAL PATIENTS

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Introduction. Infections are frequent complications in patients affected by hematological disorders and may arise from both treatment and both disease-related immunosuppression. Data about home care managed patients with hematological illness are scarce and sparse. Aim. To evaluate the rate, type, etiology, duration and outcome of infectious complications in patients during the course of home care management. Material and methods. Between January and December 2008 infections development was monitored monthly, by registering the following variables: onset data, site, etiology and treatment outcome. Results and Conclusions. There were 127 patients (65 males) with a median age 78 (range:) years. The hematological diagnoses were as follows: 39 (31%) myelodisplastic / myeloproliferative disorders, 35 (28%) acute leukemia, 26 (20%) lymphoma, 18 (14%) multiple myeloma, 9 (7%) non-neoplastic disorders. At the beginning of the home care program, patients were in the following disease phases: advanced / terminal 64 (50%), supportive care 49 (39%), active treatment 14 (11%); 58/127 (45,7%) patients developed infectious complications at home where a total amount of 64 infectious events were recorded. According to infection type and site, the following event were identified: 25 (39%) fever of unknown origin, 13 (20%) pneumonia, 9 (14%) urinary tract infections, 7 (11%) skin infections, 2 (3%) bronchitis, 2 (3%) colitis, 1 (1.6%) oral cavity infection, 1 (1.6%) sinusitis, 1 (1.6%) otitis, 1 (1.6%) faringitis, 1 (1.6%) central venous catheter related infection and 1 (1.6%) CMV reactivation. Microbiological etiology was identified in 13/64 (20%) episodes: Gram negative in 10 (15.5%) events (E. coli in 4, Pseudomonas/Stenotrophomonas in 3, Serratia in 1, Pseudomonas+Escherichia Coli in 1, unspecified in 1); Gram positive in 1 (1.5%) case only (S. aureus); virus in 2 (3%) events (CMV e VVZ). Outcome was: resolution in 31 (48%), death in 3 (5%), persistence at the end of observation period 4 (6%) or unspecified in 26 (41%). Infectious episodes resolved after a median of 8 (3-100) days. Conclusions. In our experience, infectious complications affected one quarter of home cared managed patients, thus representing an uncommon cause of death, but an important cause of morbidity, quality of life impairment and cost increase.

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SEPSIS CAUSED BY NOT DIPHTHERIAE CORYNEBACTERIUM ARISING FROM SKIN LESIONS IN ACUTE MYELOID LEUKEMIA (AML): CLINICAL DESCRIPTION OF TWO CASES AND REVIEW OF LITERATURE

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Introduction.: We report two cases of sepsis from Corinebacterium striatum (CS) and Corinebacterium JK (CJK), starting in the skin, in two patients with AML. Case 1: A 69 years old man with AML at day +5 after chemotherapy presented fever. Blood cultures were obtained peripherally and from a central line and empirical administration of Piperacillin-Tazobactam and Amikacin was started without defervescence. At day +10 a painful, erythematous-papular eruption appeared on forearms and the back of the right hand. As an infection from Gram + bacteria was suspected, Amikacin was discontinued and Vancomycin was added. A day +16 blood culture grew vancomycin-sensitive CJK. At day +18 the fever disappeared and blood cell count showed PMN>500/mcl; at discharge, skin lesions were healing. Control blood and central venous catheter (CVC) tip cultures were negative. Bone marrow examination showed Complete Remission. Case 2. A 63 years old woman with AML, during induction chemotherapy, presented fever resolving after empirical Ceftazidime. At day +7 she had a new febrile episode; prior to empirical addition of Amikacin, blood cultures were obtained peripherally and from a central line. Thorax CT scan showed a parenchymal consolidation of the middle lobe, then Tigecycline was added. Due to persistence of fever Caspofungin was added. A g +15 CVC was accidentally removed. At day +16 Amikacin and Tigecycline were discontinued and Meropenem was added. Blood cultures grew Vancomycin-sensitive CS: vancomycin was added. Despite the antibiotic therapy the fever did not disappear. At day +22 an erythematous papular skin lesion on the left hand appeared. PMN were> 500/mcl. Culture of purulent material from the skin lesion grew vancomycin-sensitive CS microbiologically similar to the one isolated in blood. In the following days the patient experienced improvement of the fever and of the lung consolidation. The patient died at day +48 presumably due to viral encephalitis. Conclusions: The non-diphtheria corynebacteria in immunosuppressed patients with compromised skin barrier integrity, may lead to sepsis (increased in recent years) starting from skin lesions. They are often antibiotic-resistant but Vancomycin-sensitive.

P167

GRANULOCYTE TRANSFUSIONS AS ADJUNCTIVE TREATMENT OF INFECTIONS IN NEUTROPENIC PATIENTS

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Background: The degree and duration of neutropenia are crucial prognostic factors in hematological patients (pts) with invasive infections. Since the introduction of granulocyte colony stimulating factor (G-CSF), there has been a renewal of interest in granulocyte transfusions (GTX). Aims: to evaluate feasibility, efficacy and safety of GTX as adjunctive treatment for neutropenic fever unresponsive to antimicrobial therapy. Methods: retrospective analysis on adult pts with hematological malignancies (HM) and fever during neutropenia (ANC<500x106/l and anticipated duration >7days) who received GTX after no clinical response to antimicrobial therapy. Volunteer donors received G-CSF 12h before the first of 2 consecutive collection procedures (5 g/kg). All of them had signed an informed consent for G-CSF administration and leukapheresis. Results: During a 7-year period (2004-10) 46 courses of GTX were administered. Pts were suffering from acute leukemia (30 myeloid and 5 lymphoid), lymphoma (9), multiple myeloma (2). Overall, 209 GTX were administered, with a median of 4 GTX per episode of infection (range 1-20). Infections causing fever were identified in 41 episodes: 17 bacterial sepsis, 23 invasive fungal diseases (IFDs) and 1 mixed bacterial/fungal sepsis. Remaining 5 cases were classified as fever of unknown origin (FUO). IFDs included 16 cases of pulmonary aspergillosis (proven/probable), 5 candidemia, 1 invasive zygomycosis, 1 invasive fusariosis and 1 infection due to Blastoschizomices capitatus. Donors' mean white blood cell (WBC) count at 1st leukapheresis was 27x109/l (range 13-45); at 2nd procedure WBC count was lower (15x109/l, range 8-33), as expected. The mean yield was 25.6x109 PMN (range 3.5-75.8) at first procedure and 11.1x109 PMN at the second one (range 0.6-42.4). Mean transfused dose was 3.7x109/kg at first day (range 0.6-9.6) and 1.4x109/kg at second day (range 0.1-4.7). The combination of antimicrobial therapy with GTX led to a favourable clinical response in 33 of 46 valuable pts (72%); the acute infection-attributable mortality rate at 30th day after the last GTX was 29% for sepsis, 22% for IFD and 40% for FUO. Conclusions: at preliminary analysis GTX may be safe and efficacious in HM with severe infection to bridge the period of deep neutropenia, when antimicrobial therapy has failed. Controlled studies are needed to confirm this datum, and to define the proper role of this procedure and the optimal schedule for HM.

P168

PROSPECTIVE REGISTRY OF INVASIVE FUNGAL DISEASES IN ACUTE MYELOID LEUKEMIA: PRELIMINARY RESULTS ON 142 CASES

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Aims: To evaluate epidemiological characteristics, treatments and outcome of invasive fungal diseases (IFDs) in acute myeloid leukemia patients (AMLs). Methods: From January 2010 to March 2011, 31 Italian participating centers registered all consecutive cases of IFDs in adult AMLs at first induction (until 30th day from the end of chmotherapy). The parameters we analyzed were: age, sex, severity and duration of neutropenia, antifungal prophylaxis, certainty of IFD diagnosis, empirical/pre-emptive therapy, target therapy, etiologic agent, outcome. Response rate to antifungal therapy and mortality rate were thus analyzed. Results: over a 15 month period, 142 IFDs were collected in 593 newly diagnosed AMLs (incidence 23.6%). Median age was 60 (range 18-81), with a male/female ratio of 1.6/1. The most part of IFDs (128, 90%) occurred in pts who had received conventional chemotherapy (128/498, incidence 25.7%). As expected, IFDs incidence was lower in those receiving either supportive or low dose therapy (14/95, 14.7%). Probable and proven IFDs were 37 and 14, respectively; remaining cases were classified as possible IFDs (91, 64%). A deep neutropenia (PMN count <500/ l) lasting for at least 7 days occurred in 129 of them (91%). Antifungal approaches are reported in the table. Most of pts had received systemic antifungal prophylaxis (120/142, 85%), more frequently with posaconazole. Liposomal AmB and caspofungin were the most frequently employed drugs, as empirical/pre-emptive therapies. Of 51 proven/probable IFDs, the majority were mold infections (36, 69%), with a mold/yeast ratio of 2.4/1. Among molds, aspergillosis (IA) were predominant (27, 75%). Four cases of rare fungal agents were identified (1 Fusarium, 1 Blastoschizomices, 1 Geotrichum and 1 Trichosporon). At 30th day, 104 pts had achieved a favourable response; the overall response rate was 73%. IFD-attributable mortality rate (AMR) was 11.3%, ranging from 5.5% for possible to 21.6% for proven/probable cases. Conclusions: IFDs continue to be a challenging complication in high risk patients. Our results confirm the recently reported trend in reduction of IFD-AMR. On the contrary, cases with unidentified origin continue to be the most frequent. This datum makes it necessary to improve our diagnostic work-up to better target treatment and preventive strategies, and to reduce the risk of overtreatment.

	ALL CASES (142)	Possible IFDs N° cases (91)	Proven/probable IFDs N° cases (51)	Molds (36)	Yeasts (15)
Systemic antifungal prophylaxis					
Administered	**120 (85%)**	78	**42 (82%)**	30	12
Not administered	**22 (15%)**	13	**9 (18%)**	6	3
Prophylactic systemic drug					
Itraconazole	**34 (28%)**	22	**12 (29%)**	9	3
Fluconazole	**28 (23%)**	14	**14 (33%)**	9	5
Posaconazole	**55 (46%)**	40	**15 (36%)**	12	3
Other	**3**	2	**1 (2%)**	0	1
Empirical/pre-emptive					
Performed	**124**	85	**39 (76%)**	31	8
Not performed	**18**	6	**12 (24%)**	5	7
Empirical/pre-emptive drug 1					
Caspofungin	**25 (24%)**	17	**8 (23%)**	6	2
L-AmB	**51 (48%)**	37	**16 (46%)**	11	5
Voriconazole	**17 (16%)**	8	**8 (23%)**	7	1
Abelcet	**8 (7.5%)**	7	**1 (3%)**	1	0
Other	**5 (4%)**	3	**2 (5%)**	1	0
Drug in 1° line target therapy 2					
L-AmB			**11 (27%)**	8	3
Caspofungin			**5 (14%)**	0	5
Voriconazole			**20 (48%)**	17	3
Combined			**3 (7%)**	0	3
Other			**2 (5%)**	1	1
Favourable responses (RR)	**104 (73%)**	72 (79%)	**32 (63%)**	25 (69%)	7 (47%)
IFD attributable deaths (AMR)	**16 (11.3%)**	5 (5.5%)	**11 (21.6%)**	5 (13.9%)	6 (40%)

Table: antifungal treatments and outcome of possible and proven/probable IFDs registered among 593 AMLs

[1]data available for 106 of 123 cases and for 35 of 39 proven/probable cases
[2]Data applicable to proven/probable cases only; 10 patients excluded [3 with incomplete data and 7 for early death (while on empirical/pre-emptive)].

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CLINICAL EFFECTIVENESS OF POSACONAZOLE VERSUS STANDARD AZOLES FOR ANTIFUNGAL PROPHYLAXIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA: A COMPARATIVE RETROSPECTIVE SINGLE-CENTER STUDY

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Background. Invasive fungal infections (IFI) are an important cause of morbidity and mortality in haematological patients receiving induction therapy for acute myeloid leukaemia (AML); large randomized controlled trials have shown favourable results with posaconazole prophylaxis (PP), however the clinical effectiveness of this approach may vary depending on local epidemiology and the routine use of PP may yield different results than those from clinical trials. Aim of the study was to investigate the impact of PP on the incidence of IFI in patients with AML treated in a single Hematological unit. Methods: We retrospectively analyzed 197 adult AML patients receiving an induction chemotherapy between January 2001 and December 2010; 136 patients admitted from 2000 to 2008 received standard azoles (Itraconazole in 120 pts, Fluconazole in 12, and topical prophylaxis in 4) (group 1), while 61 patients admitted from 2009 received 200 mg of oral Posaconazole TID (group 2); other diagnostic and therapeutic standard operating procedures remained unmodified including the induction chemotherapy regimens.

Clinical characteristics were well balanced between groups: mean age was 50 and 51 years (p=0.94), and mean duration of neutropenia was 23 and 22 days (p=0.76), for group 1 and 2 respectively. Results: mean duration of antifungal prophylaxis was 19 and 21 days (p=0.13), for group 1 and 2, respectively. 88% of the patients in both groups developed fever and the median duration of fever was 8 days (2-24) in group 1 and 7 days (1-20) in group 2. No significant differences were observed between the two groups for the use of empirical antifungal therapy (group 1: 37%; group 2: 38%) with a mean duration of 7 days in group 1 and 5 days in group 2 (p=0.22). None of the patients receiving PP developed probable or proven IFI compared to 10 (7%) of the 136 patients receiving standard azoles (p=0.03): aspergillosis occurred in 6 patients, invasive candidiasis in 3 and fusariosis in 1 pt. Overall survival was 93% for patients receiving standard azoles compared to 92% for pts receiving PP. Conclusions: our data add further evidence to the advantage of Posaconazole prophylaxis in patients receiving induction chemotherapy. Additional studies are warranted to confirm our preliminary observation.

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EVALUATION ON REAL LIFE PRACTICE OF ANTIFUNGAL PROPHYLAXIS IN HIGH RISK PATIENTS: PRELIMINARY RESULTS FROM A PROSPECTIVE SURVEY

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Background/Aims: To describe the current use of antifungal (AF) prophylaxis in adult acute myeloid leukemia patients (AMLs) at first induction of remission and to analyze the efficacy of prophylaxis with posaconazole (POS) when compared to old azoles. Methods: from January 2010 to March 2011, all newly diagnosed AMLs have been consecutively registered and prospectively monitored in 31 Italian participating centers. Only adult cases that received conventional chemotherapy were included in the present study. Principal demographic and clinical data, as well as antifungal treatments were collected. In particular we analyzed data about systemic AF prophylaxis: the drug of choice, the duration of treatment, and its efficacy were thus evaluated. To determine prophylaxis efficacy, incidence of IFDs was assessed at 30th day from the end of chemotherapy. IFDs outcome was also evaluated. Results: 498 pts received conventional chemotherapy as first induction for AML. Median age was 60 (range 18-81), with a male/female ratio of 1.6/1. The most part of them (448, 90%) received systemic antifungal prophylaxis. POS was the most frequently employed drug (224/448, 50%), followed by fluconazole (128, 29%) and itraconazole (86, 19%). When comparing the POS group (224 pts) to those receiving itraconazole or fluconazole (214 pts) (FLU/ITRA) no significant differences emerged in terms of the main risk factors for IFDs (table). In particular the 2 groups resulted to be comparable in terms of age, sex, frequency and duration of deep neutropenia, days of prophylaxis. On the contrary, there were significant differences in breakthrough IFDs (6.2% in POS vs 11.7% in FLU/ITRA, p-value 0.04). Not only molds, but also yeasts were more frequent in the FLU/ITRA group. Caspofungin and amphotericin B compounds were the most frequently employed drugs, as empirical/pre-emptive treatments. There were no significant differences in the response rate, nor in the IFDs attributable mortality rate. Conclusions: during the last few years the use of POS prophylaxis in high risk pts has significantly increased. Although not randomized, our study demonstrates in a "real life" setting the higher efficacy of POS prophylaxis, when compared to FLU/ITRA. Only 14 patients developed a breakthrough IFDs. Suprisingly, POS superiority emerged for both molds and yeasts infections. Previous AF prophylaxis do not seem to impact IFDs outcome.

	FLU/ITRA	POS
	214	224
Mean age (years)	57.7	53.5
Sex		
- male	112 (52%)	101 (45%)
- female	102 (48%)	123 (55%)
Deep neutropenia	210 (98%)	213 (95%)
Urinary catheter	119 (56%)	143 (64%)
Mean duration of deep neutropenia (days)	19.5	21.5
Median AF prophylaxis duration (days)	20	20
Empirical/pre-emptive therapies	59 (28%)	71 (32%)
Most used drugs as empirical/pre-emptive therapy	- L-AmB 18 (30%) - Caspo 17 (29%) - Vorico 8 (14%) - CL-AmB 8 (14%) - Others 8 (14%)	- L- Amb 39 (55%) - Caspo 12 (17%) - Vorico 6 (8%) - CL-AmB 2 (3%) - Others 12 (17%)
Proven/probable IFDs	25 (11.7%)	14 (6.2%)
- moulds	- 17 (7.9%)	- 11 (4.9%)
- yeasts	- 8 (3.7%)	- 3 (1.3%)
Molds/yeasts ratio	2.1/1	3.7/1
Favourable responses (RR)	14/25 (56%)	11/14 (79%)
N° deaths (AMR)	7/25 (28%)	2/14 (14%)
- moulds	4	1
- yeasts	3	1

Table 1: comparison between POS and FLU/ITRA groups

Legend: AF: antifungal; IFD: invasive fungal disease; L-AmB: liposomal Amphotericin B; CL-AmB: lipid complex Amphotericin B; RR: response rate; AMR: attributable mortality rate; pos: posaconazole; flu: fluconazole: itra: itraconazole.

P171

POST-TRANSPLANT CMV REACTIVATION AFTER CBT: THE ROLE OF DELAYED IMMUNE RECONSTITUTION OR DONOR'S SERONEGATIVITY?

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Allogeneic cord blood transplantation (CBT) is being increasingly used but immune recovery is reported as delayed. The aim of this study is to compare CMV infections in CBT recipients with transplants from unrelated or family mismatched donors (referred to as alternative donors). CMV infection was monitored by pp65 antigenemia, late infection was defined as > 100 days after HSCT. The cumulative incidence (CI) and survival were calculated by means of Kaplan-Meier method, log rank test. Overall, 136 consecutive transplants in seropositive recipients were identified and divided according to donor type ad serostatus into: 1) 38 D+/R+ alternative transplant, 2) 29 D-/R+ alternative transplant and 3) 69 D-/R+ CBT. Median follow-up was 257 days (1-1328). CI of CMV infection was slightly higher in D-/R+: D-/R+ alternative 69%, D-/R+ CBT 72.5% and D+/R+ alternative 55.3%, p=0.2). Late infection was significantly more frequent in D-/R+ group: D-/R+ alternative 60%, D-/R+ CBT 67.4% and D+/R+ alternative 7.4%, p<0.001, figure 1). The time from the first to last positive antigenemia was 11 days (range, 1-249) for D+/R+ alternative group versus 126 days (range, 1-794) for D-/R+ alternative group (p=0.006), and versus 106 days (range, 1-674) for D-/R+ CBT (p<0.0001). There was no difference in overall survival between D+/R+ and D-/R+ groups (p=0.98). In conclusion, characteristics of CMV infection were similar in D-/R+ CBT and D-/R+ alternative transplant group, and differed importantly from D+/R+ alternative transplant group. The burden of CMV morbidity, related mostly to late infection, seems to be associated rather with donor serostatus than with CBT-related immunodeficiency.

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ANTIFUNGAL PROPHYLAXIS WITH POSACONAZOLE IN HIGH RISK HEMATOLOGICAL PATIENTS: OUR EXPERIENCE

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Invasive fungal infections are a main cause of morbidity and mortality in hematological patients undergoing intensive chemotherapy regimens. Prophylactic and early treatment is mandatory to improve survival. Antifungal prophylaxis refers to the prevention of invasive fungal infections. Effective prophylactic antifungal agents are: fluconazole, itraconazole and posaconazole. Many hematological centers use fluconazole prophylaxis during neutropenia in patients with acute leukemia. Recent randomized trials suggest that prophylaxis with the mold-active triazole posaconazole is more effective in patients with prolonged neutropenia. In our hematology division, between January 2010 and November 2010, nr. 22 patients (median age 48 years) with acute myeloid leukemia were treated with nr 41 total cycles of chemotherapy (nr 20 induction chemotherapy, nr. 21 consolidation chemotherapy) and nr 41 cycles of antifungal prophylaxis. Antifungal prophylaxis was posaconazole in 22/41 (54%), itraconazole in 7 (17%) and fluconazole in 12 (29%). We evaluated: oral mucositis, intestinal mucositis, ileo typhlytis, fever and switch of prophyilactic antifungal agent to the empirical treatment. The median duration of antifungal prophylaxis was: nr. 23 days (r. 15-31) with posaconazole, nr. 17 days (r 23-25) with itraconazole and nr. 13 days (r 7-27) with fluconazole. Oral mucositis was detected in nr. 8 of 41 prophylactic treatment; nr. 1 case (grade III) was with itraconazole; nr. 7 cases were with fluconazole; no cases with posaconazole. Intestinal mucositis was detected in nr. 11 (27%)of 41 treatment: nr. 1 case (grade II) with itraconazole, nr. 7 cases with fluconazole, no case with posaconazole. Ileotyphlytis was detected in nr. 3 (7%) of 41 treatment: nr.1 case with itraconazole, nr. 2 cases with fluconazole, no case with posaconazole. Fever of unknown origin (FUO) was detected in 31 (75%) of 41 treatment; nr. 6 days (r 1-19) was the median duration of fever. The switch of antifungal prophylaxis in to the empirical treatment was in nr. 11 of 31 episodes of fever: fluconazole was replaced in nr.8 cases of 11, itraconazole in nr. 2 and posaconazole in nr. 1. In our experience, the antifungal prophylaxis of high risk neutropenic patient with posaconazole reduced the number and severity of mucositis and the progress of FUO with lower price of global management of patients.

CHRONIC MYELOPROLIFERATIVE DISORDERS

P173

ANAGRELIDE AND FIBROBLAST GROWTH FACTOR-2 LEVELS IN ESSENTIAL THROMBOCYTHEMIA

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The essential thrombocythemia (ET) is characterized by platelet endothelial activation that releases fibroblast growth factor-2 (FGF-2) inducing myeloproliferation and fibrosis. Anagrelide (ANA) is a cytoreductive drug inhibiting platelet activation. Therefore, we evaluated platelets, platelet factor 4 (PF4), tissue factor pathway inhibitor (TFPI), tissue factor (TF), von Willebrand factor (vWF), FGF-2, white blood cell count (WBC), haemoglobin (Hb) and reticulin in thrombocythemics on ANA. We recruited 21 thrombocythemic (13 man, 8 women; mean age, 54 years; range, 28-77 years) who fulfilled WHO. Their mean duration of disease was 9 years (range, 4-21 years). None had splenomegaly or mutational status. The mean dose of ANA was 2.1 mg/day. All patients were on aspirin. None had thrombophilia or previous thrombosis. All had bone marrow biopsy at diagnosis and underwent follow-up trephines every 2 years. Thirty subjects with reactive thrombocytosis served as controls. Platelets, WBC and Hb were measured by automated analyzer. PF4, TFPI, TF, FGF-2 and vWF were assayed by ELISA and immunoturbidimetric assay, respectively. Considering that FGF-2 may be produced by platelets, we adjusted FGF-2 per platelet (FGF PLT pg/106). Before treatment, all patients had thrombocytosis (1005±314x10^9/L vs 510±25x109/L) (p<.0001), high PF4 (127±45 IU/ml vs 4.1±2.4 IU/ml) (p<.0001), TFPI and TF (156±64 ng/ml and 235±287 pg/ml vs 94±10 ng/ml and 4.4±2.7 pg/ml, respectively) (p<.0001 and p<.0001), low vWF (23±7.8 % vs 77±18%), (p<.0001), high FGFPLT (0.08±0.08 pg/10^6 vs 0.01±0.001 pg/10^6) and WBC and Hb (10.5± 2.4x10^9/l and 14±1.4 g/dl vs 5.2±1.1x10^9/L and 12±0.4 g/dl, respectively) (p<.0001 and p<.0001) and a median reticulin level of 1.2. After treatment, all patients had platelets <400x10^9/L (386±55x10^9/L) and normal PF4, TFPI, TF ,vWF, FGFPLT , WBC and Hb (8.5±3 IU/ml, 100±49 ng/ml, 8.7±0.8 pg/ml, 91±35 %,0.01±0.0 pg/10^6, 7.4±1.4x10^9/L and 12.6±1.1 g/dl) and the reticulin level was 0 (Table). A correlation there was between PF4 and platelets (p<.0001), PF4 and TFPI and TF and vWF (p=0.005 and p=0.014 and p<.0001, respectively), PF4 and FGFPLT (p<.0001), TFPI and TF and vWF and FGFPLT (p=0.003 and <.0001 and p<.0001, respectively), FGFPLT and WBC and Hb (p=0.002 and p=0.004, respectively) and FGFPLT and reticulin (p<.0001). These data suggest that FGF-2 may be a prognostic marker that normalizes after ANA ameliorating the outcome of ET.

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IMMUNOPHENOTYPIC CHARACTERIZATION OF PERIPHERAL BLOOD CD34+ CELLS IN IDIOPATHIC MYELOFIBROSIS

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Idiopathic Myelofibrosis (IMF) is chronic myeloproliferative neoplasm characterized by constitutive mobilization of hematopoietic stem cells (HSC) and progenitor cells (HPC) into the peripheral blood (PB). The interaction between the chemokine CXCL12 and its receptor CXCR4 plays a pivotal role in determining the trafficking of CD34+ cells between the bone marrow (BM) and the PB. IMF is associated with downregulation of CXCR4 by CD34+ cells due to epigenetic events. Altered gene expression was corroborated by the detection of abnormally high CD9 or CD164, and low CXCR4, membrane protein expression in IMF CD34+ cells. Moreover, endothelial precursor cells (CD34+/ CD133+) are increased in the blood of a subset of patients with IMF, and peripheral endothelial cells bear the same molecular markers as hematopoietic cells, suggesting a primary role of pathological endothelial cells in this disease. We evaluated, by flow cytometry, the number of CD34 positive cells in peripheral blood and the expression of CXCR4, CD9, CD117 and CD133 on these cells. In our institution we are following 20 patients affected by IMF, according to WHO criteria (M: 12, F: 8;

median age: 55 years, range: 48-62 years). In all patients, at diagnosis, we found a high count of CD34+ cells in PB (greater than 15x10(6)/l; median: 2,4x10(6)/l, range: 1,8-3,2x10(6)/l) compared with normal controls and other chronic myeloproliferative diseases. In all cases CD34+ cells were negative for CXCR4 while expressing high intensity CD9. About 40% of CD34+ cells expressed CD133, while 20% expressed CD117 at low intensity. In no case was detected coexpression of CD133 and CD117, suggesting a simultaneous presence of two distinct hematopoietic progenitors, endothelial progenitors and myeloid progenitors. We monitored every 6 months the phenotypic pattern of CD34+ cells, and after 36 months have seen an increase of myeloid precursors (CD34+/CD117+: 45,7%) compared with a reduction of endothelial precursors (CD34+/CD133+: 15,3%). By comparing these findings with other clinical data, our results seem to confirm that according to the natural history of disease, from an initial stage towards a fibrotic phase (pancytopenia and/or splenomegaly), there was a change in PB CD34+ cells. In conclusion, immunophenotypic profile of PB CD34+ cells is associated in IMF with patients' clinical characteristics and may have potential prognostic application.

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ANAGRELIDE IN THE TREATMENT OF ESSENTIAL THROMBOCYTHAEMIA (ET)

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Essential thrombocythaemia (ET), the most often occurring myeloproliferative disorder is a clonal malignant disorder arising from stem cell. The course of the disease is complicated by some severe thrombotic events and far less commonly by haemorrhagic phenomena. Treatment of ET consist of antiplatelet drugs (e.g. aspirin) and lowering platelet count (hydroxyurea or interferon alpha). Anagrelide is an oral imidazoquinazoline agent which is indicated in Europe for the reduction of elevated platelet counts in at-risk patients with essential thrombocythaemia who are intolerant of or refractory to their current therapy. In our institution we are following 120 patients affected by ET (71 females and 49 males; median age 48 years, range: 27-82) according to PVSG criteria and WHO classification. 67 out of 120 patients were classified as low-risk, 28 as intermediate-risk and 25 as high-risk. The Val617Phe point mutation of Janus Kinase 2 gene (JAK2V617F) was found in 69 patients. Anagrelide was used in 15 patients with ET from Jan. 2010 to April 2011. Anagrelide, in the average dose of 2,0 mg (range: 1,0-3,5 mg) reduced platelet count in all patients. Median time of response was 3-4 weeks. Complete remission (platelet count < or = 450x109/l) was achieved in 14/15 patients, and only one patient had platelet count slightly above 450x109/l (but less than 600 x109/l). During the first two months of treatment with anagrelide some mild and transient side effects were noticed, e.g. headache in 7 (47%), fluid retention in 4 (27%), palpitations in 2 (13%), and diarrhoea in 2 (13%) patients, but all of them continued therapy. We have found no case in thrombotic events. Anagrelide proved to be a safe and effective drug for pre-treated ET patients. The positive results described in several studies may well lead to the next use of anagrelide as first line treatment in patients with high risk ET.

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MYELOPROLIFERATIVE NEOPLASMS (MPN) PHILADELPHIA NEGATIVE DIAGNOSTIC WORK UP

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Introduction. The aim of this study was to create a diagnostic itinerary for MPN Philadelphia negative in the Haematology Unit of Florence. Methods. From April 2008 to April 2011 we collected a total of 313 cases,219 cases resulted MPN,188 male (60%),125 female(40%) (Range 25-82 years, median age 50 years). The diagnostic itinerary included the following tests: physical examination, several blood tests, (including serum tryptase in suspect of mastocytosis), X-ray chest scan, abdominal ecography scan, bone marrow biopsy, karyotype, evaluation of BCR/abl by FISH analysis, flow cytometry (for mastocytosis), molecular biology (JAK2 mutation, MPL, cKit, FIP1L/PDGFRa). In all the bone marrow specimens we performed both a morphological study(GIEMSA, H&E), istochemestry(PERLS and Gomori silver stain) and immunohistochemestry analysis(GPA, CD34, CD61, MPO, tryptase for mastocytosis). Expression rate, growth and features of dysplasia were evaluated for all haematopoietic series (granulocytic, erythroid, megakaryocytic). For megakaryocytic lineage, megakaryocytes size, istotopography and the presence of clusters were analized. Marrow microenvironment, was characterized performing analysis of fibrosis grading, presence of sinusoids, lymphoid aggregates and fat. Results. On the basis of BM evaluation we found the following diagnosis:80 PMF(25%), 48 PV(26%), 70 ET(36%), 21 SM(13%). The median expression of CD34+ cells was: 2,9% in PMF, 2,6% in PV, 2,2 % in ET, 2.6% in SM. All showed a different grade of fibrosis, while only in two cases of PV, three cases of ET and ten cases of SM some degree of fibrosis was detected. Bone marrow vascularisation was present in 54/80PM cases, 7/48PV, 5/70TE, 4/21SM. We analized the correlation between bone marrow results and other tests, finding a discordance with the final diagnosis in 13 cases on 219 (7PV and 6ET), corresponding to 6% of cases. In that discordants cases, the bone marrow biopsies resulted only in a moderate lineage hyperplasia. However, all the seven biopsies with erythropoietic hyperplasia were JAK2+, while 1/6 ET was JAK2+,and 6/6 MPL negative. In the five JAK2 negative ET, the final diagnosis was based on minor criteria. All mastocytosis showed a multifocal, dense infiltration of mast cells(>15%)strongly tryptase positive, and 17/21 presented cKit D816V mutatioN(83%). Conclusions. Our results showed the benefit of a diagnostic work up in terms both of diagnostic accuracy and patient satisfaction.

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A CASE OF EVOLUTION OF CUTANEOUS MASTOCYTOSYS INTO INDOLENT SYSTEMIC MASTOCYTOSIS WITHOUT EVIDENCE OF C-KIT MUTATION ASP-816-VAL

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Mastocytosis comprises a heterogeneous group of disorders characterised by abnormal proliferation and accumulation of mast cells in various tissues and organs. The WHO classification includes seven main categories of mastocytosis, namely cutaneous mastocytosis, indolent systemic mastocytosis usually presenting with cutaneous involvement, systemic mastocytosis with an associated clonal haematological non-mast cell lineage disease, aggressive systemic mastocytosis, mast cell leukaemia, mast cell sarcoma and extracutaneous mastocytoma. For the discrimination of systemic mastocytosis from cutaneous mastocytosis and other pathological conditions with a marked increase of reactive mast cells, certain criteria were defined: the major diagnostic criterion is the histological evidence of multifocal compact tissue infiltrates consisting of at least 15 mast cells. Minor criteria include the demonstration of (a) >25% spindleshaped mast cells; (b) c-kit mutations at codon D816V; (c) expression of CD25 or CD2 by mast cells; and (d) a persistently raised serum total tryptase level. Clinical manifestations of mastocytosis range from disseminated maculopapular skin lesions that may spontaneously regress to highly aggressive neoplasms like mast cell leukemia or mast cell sarcoma. Recently, it could be shown that systemic mastocytosis is a clonal disorder often exhibiting mutations of c-kit, a protooncogene encoding the tyrosine kinase receptor for stem cell factor. Mutations of c-kit are considered to play a key role in the pathogenesis of mastocytosis Therefore, we investigated the unique case of a 38 year-old woman patient with indolent systemic mastocytosis evolving from UP by means of histology, immunophenotyping and molecular biology. At the time of initial diagnosis physical examination revealed urticaria pigmentosa-like skin lesions over the extremities. There was no evidence of organomegaly or lymphadenopathy A complete blood cell count and the biochemical profiles were within normal limits. The histological examination of a biopsy taken from the back revealed a sub-epidermal bulla with a dense infiltration of mast cells and some eosinophils in the upper dermis The toluidine blue and Giemsa stains showed that almost all of the infiltrating cells in the dermis were mast cells. The bone marrow showed only a mild diffuse increase in mast cells but compact infiltrates were missing. The serum tryptase levels were normal. Five years later, however, the bone marrow histology displayed patchycompact mast cell infiltrates, which now allowed to establish the diagnosis of an

ISM. The serum tryptase levels at this time were markedly elevated. At both time points, mast cells were analyzed by immunohistochemistry using anti-tryptase antibody AA1, by flow cytometry using antibodies against CD2 and CD25, and nested polymerase chain reaction on laser-microdissected, single pooled mast cells. Immunohistochemistry revealed strong tryptase-positivity of mast cells in both cutaneous and bone marrow infiltrates. Flow cytometry yielded an aberrant expression of CD2 and CD25 on bone marrow mast cells. However, repeated thorough PCR analysis failed to unveil c-kit mutation in atypical mast cells of skin and bone marrow samples of both dates. These findings clearly show that ISM can evolve from UP. Moreover, our study provides further evidence that the c-kit mutation Asp-816-Val is not invariably present in ISM. A follow-up for systemic involvement is required.

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PREGNANCY COMPLICATIONS ARE COMMON IN FEMALES AFFECTED BY ESSENTIAL THROMBOCYTHEMIA WITH THROMBOTIC COMPLICATIONS

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Approximately 1/3 of pregnancies of females with essential thrombocythemia (ET) end up in fetal loss. Thrombotic occlusion of placental circulation is considered an important mechanism of pregnancy morbidity. Within the various risk factors evaluated, JAK2V617F mutation was identified as an independent predictor of miscarriages. Thrombotic complications are the main causes of morbidity and mortality in ET usually affecting patients over 60. However, young ET patients are prone to develop unusual vein thrombosis (splanchnic veins and cerebral sinuses). The aim of this study is to evaluate a large retrospective cohort of patients supposing that both pregnancy and thrombotic complications occur in the same setting of young females affected by ET. Our study includes 57 consecutive pregnancies in 34 young females affected by ET, (mean age 29.4±5.8y at diagnosis and 32.5±5.4y at conception). JAK2V617 mutation was searched with allele specific PCR. JAK2V617F allele burden was determined with PCR real time. Statistical analysis was performed with X2 test with Yates correction. 13 pregnancies ended in first trimester abortion and 8 in later fetal loss. Eight females had one normal baby after 1 (6 cases) or 2 (2 cases) abortions. Fourth pregnancies had the JAK2V617F status: 27 (13 uneventful and in 14 fetal loss) (67.5%) pregnancies occurred in JAK2V617F positive females (13 pats) and 13 (11 uneventful and 2 miscarriages) (32.5%) in JAK2 wild-type (p=0.04). Mean allele burden was 31.2±15.1%. Thrombotic events occurred in 9 females (1 stroke, 1 myocardial infarction, 1 cerebral sinuses, 4 Budd-Chiari and 2 portal vein) who had a total of 18 pregnancies. JAK2V617F mutation was found in 5 patients out of the 6 evaluated. Eight out of the 9 females (89 %) with thrombosis had at least one complicated pregnancy while only 1 patient out of females with one or more normal deliveries had a cerebral vein thrombosis (Tab I)(p< 0.01). JAK2V617F mutation is confirmed as a negative prognostic factor for pregnancy complications in agreement with the data of the literature. Our study shows that when a female affected by ET has pregnancies negative outcomes commonly has also thrombotic complications mainly affecting splanchnic veins. Considering that placental thromboses have a basic role in pregnancy failure, we can suppose that abortions may be considered as thrombotic events that is a validate risk factor for risk stratification of patients with ET.

	Thrombotic complications	No thrombotic complications	Total
Uneventful delivery	7	29	36 (64%)
Fetal loss	11	10	21 (36%)
Total	18	39	57

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CLONAL MAST CELL DISORDERS: ROLE OF MULTICOLOR FLOW CYTOMETRY IN THE DIAGNOSTIC WORK-UP OF INDOLENT FORMS

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Mastocytosis is a group of diseases characterized by increased mast cells (MC) in different tissues, mainly skin and bone marrow (BM). Mastocytosis may be limited to the skin (cutaneous mastocytosis - CM), or may involve one or more extra- cutaneous (EC) organs (systemic mastocytosis - SM), with or without skin involvement. The presence of the so-called B- and C-findings further distinguishes SM in indolent (ISM), smouldering and aggressive forms. Patients with SM often experience symptoms due to inappropriate release of MC mediators, such as flushing, urticaria, diarrhoea, and unexplained or recurrent anaphylactic reactions (mainly after hymenoptera sting) and may suffer from severe osteoporosis. According to the WHO classification the diagnosis of systemic mastocytosis (SM) relies on bone marrow (BM) examination and is based on one major and four minor criteria. Herein, we used WHO criteria to compare flow cytometry (FC) with other available techniques in the diagnosis of SM after BM examination. Methods. We analyzed a cohort of 95 patients with suspect SM. All patients underwent comprehensive BM examination by using cytology, immunohistochemistry (IHC), FC and molecular study for mutation of c-Kit and serum tryptase dosage. FC evaluation was based on a combination of monoclonal antibodies, specifically CD25/CD2/CD45/CD34/CD117. 74 out of 95 patients were diagnosed with indolent SM (n = 59) or monoclonal mast cell activation syndrome (n = 15) because satisfying less than 3 minor criteria. 39 out of these 74 patients fulfilled the major histological criterion, whereas the presence of a minor criterion was assessed by FC, molecular study, cytology and tryptase level in 70/74, 52/67, 56/74 and 42/74 patients, respectively. FC showed higher sensitivity than IHC in detection of CD25+ mast cells (MC) (92.9% vs. 73.8%; p=0.019), especially in the absence of the major histological criterion (90.5% vs. 47.6%; p=0.003). Moreover, CD2 expression was documented by FC and IHC in 97.1% and 35.3% of cases, respectively (p<0.001). FC showed the best sensitivity for identifying abnormal MC compared to other techniques, especially in cases with low MC burden. Therefore, we hope for a major role of FC in the diagnostic work-up of clonal MC disorders.

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CLINICAL FEATURES AND TREATMENT OF YOUNG PATIENTS (UNDER 40 YEARS OF AGE) WITH CHRONIC PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE DISORDERS: A SINGLE CENTRE EXPERIENCE

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Background: Chronic Philadelphia negative myeloproliferative disorder (CMD) are typical of middle age. However, it has also been observed in children and young adults. Major thrombotic episodes and microvascular disturbances have been described in young setting but the real risk for vascular complications has not been clearly established, and different specific therapeutic approaches have been investigated Methods: We retrospectively analyzed a cohort of 37 CMD patients (28 Essential Thrombocythemia, 5 Polycythemia Vera and 4 Idiopathic Myelofibrosis), younger than 40 years at diagnosis, in order to evaluate the features at presentation, different options of treatment and outcome in term of incidence of thrombotic and hemorrhagic complications Results: Between 1992 and 2010, 37 consecutive patients (pts) under 40 years of age (23 males, 14 females; median age: 35 years), have been diagnosed as CMD in our Institute. Laboratory features at diagnosis showed median platelet count of 702x10^9/L (range 452–2045 x10^9/L) with 8 pts with a platelet count > 1000x10^9/L; median leukocyte count of 8.27x10^9/L (range 4–19.6x10^9/L) and median Hb level of 14,2g/dl were also recorded. The diagnosis was carried out according to the PVSG or the WHO criteria. JAK2V617F mutation was present in 15 (40%) pts. At presentation 16 pts (43%) were experiencing vasomotor symptoms, including headache (10), dizziness (5) or visual disturbances (1). Major thrombotic and hemorrhagic events occurred in 4 (11%) and in 2 (5%) pts, respectively. All 4 thrombotic complications were arterious and occurred at diagnosis (1) or during follow-up (3). Concomitant thrombophilia or cardiovascular risk factors were present in 59% of cases. Majority of pts (92%) received antiplatelet drugs from diagnosis; cytoreduction was

performed in 15(40%) pts, after first thrombotic event or to control of myeloproliferation. It was based on the discretion of the physician: in particular Hydroxyurea was used in 12(80%), interferon-alpha in 5(33%) and anagrelide in 3(20%) pts, respectively. 2 pts also received long term anti-coagulation therapy after major thrombotic event. No patient experienced leukemic transformation Discussion: In our cohort of young CMD pts, vasomotor symptoms at presentation are more frequent (43%) and the incidence of thrombosis is high (11%). 40% of pts need cytoreductive therapy for thrombotic events or to control myeloproliferative features. Majority of pts (80%) receive hydroxyurea with primary option of treatment after first thrombosis

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A RETROSPECTIVE ANALYSIS ON 285 PATIENTS WITH IDIOPATIC MYELOFIBROSIS FOLLOWED IN THE LAZIO REGION DURING 20 YEARS: IMPACT OF PROGNOSTIC FACTORS ON SURVIVAL AND PROGRESSION TO AML

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Primary myelofibrosis (PMF) is a rare chronic myeloproliferative neoplasm (MPN) characterized by a heterogeneous clinical presentation, a relatively shortened survival and a propensity to develop acute myeloid leukemia (AML). To correlate the clinico-biologic features at presentation with outcome of IM cases, we retrospectively collected the clinical records of 285 patients followed in 12 hematologic units of Lazio diagnosed between 1981 and 2010. Diagnosis was made according to the criteria accepted at the time when the patient was analysed. One hundred seventy seven (62%) patients were males, 108 females (38%) (M/F= 1.63). Patients mean age was 67 years. Hb < 10 g/dl was present in 85/274 (31%) of patients. Eigtheen/270 (6.6%) patients presented a WBC count >25 x 10^9/L. The presence of JAK2V617F mutation was checked in 143 patients. However, only 83 patients had JAK2 V617F mutation assessed within 1 year from diagnosis. Among these ,51 (61%) were JA2V617F-positive. 190 (70%) patients received conventional chemotherapies. At time of analysis, 152 patients were alive, 65 dead and 62 lost to follow-up. With a median follow-up of 48.4 months (range: 1-252) the actuarial survival rates at 5, 10 and 20 years were 70%, 50% and 43%, respectively. The univariate and multivariate analyses show that Hb <10 gr/dl (p<.0001), WBC >25 x 10^9/L (p=.045) and age < 50 years (p<.018) were independent prognostic factors negatively affecting the overall survival rates. Twenty-four of 285 patient (8.4%) progressed to AML after a median time from diagnosis of 92 months (range: 2-250 months) for a cumulative incidence rate of 11%, 22% and 30% at 5, 10 and 20 years, respectively. At univariate and multivariate analyses only Hb < 10gr/dl resulted as an independent prognostic factor significantly affecting the AML occurrence. JAK2V617F did not impact on survival or on progression to AML. In conclusion, our retrospective analysis on a large series of patients demonstrates that survival and progression to AML of PMF patients may be predicted by easily assessable factors such as anemia, age and leukocitosis. Interestingly, anemia is also significantly associated to AML occurrence. These data, helping risk stratification and decision making for PMF patients, may be of paramount relevance particularly in view of the forthcoming availability of new targeted drugs.

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DRUGS TARGETING THE MTOR PATHWAY EFFECTIVELY INHIBIT CELLS OF MYELOPROLIFERATIVE NEOPLASMS

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Dysregulated signaling of the JAK/STAT pathway due to JAK2V617F mutation is found in myeloproliferative neoplasms (MPN).We explored the contribution of AKT/mammalian target of rapamycin (mTOR) pathway to MPN pathogenesis by means of an allosteric (RAD001) and an ATP-competitor (PP242) mTOR inhibitor. We found that JAK2V617Fmutant HEL and SET2 cells were more sensitive to mTOR inhibition than control K562 cells. We also investigated the effects of mTOR inhibitors in JAK2 wild- type murine IL-3 or EPO-dependent (Ba/F3 and Ba/F3-EPOR) cells or the cytokine-independent JAK2V617F counterpart that were more sensitive plus/minus IL-3 or EPO in the culture medium. Both drugs increased the fraction of G0/G1 cells but they were not effective in inducing cell apoptosis. Next, we analyzed the JAK1/JAK2 inhibitors AZD1480 or INC424 (AstraZeneca, USA) and the histone deacethylase (HDAC) inhibitors ITF-2357 (Givinostat,Italfarmaco) or LBH589 (Panobinostat,Novartis). Those molecules were all growth inhibitory in HEL and SET2 cells at IC50 concentrations significantly lower than in K562 cells. However, unlike mTOR inhibitors, they were dose-dependently potent inducers of cell apoptosis. V617F Ba/F3 and Ba/F3-EPOR cells were more sensitive to AZD1480 or INC424 that wt counterpart but, addition of cytokine to culture medium abrogated the preferential growth inhibitory effect. Co-treatment of mTOR inhibitor with JAK1/JAK2 inhibitor resulted in synergistic inhibition of cell proliferation (Chou-Talalay software). Treatment of SET2 cells with mTOR inhibitor dose-dependently reduced phosphorylation of the mTOR target 4EBP1 and also of STAT5, while both phosphorylated and total JAK2 resulted unaffected. In comparison, JAK1/JAK2 inhibitors markedly and dose-dependently reduced phosphorylation of JAK2 and STAT5 leaving unaffected 4EBP1. The HDACi dose-dependently reduced phosphorylated and total JAK2, pSTAT5 and showed a modest effect on p4E-BP1, particularly Givinostat. Indeed, antisense RNA-mediated silencing of mTOR resulted in effective prevention of STAT5 phosphorylation, suggesting an interplay between these signalling pathways. Colony formation from MPM CD34+ cells was inhibited at concentration of mTOR and JAK1/JAK2 inhibitor significantly lower than controls, and RAD001 efficiently prevented the growth of EPO-independent colonies from PV patients. In aggregate, our data indicate that targeting the Akt/mTOR pathway has potential for treatment of MPN.

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RISK ASSESSMENT OF NON HEMATOLOGICAL SECOND MALIGNANCIES IN 733 PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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It is commonly held that patients with Philadelphia-negative myeloproliferative neoplasms (MPN) have an increased incidence of secondary cancers or of another neoplasm of the hematopoietic system (Vannucchi et al, 2009), but wide epidemiologic studies at this regard are scarce. The aim of this study was to assess the risk of subsequent non hematological malignancies in patients with MPN compared to the general population. We performed a retrospective chart review of all consecutive patients diagnosed with Polycythemia Vera (PV), Essential Thrombocythemia (ET) or Primary Myelofibrosis (PMF) between 1980 and 2006 and regularly followed in our institution. The identification of solid cancer cases was obtained through linkages with hospital discharge system, pathology department registries, with the Regional Cancer Registry and the Regional Mortality Registry. Expected numbers of incident cases of second tumors were calculated based on 5-year age group, gender, and calendar time–specific cancer incidence rates in the general population of the same area. The relative risk was computed by standardized incidence ratios (SIRs). Analyses were carried out for the whole series and separately for PV and ET, for gender and for JAK2V617F genotype. The overall cohort of the study included 733 MPN cases, 302 (41.2%) PV, 375 (51.2%) ET and 56 (7.6%) PMF patients; 372 were females (50.75%), median age at diagnosis for PV, ET and PMF was 59, 57 and 67 yrs respectively.The mean follow-up period was 6.45 yrs with a total of 4724.72 person-yrs. In the entire cohort 49 (6.7%) non-hematological cancers subsequent to MPN diagnosis were recorded: results showed the lack of a specific risk pattern for all cancer-sites (SIR=0.87, 95% CI:0.64,1.14) while a significantly increased risk for melanoma (SIR=3.69, 95%CI:1.39,9.64) was observed. The analyses stratified by gender and PV or ET did not highlight a specific pattern of risk: the only increased risk was confirmed by 2 cases of melanoma associated with PV (SIR=4.26, 95% CI:1.06,17.04). Sixty percent of the patients received a cytotoxic therapy (hydroxyurea in >85%); after adjustment for sex and age at diagnosis, the risk of second cancers was not increased compared to control population (HR = 0.935,95% CL:0.462,1.891;p= 0.85).

Finally, considering the 477 (65.1%) patients with known JAK2V617F genotype, we did not identify increased risk for all cancer sites (SIR=0.86,95% CI:0.51,1.35) in the 340 JAK2V617F positive cases.

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CYTOGENETIC ABNORMALITIES IN ESSENTIAL THROMBOCYTHEMIA: EXPERIENCE OF THREE HEMATOLOGICAL CENTRES

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Cytogenetic abnormalities in patients with Essential Thrombocythemia (ET) are rare and heterogeneous. Their frequency at diagnosis varies from less than 5 to 10% and includes structural changes, numerical gain and losses, unbalanced traslocations. The more frequent abnormalities are trisomy 9, trisomy 8, abnormal chromosome 1, abnormal chromosome 11, del(5q), del (20), del(6). The prognostic role of cytogenetics has not be established, survival of patients with cytogenetic changes at presentation did not differ from that of the patients with normal karyotype. The authors report association between transformation to acute myeloid leukemia (AML) and unfavorable survival with de novo appareance of cytogenetic abnormalities, instead majotity of patients who transform to Myelofobrosis (MF) do not acquire cytogenetic abnormality. In this study we reports the cytogenetic results of 342 consecutive patients at three hematological institutions between 1987 to 2010. The JAK2V617F mutation was documented in 176 patients. Thirty-nine patients had no adeguate metaphases for interpretation. Only 15/342 (4.3%) had an abnormal karyotype at diagnosis. Our cytogenetic abnormalities were –Y (in 6 patients), del (20) (n=2), del (11) (n=1), trisomy 9 (n=1), trisomy 20 (n=1), del(5q) (n=1), del (22) (n=1), del (13) (n=1), add (15p) (n=1). Furthermore we found the chromosome aneuploidy in ten patients. Follow-up cytogenetics studies were performed in 18 of the 342 patients with normal karyotype at diagnosis and five patients showed acquired new cytogenetics abnormalities, including del(11), –Y, t(1:9), chromosome aneuploidy and complex karyotype. Disease transformation to acute leukemia was documented in two patients; these patients had normal karyotype at diagnosis and at transformation showed del(11) and t(1:9). No patients with disease transformation to myelofibrosis had cytogenetics abnormalities. There is no difference in survival of patients with cytogenetic abnormalities and those with normal karyotype in our centres. In conclusion detection of cytogenetic abnormalities at diagnosis are rare and heterogeneus in ET patients, and in this study are less than 5%. These anomalies are no specific and have no clinical and prognostic significance and also in this study is no predictor of evolution into AML or MF, and have no impact on overall survival.

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A RETROSPECTIVE ANALYSIS OF 990 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA(ET) FOLLOWED IN THE LAZIO REGION FROM 1979 TO DATE: DEFINITION OF PROGNOSTIC FACTORS ON THROMBOSIS-FREE SURVIVAL (TFS) AND OVERALL SURVIVAL (OS)

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ET is a myeloproliferative neoplasm characterized by a clonal expansion of megacaryocytes with persistent peripheral thrombocytosis that is complicated by thromboembolic events. We have collected the data of 990 patients (pts) followed in 11 Hematological centers of our region from 1978 to December 2010. The diagnosis was made according the PVSG criteria, the WHO 2001 and 2008 criteria, respectively for the diagnoses made until 2000, 2007 and to date. The main epidemiological and clinical features of all pts are reported in the table: Pts number 990 Age (years, median, range) 63 (17-93) Gender, F/M number, (%) 620/370 (62,7/37,3), WBC (x 109/L, median, range) 8,8 (1,2-57,7) Hb (g/dL, median, range) 14,0 (6,0-18,9) PLT (x 109/L, median, range) 807 (307-3582) JAK2 V617F mutated/all performed, (%) 372/627 (59,3) Quantitative JAK2 V617F (%), median 20,3 (0,2-99,9) Splenomegaly number,(%) 180/916 (19,6) Previous thrombosis number, (%) 184/990 (18,75 %) Arterious /Venous number,(%) A: 140 (14,1 %)/ V: 44 (4,4%) Of 963 valuable pts, 90 (9,3%) resulted deceased, 120 (12,5%) lost at follow up and 753 (78,2%) alive at the time of evaluation. The median follow up of living pts was 5,08 years. The thrombotic events during follow-up were 91 (9,17 %): the arterial events were 51 (5,15%), the venous events were 40 (4,0 %). The rate of thrombosis (patients/year) was 1,4%. At the univariate analysis, the risk factors at diagnosis that resulted statistically significant for Thrombosis-Free Survival (TFS) were: age (> 60 yrs, p < 0,0001), previous thrombosis (p= 0,0027) and the presence of cardiovascular risk factors (p= 0,033). PLT count (either > 807 x 109/L or > 1000 x 109/L), Hb (<14 g/dL), WBC count (either > 8,8 x 109/L, or >15 x 109/L), JAK2 V617F mutated, JAK2V617F allele burden >50% did not reach the cut-off value of significance (p<0,05). At the multivariate analysis, performed according the Cox proportional hazards model, only age (>60 yrs, p= 0,046) and previous thrombosis (p= 0,043) maintained the significance level (p< 0,05). The risk factors for Overall Survival (OS) that reached at univariate analysis the statistical significance were: age >60 yrs, p <0,0001), WBC > 15 x 109/L (p =0,0009) and Hb level below normal values (< 12,5 g/dL in females and < 13,5 g/dL in males, p= 0,027). All other variables tested for OS (PLT count, previous thrombosis, JAK2 V617F mutated, JAK2 V617F allele burden >50% and presence of cardiovascular risk factors) failed to obtain the statistical significance. At the multivariate analysis, age > 60 yrs (p <0,0001), Hb level below normal values (p =0,0002) and WBC count > 15 x 109/L (p = 0,0082) maintained their prognostic impact on OS. In conclusion, our analysis confirms standard clinical risk factors for thrombosis: OS seems to be affected mainly by hematological features at diagnosis. Mutational JAK-2 status does not seem to affect thrombotic risk and OS, as reported by other studies.

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OUR MANAGEMENT OF PREGNANCIES IN ESSENTIAL THROMBOCYTHEMIA PATIENTS: EXPERIENCE OF CAMPANIA CENTRES

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Pregnancies in Essential Thrombocythemia patients is associated with complications such as spontaneus abortion during the first trimester. Moving from data of an observational retrospective study performed to investigate pregnancy outcome in patients of the centres of the Italian Thrombocythemia registry (RIT), we collected the data from four centres in Campania, a geographical area in South Italy where the birth rate is very high. The study refers to 15 pregnancies in 13 women with ET consecutively observed during the years 2007-2011. One patient is pregnancy at drafting. Other nine pregnancies in patients in the same four centres, have been reported in the previous study. We considered only the pregnancies occurred after the diagnosis of ET. Fourteen patients started pregnancy after the diagnosis of ET and 1 patient received diagnosis of ET during pregnancy. The median age of the patients at diagnosis was 26 (range 14-32), and 31 at conception (range 21-39); the age at conception was over 35 years in one patient. The median Plt count was 763x109/L (range 445-1613) at diagnosis, 619 (range 412-1034) at conception and 450 (range 288- 658) at childbirth. Two patients had a plt count over 1000x109/L at diagnosis and one at conception. The JAK2V617F mutation was documented in 7 patients. A IFN treatment at the time of the conception was reported in two patients and IFN was given throughout pregnancy. 13 out of 15 pregnancies ended in live births, 11 deliveries were full term with normal fetal growth and there was one preterm with IUGR. There was a spontaneus abortion during the first trimester. No patients had maternal complications. ASA (dose 100mg/die) was given during pregnancies in all patient. Low molecular

weight heparin was given in 8 patients during the last week of pregnancy and for four week post partum, and in one patient with Budd-Chiari syndrome was given throughout pregnancy. In these retrospective study ASA confirms a favorable effect on pregnancy outcome. One pregnancy managed with IFN ended in live birth and 1 is ongoing at 28 weeks. Only 2 patients with JAK2V617F mutation had complications (a preterm childbirth with IUGR and a spontaneus abortion). In conclusion we report other pregnancies with high live birth, we reinforces the opinion that the pregnancies in ET patients can have a successful outcome, the IFN citoreductive treatment can be an useful and safe tool. The JAK2V617F mutation is a predictor of unfavorable pregnancy outcome.

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AUTOCRINE REGULATION OF MEGAKARYOCYTE FUNCTION BY TGF-BETA: A POSSIBLE ROLE IN THE PATHOGENESIS OF PRIMARY MYELOFIBROSIS

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Background. Megakaryocytes(MKs) are large bone marrow cells that release platelets into the blood stream by elongating proplatelets. Recent studies pointed to a key role of abnormal megakaryocytopoiesis in the pathogenesis of primary myelofibrosis (PMF), however little is known about the underlying mechanisms. Aim. We studied the expression and the role of Trasforming Growth Factor beta (TGF-beta) and its receptors on MK development in vitro. Methods. Human MK were differentiated from peripheral blood progenitors of healthy controls and PMF patients for 14 days in the presence of thrombopoietin. Results. Control and PMF derived MKs equally expressed the TGF-beta R1 and R2 receptors and constitutively released TGF-beta, which peaked in the culture medium earlier in PMF MKs (day 7) than control MKs (day 10). Moreover, the total bioactive TGF-beta released during MK cultures was increased in PMF with respect to control. In order to explore a possible role of released TGF-beta on MK function, activation of downstream signaling, through phosphorylation of Smad2 and Smad3, was analyzed by a time course western blot analysis. Smad2 and Smad3 started to be phosphorilated at the same time points of TGF-beta picks in MK culture supernatants, thus indicating a sort of autocrine stimulation of released TGF-beta on MKs. Interestingly, this activation seemed to be TGF-beta concentration dependent as an increase of Smad2 and Smad3 phosphorilation was observed in PMF derived MKs at the end of the culture. Finally, the specificity of the signalling was demonstrated by the decrease of Smad2 and Smad3 phosphorilation upon treatment with the TGF-beta R1 receptor inhibitor SB431542 (10 microM). Conclusion. The results of our study demonstrate that the interaction of constitutively released TGF-beta with its receptors contributes to activation of specific downstream signaling in MKs cultured in vitro. Increased levels of TGF-beta and abnormal activation of the downstream pathway have been observed in PMF derived MKs, the clinical relevance of this previously undescribed physiological role of TGF-beta needs to be further explored.

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RETROSPECTIVE EVALUATION OF VALIDATED AND CANDIDATE THROMBOTIC RISK FACTORS IN ESSENTIAL THROMBOCYTHEMIA: PRELIMINARY ANALYSIS OF THE REGISTRO ITALIANO TROMBOCITEMIA (RIT)

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Background. Age over 60 y and history of thrombosis are validated risk factors for thrombosis at onset and during the follow-up in essential thrombocythemia (ET)patients. The thrombocytosis, constitutive abnormality in ET, is associated with both thrombotic and hemorrhagic complications, while JAK2 mutation and leukocytosis have been reported as associated with high rate of thrombosis. Aims. To evaluate in ET the thrombotic prognostic value of JAK2 mutation, PLT count, WBC count, and other clinical and biological parameters. Methods. A cohort of ET patients of the Registro Italiano Trombocitemia (RIT)was retrospectively analysed. Results. A total of 977 patients, 387 males and 590 females, presented at diagnosis: median age 55 y, median PLT count 783 x 10^9L, median WBC count 8.8x10^{9}L, median Hb 14.1 g/dL, history of thrombosis in 189 cases (19.3%), history of hemorrhage in 49 cases (5.0%). The patients at high risk (age over 60 y and/or history of thrombosis) were 511 (52 %). During the follow up (4088 pt-y), thrombotic events were reported in 35 patients (3.6%). The thrombotic events at onset of disease were significantly related to: age over 60 (p 0.001), male gender (p <0.05), lower grade of thrombocytosis (PLT < 783 x10^9/L, p 0.001), higher grade of leukocytosis (WBC > 8.8 x10^9/L, p 0.01), and JAK2 V617F mutation (p 0.06). No relationship was found with bone marrow fibrosis grade. The 977 patients were subdivided in four groups: lower thrombocytosis and higher leukocytosis (group 1: 202 pts); lower thrombocytosis and lower leukocytosis (group 2: 270 pts); higher thrombocytosis and higher leukocytosis (group 3: 272 pts); higher thrombocytosis and lower leukocytosis (group 4: 197 pts). In those patients, the rate of thrombosis at onset was: 26.7% in the group 1(both risk factors); 24.1% in the group 2 (PLT risk factor); 20.2% in the group 3 (WBC risk factor); 7.6% in the group 4 (no risk factors). The rate of thrombosis in patients with one or two of these risk factors was significantly (p 0.001) higher than in patients with no risk factors. The thrombotic events during the follow up are still object of analysis. Conclusion. In this cohort of ET patients the rate of thrombosis at onset of disease has been confirmed to be related to age over 60 y. Moreover, a significant relationship has been found with male gender, JAK2 mutation, WBC count over the median value (8.8 x10^9/L), and PLT count below the median value (783 x10^9/L).

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SPLEEN VOLUME IS THE MOST ACCURATE PARAMETER TO AVOID UNDERSTIMATION OF 25% SPLENOMEGALIC PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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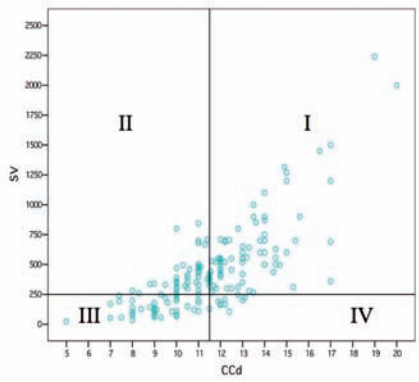
Introduction: Essential Thrombocythemia (ET) is a Ph- MPN; the spleen can be involved in disease. The definition of palpable splenomegaly (WHO 2008 diagnostic criteria for PMF) seems to be too variable and inaccurate. Evaluation of spleen size by imaging methods (European Leukemia Net criteria for response to therapy in ET patients) comprises only the measure of craniocaudal diameter (CCd). This study aimed to investigate whether the measure of spleen volume (SV) may be more accurate than CCd to establish the presence of splenomegaly in ET patients. Methods: Both SV and CCd were measured by ultrasound scan in patients with ET according to the WHO criteria. A single operator performed all the measurements using a Philips IU 22 instrument with a 2-5 MHz broadband curvilinear probe. Perimeter, longitudinal diameter and area, defined as maximum measurements with splenic borders and angles clearly defined, were measured, and volume was calculated automatically. Results: In our cohort of ET patients, median SV and CCd at presentation were 380 mL (normal value ≤250 mL) and 11,5 cm (normal value ≤11,5cm), respectively. As a whole, 69% of ET patients showed a SV higher than 250 mL, and were classified as splenomegalic, while only 46% of them could been classified splenomegalic using the CCd measure. Although there was a correlation between SV and CCd (Pearson 0.750, r2=0.339), 25% of patients showed normal or reduced CCd in spite of a SV enlargement. These data clearly show that the most common measurement of CCd frequently fails to detect splenomegaly. No correlations were found between SV, CCd and Hb levels, platelet and WBC count and JAK2 mutational status. The correlation between SV and CCd was confirmed during the follow-up (Pearson 0.864, r2=0.511). Interestingly, at this time, in about 20% of patients, the CCd decreased, defining an apparently splenomegaly reduction or normalization, while the SV clearly defined spleen enlargement. Therefore, SV is a more accurate measure for monitoring splenomagaly over time

in ET patients. Conclusion: Although CCd measure by ultrasound scan is more accurate than palpation to detect splenomegaly in ET patients, however its diagnostic sensitivity is significantly inferior to the evaluation of spleen volume. Therefore, the best method to assess splenomegaly in ET patients is spleen volume definition by ultrasound scan, that we also suggest as a reference parameter for splenomegaly monitoring.

Figure 1.
In the II quadrant 43/170 patients showed SV enlargement, despite a normal or reduced CCd.



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HIGH RATE OF COMPLETE RESPONSE WITH ANAGRELIDE MONOTHERAPY OR COMBINED WITH HYDROXYUREA IN ESSENTIAL THROMBOCYTHEMIA

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Introduction: Hydroxyurea (HU) and Anagrelide (Ana) are commonly used treatments for Essential Thrombocythemia (ET). Despite Ana effectiveness in reducing platelet count, it was registered by the EMEA as second line treatment for ET patients resistant or intolerant to first line therapy. Here we describe our experience with these drugs. Patients and results: We evaluated the hematological response of 113 ET patients treated with HU, Ana or a combination of both. Twelve patients were on monotherapy with anagrelide (Ana); of these, 10 were resistant to first line treatment after a median of 43 months; 2 patients asked for Ana as first line therapy. After 21 months of therapy, median values of Hb, PLT and WBC were respectively: 13,6 g/dl, 449 x103/mm3 and 8665/mm3 (data calculated as the median of these parameters for each patient, based on determinations made every two months). Six patients (50%) obtained a complete response (CR) with PLT normalization; doses ranged from 1,5 to 1 mg daily. Five patients experienced partial response (PR), with PLT < 600 x103/mm3; only one was resistant. Mild side effects (transient headache and palpitation) were observed in 30% of them. We used also combined HU and Ana therapy in 8 patients resistant to multiple line treatment, included busulfan (2 patients). We used 1 g daily of HU plus escalating Ana doses (from 1 to 2 mg daily), on the basis of their individual hematological responses. After a median of 23 months Hb, PLT and WBC median value were respectively 13,7 g/dl, 456 x103/mm3 and 7355/mm3. Five patients (62,5%) achieved a CR and 3 PR (HU 1 g plus Ana 2 mg daily). Ninety-three patients received HU as first line therapy: 23,6% achieved CR, 55,4 % PR and 21% were resistant to HU. Hb, PLT and WBC median value were respectively 13,5 g/dl, 406 x103/mm3 and 6570/mm3. Conclusion: Although groups treated with Ana plus HU or Ana as monotherapy, could suggest a selection bias because they are composed of patients resistant to previous treatment, the rate of CR is higher in these groups, compared to those on HU therapy. Despite resistance to previous treatment Ana seems effective, with only one patient being resistant to Ana. Nevertheless, Ana plus HU is more useful in controlling WBC count compared to Ana monotherapy. Combined therapy or Ana monotherapy should be considered also in patients with PR to HU, to try to achieve CR and the best control in platelet lowering.

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SPLEEN IS THE DARK SIDE OF ESSENTIAL THROMBOCYTHEMIA

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Introduction: Splenomegaly occurs in about 60% of Essential Thrombocythemia (ET) patients, and normalization in spleen size is one of the clinical-hematological of the criteria for assessment of response therapy according to the European Leukemia Net. The aim of this study is to investigate the correlations between spleen volume (SV) and other clinical findings, in order to predict the reciprocal impact of splenomegaly and clinical outcome. Methods and results: We analyzed 83 ET patients, whose median SV at diagnosis, evaluated by ultrasound scan, was 330 mL; 61% of them had SV >250 mL. At follow up (median 56 months), they showed significantly increases of SV ($p<0.001$), median SV being 550 ml, with a 92% average increases. Hematological response to treatment did not correlated to SV modifications: we observed increases of SV in patients with absence, partial or complete response (CR), even if enlargement in the first group seemed higher. On the other hand, treatment seemed to influence SV independently to the response. IFN treated patients showed the highest increase (mean=670 mL; $p=0.001$), followed by anagrelide (mean = 639 mL; $p=0.017$) and untreated (mean = 481 mL; $p=0.023$) patients. A not significant enlargement was observed in HU treated patients, $p=0.1$. SV increased both in JAK2V617F and JAK2WT patients, $p<0.001$ and 0.002 respectively. We evaluated MF grading in 44 patients at follow up: in 34.1% the condition worsened, in 36.4% it was stable and in 29.5% it improved. All patients had SV enlargement but the first group showed the highest increase. Patients in whom MF increased, had the highest SV at baseline ($p=0.004$). Patients with CR showed the lowest rate of MF worsening grade. In 55.5% of anagrelide treated patients MF improved, against 33.3% of those on HU and 26.7% of those on IFN treatment. Conclusion: Although all the traditional ET treatments have proven efficacy in normalizing PLT, WBC and disease related symptoms, these treatments usually fail to reduce spleen size in patients, therefore normalization of the volume should be considered anecdotic. There is a difference in spleen response to different treatment and this finding should be better evaluated, in view of the newly available drugs that reduce spleen size. Further, highest SV at diagnosis should be considered be prognostic of evolution tendency of disease toward worsening of MF grade. This suggests the need for accurate monitoring in these patients.

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TRANSITION INDEX: NEW ORIGINAL TOOL TO IDENTIFY SUBSETS OVERLAPPING MPN PHENOTYPES

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Introduction: PV, ET and PMF are the three majors Ph- MPN. These diseases share numerous phenotypic similarities and, despite the WHO 2008 diagnostic criteria, there is not a standard approach to discriminate patients showing overlapping clinical features but different outcomes. Indeed, pure ET, ET with the PV-like phenotype, prefibrotic stage, and early PMF could be considered as a continuum, it might be useful to attribute a different prognostic score to each clinical entity. Methods: We analyzed 197 ET patients (diagnosis according to WHO 2008) according to a factorial plan defining the following four modalities: BM reticulin grade; clustering of megakayocytes (MK) and/or atypical MK; WBC

count (<7, 7-8.5, 8.5- 10, 10-12, >12 x 103/mm3); and dacryocytes and/or leukoerytroblastosis. The analysis has led to new variables, which have been determined by linear combinations of the initials. Among them we considered the one that better describes the variability of the "unit point" (i.e. patients in our model). We called this new variable Transition Index (TI). This term was also inspirited to define an overlapping tendency of different ET phenotypes. Thereafter, hierarchical cluster analysis creates has created a hierarchy of clusters with different TI, represented as a dendrogram. We identified three main clusters: low TI (n=69), intermediate TI (n=112), high TI (n=16). Results: TI correlates with the percentage of patients harboring JAK2V617F mutation, spleen volume at the time of diagnosis and its enlargement at follow-up, CD34+ cells count in the peripheral blood, iron stores, LDH, beta2-microglobulin and low Hb level. No correlation was found between TI and response to treatment. Furthermore, a higher TI is associated with a greater frequency of thrombotic events (Low TI 12,3% vs Intermediate TI 17,8% vs High TI 37,5%). Conclusion: TI index identifies those patients with a peculiar clinical phenotypes, comprising features similar to PV or PMF but not matching all the established criteria for the diagnosis, and help to refine their prognosis. Indeed, the index could represent an additional tool to better define clinical outcome among a continuum of overlapping and not well defined Ph- MPN; it correlates with a worse prognosis and its value could suggest a more frequent follow up or new therapeutic strategies.

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MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASM: A FUTURE THERAPEUTIC PERSPECTIVE

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Myelodisplastic/myeloprolifertive neoplasm unclassifiable (MDS/MPNs U) are rare de novo myeloid neoplasms that exhibit hybrid dysplastic and proliferative features at presentation and are characterized by proliferation of one or more myeloid lineage that is ineffective, dysplastic or both and, simultaneously, by effective proliferation with or without dysplasia, in one or more of the other myeloid lineages either as thrombocytosis (platelet count ≥450x10^9/L) or leukocytosis (white blood cell count ≥13x10^9/L). Immunophenotype may be similar to finding in MDS and/or MPN. The cytogenetic is no specific in this group. They are rare diseases and there is no data about the specific therapy, and the only therapeutic chance is Idroxyurea therapy or low-dose araC and transfusion support. The study that evaluated the efficacy and safety of Azacitidine (AZA), showed that the AZA treatment significantly improved median OS in MDS high risk. Moving from these data and those of encouraging results in Ph-negative MPN having progressed to AML or MDS, we have treated 4 male patients affected by MDS/MPNs U. At diagnosis all patients showed leukocytosis, anaemia with macrocytosis and thrombocytopenia, blast account was <20% in bone marrow (BM). BM biopsy specimens showed hypercellular with proliferation of all myeloid lineage. Immunophenotype showed dysplastic features. There was no molecular genetic finding: BCR-ABL1 fusion gene and JAK2V617F mutation were excluded. The cytogenetic assessment showed in one patient del20q. All patients had splenomegaly. Three patients were treated with 6 cycles of AZA and 1 patient stopped therapy after 3 cycle for cerebral hemorrhage after accidental fall. All patients had response with decrease of transfusion need and reduction of spleen size. Even the fourth patient treated with only three cycle. For this patient the response duration was short and need hydroxyurea therapy for leukocytosis. The patient died for consequences of stroke 6 months after stop AZA. Two patients died for Infectious complications after 2 and 6 months of treatment. One patient is alive, without transfusion and therapy needs. Our patients had normal or good prognostic karyotype but the molecular reasons for response to AZA, although short, are not jet known. But the study of methylation (Ten-eleven-translocation 2 (TET2) status) can be a useful tool to decide which patients to treat. Therapeutic schedule and consolidation treatments have to be evaluated.

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INCREASED PHOSPHO-MTOR EXPRESSION IN AN EX VIVO MEGAKARYOCITIC UNILINEAGE SYSTEM DERIVED FROM CD34+ CELLS ISOLATED FROM PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND MYELOFIBROSIS

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The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that functions as a key regulator of cell growth, protein translation and metabolism. Consistent with its role as a growth-promoting factor, numerous studies have found increased mTOR signaling in a broad spectrum of human cancers. Essential thrombocythemia (ET) and myelofibrosis (MF) are BCR-ABL-negative chronic myeloproliferative disorders characterized by megakaryocytic bone marrow hyperplasia and a sustained elevation of platelet number with a tendency for thrombosis and hemorrhage. However, the molecular mechanisms underlying the pathogenesis of these diseases are still poorly understood, delaying the development of effective targeted treatments. We wanted to establish the role of mTOR on the proliferation and differentiation of megakaryocytic (MK) cultures derived from the peripheral blood of either healthy individuals or patients diagnosed with ET or MF. We found that mTOR activation was increased during MK differentiation in both ET and MF patients compared to healthy donors where mTOR staining was barely detectable. Immunohistochemical analysis of phospho-mTOR confirmed high expression levels in ET and MF patients in contrast with the negative staining observed in healthy individuals. Taken together, our data suggest that induction of the mTOR pathway is involved in the MK differentiation of samples derived from ET and MF patients. Our findings suggest that mTOR could represent an attractive molecular target for the treatment of ET and MF patients failing previous lines of treatment.

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IDENTIFICATION AND ANNOTATE OF SOMATIC MUTATIONS DERIVING FROM NEXT-GENERATION SEQUENCING DATA: A COMPUTATIONAL PROCEDURE

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The use of next-generation sequencing instruments to the study of hematological malignancies generates a tremendous amount of sequencing data. This leads to a challenging bioinformatics problem to store, handle and analyze terabytes of sequencing data often generated from extremely different data-sources. Our project is mainly focused on the sequence analysis of human cancer genomes, in order to identify the genetic lesions underlying the development of tumors. However, the automated detection procedure of somatic mutation and a statistical based testing procedure to identify genetic lesions are still an open problem. Therefore, we propose a computational procedure to handle large scale sequencing data in order to detect exonic somatic mutations in a tumor sample (Figure 1).

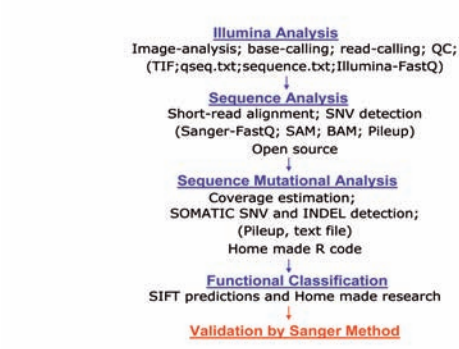

Figure 1: The basic workflow of Computational Procedure: from input Illumina sequences data; preprocessing analysis; somatic variations detection and functional annotation of coding variants, to candidate somatic mutations to validate by Sanger method.

The proposed pipeline includes several steps based on open-source softwares and R language: alignment, detection of mutations, annotation, functional classification and visualization of results. We analyzed whole exome sequencing data from 3 leukemic patients and 3 paired controls plus 1 colon cancer sample and paired control. The results were validated by Sanger method.

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THE CONSTITUTIVE ACTIVATED V617F JANUS KINASE 2 (JAK2) INDUCES CENTROSOME ABNORMALITIES

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The JAK2 tyrosine kinase leads to activation of many signaling pathways critical for cell growth and differentiation. A recurrent mutation in the JAK2 (V617F) has been described in the myeloproliferative neoplasm (MPN), giving proliferative and survival advantages to hematopoietic precursors. We previously demonstrated a nuclear localization of JAK2 protein, suggesting a new unexplored JAK2 signaling (Blood. 2010 Dec 23; 116(26)). Here we measured the nuclear and cytoplasmic distribution of JAK2 protein in K562 (JAK2WT) and in HEL (homozygous for JAK2-V617F mutation) cell lines by confocal immunofluorescence (CIF) microscopy. In K562 cell line the total JAK2 protein density was 0.65±0.13 Million pixels of which 50% was a nuclear signaling. The total JAK2 protein density was higher in HEL than K562 cell lines (1.28±0.27 Million pixels) with 20% of protein localized in the nucleus (0.27±0.07 Million pixels). We observed a distinct perinuclear localization of JAK2 in a characteristic spot signal similar to centrosome structure in both cell lines. The centrosome is a small cellular organelle essential for microtubule organization that has been reported to be altered in many MPNs. We hypothesize that the constitutive phosphorylation at this site may alter centrosome function. Therefore, we performed co-immunoprecipitation and co-immunofluorescence assays, by anti-Tubulin (a characteristic centrosome protein), to evaluate whether JAK2 associates to the centrosomes. In addition, CIF microscopy showed that wt JAK2 colocalizes with Tubulin in 92% of K562 cells with normal centrosomes, in a cell cycle independent manner. Notably, we observed an altered number of centrosomes in all remaining K562 cells lacking of JAK2- Tubulin colocalization. By contrast, only 18% of HEL cells had a JAK2- Tubulin colocalization. The remaining HEL cells, lacking of JAK2-Tubulin colocalization in centrosomes, were characterized by structural or numeral centrosomes abnormalities. Interestingly, when we forced the expression of JAK2V617F in K562 cell line by transfection with vector containing the mutant form, the JAK2- Tubulin colocalization was significantly reduced and was restricted to the cells with normal centrosomes. This data strongly suggest that JAK2 protein interacts with centrosome structure and that JAK2V617F is associated with centrosome abnormalities.

LYMPHOMAS II

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IGEV +/- BORTEZOMIB (VELCADETM) AS INDUCTION BEFORE HIGH DOSE CONSOLIDATION AND STEM CELL TRANSPLANT (ASCT) IN RELAPSED/REFRACTORY HODGKIN'S LYMPHOMA (HL) AFTER FIRST LINE TREATMENT : A RANDOMIZED PHASE II TRIAL OF FIL (FONDAZIONE ITALIANA LINFOMI)

Balzarotti M, Brusamolino E, Angelucci E, Pulsoni A, Carella AM, Vitolo U, Spina M, Merli F, Rossi G, Levis A, Morra E, Stelitano C, Massidda T, Botto B, Re A, Cascavilla N, Mazza R, Rusconi C, Congiu A, Gaidano G, Annechini G, Zinzani PL, Giordano L, Santoro A

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Background: Complete remission (CR) status after induction chemotherapy before ASCT in relapsed/refractory HL is the mainstay of therapeutic success of the whole program. IGEV regimen (Santoro 2007) seems very promising with a CR rate approaching 50%. Data on solid tumours suggested a synergism between bortezomib and gemcitabine. Thus , we combined IGEV with bortezomib (BIGEV) with the aim to increase CR rate in these patients (pts). METHODS: In 15 italian centers, 80 pts with relapsed –refractory HL after one line, age 18-65, meeting criteria for phase II trial, were randomly assigned to receive 4 courses of IGEV alone or combined with bortezomib at 1,3 mg/mq on day 1,4 & 8.Peripheral stem cell harvest was planned after third course in responding pts. Both regimens were administered every 3 weeks and supported by GCSF. The primary endpoint was CR rate after four cycles, defined as FGD-PET negativity. Secondary end-points were comparative toxicity, mobilization potential and survival rates. Results were analysed on an intention-to-treat basis. Results : From Feb 08 to Feb 10, 80 pts were enrolled , 40 assigned to IGEV and 40 to BIGEV . Main clinical characteristics were well balanced between the two groups: median age 35.5 (range 18-64) , Sex M/F 46/34 , relapsed /refractory 43/37, stage at relapse I-II/III-IV 38/42 , bulky disease 35. At the end of induction, of 75 pts evaluable for response in the whole series, ORR was 59% with 43% CR. In the IGEV arm, 17 /38 (44.7%) obtained CR and ORR was 63%. In the BIGEV arm , CR rate was 40.5% (15/37) and ORR was 55%. BIGEV was globally well tolerated and no substantial differences from IGEV were documented. Neither life threatening toxicity nor treatment related death occurred . Peripheral neuropathy and febrile neutropenia never exceeded grade 2 . No hospital admission was registered for toxicity management. Thirty-six pts received red blood cells and/or platelet transfusion. Thirty-seven out of 75 evaluable pts underwent ASCT after IGEV or BIGEV induction. One-year PFS and OS for the whole series are 47.8% and 93.7% , respectively . Conclusions : These data data confirm the IGEV activity as pre-transplant induction in relapsed or refractory HL. The addition of bortezomib to IGEV did not improve the response rate compared to IGEV alone. Good safety profile and acceptable transfusion need may prompt testing new different active drugs in HL to possibly improve on the efficacy of IGEV alone.

P198

TUMOR BURDEN AT DIAGNOSIS AND RISK OF RESISTANCE TO ABVD PLUS RADIOTHERAPY ARE RELATED IN HODGKIN LYMPHOMA

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Background. Early resistance to treatment is an infrequent but unforseeable event in Hodgkin lymphoma. Resistance can be clinically expressed by either incomplete response to a correct first-line treatment or early relapse, occurring within 12 months after the end of therapy Materials and methods. A number of staging clinical characteristics, with inclusion of relative tumor burden (rTB), were analysed in 246 patients with Hodgkin lymphoma in relation to the failure in achieving complete remission or to the occurrence of early relapse. One hundred twen-

ty-nine patients presented early unfavorable-stage disease and were treated with ABVD 4-6 cycles + involved field radiotherapy; in particular, a restaging was established after the third cycle and one further cycle was added in case of complete response, while three more cycles were administered in case of partial response. Other 117 patients, who had advanced-stage disease, received ABVD 6 cycles + optional irradiation of no more than 2 sites. The rTB were measured through the evaluation of all the slices of the staging computed tomography. The relationship with early resistance was analysed with logistic regressions. Results. The rTB was the relatively best predictor of early failure in both groups of patients, superior to the multiparameter IPI score; it showed a significant relationship with the relative risk of early failure, and, with consideration of the extranodal involvement, a single function proved to be statistically adequate for the prediction of early outcome both in early unfavorable-stage and in advanced-stage patients. The treatment differences of these two distinct groups of patients were not so heavy and did not prevent them from sharing a prevalent risk factor. Conclusion. The rTB is the relatively best pre-treatment factor related to the risk of resistance to combined ABVD + radiotherapy, and such relationship can be mathematically expressed. It might be interesting to study how the dependence of risk on rTB changes with different treatments. A simplified method for the assessment of rTB is desirable.

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TUMOR BURDEN CAN BE ESTIMATED FROM SIMPLE STAGING PARAMETERS IN HODGKIN LYMPHOMA

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Background. The relative tumor burden (rTB), i.e. the TB normalized to body surface area, has a primary prognostic role and a distint clinical importance in Hodgkin lymphoma. It shows to be better predictive than the IPI score and demonstrates interesting relationship with treatment outcome. However, the measurement of rTB is rather complicated and its bedside computation improbable. So, while searching for a novel, direct assessment technique, we investigated the possibility of estimating rTB through elementary parameters of the initial clinical staging. Patients and methods. In the last twelve years we measured the rTB of 507 patients, treated with different therapeutic protocols of the Gruppo Italiano Studio Linfomi according to their staging characteristics (VBM + IF-RT in early favorable stages, ABVD + IF-RT for early unfavorable stages, ABVD or M(C)OPPEBVCAD or BEACOPP + limited and optional RT for advanced stages). The rTB were measured through the evaluation of all the slices of the staging computed tomography. The relationships among rTB and staging characteristics were analysed by means of simple and multiple regressions. The population of the study was divided into two well- balanced groups: 254 patients was the training sample for the selection of the best paramaters, and 253 subjects formed the test sample for validation of the results. Results. Three best variables related to rTB were selected from the 20 analysed: IPI score, bulky mass and number of involved sites. The relationship between rTB and IPI score was not linear, being better represented by a polynomial regression of second order (IPI2); the presence of bulky mass showed to correspond, on the average, to three involved sites in addition to those actually recorded. The resulting final equation {[estimated rTB = – 4,3 + 8,3 x IPI2 + 22,7 x [No. involved sites (+ 3 if bulky mass is present)]} allowed the maximal approximation to the measured rTB. Its R2 of 0.671 indicates that little more than 2/3 of the variability of the rTB is explaind by the function.The validity of the equation was confirmed on the test sample in which the predictive superiority of the estimated rTB over IPI is still evident in terms of failure-free survival. Conclusion. The estimated rTB shows a sufficient accuracy to retain some advantage compared to IPI score. It can be simply calculated and might be a valid approximation of the measured rTB to expand the clinical experience with such a parameter.

P200

INTERIM PET 2: IS IT A REAL PROGNOSTIC FACTOR IN SELECTING TWO DIFFERENT SUBSETS OF HODGKIN DISEASE PATIENTS?

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The role of 18F-flourodeoxyglucose (FDG)–positron emission tomography (PET) in Hodgkin Lymphoma (HL) patients before, during, and after therapy has developed dramatically during the last decade and it is nowadays well established. About 20-25% of patients with HL failed to achieve a complete remission (CR) with first-line standard chemotherapy, while 10 to 50% of patients who obtain a CR, may relapse within a year. Among 301 patients with HL previously treated at our institutes we isolated 2 subgroups of patients, one made of 19 HL patients and the other made of 13 HL patients presenting a positive interim PET (the first group) and a negative interim PET (the second group). First patient group, despite an intermediate-positive PET reached and maintained a CR at the and of treatment, while the other group with a negative PET after 2 cycles, early relapsed at the end of treatment or experienced a progressive disease. Both groups of patients were treated at our institutes and underwent a central histological review. Demographic and therapeutic data of these patients are shown in TABLE 1. At a median follow up of 4.8 years all the 19 patients of the positive interim PET reached and still maintain a CR, while 3 patients of the negative interim PET group died of the disease, 2 had a partial response and 1 a stable disease. Our previous experience with 301 patients confirms the highly predictive value of a negative early PET during HL therapy, in terms of response to the therapy and progression free survival, however 32 (10.2 %) HL patients experienced a disease history apparently not PET-related; therefore we described a small but consisting percentage of HL patients in whom interim PET does not correlate with the outcome.

POSITIVE INTERIM PET	N	NEGATIVE INTERIM PET	N
Total	19	Total	13
M	9	M	9
F	10	F	4
Median age	32.2	Median age	44.7
stage 1	1	stage 1	0
stage 2	10	stage 2	4
stage 3	6	stage 3	8
stage 4	2	stage 4	1
NO symptoms	9	NO symptoms	7
B symptoms	10	B symptoms	6
non bulky	8	non bulky	8
bulky (mediastinum)	10	bulky (mediastinum)	4
bulky (nodal)	2	bulky (nodal)	1
THERAPY		THERAPY	
ABVD	12	ABVD	12
ABVD + RT	7	MBVD	1

Table 1

P201

RETROSPECTIVE ANALYSIS OF SALVAGE REGIMEN FOR RELAPSED OR REFRACTORY CLASSICAL HODGKIN LYMPHOMA

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We retrospectively reviewed the outcome of 22 patients (pts) referred to our Center from 2001 to 2009, affected by relapsed or refractory classical Hodgkin lymphoma (CHL) after first line therapy with ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) associated or not with involved-field radiotherapy (IFRT) and then treated with IGEV (methyl-

prednisolone, ifosfamide, gemcitabine and vinorelbine). The main features of the pts were the following: median age was 27 years (15-57), M:F ratio 0.7; 18 pts presented nodular sclerosis and 4 mixed cellularity; 11 pts were stage II, 5 stage III, 6 stage IV and 11 were B symptomatic; 7 pts presented bulky disease. All of them were treated with a median number of 6 cycles of ABVD (range 2-8), followed by IFRT in 10 patients; 12 pts resulted refractory and 10 relapsed after a median time of 22 months (range 6-72). All of the 22 pts were treated with IGEV as first salvage chemotherapy with the intention to perform an autologous stem cell transplantation (ASCT). The response was evaluated according to conventional criteria including PET. After a median of 3 cycles of IGEV (range 2-4), 8 pts were in complete remission (CR), 9 in partial remission (PR), 3 in stable disease (SD) and 2 in progressive disease (PD). Eleven pts (8 pts in CR and 3 in PR) underwent ASCT immediately using BEAM (BCNU, etoposide, Ara-C, and melphalan) as conditioning regimen: all of them obtained CR, except one that confirmed the PR. Of remaining 11 pts that did not obtained CR to IGEV (6 PR, 3 SD, 2 PD), 10 were treated, as second line salvage therapy, with standard BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone). After a median of 3 BEACOPP cycles (range 1-4), 5 pts obtained CR, 2 PR, 1 SD and 2 PD. All of them underwent ASCT. Ten of 11 pts were evaluable: 5 were in CR, 2 in PR, 2 SD and 1 PD. After IGEV ± BEACOPP 13/22 pts obtained pre-ASCT CR. After a median follow-up of 62 months (range 17-129), 16 pts are alive, 13 in CR, 2 in PD and 1 pts developed non-Hodgkin lymphoma (NHL) 16 months after ASCT. Although the limited number of pts involved and the retrospective analysis, the study demonstrates that BEACOPP is feasible and effective salvage regimen for pts with CHL not completely responder to IGEV chemotherapy. In fact, 50% of patients refractory to IGEV treated with BEACOPP obtained a CR and could be consolidated with ASCT.

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BONE MARROW BIOPSY IN STAGING HODGKIN'S LYMPHOMA: IS STILL MANDATORY?

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Background & aim: An accurate staging of Hodgkin's Lymphoma (HL) is mandatory to plan a risk-adapted treatment. Bone marrow involvement (BMI) is rare in HL patients and its incidence varies from 4 to 14%. Bone marrow biopsy (BMB) is able to explore a limited part of bone marrow. According to the update Cotswolds criteria, a bone marrow biopsy from at least one site is recommended for clinical stages III/IV as well as clinical stage II with adverse features. Despite these formal recommendations bone marrow biopsy is still performed in almost all patients. In this study we reviewed 330 cases of Hodgkin's Lymphoma in our Institution to evaluate the impact of BMI on upstaging and treatment decision. Design & Methods: We reviewed the clinical characteristics (Age, Histology, Staging by CT-Scan, Systemic symptoms, Blood chemistry) and bone marrow trephine biopsy assessment at diagnosis of 342 consecutive, unselected patients, treated for Hodgkin's Lymphoma at Humanitas Cancer Center, between August 1997 and March 2011. Results: Of 330 patients with BMB at diagnosis, 314 were negative (95%) and 16 had BMI (5%). All 16 patients with BMI had advanced disease, 11/16 had Stage IV due to lung, liver and/or skeletal HL involvement. BM positivity changed the disease stage in 4 patients: from III to IV in 2, from IIIs to IV in 3. B symptoms were seen in 11/16, Bulky disease in 2/16 patients. Four had Mixed cellular (MC), 12 had Nodal Sclerosis (NS) histology. Two patients with NS had a Compositum Lymphoma (HL plus DBLCL). Median age was 40 years (range:22-76). IPS system was available in 12/16 patients, 6/12 had at least a score of 3. Of all patients 2 died before starting chemotherapy and 1 died after II cycle. One of 13 patients who completed first line chemotherapy is alive NED; 12/13 were refractory or relapsed. BMB was assessed in all the patients before salvage treatment while BMI was confirmed in 2/12 cases. BMI Patients characteristics are reported in Table 1. Statistical analysis including comparison with advanced Stage without BMI is ongoing. Conclusions: In this retrospective analysis we found that BMB is superfluous in clinical early stages (IA/IIA & IB/IIB) to improve the upstaging. Our data also suggest that BMB does not appear necessary in advanced Stages when selecting risk-adapted treatment.

Pts	Age	Stage without BMB	Histology	Bulky	IPS	Fist line CT	REL/REF	BMB at REL/REF
1	27	IIIB	MC	Yes	2	Yes	Yes	Neg
2	52	IVA liver	NS/DBLCL	No	3	Yes	No	na
3	64	IVB lung	NS	No	5	No	na	na
4	19	IVB skeletal	NS	No	2	Yes	Yes	Neg
5	34	IVB liver skeletal	NS	Yes	2	Yes	Yes	Neg
6	22	IVA lung	NS	No	2	Yes	Yes	Neg
7	35	IVA skeletal	NS	No	4	Yes	Yes	Neg
8	30	IVB lung	MC	No	2	Yes	Yes	Neg
9	24	IVB liver	MC	No	na	Yes	Yes	Neg
10	20	IVB liver	NS	No	2	Yes	Yes	Neg
11	76	IIIsA	NS	No	4	Yes	Yes	Pos
12	30	IIIA	MC	No	na	Yes	Yes	Neg
13	70	IVB skeletal colon	NS/DBLCL	No	5	Yes	Yes	na
14	50	IIIsB	NS	No	na	Yes	Yes	Pos
15	45	IIIsB	NS	No	5	No	na	na
16	47	IVB liver	NS	No	na	Yes	Yes	Neg

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FEASIBILITY AND EFFICACY OF BCVPP REGIMEN IN VERY ELDERLY PATIENTS WITH HODGKIN'S LYMPHOMA

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Background: While some age-adapted modifications of ABVD regimen have been proposed for elderly Hodgkin's lymphoma (HL) patients (pts), no specific chemotherapy regimens have yet been recommended for very elderly or elderly pts with severe comorbidities. The five-drug-combination BCVPP (Carmustine, Cyclophosphamide, Vinblastine, Procarbazine and Prednisone) has the advantage of not containing anthracyclin and being potentially effective and tolerated in HL pts. AIM: We report on the therapeutic efficacy and safety of BCVPP in a group of very elderly (≥80y) and elderly pts with severe comorbities unfit for anthracyclin-containing chemotherapy. METHODS: Eligibility criteria included age older than 80, severe comorbidities (one grade 4 or at least 3 grade 3 according to Cumulative Illness Rating Score for Geriatrics - CIRS-G), relapse/refractoriness to ABVD or other regimens. BCVPP (Carmustine 100 mg/sqm iv d 1, Cyclophosphamide 600 mg/sqm iv d 1, Vinblastine 5 mg/smq iv d 1, Procarbazine 100 mg/sqm d 1-10 po, Prednisone 60 mg/sqm d 1-10 po) was administered every 42 days for a maximum of 6 cycles. Reduction of the doses was applied for adverse events and intolerance. Results: Twelve pts have been treated (M/F ratio 1/2). Median age was 77y (68-88). Seven pts were unfit for comorbidities (1 dementia, 5 grade 4 and 1 multiple grade 3 comorbidities according to CIRS-G), 5 were aged ≥80y. Ten pts were treated upfront, while one was refractory /intolerant to low dose ABVD and one relapsed after MOPP. Histology was nodular sclerosis in 8 pts, mixed cellularity in 3, and lymphocytic depletion in 1. EBV LMP-1 was positive in RS cells of 3/5 cases. Ten pts (83%) had advanced disease (6 stage III and 4 stage IV). Eight pts (66%) presented with B symptoms and 5 pts with International Prognostic Score>2. The median number of cycles administered was 5 (range 4-6). Hematologic grade 3-4 toxicity was seen in 5 (42%) pts. Pneumonia was seen in 2 pts, causing death in 1 case. In 7/12 (58%) pts doses were reduced. The mean administered dose of any drug was >70%.ORR was 83% (CR/Cru in 5/11 pts, PR 5/11, 1 NR and 1 toxic death). Relapse occurred in 2 pts, who died of lymphoma. One pt died of unrelated causes. Seven are alive and disease-free after 7-171 months. The 3-years EFS and OS were 71% and 65% respectively. Conclusions: BCVPP regimen provides an effective and well tolerated therapeutic option for very elderly patients with HL.

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ESR AND FERRITIN EVALUATION AT DIAGNOSIS CAN IMPROVE THE CHEMOSENSITIVITY AND RESPONSE ASSESSMENT OF EARLY- AND ADVANCED-STAGE HODGKIN LYMPHOMA

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In Hodgkin Lymphoma (HL) inflammation plays a key role in tumor

growth and progression. Interim PET (after 2 cycles of chemotherapy) is the most important prognostic factor and is probably linked to the persistence of the inflammatory microenvironment. In this study we evaluated the prognostic significance of 2 marker of inflammation, ferritin and ESR. One hundred and one patients, 71 at early-stage and 30 at advanced-stage, were treated with ABVD as first line. Ten patients (10%) had a positive interim-PET and were switched to BEACOPP Among patients with negative interim-PET, 11 relapsed. The remaining 81 patients with negative interim-PET are still free of disease. The median follow-up was 26.43 months with a range between 2.56 and 86.33 months. Patients were divided into four different groups: 1. patients with negative interim-PET in CR after first line of treatment 2. patients with positive interim-PET in CR after early switch to BEACOPP 3. patients with negative interim-PET in progression or relapse after first line treatment 4. patients with positive interim-PET in progression or relapse notwithstanding early switch to BEACOPP Results On the whole, Ferritin levels showed a wide variation with a median of 167 ng/mL and a range between 11.3 and 1530 ng/mL. Patients that had an interim-PET positive, but not-responder to BEACOPP early-switch, had levels of ferritin at diagnosis higher than patients with interim-PET positive and responder to treatment (581±147.5 vs 233.6±133.3; p=0.038). With a ROC curve with a p value equal to 0.04, we set the cut-off at 400 ng/mL, with 100% sensitivity and specificity. No differences were found between patients in complete remission and patients with progression/relapse of disease that had an interim-PET negative. Although in the entire series ESR levels were not different between interim-PET positive and negative patients, male patients at early-stage with a positive interim-PET had at diagnosis levels of ESR higher than patients with negative interim-PET (76.75±24.66; 36.52±27.16; p= 0.01). In this subset, ROC analysis with a p value of 0.02, estimated a cut-off at 45 mm/hr, with a sensitivity of 100% and a specificity of 65%. Conclusions Patients with very poor prognosis have the highest ferritin levels at diagnosis. However, ferritin levels are not able to predict interim-PET outcome. On the other hand, ESR levels at diagnosis seem to be related with the interim-PET in male patients at early-stage.

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FUSED 18F-FDG PET/CONTRAST-ENHANCED CT DETECTS OCCULT SUBDIAPHRAGMATIC INVOLVEMENT OF HODGKIN LYMPHOMA THEREBY IDENTIFYING PATIENTS REQUIRING SIX CYCLES OF ANTHRACYCLINE-CONTAINING CHEMOTHERAPY AND CONSOLIDATION RADIATION OF SPLEEN

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Spleen and liver assessment for occult involvement of Hodgkin lymphoma (HL) challenges current staging procedures. We prospectively evaluated event-free survival (EFS) in 103 HL patients staged with fused fluorodeoxyglucose positron emission tomography (PET)/contrast-enhanced computed tomography (CT) to identify those at greatest risk of abdominal relapse. The EFS of this series was compared to that of a historical cohort of 100 HL patients staged with separate PET and diagnostic CT acquisitions. 31/103 patients staged with PET/contrast-enhanced CT were found to have spleen involvement and 10 patients liver involvement, whereas 14/100 patients staged with separate procedures were found to have spleen involvement and 3 patients liver involvement. There were significantly more intensive treatments (six courses of anthracycline-containing chemotherapy and spleen radiation) in the fused PET/CT group than in the historical cohort ($P \leq 0.04$). At a median follow-up of 27 months, five events occurred in the fused PET/CT group (HL relapse, 4 patients; carcinoma, 1 patient) and 19 events in the historical cohort (HL relapse, 18 patients; acute promyelocytic leukemia, 1 patient). Ten of the 18 relapses in the historical cohort were localized in the spleen and/or liver area. None of the four relapses in the fused PET/CT group was localized below the diaphragm. Thus, PET/contrast-enhanced CT-guided treatment resulted in a 95% EFS, whereas separate PET and diagnostic CT-guided treatment in an 81% EFS ($P = 0.002$).PET/contrast-enhanced CT is an accurate front-line single imaging diagnostic tool enabling effective tailored treatment in HL patients.

P206

EFFICACY AND SAFETY OF RITUXIMAB TREATMENT IN PATIENTS WITH PROGRESSIVE TRANSFORMATION OF GERMINAL CENTERS AFTER HODGKIN LYMPHOMA IN COMPLETE REMISSION POST-INDUCTION CHEMOTHERAPY AND RADIOTHERAPY

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Because the lymphatic tissue of Progressive Transformation of Germinal Centers (PTGC) expresses CD20, rituximab treatment may prevent transformation to lymphoma of this rather atypical entity. We prospectively evaluated the efficacy of immunotherapy with rituximab (375 mg/m2i.v. weekly for 4 consecutive weeks, followed by a single i.v. infusion of 375 mg/m2 every 3 months for 2 consecutive years) in 48 patients with biopsy-proven PTGC after Hodgkin lymphoma in complete remission post-induction therapy (4-6 courses of anthracycline-containing chemotherapy with radiotherapy). The event-free survival (EFS) of this series was compared with that of a historical cohort of 48 patients with PTGC, developing after Hodgkin lymphoma in complete remission post-induction therapy, who underwent observation. At a median follow-up of 40 months, histology showed a malignancy in 27% of patients in the observation group (Hodgkin lymphoma, 13 patients) and in 2% of patients in the rituximab-protected group (non Hodgkin lymphoma, 1 patient) (P= 0.001). Rituximab was well tolerated in all treated patients (3 patients with cutaneous herpes zoster infection). All relapses in the group nonprotected by immunotherapy involved the PTGC regions and noncontiguous nodal sites, which suggests that PTGC is a reservoir for malignant transformation and dissemination. The number needed to treat for rituximab to avoid one Hodgkin lymphoma relapse was four. In spite of the limitations (single centre nonrandomized study with a small number of events, possible overestimation of the diagnoses of PTGC, cost-effectiveness for the prolonged treatment with rituximab), our study shows that prophylaxis with rituximab helps improve EFS in patients with PTGC and a history of Hodgkin lymphoma.

P207

ROLE OF POSITRON EMISSION TOMOGRAPHY IN INDOLENT NON HODGKIN LYMPHOMAS

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Fluorodeoxyglucose positron emission tomography (FDG-PET) is a established functional imaging technique used in the staging and evaluation of response in Hodgkin lymphoma and diffuse large B cell lymphomas. Its role in other subset of non Hodgkin lymphoma (NHL) and in particular in low grade NHL has been poorly investigated. We retrospectively analysed 53 PET scans of patients affected by low grade NHL and we compared them with a concomitant standard contrast-enhanced computerized tomography (CT) in order to define the degree of concordance of this two imaging techniques. Our series was composed by 19 marginal zone (MZL), 26 follicular (FL) and 8 mantle cell lymphomas (MCL). We compared PET and CT scans at diagnosis for MZL, FL and MCL and at the end of first line therapy before high dose therapy (HDT) only for MCL. Among MZL, PET and CT were concordant in 7 cases. Considering discordant cases, 7 presented CT positive with PET negative (in 4 cases the difference was related to the presence of splenic involvement of disease) while in 5 patients the discrepancy consisted in more sites of disease detected by PET. Among FL, PET and CT were concordant in 14 cases. Considering 12 discordant cases, one presented only CT positivity; in the remaining PET was positive in a higher number of sites than that detected by CT. Among MCL patients, only in three cases PET and CT were concordant. In the remaining discordant cases PET was positive in more sites of disease than CT and in all of them the stage was modified by PET results. At the evaluation at the end of first line therapy before HDT, 7/8 cases were in complete remission (CR) considering both CT and PET. In conclusion, the sensitivity of PET seems to be higher than CT even in low grade NHL. In particular, in more than 90% of FL and MCL discordant cases PET was more sensitive than CT.

In MZL the role of PET is not so evident because it frequently failed to detect diffuse splenic involvement. Anyway, even in this subset of NHL, it could be useful in the detection of disease in soft tissues.

P208

SAFETY AND ACTIVITY OF INTENSIVE SHORT-TERM CHEMOIMMUNOTHERAPY IN HIV-POSITIVE (HIV+) PATIENTS (PTS) WITH BURKITT LYMPHOMA (BL)

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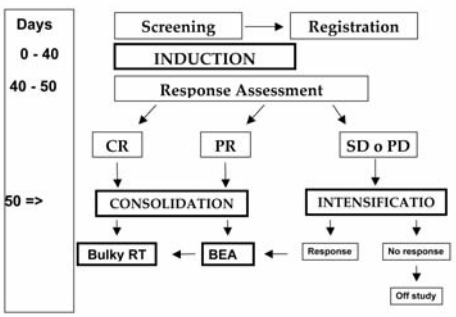
Background: Worldwide experience with intensive chemotherapy plus HAART in HIV+ pts with BL is still limited. We adapted an intensive short-term chemotherapy program used for HIV-negative childhood and adult pts with BL to treated HIV+ pts. Herein, we report feasibility and activity results of a multicenter pilot experience. METHODS: Consecutive HIV+ pts with BL, age ≤65 yrs and ECOG-PS ≤3 were treated with a 38-day Induction Phase (IP – Figure) of sequential doses of methylprednisolone, cyclophosphamide, vincristine, rituximab, methotrexate, VP-16, doxorubicin, with intrathecal prophylaxis. After IP (Fig), pts in CR received consolidation phase (CP; cytarabine+cisplatin+rituximab); pts in PR received CP followed by BEAM+ASCT; pts with SD or PD received intensification phase (R-ICEx2+HD-CTX+HD-araC+BEAM+ASCT). Leukaphereses were performed after CP. Pts with residual or bulky disease received consolidation radiotherapy. Results: 13 pts (median age 42 yrs, range 27-63; all males; ECOG-PS >1 in 5) were considered. Most pts had advanced stage, increased LDH, B symptoms, bulky mass and extranodal disease (meningeal in 2). Eight pts received HAART before BL; median CD4+ cells was 272 (range 17-858), 4 pts having <200. Eleven pts completed IP (median duration 49 days; range 38-86), treatment is ongoing in 1 pt and 1 pt died of sepsis. Dose reductions were not indicated.



CR= complete remission; PR= partial response; SD= stable disease; PD= progressive disease. RT= radiotherapy.

During the IP, G4 haematological toxicity was observed in all pts, with neutropenia in 11, thrombocytopenia in 5 and anaemia in 5. Eleven pts had infections, with CMV reactivation in 5, and multiple agents in 3. Only 1 pt had G4 non-hematological toxicity. Response to IP was CR in 5 pts and PR in 6 (ORR=85%, 95%CI: 66-100%). Nine pts were referred to CP; the first 4 experienced prolonged G4 neutropenia and infections. Thus, the following 5 pts were treated only with cytarabine-rituximab and did not experience infectious events. Leukapheresis was successful in 6 of 8 referred pts. Response after CP was CR in 6 pts and PR in 2; 1 pt experienced PD. Per protocol, 2 pts were referred to BEAM+ASCT and 1 to intensification. At a median follow-up of 12 months (range 1-20), 11 pts are alive (8 in CR), 1 pt died of BL and 1 of sepsis. Conclusions: This pilot experience suggests that the modified intensive short-term program is feasible and active in HIV+ pts with BL and may compare favourably with more demanding and resource consuming regimens. A multicenter prospective phase II trial is ongoing.

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SHORT-TERM, INTENSIVE CHEMOTHERAPY REGIMEN IS EQUALLY EFFECTIVE IN BURKITT LYMPHOMA (BL) AND IN THE NOVEL WHO 2008 ENTITY "B-CELL LYMPHOMA, INTERMEDIATE BETWEEN DLBCL AND BL"

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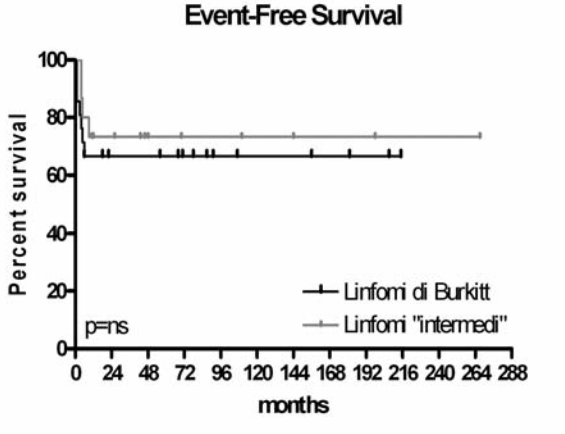
The diagnosis of Burkitt Lymphoma (BL) relies on precise histologic, immunophenotypic, cytogenetic and molecular features. The 2008 revision of the WHO Classification of Lymphoid Tissue Tumors defined a new provisional entity termed "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL" (hereafter intermediate) to define cases of high-grade, mature B-cell NHL only partially fulfilling the criteria for BL (i.e. higher morphological heterogeneity, variable BCL2 expression, non-homogeneous Ki-67 staining, other immunophenotypical aberrations). The optimal treatment of very aggressive B-NHL is still undefined. Standard chemotherapy regimens (i.e. CHOP) are clearly inadequate to face these high kinetic lymphomas. Better results were obtained also in adults with the use of short-term, pediatric-inspired, intensive chemotherapy regimens, and recently with the addition of Rituximab. However, informations about the treatment of intermediate NHL are lacking. Thirty-eight patients (29 males, aged 17-77 years, median 34) with very aggressive, advanced B-NHL were homogeneously treated from 1988 to March 2011 at our Institution with a short-term intensive protocol alternating fractionated cyclophosphamide, vincristine and adryamicine (cycle A) with high-dose methotrexate and cytarabine (cycle B) plus intrathecal prophylaxis. Rituximab was added since 2002 in 26 patients. After revision according to the WHO 2008 criteria, 22 cases were confirmed as BL and 16 were reclassified as intermediate lymphoma. Clinical presentation was similar in both groups. The large majority of patients had advanced disease (Ann Arbor III-IV 34/38, 89.5%) and very high kinetic (Ki-67≥90% 27/36, 75%). A leukemic phase was present in 5/22 BL (22.7%) and in 6/16 intermediate lymphomas (37.5%). Of the 36 evaluable patients, 33 (91.7%) achieved the complete remission, 2 did not respond or had progressive disease (5.5%). We observed 2 toxic deaths (5.5%). Relapses occurred in 3/18 BL (16.7%) and 3/15 intermediate cases (20%). After a median follow-up of 74 months (range 6-267), the Event-Free Survival rate is 69.4%, without any significant difference between BL and intermediate lymphomas. The addition of Rituximab did not significantly influence the outcome. Our data suggest that intensive, pediatric-inspired, short-term regimen is a valuable option for treating very aggressive B-NHL and determines comparable results in BL and in intermediate lymphomas.



P210

RESULTS OF A MULTICENTER ITALIAN POOLED ANALYSIS: IMPACT OF DIFFERENT THERAPIES ON SURVIVAL IN 309 YOUNG PATIENTS AFFECTED BY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) AT POOR PROGNOSIS

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Background. Poor-risk DLBCL had dismal prognosis. Aim of this pooled analysis was to test the role of high-dose chemotherapy plus autologous stem-cell transplant (HDC+ASCT), Rituximab (R), dose-dense intensified-CHOP (iCHOP), third generation regimens and involved-field radiotherapy (IF-RT) as first line treatment in young poor-risk DLBCL. Methods. 309 untreated DLBCL<61 years with age-adjusted International Prognostic Index (aa-IPI) 2-3 were enrolled from 1986 to 2006 into four consecutive multicenter trials; 42 into a phase II study, treated with 12 weekly infusion of MACOP-B (Vitolo, JCO 1992); 40 into a phase II trial with 8 weekly MACOP-B followed by HDC+ASCT (Vitolo, JCO 1997); 107 into a phase III randomized trial, 48 treated with HDC+ASCT and 59 with 6 iCHOP (Vitolo, Haematol 2005); 120 into a phase II trial with 4 courses of R-iCHOP followed by HDC+ASCT (Vitolo, Haematol 2009). IF-RT was performed as consolidation of bulky disease or residual mass. A Cox model was performed for Overall Survival (OS) and Progression-Free Survival (PFS) to estimate the Hazard Risks (HR) of four variables(with/without R, HDC+ASCT, new regimens vs MACOP-B, IF-RT yes/no), adjusted by aa-IPI score, age and sex. Results. Clinical characteristics were shown in Table 1. R was performed in 39% of patients; IF-RT in 35%; new generation regimens in 73% and MACOP-B in 26%. ASCT was performed in 171 (82%) of 208 scheduled; 39 did not because of progressive disease in 22, toxicity in 9 and poor mobilization in 6. Response rate was: complete response 69%, partial 7%, 18% not response and 6% toxic deaths. With a median follow-up of 10 years, 10-year OS and PFS were: 59% (95%CI: 53-65) and 48% (95%CI: 41-55). Secondary haematological malignancies or solid tumour were observed only in 3 patients. The Cox's multivariable model showed an improvement of the outcome with the use of R (HR 0.36, 95%CI: 0.21-0.63, p.0003) and IF-RT (HR 0.42, 95%CI: 0.27-0.66, p.0002), while no clear benefit was represented by new regimens (HR 0.86, 95%CI: 0.57-1.31, p.482) and by HDC+ASCT (HR 0.94, 95%CI: 0.62-1.41, p.751). However, in this retrospective setting, the role of Rituximab+HDC+ASCT was not tested. Conclusions: with the limit of a retrospective study, Rituximab and IF-RT seems to represent an important role in young untreated DLBCL at poor-prognosis. The ongoing randomized phase III trial DLCL04 by FIL will clarify the real impact of Rituximab plus HDC+ASCT versus dose-dense chemoimmunotherapy.

Table 1.

Clinical characteristics N=309		Median age 44 years (18-60)	
Male/Female	178/131	Stage II-III-IV	12/19/69 %
LI-IH-H risk	10/51/39%	BM involvement	31 %
PS>1	59 %	N extranodal sites > 2	34 %
LDH> normal	84 %	Bulky disease	53 %

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TREATMENT OF HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA WITH INTENSIFIED INDUCTION THERAPY AND HIGH DOSE SEQUENTIAL THERAPY: LONG TERM FOLLOW-UP

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The standard first-line treatment for DLBCL is CHOP, but its results in patients (pts) with aaIPI 2-3 is still not satisfactory. The use of HDST in first-line treatment led to discordant results. We have treated in pre-rituximab era, young high-risk pts with DLBCL with an intensified induction therapy followed by HDST. Pts with DLBCL, age less than 50 years, and intermediate-high with bulky or high-risk IPI were eligible for this study. Treatment consisted of 3 cycles of an intensified CHOP (MEGA-CHOP: cyclophosphamide 3 g/m2; doxorubicin 75mg/m2; vincristine 1,4 mg/m2 and prednisone 100 mg for 5 days) followed by high dose sequential therapy (Cyclophosphamide 4 gr/m2; Methotrexate 8 gr/m2 and Etoposide 2 gr/m2) and autologous stem cell transplantation (ASCT) with Melphalan-Mitoxantrone induction therapy. Since March 2002 to September 2005 we enrolled 15 pts to receive this treatment. Fourteen pts completed the scheduled treatment, while 1 died during the 1st phase for a sepsis. At the end of therapy 12 pts obtained a complete remission (CR) (83%), 2 pts were in partial remission (PR) one of these obtained a complete remission after radiotherapy. After the 1st phase 87% of the pts obtained a PR , 23% of pts obtained a CR after the 2nd phase and 13 pts (87%) have obtained the CR at the end of 3rd phase. After a median follow-up of 8 years (range 2-126 months) 13 pts (87%) were alive; 2 pts died (one for a sepsis and one for disease progression). After a median observation of 7 years range 2-104 months), progression free survival was 80%. Neutropenia grade 3-4 was recorded in 21% of the total number of MEGA-CHOP courses delivered and for platelets and hemoglobin occurred in less than 8% of the courses. One (7%) episodes of acute severe infection were reported in the schedule of treatment . So far, no cases of secondary acute myelogenous leukemia, myelodysplastic syndrome or solid tumor have occurred. The main late toxic effect was amenorrhea in all young female. We can conclude that, in pre-Rituximab era, the intensified induction therapy could represent an attempt to improve the results of HDST either according to OS and PFS in high risk DLBCL pts. As confirmation of this study hypotesis, recent randomized studies has at least one arm with intensified induction therapy associated with rituximab.

P212

IMPACT OF DOSE-DENSE IMMUNOCHEMOTHERAPY ON PROGNOSIS OF GERMINAL CENTER AND NON GERMINAL CENTER ORIGIN OF DIFFUSE LARGE B CELL LYMPHOMA

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Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma. Despite the high percentage of complete response, a significant percentage of patients has a more aggressive disease, with partial response or not-response to the first-line therapy, or a relapse within the first two years from CR. Gene-expression profiling in DLCBL has brought an insight into the biological heterogeneity of the disease. Two major subgroups were identified: germinal centre B (GCB) cell and non-germinal centre (non-GCB). In 2004, Hans' paper demonstrated that was possible to identify the same subgroups by using an immunohistochemistry panel. GCB patients showed to have a better survival. This study was to define retrospectively by immunohistochemistry the B-cell origin of 69 adult patients treated with R-CHOP14 in first line, and to evaluate if the dose-dense therapy could improve their clinical outcome. According to immunohistochemistry analysis, 28 patients were GCB and 41 were non-GCB. The complete response (CR) to treatment was obtained in 55 patients (80%) and a partial response (PR) in 11 patients (16%). The overall response rate (CR+PR) to treatment was 96%. At the last follow up 54 patients were alive (40 free from disease) and 15 died, all but one due to disease progression. Among 55 CR patients, 39 manteined CR (73%) and 16 relapsed (27%). After a median period of observation of 46 months (range 3-101 months) the overall survival (OS) was 75%, and the progression free survival (PFS) was 53%; with a median observation period of 34 months, the disease-free survival (DFS) rate was 68%. No differences were observed according to cell origin. In multivariate analysis, IPI and the response to treatment were two strong independent prognostic factors for OS. In this study an higher percentage of response to therapy and improvement in overall survival were evident in the subgroup not derived from germinal centre compared with literature data. Moreover in our experience the achievement of

complete remission was not associated with histological origin of lymphoma. In conclusion we can point out that intensification could enhance the efficacy of R-CHOP regimen improving the overall survival in patients with non-GCB.

P213

RITUXIMAB PLUS LOW-DOSE ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE AS FIRST-LINE TREATMENT OF ELDERLY PATIENTS WITH INDOLENT NHL

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Background: Low-Dose Fludarabine (FLU) and Cyclophosphamide (CY) combination has resulted as an effective therapeutic strategy for patients with chronic lymphocityc leukemia (CLL) or indolent non Hodgkin lymphomas(iNHL), both as first line and as salvage treatment; it is associated with lower immunosuppression and lower myelotoxicity than FLUCY at standard doses particularly in elderly and unfit patients, although response rates remained very high. The addition of Rituximab (R) to FLUCY results into superior efficacy than chemotherapy alone either in CLL and low-grade NHLs Aims: endpoints were 1) to evaluate response rates and toxicity in patients with low-grade NHL receiving Rituximab plus low-dose oral FLUCY combination (R-ldFLUCY) and 2) to evaluate EFS in the same group of patients. Patients and Methods: in a multicenter phase 2 clinical trial, 25 elderly patients (median 76 years, range 64-86) with iNHL requiring treatment for the first time received standard-dose rituximab (375mg/m2 d 1) plus low-dose oral fludarabine (25 mg/m2/d for four consecutive days) and cyclophosphamide (150 mg/m2/d for four consecutive days), for 4 monthly cycles; treatment was interrupted after 2 cycles in case of non response and at any time in case of unacceptable toxicity.Efficacy was assessed after 2 and 4 cycles respectively, in respect to the NCI criteria. Response, toxicity and EFS were also compared with the ones reported in a previous trial in which we treated a similar cohort of patients with oral ld-FLU-CY. Toxicity was evaluated after each cycle and reported according to CTCAEv.3. Results: All patients were evaluable for all endpoints. Twelve out of 25 evaluable patients (48%) achieved a CR and 11 of them (44 %) PR, while 2 patient (8 %) were non responder. Nineteen out of 25 patients (76%) completed the treatment, while 6 (24%) interrupted the treatment because of toxicity (3 cases), refractoriness (2 cases) and patient refusal (1). Severe toxicities (grade 3-4) was documented in 3 (11%) patients only; toxicity was also similar to the historical cohort of patients that had not received Rituximab. After a median follow-up of 22 months (range 9-40 months), 21 patients (84%) are alive and four of them (16%) died, three due to progressive disease and a patient, who was in CR, of aortic aneurism dissection. Median EFS was not reached. Remarkably, patients which we previously treated with ldFLUCY alone, had a significantly shorter EFS (median 20 months, p=0.02). Conclusions: Compliance to treatment was good and toxicity was mild. This prospective multicentric experience confirmed that oral ldFLUCY is an effective and easy to administer treatment for elderly patients with iNHL; most importantly the addition of rituximab results into a prolonged EFS without affecting toxicity.

P214

HEPATITIS C VIRUS POSITIVE STATUS IS ASSOCIATED WITH A SIGNIFICANTLY LONGER REMISSION DURATION IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Introduction: Diffuse large B cell lymphoma (DLBCL) is a heterogeneous disease and can show variable aggressiveness, with more indolent cases relapsing late after remission and cases relapsing early and running an unfavourable disease course. Prognostic factors able to predict such differences are limited. The association between hepatitis C virus (HCV) and B cell lymphoma has been clearly established, with highest frequency in marginal zone lymphoma (MZL) and about 15% frequency in DLBCL. We have analysed cases of DLBCL according to HCV status and time of relapse. Methods: Of the 597 DLBCL patients seen in our Department between 1994 and 2009, 468 were evaluable for HCV serology and 83 (18%) were HCV+. Relapse or progression occurred in 131/468 (28%) pts. Twenty-three received palliative treatment because unfit for age or comorbidities . The other 109 pts received antracyclin-containing regimens (standard or intensified CHOP/CHOP like) w/wo rituximab. Relapse occurred two or more years after remission in 27 pts, considered "late relapser" (LR) and within 2 years from remission in 82 "early relapser" (ER) pts. Intensified treatment and rituximab were given respectively to 50% and 33% LR patients and in 38% and 44% of ER patients (P=NS). HCV serology, clinical characteristics, and outcome were compared in both groups. Results: The two groups did not differ in any pre-treatment prognostic variable, including IPI, stage and B symptoms except for age, that was significantly lower in LR patients at diagnosis (median age 53 vs 60 years; p 0.01); but not at relapse (57 vs 61 years). HCV infection was present in 17% ER and in 48% LR patients (p 0.002) with a progressive increase of incidence according with the longer interval of remission (HCV+ in relapsing pts: 41% 3-5 years; 56% 6-10 years and 66% >10 years disease free interval, respectively) . Outcome was significantly better in LR. With a median follow up of 70 months, 5y OS was 55% in LR vs 13% in ER patients (p 0.0003), independently of HCV status. Conclusions: HCV+ status is associated with a lower risk of early relapse suggesting a more indolent DLBCL subgroup. Since HCV+ is particularly frequent in MZL which may sometimes be difficult to distinguish from DLBCL on histological grounds it may be hypothesized that a proportion of HCV+ DLBCL may be misdiagnosed MZL. Histological revision of all cases is being conducted in order to identify potentially discriminating characteristics.

P215

INTERIM 18FDG-PET FOR AGGRESSIVE NON-HODGKING'S LYMPHOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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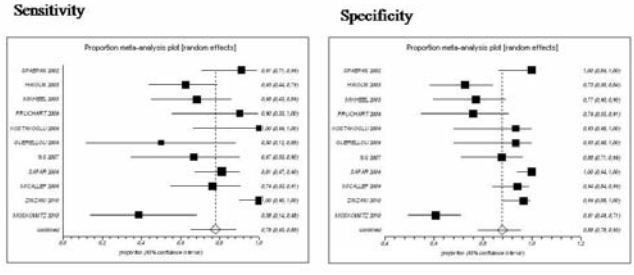
Background: The advantage of using interim 18F-fluorodeoxyglucose positron- emission tomography (FDG-PET) scan in the clinical work-up of patients with non- Hodgkin's lymphoma (NLH) is unclear. Data from meta-analyses are inconclusive, mainly because of the low number of patients evaluated and heterogeneity among studies. New clinical investigations, focused on this topic, have been recently published. We therefore conducted an updated systematic review on the role of interim 18FDG-PET in patients with aggressive NHLs. Materials and Methods: Medline, Embase, Scopus and Databases were searched for relevant studies through March 2011. We included studies that evaluated 8FDG-PET performed between the first and the fourth cycle of first-line chemotherapy. Patients were selected when they were evaluated for response assessment with interim 18FDG-PET. For each study, we constructed a 2x2 contingency table consisting of true positive, false positive, false negative, and true negative, where all patients were categorized according to whether they were PET positive or negative, and whether they experienced treatment failure.



A meta-analysis of the prognostic accuracy was performed. Results: We selected 11 out of 38 studies, involving 786 patients with aggressive NHLs (80% with Diffuse Large B-Cell Lymphoma); 687 patients met our inclusion criteria and were considered for the final analysis. Interim 18FDG-PET, performed after a median of 3 cycles of chemotherapy

(range 1-6 cycles), gave true and false negative results in 353 (51 %) and 72 (10%) patients, respectively. Therefore, 18FDG- PET had an overall sensitivity of 0.78 (95% CI, 0.65 to 0.88) and a specificity of 0.88 (95% CI, 0.78 to 0.95) (figure). Heterogeneity among studies was substantial. Conclusion: Because of data heterogeneity, interim 18FDG-PET for aggressive NHL patients remains an unproven test for routine clinical practice. Its role should be further evaluated in clinical researches with homogeneous treatments and standardized imaging.

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INTRATHECAL LIPOSOMAL CYTARABINE (LIP-ARA-C) DURING CNS PROPHYLAXIS IN PATIENTS WITH NON HODGKIN LYMPHOMA (NHL)

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Lymphomatous meningitis (LM) develops in at least in 4%-8% of patients with NHL. Risk factors are not completely standardized in the different guidelines and the incidence of the event depends also on intensity and efficacy of front-line CNS prophylaxis. The intrathecal (IT) injection of MTX or Ara-C is currently the most frequent prophylaxis. However, due to their pharmacocynetic, multiple and frequent lumbar punctures are necessary to achieve cytotoxic concentrations. Lip Ara-C (Depocyte) is a sustained-release formulation of ara-C developed for IT administration, ensuring prolonged cytotoxic drug concentrations (at least 14 d) of Ara-C in CSF with a homogeneous distribution in the neuraxis. Lip Ara-C has shown activity in the treatment of LM, but there are limited data for prophylaxis. The present retrospective study aims to evaluate the safety and tolerability of lip araC in the CNS prophylaxis of LM. Sixty-seven patients aged 16-77y (median 43,9) have been preventively treated with a total of 288 (range: 1-8) doses of lip araC 50 mg. Diagnosis consisted on 67 NHL: 42 high risk-CNS DLBCL according to SIE guidelines, 10 BL, 3 blastoid mantle cell, 9 lymphoblastic, 1 gastric marginal zone lymphoma, 1 anaplastic, 1 follicular. All patients were treated according to the standard protocols in use for their disease in particular, all received RCHOP-like treatments, a part of 10 BL (RCODOX-M/R-IVAC-like therapy) and 9 lymphoblastic (hyperC VAD/HD MTX/araC); All patients received lip araC 50 mg every 2, 3, 4 or 8 wks, excepted 2 receiving 30 mg. All patients had steroids for prevention of chemical aracnoiditis.The toxicity was headache (G1 e G2) nausea/vomiting (G1,)localized or diffuse bone pain. In conclusion, no severe toxicity has been observed and none of our patients developed unexpected long term neurological side effects. IT lip araC therapy with concomitant corticosteroids appears to be feasible and well tolerated in the prophylactic setting, reducing the number and the disadvantages of IT drug administration including the pain and inconvenience associated with the procedure. Because only few patients received CNS-directed concomitant therapy, lip araC appears effective towards CNS recurrence in the high risk NHL. More randomized studies are warranted.

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DOSE INTENSE RAPID-CYCLING CHEMOTHERAPY ASSOCIATED WITH RITUXIMAB AND INTRATHECAL CNS PROPHYLAXIS IN PATIENTS WITH BURKITT LYMPHOMA (BL) AND INTERMEDIATE UNCLASSIFIABLE DIFFUSE LARGE B CELL LYMPHOMA/BL: REPORT OF A SINGLE INSTITUTION EXPERIENCE

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Background. Burkitt Lymphoma (BL) and B-cell lymphoma, unclassifiable, with features intermediate between BL and diffuse large B-cell lymphoma (intermediate DLBCL/BL) are mature B-cell non-Hodgkin lymphomas with an aggressive clinical course. With the introduction of dose intense, rapid-cycling chemotherapy (Magrath 1996), mainly when supplemented with Rituximab, the prognosis of BL was improved. Conversely, the issue of intermediate DLBCL/BL treatment is still a matter of debate. Aim: to investigate the outcome of adult patients with BL and intermediate DLBCL/BL who received an intensive immunochemotherapy (R-HDCT) in a single hematological center. Methods. We retrospectively analyzed 23 adult patients divided in two groups. Group 1: 18 adult BL patients, including three with a diagnosis of L3 acute lymphoblastic leukemia, with a median age of 45 years (29-74) were treated according to CODOX-M/IVAC regimen including Rituximab and intrathecal liposomal Cytarabine (R-CODOX-M/IVAC), between 2006 and 2011. Group 2: five intermediate DLBCL/BL adult patients, median age 47 years (range 32-58) were treated, between 2008 and 2011, with Rituximab intensified CHOP with intrathecal Methotrexate followed by high dose Cytarabine and Mitoxantrone and HDCT with autologous stem cell transplantation in two patients and R-CODOX-M/IVAC regimen in three. Results. Group 1: 15 patients are in persistent complete remission (CR) and three died of progressive disease. With a median follow-up of 70.3 months, progression free survival and overall survival were 76.5% and 80.3%, respectively. There were no significant acute and late treatment related toxicities and no toxic deaths. In the second group the two patients treated with R-HDCT died of progressive disease; of the three patients treated with R-CODOX-M/IVAC regimen, one died of early relapse disease occurred three months after achieving CR and two are still on therapy. Conclusions. Our data suggest that in BL R-CODOX-M/IVAC is a safe and highly effective therapeutic regimen providing a high rate of persistent CR. Within the limits of a small sample size, in our experience, patients with intermediate DLBCL/BL have a clinical aggressive disease with a bad prognosis regardless of the type of treatment. Additional studies are warranted to clarify the behavior of this new histological entity and develop novel and efficacy therapeutic approaches.

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RITUXIMAB MONOTHERAPY FOR SPLENIC MARGINAL ZONE B-CELL LYMPHOMA (SP-MZL): REPORT ON OUTCOME OF 19 CASES.

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SP-MZL is a rare subset of MZL characterized by a primary splenic infiltration, frequent bone marrow (BM) involvement and leukemic manifestation. We analized 19 cases of SP-MZL: 6 males, 13 females, 47 to 83 years (median: 69 years). 31% of cases expressed CD5+. Anemia (hemoglobin level<100g/L) was present in 53% and thrombocytopenia (platelet count<150x10^9/L) in 42%. The lymphocyte count ranged from 1.2x10^9/L to 26x10^9/L (median: 15.7x10^9/L). High LDH and B2-microglobulin levels were observed in 63% and 79% of the patients, respectively. B symptoms were presents in 31%. No bulky disease. Splenomegaly and involvement of BM were present at diagnosis in all patients. 21% of patients was HCV+ and we observed two cases of severe Coomb's positive autoimmune hemolytic anemia. SP-MZL patients were treated with Rituximab 375 mg/m^2/weekly x 4 weeks (37%) or x 6 weeks (58%). One patient was treated with only two cycles of Rituximab for intollerance. In our serie the most rilevant characteristics were: splenomegaly and BM infiltration in all patients and lymphocitosis in most patients. SP-MZL does not correlate with HCV+ in our experience. Even though CD5+ and CD5- SP-MZL show similar clinical characteristics, we observed a higher lymphocyte count at diagnosis and a more frequent diffuse pattern of BM infiltration in subgroup of CD5+. After treatment with Rituximab, ORR was 100%: 84% of CR and 16% of PR. Two patients in PR received 6 cycles of Rituximab and one patient in PR only 2 cycles for intollerance and was submitted to splenectomy. The two cases of hemolytic anemia, after treatment, obtained complete remission and partial remission, respectively. Treatment was well tolerated and reported only hematologic toxicity (grade I-II WHO) in 16% and diarrea (grade I-II WHO) in 10%. No differences in terms of toxicity or outcome were found in relation to 4 vs 6 cycles of immunotherapy. After a median follow-up of 23 months (range: 1-44 months), 95%

of patients are in CR and alive. During follow-up clinical progression was observed in 5%: one patient in PR after 6 cycles of therapy presented PD after 1 month and exitus shortly later. Immunotherapy with rituximab is highly effective and safety for treatment of SP-MZL. We didn't observe differences in outcome related to CD5 expression or other biological and clinical parameters in this subset of patients. At last, we underline the efficacy of rituximab in autoimmune hemolytic anemia associated to SP-MZL.

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CHARACTERISTICS OF CD5+ MARGINAL ZONE LYMPHOMA: CLINICAL, FLOW CYTOMETRY FINDINGS AND OUTCOME OF 18 CASES.

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Marginal zone lymphoma (MZL) is a low-grade B-cell limphoma.CD5 is expressed to varying degrees by mature B-cell neoplasms. MZL is characterized by the absence of CD5 antigen. CD5 expression has been reported in a few cases of MZL and more recently in less than 20-25% of SP-MZL. We analized 66 cases of MZL observed from 2003 to 2011. 18/66 cases (29.8%) expressed CD5+ (9 males, 9 females, median age 67 years): 8 nodal, 9 SP and 1 extranodal MZL. Four cases presented with anemia (hemoglobin level<100g/L),10 cases with thrombocytopenia (platelet count<150x109/L). The lymphocyte count ranged from 3.4x109/L to 90x109/L. High LDH and B2-microglobulin levels were observed in 67% of the patients.B symptoms presented in 16%. Splenomegaly in 72% of cases. No immunological events were observed. In this series, 11% patients was HCV+. All patients showing CD5+ B-cells in their peripheral blood and involvement of BM in 83% of cases. Flow cytometric analysis of mononuclear cells of PB and BM was performed using MoAb against CD5, CD19, CD20, CD22, CD23, CD38, CD10, HLADR, CD11c, FMC-7, CD25, CD103, and . Splenectomy was performed at time of diagnosis in 1 patient. SP-MZL were treated with Rituximab 375 mg/m2/weekly x 4-6 weeks and 8/9 patients obtained CR while 1/9 patient with higher lymphocyte count (90x109/L) at diagnosis obtained PR. Nodal and extranodal MZL were treated with 6 cycles of R-CHOP or R-CHOP-like regimens or R-FN and all patients obtained CR.The main differences between CD5+ and CD5-MZL were a higher lymphocyte count at diagnosis (28x109/L versus 3.90x109/L) and more frequent diffuse pattern of BM infiltration (30% versus 7%) in CD5+. Flow cytometry analysis showed light chain + in 56% and light chain + in 44%, CD23 dimly+ in 33%. CD20 and CD22 both strongly expressed in all cases. CD38 dimly, CD25, CD103 and HLADR expressed respectively in 39%, 28%, 5% and 56% of cases. No case was CD10+. The median follow-up was 26.5 months (range, 0.76 – 56 months). Clinical progression was observed in 28% of CD5+ and in 12% of CD5-. 11% and 8% of patients died for PD, rispectively in CD5+ and CD5-group. We confirm the existance of CD5+ MZL (29.8% in our serie).These cases are closely related to classical MZL CD5- but present more aggressiveness in term of biological parameters (LDH, B2-microglobulin) and long term outcome. CD5 expression could be a marker of aggressiveness but whether or not CD5+ MZL constitutes a true subset requires the study of more cases.

P220

A SINGLE CENTER ANALYSIS OF CD23 EXPRESSION IN MANTLE CELL LYMPHOMA (MCL): BIOLOGIC AND CLINICAL CORRELATIONS

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Background: MCL is typically: CD5+CD20+CD10 CD23 FMC7+ cyclinD1+. A minority of MCL express CD23. MCL is an aggressive disease, but some cases display indolent behaviour. Previous reports showed that extranodal presentation, hypermutated IGVH, absence of SOX11 expression are qualities of these latter type. Some studies have described a better outcome of MCL with CD23 expression, but others found no prognostic value of CD23 expression. Aim of our study was to investigate CD23 expression in MCL and correlate this expression with pathologic and clinical parameters. Methods: We retrospectively investigated CD23 expression by flow cytometry in bone marrow (BM) and peripheral blood (PB) samples in 57 patients (pts) with MCL, and correlated CD23 expression with biologic and clinical parameters. Pts median age was 62 years (range 31-83), male/female ratio 40/17, 49 pts (86%) had stage III-IV, 45 (79%) BM and 24 (43%) PB involvement, 20 (35%) had extranodal disease, 9 (16%) splenic involvement, 28 (50%) B symptoms and 23 (41%) a MIPI score ≥ 5. In 48 (85%) pts Ki67 was >= 20, in 20 (35%) LDH and in 48 (85%) 2 globulin was high. CD23 was positive in 14 (24%) pts. No statistically significant different features were observed in the two groups, CD23 pos and neg. Forty-eight (84%) pts received CHOP like chemotherapy, 9 (16%) HyperCVAD. Results : CD23 expression was present in 14 (24%) pts; 11 (78%) CD23+ and 39 (91%) CD23- pts were responsive to treatment (p: n.s.):4 (28%) and 11 (25%) achieved Complete Remission (CR) and 7 (72%) and 28 (65%) Partial Remission, respectively. Three (21%) CD23+ and 4 (9%) CD23- pts had progressive disease. At a median follow-up of 30 months (range 4-128) Overall Survival (OS) is 58% and Progression Free Survival (PFS) 30%. Parameters that had an impact on the overall Response Rate (ORR) were: III-IV stage, 2 globulin, Bone Marrow involvement. No significant differences between the two groups were observed for ORR, OS, PFS and prognostic parameters. Conclusions: Some studies have reported a better prognosis with CD23 expression in MCL, but the correlation between CD23 expression and outcome is not clear. In accordance with others papers, in our study no significant differences in terms of ORR, OS and PFS were observed between the two groups CD23+ and CD23- MCL. Further biological studies are needed to explain the prognostic value of CD23 expression in MCL and establish whether CD23+ MCL is a distinct pathologic entity.

P221

PROGNOSTIC SIGNIFICANCE OF THE KI 67 INDEX FOR DLBCL TREATED WITH IMMUNOCHEMOTHERAPY

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous entity and patients exhibit a wide range of outcomes. The addition of Rituximab to chemotherapy has led to a marked improvement in survival and has altered the significance of previously recognized prognostic markers. Tumor cell proliferation, as assessed by the ki67 index, has been shown to yield prognostic information on DLBCL. Aim: we performed a retrospective analysis of 92 patients with de novo DLBCL, treated with immunochemotherapy, to evaluate the impact of the Ki67 index on Response Rate (RR), Overall Survival (OS) and Progression Free Survival (PFS). Methods: Ki67 was immunohistochemically assayed in tissue samples of 92 patients with newly diagnosed DLBCL. Ki67 values of > 80%, versus 60%-79%, were considered to define the two risk groups. Survival and Response Rates of 34 DLBCL patients with > 80% were compared with Survival and Respone Rates of 58 patients with Ki67 in the 60%-79% range. The two patients groups were similar with regard to pretreatment clinical variables. All patients were treated with Rituximab in association to CHOP chemotherapy. Results: The RR (Complete Remission + Partial remission) was 62% (21/34) in the Ki 67 > 80% group and 84% (44/54) in the 60%-79% Ki 67 group (p< 0,05). At a median follow up of five years, 13 patients died in the Ki 67 > 80% group and 10 in the 60%-79% Ki 67 group. The OS at 5 years was 62% in the Ki 67 > 80% group and 78% in the 60%-79% Ki 67 group (p< 0,05); the PFS at 5 years was 40% in Ki 67 > 80% group and 60% in the 60%-79% group (p< 0,05). Conclusions: Our data show that the Ki67 proliferative index has a prognostic significance in DLBCL treated with immuno-chemotherapy, and a ki67 > 80% may identify a high risk subgroup of newly diagnosed DLBCL. Further large-scale and prospective studies will be required to confirm these results.

P222

MARGINAL ZONE LYMPHOMA: A FAVOURABLE SUBSET OF LYMPHOMA. MONOCENTRIC EXPERIENCE

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Marginal zone lymphoma (MZL) is an indolent B-cell limph-proliferative disease. WHO classification recognizes splenic marginal zone B-cell Lymphoma (SP-MZL), extranodal B-cell MZL and nodal B-cell MZL.

We analized 66 cases of MZL observed from 2003 to 2011 (34 males, 32 females, median age: 63 years): 30 nodal MZL (45%), 27 SP-MZL (41%) and 9 extranodal MZL (13%). Eextranodal sites: skin, stomach, pleura. 21% of cases presented B symptoms, 51% thrombocytopenia (platelet count<150x109/L) and 21% anemia (hemoglobin level<100g/L). High LDH and B2-microglobulin levels were observed in 57% and 65% of the patients, respectively. Splenomegaly was present at diagnosis in 57 % of patients. Bulky diseases was observed in 3%. Histology: we observed 5 MALT-type and 3 cases with plasmacellular differentiation. Staging: 86% of patients presented IV stage at diagnosis. Autoimmune hemolytic anemia was observed in 4.5% of cases. 10% of patients were HCV+. CD5+ B-cells were observed in 29.7%. Treatment: splenectomy was performed at time of diagnosis in 4.5% of cases (1 nodal MZL for spleen break, 2 SP-MZL for severe splenomegaly troubles) with PR as outcome; than, the patients underwent immunotherapy with rituximab, obtaining CR; a w/w strategy was choosen in 4,5% (1 SP-MZL and 2 nodal MZL with very low burden); 3% (extranodal MZL) were treated with surgery plus RT. 88% were treated with Rituximab 375 mg/m2/weekly x 4-6 cycles (SP-MZL) or 6 cycles of R-CHOP - R-CHOP-like or R-FN (nodal and extranodal MZL). Global ORR was 87%. The rates of response were: 84% CR and 16% PR (SP-MZL); 70% CR, 27% PR and 3% SD (nodal MZL); 75% CR and 25% SD (extranodal MZL). The median follow-up was 18 months (range, 1-70 months) for SP-MZL, 14.5 months (range, 1-87 months) for nodal MZL and 9.6 months (range, 1-60 months) for extranodal MZL. Treatments were well tolerated. We observed few cases of hematologic toxicity of III-IV grade WHO, only after chemoimmunotherapy. One case of nodal MZL suffered from anaphylactic shock after immunotherapy with rituximab and continued only chemotherapy obtained CR. No other toxicity III-IV grade WHO. No treatment related mortality. DFS was 17.3 months for SPMZL, 23.4 months for nodal and 20 months for extranodal ones. Median OS was 19.5 months for all groups. We confirm the favourable outcome of Marginal Zone B-cell Lymphoma, especially after the introduction of Rituximab in therapeutic protocol.

MULTIPLE MYELOMA II

P223

TWENTY-FIVE MG LENALIDOMIDE EVERY OTHER DAY: FEASIBILITY AND EFFICACY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND RENAL FAILURE

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Lenalidomide administered orally is rapidly absorbed, reaching its maximal plasma concentration after a median time of 0.6-1.5 h.. With normal renal function, it is eliminated through glomerular filtration and active tubular secretion in 3 to 4 hours. Serum half life increases up to 9 hours if moderate/severe renal impairment is present (creatinine clearance <50 or <30 mL/min, respectively). In the latter cases a reduction of the daily dose is recommended. However, there is no theoretical assumption that protracting the full standard doses could be equally effective and tolerated in patients requiring reduced doses.Ten patients (6 f, 4 m; mean age 60 yrs.; r.: 49-81) affected by advanced resistant and progressive multiple myeloma (mean number of previous treatment lines: 3, r.:0-5, all including bortezomib) with concomitant renal failure (mean calculated creatinine clearance 46 ml/min, r.: 18-119) were treated with monthly 21-day courses of 25 mg lenalidomide e. o. d. and dexamethasone (low-dex.). Disappearance of urinary light chain and reduction of serum creatinine were detected in two patients; four patients were considered as partial responders, and two in stable disease, whereas two patients had signs of progressive disease. No patient experienced significant myelotoxicity; four patients required red cell transfusions. No SAE occurred during treatment. These preliminary observations point to a significant therapeutic effect in more than half of a small population of patients with particularly advanced disease. However, these results have to be validated by controlled studies involving larger number of patients.

P224

TREATMENT WITH BORTEZOMIB-CONTAINING REGIMENS IN PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS: A SINGLE CENTER EXPERIENCE

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Treatment of patients (pts) with systemic AL Amyloidosis remains challenging with organ dysfunctions improving in not more than 1/3 of cases with standard treatment. Bortezomib has been reported to have activity in this disease, where the misfolded protein may render the amyloidogenic plasma cells particularly vulnerable to proteasome inhibition. To evaluate the feasibility and efficacy of Bortezomib, we report our single center experience with Bortezomib-containing regimens in pts with AL Amyloidosis. Hematologic response (HR) and functional organ response are evaluated according to the 2005 Int. Soc.of Amyloidosis criteria (Gertz, Am J Hematol 2005). Complete HR is defined as normalization of the free light chain ratio with no evidence of a monoclonal protein by immunofixation and partial HR as a 50% reduction in M-spike or absolute light chain level. Since May 2010, 13 consecutive pts with AL Amyloidosis received Bortezomib-containing regimens at our center: Bortezomib-Dexamethasone (Vel-D),5 pts; Cyclophosphamide-Bortezomib-Dexamethasone (CyBOR-D),6 pts; Bortezomib-Melphalan-Prednisone (VMP),2 pts. Median age was 62 (52-72). 9 pts were treated upfront, while 4 had relapsed or refractory disease. 3 pts have been previously treated for multiple myeloma. According to the cardiac staging system based on Nt-proBNP and troponin I, 6 pts were stage 1, 5 stage 2 and 2 stage 3. Treatment history, organ involvement, regimen received, HR and organ response are shown in table1. 5 pts are still on treatment and 2 are not yet evaluable for response. 3 pts (27%) had complete HR and 7 (64%) partial response. Only 1 pt, treated for second relapse, had progressive disease. Median time to response was 2 ms (2-4). 1 pt received ASCT after Vel-D and 3 pts had PBSC collected and cryopreserved. Six of 10 evaluable pts (60%) had organ response (kidney in 4 cases,heart in 2). Hematologic toxicity was negligible;5 pts had significant extra-hematologic complications, including 3 heart failure, 2 interstitial pneumonia, 1 Staph.sepsis, 1 H1N1 virus infection, 1 CMV reactivation, 1 grade 3 neuropathy and 1 grade 3 diarrhoea. In 3 pts the dose of Bortezomib and in 4 the dose of Dexamethasone was reduced. In this unselected series of systemic AL Amyloidosis, Bortezomib-containing regi-

mens produced rapid HR in the great majority of pts, with and an encouraging high rate of organ response. Bortezomib represents a major advance in the everyday clinical management of this disease.

Pt	Treatment* history	Organ involvement^	Regimen received°	N.of cycles	Hematologic response	Organ response
1	VAD, MD, ASCT	R,C	Vel-D	8	Complete	Yes
2	Naive	C	VMP	5	Partial	Yes
3	Naive	R	Vel-D	4	Partial	Yes
4	VAD, MPT	R,C,H,G,N	Vel-D	5	Non Response	No
5	Naive	R,C	Vel-D	8	Partial	Yes
6	Naive	R	CyBor-D	8	Partial	Yes
7	Naive	R	CyBor-D	4	Complete	No
8	Naive	R,C	VMP	6+	Partial	Yes
9	ASCT	R,N	CyBor-D	4+	Partial	No
10	Naive	R	CyBor-D	4+	Partial	No
11	VAD, ASCT, INF, TD, VTD	R,C	Vel-D	4	Complete	Not eval.
12	Naive	C, N, S	CyBor-D	2+	Not eval.	Not eval.
13	Naive	S	CyBor-D	2+	Not eval.	Not eval.

Table 1. Previous treatment history, organ involvement, regimen and number of cycles received, hematologic and organ response for 13 patients with AL Amyloidosis

* VAD = Vincristine, Adryamicin, Dexamethasone; MD = Melphalan, Dexamethasone; ASCT = autologous stem cell transplant; MPT = Melphalan, Thalidomide, Prednisone; INF = Interferon; TD = Thalidomide, Dexamethasone; VTD = Bortezomib, Talidomide, Dexamethasone.
^ R = renal; C = Cardiac; H = Hepatic; G = Gastrointestinal; N = Nerves; S = Soft tissues.
° Vel-D = Bortezomib, Dexamethasone; VMP = Bortezomib, Melphalan, Prednisone; CyBor-D = Cyclophosphamide, Bortezomib, Dexamethasone.

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HOME SUBCUTANEOUS SELF-INJECTION OF BORTEZOMIB TO TREAT MULTIPLE MYELOMA IS SAFE AND EFFECTIVE

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We have recently demonstrated that a solution of bortezomib powder in normal saline stored at 4°C remains stable for nearly one month 1. This allows home intravenous administration of the drug to adequately informed patients. Since 2009, in our unit all patients requiring bortezomib for the treatment of multiple myeloma perform intravenous injection of the drug at home, after having been supplied with the exact dose in saline solution, in ready-to-use plastic syringes, appropriately prepared under hood in sterile conditions. This procedure reduces the time spent by patients in hospital, with positive consequences on patients' discomfort, nurses' overwork, and drug waste, as well. However, the intravenous administration must be carried out by professionals, and, furthermore, in some patients venous access may be difficult or sometimes unfeasible. As the drug is not histotoxic, subcutaneous administration is also feasible, and its efficacy is currently being evaluated 2. Hence, the possibility to receive bortezomib subcutaneously appears particularly attractive in domestic settings, provided that its efficacy be demonstrated compared to the standard intravenous administration. We are currently verifying the safety and efficacy of subcutaneous injections of bortezomib at the same dose as i.v. administrations (1mg/sm, days 1,4,8,11), but dissolved in smaller saline volume (max. 1ml), in association with dexamethasone 20mg/dd. 1-2,4-5, 8-9, 11-12, in patients affected by multiple myeloma, who lack easy venous access. The efficacy is evaluated based on the reduction of the monoclonal component during the i.v. period vs. the s.c. period, if the time elapsed between the two periods is less then two months. The table shows the main characteristics of the patients and the outcome. No evaluation of its efficacy can be made based on these preliminary results, but they seem to indicate an equivalence between the two administration modalities, as demonstrated by a prospective controlled study2. No adverse events have ever been associated with the home subcutaneous administration of bortezomib. 1. A Bolognese, A Esposito, M Manfra, L Catalano, F Petruzziello, MC Martorelli, F Pagliuca, V Mazzarelli, M Ottiero, M Scalfaro, B Rotoli. An NMR study of the bortezomib degradation under clinical use conditions Advances in Hematology, ID 704928, doi:10.1155/2009/704928 2. P Moreau, V Coiteux, C Hulin, X Leleu, H van de Velde, M Acharya, J Harousseau. Prospective comparison of subcutaneous versus intravenous administration of bortezomib in patients with multiple myeloma. Haematologica 2008; 93:1908-11.

Pat, G, age	dg	previous treatm.	No. of I.V bort.courses	% MC reduction	No. of S.C bort.courses	% MC reduction
F.S., M, 54	Ig-G-k IIIA	TD; AUTOBMT	1	0	1	0
R.L., F, 70	IgG-λ IIIA	MD; TD; VD	2	50	2	20
I.T., F, 81	IgG-λ IIA	MP; TD	1	0	1	ongoing
M.G., M,74	IgA-λ IIIB	MP; TD; VD	1	78	1	ongoing
M.G., F, 61	IgG-k IIA	TD	1	4	1	11
DR.O., M, 55	λ IIIB	VD	1	0	1	0

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LONG-TERM FOLLOW-UP AND ROLE OF SALVAGE THERAPY IN 156 PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH THALIDOMIDE-BASED THERAPY

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Treatment of newly diagnosed or relapse-refractory multiple myeloma (MM) is radically changed with the introduction of new drugs as thalidomide, lenalidomide and bortezomib. It has been suggested that the sequence of these new drugs may exert important prognostic implications. In particular, long-term use of thalidomide in the first line could significantly reduce post-relapse survival. In this retrospective study we analyzed 156 patients with newly diagnosed MM treated with protocols containing long-term thalidomide administration to assess the impact of the latter and the role of salvage therapies on the final outcome of these patients. Median age of patients was 70 years (range 31-91), 20% had IgA MM, 69% an ISS of 2-3 and 21% had a creatinine > 2 mg/dl. Only 7% underwent auto-transplant. Overall response ≥ PR and a CR wee achieved in 87% and 34% of cases, respectively. Five percent of patients progressed and 2.5% died during induction. The second-line therapy was performed in 74 patients (51 bortezomib-based: ≥ VGPR response = 27.5%; 17 lenalidomide-based: VGPR response = ≥ 41%, 6 palliative care) while 29 received a third line therapy (5 bortezomib-based, 18 lenalidomide-based and 6 palliative). After a median follow-up of 51 months (range 24-98), the overall median TTP and OS were of 30 and 56 months while the TTP in the 2nd and 3rd line were equal to 13 and 8 months, respectively without any significant difference between regimens containing bortezomib or lenalidomide. Post-relapse survival were 27 and 28 months with bortezomib and lenalidomide, respectively (p= 0.866). ISS = 1 (OR = 0.5, p = 0.035), creatinine < 2 mg / dl (OR = 0.5, p = 0.017), and the achievement of a CR (OR = 0.5, p = 0.007) were the factors affected positively overall survival whereas age (p = 0.721) and the sequence of therapeutic rescue bortezomib vs lenalidomide (p= 0.558) showed no impact. This retrospective analysis shows that in patients with newly-diagnosed MM, thalidomide containing regimens result in an increase of survival > 50% compared to conventional therapy, confirming the data of Kumar et al (Blood 2008). Moreover, the prolonged use of thalidomide does not cause a reduced post-relapse survival when bortezomib or lenalidomide was used as salvage therapy regardless of the sequence in which they are administered.

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A RETROSPECTIVE ANALYSIS ON 277 SMOLDERING MULTIPLE MYELOMA CASES: A PROPOSAL FOR A SCORE SYSTEM TO IDENTIFY SLOWLY AND HIGHLY PROGRESSIVE PATIENTS

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Smoldering multiple myeloma (SMM) is an asymptomatic plasma-cell proliferative disorder associated with a high risk of progression to symptomatic multiple myeloma (MM). Patients with SMM meet the diagnostic criteria of MM [serum M-protein (MC) higher than 3 g/l and a proportion of bone marrow plasma cells (PC) ≥10%] in the absence of clin-

ical manifestations. Prognostic factors for the progression and outcome of this disease are unclear. We propose a new score system to identify slowly and highly progressive SMM patients. We carried out a retrospective multicenter cohort study on 277 patients with SMM (M/F 123/154, median age at diagnosis 62.5 ys, (range 62.5±11.9) diagnosed between 1980 and 2010. Biological characteristics at diagnosis were as follows: haemoglobin (Hb) 13gr/dl (range,10-16.4), MC 2.6 gr/dl (range 0.4-5.7), PC 21% (range,7-70%), 2-microglobulin (2M) 2,7 (range,1-3.5), erythrosedimentation rate (ESR) 52,9 (range,2-140). The isotype of MC was as follows: IgAk 29/277, IgA 18/277, IgD 1/277,IgE 1/277, IgGk 146/274, IgG 77/277, IgG +IgA 2/277, IgGk+IgMk 1/277, monoclonal k and light chain 1/277 and 1/277, respectively. During follow-up, 98/277 (38%) developed MM with actuarial progression rate at 15 ys of 64%. We evaluated the baseline factors with respect to progression into MM. In the univariate analysis, the serum level of MC (p=<0.001), the Hb level (p=0.004), the bone marrow PC (p=0.001) and ESR (p<0.001), were significantly associated with progression. Therefore, using these three variables, we created a score system (Table 1) to identify slowly (score 0-1) or highly (score 2-3) SMM progressive patients. The median time to progression was 2.5ys and 10ys for the highly and slowly progressive group, respectively. Our results show that this score system at diagnosis is a valuable tool that could help to distinguish slowly and highly SMM.

BIOLOGICAL CHARACTERISTICS	SCORE
Hb ≥ 12,5 gr/dl	0
Hb < 12,5 gr/dl	1
Bone marrow PC ≥20%	1
Bone marrow PC <20%	0
Serum monoclonal level ≤2,5gr/dl	0
Serum monoclonal level > 2,5gr/dl	1
erythrosedimentation rate (ESR>50	1
erythrosedimentation rate (ESR)<50	0

Table 1. Score system models for SMM

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LENALIDOMIDE COMBINED WITH LIPOSOMAL DOXORUBICIN AND LOW DOSE DEXAMETHASONE (RDD) FOR RELAPSED MULTIPLE MYELOMA PATIENTS: SAFETY AND EFFICACY

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Background:Treatment of Multiple Myeloma has changed in the past decade with improvement of response rates and overall survival.Nevertheless, the best therapy for relapsed patients is not standardized. Aims: We assess safety and efficacy of Lenalidomide,Liposomal Doxorubicin and low-dose dexamethasone(RDd) regimen in relapsed patients.Methods:From June2008 to January 2011, 26patients were enrolled. Oral 25 mg/die Lenalidomide (days1-21), Liposomal-doxorubicin 30 mg/mq (day1), oral 20 mg/die dexamethazone(days1,8,15,22of28 days cycle) is administered for 6 cycles (Induction Phase,IP).Patients in CR-PR(IMWG criteria)post IP received 3 other cycles of RDd as consolidation.Responder patients allowed 10 mg/die Lenalidomide (days 1-21, every 35 days) until progression or treatment intolerance. Unresponsive patients discontinued treatment. Table1 provides patient' characteristics. 9 patients (35%) were considered as high risk because of heavly pre-treatment. 17 patients (65%) and 15 (58%) received a prior thalidomide or bortezomib containing regimen, respectively. 14 patients (54%) received a previous autotransplant. Table 2 showed type and grade of common side-effects. No grade 4 haematologycal toxicity was observed. Despite 95%neutropenia, only 2patients(8%)developed fever.The most frequent extrahaematological adverse event was neuropathy (19 patients, 73%). 4 patients (15%) developed a transitory skin rash. DVP occurred in 8% patients. 4 patients(23%)needed Lenalidomide-dose reduction. 22 of the 26 patients were assessable for response. Non-assessable patients received less 2 cycles (2 cases) and 2 are lost at the follow-up. After Induction,the overall response rate (ORR:7PR+9VGPR) was 73% (figure 1). 3 patients relapsed (18%). 10 patients completed planned treatment and are still in remission(CR,VGPR and PR in 1(10%), 6 (60%) and 3 (30%), respectively).After a median follow-up of 14 months (2-29), 4 (15%) patients died (2 for progression and 2 after transplant-TRM). 22 patients are alive: 2 (9%) CR, 5(23%) VGPR, 6(27%)PR, 2(9%) SD and 4 (18%) progression. MedianTTP is12months(2-29). Kaplan-Meyer estimated 2yTTP is 61%. MedianOS is14months(2-29). We analyzed factors influenced TTP and ORR:only previous treatment with thalidomide seems correlated to a lower TTP (p<0,04). Conclusion: Our experience suggests that RDd is tolerable and effective in relapsed myeloma patients. RDd should be considered as an appropriate treatment also in high risk patients (age>65 and more 2 previous line therapies, including thalidomide,bortezomib,transplant).

Table 1: Patient Characteristics at enrollment

Patient Characteristics	N = 26
Median age, y (range)	
At enrollment	69 (54-83)
> 75 years	5 (19%)
Type of Myeloma, no. (%)	
IgGL	4 (15%)
IgGK	10(39%)
IgAL	4 (15%)
IgAK	6 (23%)
Lambda	2 (8%)
Durie Salmon stage, no. (%)	
IIIA	21 (81%)
IIA	3 (11%)
IIIB	2 (8%)
Median Time since initial diagnosis, months	46 (4-184)
Median number of prior chemotherapy, no.(range)	2 (1-6)
cycles > 2	9 (35%)
Prior transplant, no. (%)	14 (54%)
Prior thalidomide-containing regimens, n (%)	17 (65%)
Prior Bortezomib-containing regimens, n (%)	15 (58%)

Table 2: Incidence of haematological and extra-haematological toxicity according to WHO criteria

Variable	All cases	WHO grade, n (%)			
		1	2	3	4
Haematological side effects					
Anemia	21 (81%)	13 (62%)	5 (24%)	3 (14%)	/
Thrombocitopenia	21 (81%)	13 (62%)	4 (19%)	4 (19%)	/
Neutropenia	25 (96%)	10 (40%)	8 (32%)	7 (28%)	/
Non-Haematological side effects					
Neuropathy	19 (73%)	10 (53%)	7 (37%)	2 (10%)	/
Skin rash	4 (15%)	3 (75%)	1 (25%)	/	/
Fever	2 (8%)	1	1		
Deep Venous Thrombosis	2 (8%)				

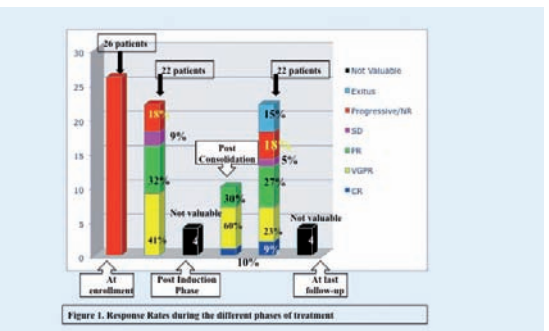

Figure 1. Response Rates during the different phases of treatment

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THE ADVERSE PROGNOSTIC IMPACT ON SURVIVAL OF CD20 IN MULTIPLE MYELOMA

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Introduction MM cells were first described as lacking CD20 expression. Subsequent experiences have shown that approximately 15% of MM are positive for CD20, generally with a weak expression (< 30% positive plasma cells). The expression of CD20 was found to be associated with a shorter survival and with lymphocytoid plasma cell morphology. More recent studies, however, demonstrated a correlation between CD20 MM cells and a favourable translocation t(11;14)(q13;q32). Thus the prognostic significance of CD20 expression in MM is unclear. Patients and Methods We describe 4 cases with expression of the antigen CD20 on plasma cells. The bone marrow aspirate and biopsy of patient 1 were extensively infiltrated by mature small plasma cells, while bone marrow aspirate and biopsy of patient 2 showed a population of immature lymphoplasmacitic cells. The bone marrow aspirate and biopsy of patient 3 presented mature malignant plasma cells including 30%

of CD20 very small plasma cells. The case 4 presented small lymphocyte-like morphologic features, mimicking mature small B-cell lymphoma and causing initial diagnostic confusion; the diagnosis of MM was supported by the presence of CD38 and CD138, and by the presence of osteolytic lesions and monoclonal paraprotein in serum and urine. Three patients out of 4 presented extramedullary localizations. Patients 1 and 2, after induction treatment according to the VDT protocol, received high-dose Melphalan with autologous stem cell (PBSCT), obtaining a VGPR lasting only some months; the patients died of progressive MM disease. Patient 3 was refractory to VTD and VTD-PACE protocols. Patient 4, considered ineligible to PBSCT both for advanced age and severe deterioration of clinical conditions (renal and hepatic failure), underwent a VMP regimen, obtaining only a marginal decrease of the monoclonal spike. Conclusions In our small experience, CD20 expression on plasma cells appears adversely to influence patients outcome, although the tendency towards a lower survival found in the CD20+ cases must be evaluated aside from the coexistence of the well-known negative prognostic factors (IgA isotype, translocation t(4;14), deletion 17p, deletion 13q, elevated creatinine, low serum albumin, high serum -2-microglobulin) in a larger series of patients.

Cases	Age (years) Sex	B2M (<2,5mg/dl)	Isotype	FISH	Distribution of % CD20 cells	ISS/Durie & Salmon's clinical classification	outcome
1.	42/M	6	IgAk	complex karyotype	Clusters	II/IIIA	dead
2.	56/M	12	IgAk	complex kariotype	Diffuse	II/IIIA	dead
3.	54/M	4,5	IgGk	/	Diffuse	II/IIA	non responder
4.	75/M	7	IgGk	t(11;14)	Diffuse	II/IIIB	dead

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A PHASE I, MULTICENTER, OPEN LABEL STUDY OF VORINOSTAT (ZOLINZA) PLUS MELPHALAN AND PREDNISONE (ZMP) IN ADVANCED/REFRACTORY MULTIPLE MYELOMA PATIENTS

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Histone deacetylase (HDAC) inhibitors represent a potential new class of antitumor agents. HDAC function is critical for Multiple Myeloma (MM) cells by actively maintaining a transcriptional program crucial for their uncontrolled proliferation and/or inappropriate resistance to pro-apoptotic stimuli. Vorinostat (suberoylanilide Hydroxamic acid) inhibits the activity of all 11 known human class I and II HDACs. Due to its ability to alter gene expression and protein activity, promoting MM cells death through multiple pathways, Vorinostat provides the framework for future clinical trials in MM. The primary objective of this phase I trial was to determine the maximum tolerated dose (DLT) of Vorinostat plus Melphalan (0.18 mg/kg for 4 days) and Prednisone (1.5 mg/kg for 4 days) followed by a 24-day rest period (days 5 through 28). The dose of Melphalan was emended at 0.13 mg/kg after the first 6 patients enrolled. Patients were sequentially planned to be enrolled into 1 of 4 escalating doses of Vorinostat using a standard "3+3" design for 6 cycles, starting at 200 mg daily on days 1-21 every 28 days (level 0). Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (Version 3.0). Nine patients were enrolled. The median age was 65 y (range 58-76 y), median number of previous therapies was 3 (1 to 4). All patients received immunomodulatory drugs, 8 out of 9 bortezomib, 89% a prevoius autologous stem cell tranplantation. Grade 3-4 anemia, neutropenia and thrombocytopenia were seen in 22%, 66% and 55%, respectively. One case of febrile neutropenia was observed, controlled with antibiotic therapy. Common non hematological toxicities of all grades regardless of attribution included fadigue (44%), nausea and gastrointestinal symptoms (44%), cutaneous rash (33%), neurotoxicity (33%) and cardiovascular toxicity (22%), most of which was grade 1 or 2 in severity. DLT were mostly related to hematological toxicities (neutropenia or thrombocytopenia) which delayed times to treatment. One patient presented a persistent enlargement of QTc as adverse event, preventing to carry on therapy. No serious adverse events were recorded. No deaths have occurred on study. These preliminary data suggest that the association of MP and Vorinostat is feasible but has significant haematological toxicity in the setting of heavily pretreated myeloma patients. A schedule with reduced doses of melphalan is under evaluation.

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BORTEZOMIB AND CYCLOPHOSPHAMYDE PLUS DEXAMETHASONE (VCD) AS SALVAGE TREATMENT FOR RELAPSED MULTIPLE MYELOMA

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Different studies demonstrated the efficacy of Velcade (V) alone or in combination with dexamethasone (D) for the treatment of patients with relapsed Multiple Myeloma (MM). Nevertheless, the relapse rate or primary resistance to VD combination is considerable. Accordingly, a number of studies have explored the efficacy of a third drug addition to the VD regimen, using either conventional cytotoxic agents or novel drugs. Here we describe our single-centre experience with the VCD combination [V, cyclophosphamyde (C) and D]. The adoption of C relies on the well demonstrated efficacy of C in combination with steroids for the treatment of advanced MM. We treated 32 patients (median age 59 years, range 41-69), with relapsed MM. Median time from diagnosis to VCD was 40 months (8-92), with a median of 1 (1-3) line of previous therapy. Upfront therapy was Thalidomide-based in 9 patients, and VAD in the remaining 24 cases. All patients had been autografted (13 single and 20 double) as part of their therapeutic program. The schedule of the protocol consisted of V 1.3 mg/sqm/ev days 1,4,8,11; C 500 mg/ev day 1,8,15; D 40 mg days 1,2,4,5,8,9,11,12; the course was repeated every 21 days for a total of 8 cycles. Twenty-six patients (81%) completed the therapeutic program; in 6 VCD was discontinued because of disease progression (n=3), toxicity (n=2, including one fatal pulmonary infection) and second neoplasia (n=1), respectively. WHO grade >1 hematologic (neutropenia and thrombocytopenia) and neurologic (peripheral neuropathy, or PN) toxicity were recorded in 12 and 15 cases, respectively. Six patients had infections requiring iv antibiotics. Overall, in 18 patients a reduction of V dose to 1 mg/sm was necessary, after a median of 5 cycles. Twenty-two out of 31 evaluable patients (71%) achieved response (11 CR, 6 VGPR and 5 PR according to EBMT criteria), 5 (16%) patients were refractory and 4 (13%) progressed. After a median follow up of 13 months (2-37), 8 patients relapsed requiring further MM treatment, while 14 are still responders. Overall, 18 patients are alive, while 14 have died (from progressive disease n=12, infection n=1, second neoplasia n=1). In conclusion, our data demostrate that the VCD combination is feasible and effective in relapsed MM patients. Hematologic toxicity is substantial, requiring growth factors administration in some cases. The incidence of PN is not negligible and results in dose reduction in more than half of the patients.

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MULTIPLE MYELOMA (MM): SERUM C-TERMINAL COLLAGEN TELOPEPTIDE TYPE I CAN ACT AS A BIOLOGICAL MARKER OF INCREASED BONE RESORPTION ?

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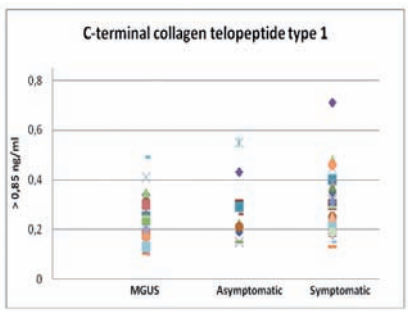
Background The skeletal involvement is one of the most important clinical characteristics of MM. Collagen type I constitutes 90% of the organic bone matrix and C-terminal telopeptide (CTX-I) together with N-terminal telopeptide are the most abundantly released products from collagen degradation. Recent studies have identified the CTX-I as a sensitive and specific biological marker of increased bone resorption. We have examined the correlation between serum levels of CTX-I and osteolytic bone lesions recognized on conventional radiography in our MM patients. Patients and Methods CTX-I has been investigated in 29 patients with Monoclonal Gammopathy of Uncertain Significance (MGUS), in 10 patients with Asymptomatic MM, in 35 patients with

untreated Symptomatic MM. The degradation products of CTX-I of type I collagen have been quantified in the serum samples of patients using a commercial immunologic test (-CrossLaps/serum Elecsys test). The monoclonal antibodies employed on -CrossLaps/serum Elecsys test recognize all fragments of type I collagen. Results Our results have showed normal CTX-I serum levels (< 0,85 ng/ml) in all study groups. In MGUS group the mean CTX-I serum level was 0,20 ng/ml (range 0,10 – 0,49), in Asymptomatic MM the mean CTX-I serum level was 0,26 ng/ml (range 0,15 – 0,55), in untreated Symptomatic MM the mean CTX-I serum level was 0,27 ng/ml (range 0,13 – 0,71). In addition, CTX-I was compared with 2-microglobulin (B2M) and sedimentation rate (ESR) and there were no positive correlations between the CTX-I serum levels and B2M and ESR. Particularly, in MGUS patients, the median values of B2M, ESR and CTX-I were respectively 1 mg/dl (range 0,8 -1,8), 7 mm/h (range 3 – 10) and 0,20 ng/ml; in Asymptomatic MM patients, the median values of B2M, ESR and CTX-I were respectively 2,44 mg/dl (range 1,92 – 4,24), 41 mm/h (range 8 – 78) and 0,26 ng/ml; in untreated Symptomatic MM patients, the median values of B2M, ESR and CTX-I were respectively 4,64 mg/dl (range 2,8 – 9), 102 mm/h (range 70 – 138) and 0,27 ng/ml. Conclusions Our data have demonstrated that CTX-I serum levels did not correlate either with increased bone resorption or biological markers related to disease activity. Therefore, markers of bone turnover including CTX-I have some disadvantages and limitations: genetic variability, disease variability, diurnal rhythm and many others. These biologic markers must therefore be validated in clinical trials.



	CTX-I (< 0,85 ng/ml)	B2M (2,5< mg/ml)	ESR (<10 mm/h)
MGUS	0,20 ng/ml (0,10 - 0,49)	1 mg/ml (0,8 - 1,8)	7 (3 - 10)
Asymptomatic MM	0,26 ng/ml (0,15 - 0,55)	2,44 mg/ml (1,92 - 4,24)	41 (8 - 78)
untreated Symptomatic MM	0,27 ng/ml (0,13 - 0,71)	4,64 mg/ml (2,8 - 9)	102 (70 – 138)

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EVALUATION OF SERUM LEVELS OF CATHEPSIN K IN MULTIPLE MYELOMA PATIENTS

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In multiple myeloma (MM), lytic bone disease occurs by means of a vicious cycle between bone destruction and myeloma cell expansion. Indeed, MM cells produce a variety of cytokines, that stimulate bone resorption by enhancing osteoclast formation and activity, and suppress bone formation by inhibiting osteoblast differentiation. Biochemical markers of bone remodelling are used in MM to assess the rate of bone turn-over and improve monitoring of bone destruction during treatment. Cathepsin K is a lysosomal cysteine protease, that is expressed by osteoclasts during the process of bone resorption. Therefore it is a potential target for anti-resorptive therapeutic intervention in the skeletal disorders characterized by altered bone remodelling. Higher circulating levels of cathepsin K have been reported in postmenopausal women with osteoporosis or Paget's disease, whereas there are no literature data in MM-bone disease. In this study, we purpoused to assess circulating levels of cathepsin K in 82 newly diagnosed MM patients (40 M/42 F, median age 72,4 years): 18 had asymptomatic MM and 64 symptomatic MM. Sera from 30 monoclonal gammopathy of undetermined significance (MGUS) patients and 22 gender- and age-matched healthy controls were tested, too. Cathepsin K levels were measured by a specific sandwich enzyme immunoassay (Biomedica, Vienna, Austria). Statistical analyses were performed by Student t test with the Statistical Package for the Social Sciences (spssx/pc) software (SPSS, Chicago, IL). Informed consent was given according to the tenets of the Declaration of Helsinki. In 8/18 patients with asymptomatic MM, we found high levels of circulating cathepsin K. These data were statistically significant (p<0,04), whereas no significant difference was found either in the other groups of patients or in the controls. Our data suggest the need of further investigations to clarify the role of cathepsin K during bone remodelling.

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CY-BOR REGIMEN FOLLOWED BY COMBINED MOBILIZATION WITH HIGH-INTERMEDIATE DOSE CYTOXAN PLUS BORTEZOBIB DOES NOT IMPAIR PERIPHERAL BLOOD STEM CELLS COLLECTION AND ALLOWS MYELOMA FREE HARVEST IN ELDERLY MULTIPLE MYELOMA PATIENTS

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Introduction.: Autologous Stem Cell Transplantation (ASCT) is a mainstream strategy in Multiple Myeloma (MM) patients (pts) under 60 years; however this strategy did not improve the outcome in MM older than 60 years. Notwithstanding, fit elderly pts can be eligible for ASTC even though a relevant percentage of them cannot proceed to ASCT, due to mobilization failure. The main causes are represented by age, previous Lenalidomide administration or advanced disease. We previously tested the feasibility of a regimen including Bortezomib (BOR), Cyclophosphamide (CY) and Dexamethasone (DEX) in fit elderly pts as first line therapy. Here we present data about PBSC mobilization in this subset. Patients and Methods: Of 31 pts evaluable for response before ASCT, 24 (77%) achieved at least PR and 22 (70%) were eligible for mobilization; the priming schedule was: BOR 1.3 mg/m2, i.v. DEX 40 mg/day (days 1-4-8-11) and i.v. CY 3000 mg/m2 (day 8). Granulocyte-Colony Stimulating Factor (G-CSF), at the dose of 5mcg/Kg/day, was administered starting from day 9 up to the last aphaeresis. Results: 20 out of 22 pts (91%) mobilized at least 3.0x10e6CD34+/kg (median 6, range 3-15) with a median number of two apheresis days (range 1-3). Of the two pts failing to harvest an adequate number of PBSC, one underwent bone marrow harvest and one was subsequently rescued with Plerixafor. The mobilizing schedule was well tolerated with only two pts experiencing grade 4 neurological toxicity, while none of them had relevant hematological toxicity; no neutropenic fever was observed and none of the pts needed hospitalization. The CD34 peak reached a median value of 84 CD34+ cells/mcl (range 23-752) and the first aphaeresis was started when the circulating CD34+ cells were >20/mcl by processing at least two blood volumes. The median number of CD34+ cells harvested was 6x10e6/Kg (range 3-15). Clonal plasmacells were evaluated by flow cytometry in 11 harvests: they were <0.01% in 9 out of 11 harvests. Conclusions: Cy-BOR regimen, followed by mobilization with CTX plus BOR is effective and well tolerated in MM pts older than 60 years. It is also characterized by an excellent mobilizing ability; indeed, up to now, 17/22 pts have been transplanted (5 are still ongoing): median time for PMN engraftment was 11 days (range 10-13) and 14 days (range 12-20) for PLT>20.000/mcl. With a median follow up of 606 days (range 129-1073) 16/17 transplanted pts are alive while one died of Disease Progression.

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CONSOLIDATION WITH HIGH-DOSE MELPHALAN (HD-MEL) AND AUTOLOGOUS STEM CELLS TRANSPLANTATION (ASCT) AFTER SECOND-LINE TREATMENT INCREASES RESPONSE RATE AND PROGRESSION-FREE SURVIVAL IN MULTIPLE MYELOMA PATIENTS RELAPSED AFTER FIRST-LINE SINGLE OR TANDEM ASCT.

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Background.Therapeutic options for patients relapsing after first-line HD-Mel and ASCT are not standardized. Few studies have addressed the potential role of ASCT consolidation after II-line treatment in patients

relapsed after first-line ASCT. Aim.To analyze the outcome of a consecutive series of relapsed MM patients receiving second-line therapy with or without further consolidation with ASCT. Methods.In 177 MM patients treated at our Institution between 1997 and 2009 with first-line HD-Mel and ASCT, relapse occurred in 121(68%).As induction therapy before ASCT, 136(77%) had received VAD-and 41(23%) thalidomide (T)/bortezomib(B)-based regimens. Ninety had received double ASCT (51%).Second-line therapy was given to 108 pts, in 70(58%) with T/B-based regimens only (TB group).Further consolidation with ASCT was given to 38 pts (31%), after TB-based regimens in 22 or chemotherapy (CT) in 16 (ASCT group).TB and ASCT groups did not differ in baseline characteristics, including age (median 60), type of first-line therapy, median follow-up from diagnosis.CR/VGPR and ORR obtained after first-line treatment (TB 51% and 91%; ASCT 68% and 95%, respectively) were also similar. Duration of first response and time to second treatment were 19 and 31 months in TB, 22 and 34 months in ASCT group, respectively. Proportion of patients receiving a double ASCT was significantly higher in TB (50%) as compared to ASCT (21%) (P=0.0039). Results. After second line therapy ORR (CR+VGPR+PR) was 84% in ASCT group, significantly better than TB group (49%) (P=0.0004).The second CR/VGPR rate was significantly higher after ASCT (42%) than after TB (21%)(P=0.027), independently from the type of second line treatment received before consolidation ASCT (ORR:TB 91% vs CT 75%; p=0.21).After a median follow-up from second-line treatment of 28 months (range 1-117mo), 2-yrs PFS was 17% after TB (median 18mo) and 30% after ASCT (median 14mo) (P=0.019).2-year OS was 65% (median 38mo) and 87% (median 45mo) after TB and ASCT group, respectively (P=0.36).Median PFS did not differ between pts receiving single or double first-line ASCT. Conclusions.The use of ASCT as consolidation after second-line treatment increased both ORR and CR/VGPR rates, independently from the type of debulking treatment used (chemotherapy versus novel drugs) and significantly impacts on PFS when compared with second-line TB-based regimens without ASCT.

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THE ROLE OF SINGLE -AGENT LENALIDOMIDE IN HEAVILY PRETREATED PATIENTS WITH RELAPSED O REFRACTORY MULTIPLE MYELOMA

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Background: Although lenalidomide (L) with dexa proved to be an effective treatment in patients (pts) with relapsed/refractory multile myeloma (MM), dexa-related toxicity (deep-vein, infections, hyperglycemia, etc.) can be dose limiting particularly in elderly or in younger pts with comorbidites.Moroever, immunomodulatory effect of L such as T cell and natural Killer cells activation, can be reduced by immunesuppressive effects of dexa. In this report, we activated this single Center study in the aim to test: 1) the efficacy in terms of Overal Response (OR), TTP and OS; 2) the safety of treatment. Patients and Methods: Between May 2008 and April 2010, at our Center, 24 pts (median age 68 y,range 51-81) with relapsed (17) and refractory (7) MM enrolled in this protocol. Our pts cohort concerned heavily pretreated pts with 3 or more prior treatments,including tandem or single autologous stem-cell transplantation (7pts), thalidomide and bortezomib. The L was administered at dosage of 25 mg/day from days 1-21 every 28-day cycle, with dose adjustement if renal failure. Toxicity L-related was evaluated according to NCIC. Thrombotic event (VTE) prophylaxis was performed with subcutaneous low-molecular weight hepaarin (22 pts) or acetylsalicylic acid 100 mg (2 pts), respectively. Response was evaluated according to IMWG criteria.TTP was defined as elapsed time between the start of L and occurence of progression and OS as the time between the start of L and death by any cause. The median duration of follow-up was 12 months (range 2-25). Eighteen pts (75%) started with full-dose L (25 mg/day). Six pts (25%)started with a reduced dose because of renal failure (10-15 mg/day or 10-15 mg every other day).The median duration of L therapy was 10 cycles (range 2-25). Results: Twenty-two pts resulted evaluable for respone. Two pts have been excluded because they were too early. OR was 59%: 9%CR, 9%VGPR, 41% PR. A median TTP was 8 months and OS was 18 months. The most common L-related side effects was neutropenia, which required dose reduction, but no pt discontinued teatment or experienced VTE or secondary cancers. At April 2011, 11 pts were still being treated: 5 in PR, 4 in SD and 2 in CR. Seven pts died and 4 pts went off therapy because of progressive disease. Conclusion: In our experience, even based on small series of pts but with poor prognosis,single agent L proved to be able to induce an OR 59% with median time to TTP of 8 months.Furthermore, no pt had side effect such as to induce treatment discontinuity.

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NEUROLOGICAL MONITORING ASSESSED BY NCI-CTC AND TNS REDUCES THE DEVELOPMENT OF SEVERE PERIPHERAL NEUROPATHY, IN MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE, BORTEZOMIB OR BOTH DRUGS

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Objective. Dose-limiting peripheral neuropathy (PN) is a disabling complication frequently reported with the use of thalidomide and bortezomib in multiple myeloma (MM) patients. The objective of this study was the monitoring of patients using the National Cancer Institute's Common Toxicity Criteria (NCI-CTC) and the Modified Total Neuropathy Score (TNS) in the early detection of PN in newly diagnosed MM patients treated with bortezomib, thalidomide or their combination. Patients: Thirty-five patients (17 men and 18 women), 11/35 treated with thalidomide (group 1), 11/35 treated with bortezomib (group 2) and 13/35 treated with both drugs (group 3), underwent neurological examination and electromyography both at baseline and at the end of treatment. Results: Cumulative incidence of PN was 71% using NCI-CTC and 74% using TNS; no grade >2 toxicities were observed. In 23/35 patients the scales agreed in terms of PN grade and their correlation was statistically significant (p<0,001). There was no significant correlation between PN and cumulative dose or dose intensity of the two drugs, treatment duration or median follow-up, while the combination of bortezomib and thalidomide (group 3) was found to be predictive of PN development (p=0.004). Conclusions: Our study showed that clinical and electrophysiological examination are both useful in the early diagnosis of PN. Careful monitoring is required to guide the clinician in dose adjusting, especially when the two drugs are used in combination, in order to avoid the development of grade 3-4 PN.

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ABSOLUTE LYMPHOCYTE COUNT AS PREDICTOR FOR SURVIVAL IN NEWLY DIAGNOSED ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background. Absolute lymphocyte count (ALC) > 1.400 x10^9/L at diagnosis has been reported to be an independent prognostic factor for clinical outcomes in patients with previously untreated multiple myeloma (MM). However, most of the patients evaluated were < 65 y.o., thus candidate to autologous stem cell transplantation. Less information is available on elderly patients who receive less aggressive treatment. We conducted a retrospective analysis on elderly patients, newly diagnosed with MM, to assess whether ALC at diagnosis, as a surrogate marker of host immune status, is associated with survival. Materials and Methods. Between 2005 and 2009, we analysed retrospectively 53 MM patients of 164 consecutive MM patients that were neither uniformly treated nor part of a clinical trail, but originally diagnosed and followed at 3 hospitals. The primary endpoint was to assess the role of ALC at the time of MM diagnosis on overall survival (OS); secondary outcome was the role of ALC to influence rate of first complete remission. Outcomes were analysed using the approach of Kaplan and Meier measured from the date of MM diagnosis to the date of death or last follow-up or first complete remission. Patients that were lost to follow-up were censored in the survival analysis. Differences between survival curves were tested for statistical significance using the two-tailed log-rank test. Results. The median age for this cohort of 53 MM patients was 73 years (range: 65–87 years). Three patients were lost to follow up. Most of patients

were treated with regimens containing Melphalan, Prednisone with/without Thalidomide. The median follow-up was 24.3 months (range: 1–81 months). At the time of the analysis 11 (20.7%) patients had died. OS of MM patients with ALC > 1.400 X109/l was 82 months vs 76 of those with ALC < 1.400 x109/l (p= 0.23). ALC, as a continuous variable, was not therefore identified as prognostic factor for OS (ALC < 1.400 x109/l vs ALC > 1.400 x109/l = HR 1.214 (95%CI: 0.222-6.650; p=0.823]). ALC did not either influence response rate of first complete remission (ALC < 1.400 x109/l vs ALC > 1.400 x109/l = HR 1.538 [95%CI: 0.662-3.570; p= 0.317]) (Figure). Conclusions. This study showed that, in newly diagnosed elderly MM patients, ALC is not an independent prognostic factor for OS and does not influence rate of first complete remission.

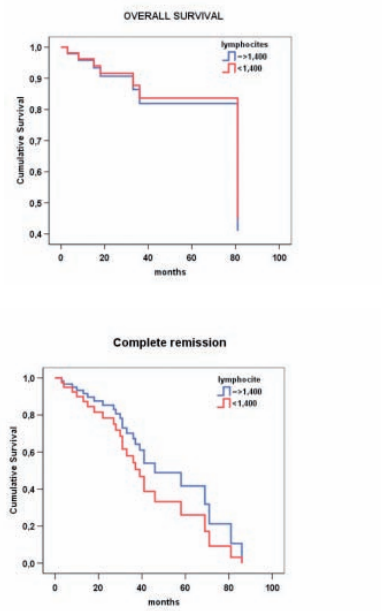

Figure. Kaplan Mayer curves comparing overall survival and complete remission rates among patients with ALC > 1.400 x109/l vs those with ALC <1.400 x10^9/L

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REAL-WORD DATA ON SUBOPTIMAL THERAPY IN OLDER PATIENTS WITH MULTIPLE MYELOMA

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The addition of a third agent to melphalan-prednisone (MP) has led to new standards of care in aged individuals with multiple myeloma (MM). All randomized studies comparing MP to MP plus thalidomide (MPT) or MP plus bortezomib (MPV) showed advantage for the 3 drug combination. However, while in the daily practice it is common to observe older patients who still receive MP, the exact number of them and reasons for suboptimal therapy are unknown. In this study, we retrospectively analyzed a consecutive series of 325 patients with MM aged over 65 years, aiming at assessing the number of patients who did not received a 3-drug regimen and the causes of suboptimal treatment. From 2008 to 2010, 325 patients over 65 years were diagnosed at our institutions. The median age was 74 years. Thirty-nine patients (12%), all aged 66-70 years, who received autologous stem cell transplantation were excluded from the analysis. Among the remaining 286, 60 patients received MP (21%), 137 MPT (48%) and 89 (31%) MPV. Among MPT and MPV patients, 66 % were accrued into prospective clinical trials; conversely, no patients within the MP subgroup were judged as eligible for any trial (p >0.001). The median age was 77 years for the MP patient subgroup, as opposed to 71 for MPV and 73 for MPT; the difference was statistically significant between MP and MPV (p=0.004) as well as between MP and MPT (p=0.007), while it was not significant between MPV and MPT (p=.14). The median number of comorbidities requiring specific treatment was 3 (range 1-5) in the MP subgroup, 1 in the MPT (range 0-1) and 0 (range 0-1) in the MPV subsets. Once again, differences between MP and MPT (p=0.03) and between MP and MPV (p=0.01) were statistically significant. The main criteria for the selection between MPT and MPV were distance from hospital, ability of the patients to travel and the period of observation (more patients received MPV after the registration as first line). We conclude that 21% of older MM patients receive suboptimal therapy (MP). Higher median age and number of concomitant severe comorbidities account for the therapeutic selection. Among patients treated with MPT or MPV, the choice is strictly related to the ability of patients to travel as well as to distance from the hematological institution. Of note, most of data refer to specialized hematological institutions; it is conceivable that in non hematological wards, the percentage of exclusion from the current standard of care can be higher.

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FDG-PET/CT FOR DIAGNOSIS, STAGING AND RESPONSE EVALUATION IN DIFFERENT MULTIPLE MYELOMA SETTING: A SINGLE CENTRE EXPERIENCE

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F-18-fluorodeoxyglucose positron emission tomography integrated with computed tomography (FDG-PET/CT) has been reported to be a widespread screening that can contribute to an accurate whole-body evaluation of multiple myeloma (MM) bone lesions and extramedullary sites, in different patients setting. However routinely application has still disappoint significance and utility. OBJECTIVES: aim of the present study was to retrospectively evaluate the results of FDG-PET/CT in different MM setting in our institution since January 2009 to March 2011: 40 patients were studied with FDG-PET/CT at least once, total number of scan 59. PATIENTS: 15 were smouldering MM (SMM), 4 were solitary plasmocitoma, 10 were MM in at least VGPR at 3 months after autologous stem cell transplantation (ASCT), 11 were in follow-up off therapy or in maintainance or reinduction therapy. Isotype was: IgG 20, IgA 11, IgD 1, BJ 5, non secretory 3. In 29 patients were available also magnetic resonance imaging (MRI) or conventional radiography. Results: in 12/15 SMM patients PET/CT was negative; in 5/12 cases whole bone conventional radiography detected doubt osteolytic bone lesions or osteoporosis. In 3 SMM patients PET/CT was positive for bone lesions and revealed a symptomatic evolution. FDG-PET/CT was negative in 7/10 patients in at least VGPR after ASCT, doubt in 2 and positive in one pt. In 7/11 patients in follow-up off-therapy or in maintainance or reinduction, FDG-PET/CT was positive revealing a disease relapse. CONCLUSION: The various application of PET/CT in MM could be useful in assessing the indolent condition in SMM, or in detecting deep response after ASCT or in revealing early relapse during follow-up even in reinduction or maintainance therapy.

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IMMUNOLOGICAL IMPAIRMENT IN MONOCLONAL GAMMOPATHIES

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Background. In some solid tumours and in experimental settings, T cell function can be modulated by regulatory myeloid cells, distinguished in immature (im-MDSC), monocytic-like (mo-MDSC) and granulocytic-like (N-MDSC). We investigated the immune function in patients affected of monoclonal gammopathies in order to identify the major player of suppression leading the expansion of neoplastic cells. Material and Methods. In peripheral blood of 45 MGUS, 45 MM patients at first diagnosis, 5 smoldering MM, 15 at follow up and 8 relapsed/refractory, we evaluated by flow cytometry circulating levels of immature im-MDSC (CD11b+, CD13+, CD14-, CD34+, CD45+), N-MDSC (CD11b+, CD13+, CD15+, CD14-, Lin-), mo-MDSC (CD14+, HLA-DR-), T-cell subpopulations, including Treg (CD4+CD25+FoxP3+) and the subset of CD62L carrying (a functional marker of migration ability expressed on

activated T cells). Results. Treg subset was reduced in active Myeloma (p=0.002), with a progressive increase during lenalidomide-based cycles of therapy, up to normalization after 4 cycles. CD8+T lymphocytes carrying on CD62L were significantly reduced in all gammopathies studied (p=0.001), with a further reduction in MM relapse compared to MM naïve patients (p<0.0001). Patients affected of MM presented higher levels of im-MDSC and mo-MDSC, but no N-MDSC, compared to MGUS and healthy subjects matched for sex and age (p<0.001). No differences were appreciable between MGUS and healthy controls. Patients with CRAB symptoms had higher levels of im-MDSC than patients without active disease (follow up of complete remission or smoldering myeloma, p=0.002). Im-MDSC levels (absolute count and percentage) in MM were negatively correlated to levels of CD62L+ CD8+ cells (R=-0.7, p=0.0023), suggesting as their expansion is linked to T cell impairment. Functional in vitro studies are ongoing to confirm this hypothesis. Conclusion. The ability to detect the myeloid impairment in premalignant phase of disease could allow for immunologic monitoring to assess the contribution of the immune system in preventing and/or inhibiting progression to MM. Moreover, the property of myeloma cells to attract infiltration by myeloid cells to facilitate their survival may provide the basis for novel approaches to myeloma therapeutics.

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PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR LIGAND INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS

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Background: Peroxisome proliferator-activated receptor (PPAR) is a transcription factor that regulates immune and inflammatory responses. It has shown that normal and malignant B cells, including multiple myeloma, express PPAR . Moreover, certain PPAR ligands can induce apoptosis in multiple myeloma cells. Because PPAR ligands can also have PPAR independent effects, the role of PPAR in B-cell malignancies remains poorly understood. To further understand the role of PPAR and its ligand in induction apoptosis in human multiple myeloma cells, we examined the functional consequences of its overexpression and activation. Methods: human multiple myeloma cell (ARH77) was incubated with rosiglitazone (1µM for 72h), a synthetic agonist of PPAR currently used to treat type 2 diabetes. After drug treatment the viability of cells was evaluated by Flow cytometer (Becton-Dickinson, San Jose, CA, USA) with annexin V and 7-Aminoactinomycin D (7-AAD) assay. By real-time PCR (7900 Fast Real Time PCR Applied Biosystems) we analyzed 96 genes involved in apoptosis and we assessed gene expression of PPAR and also of huntingtin-interacting protein 1 (HIP-1) and its molecular partner HIPPI, involved in recruitment and activation of caspases. The statistical analysis was performed using Student's t test. A value of p<0,05 was considered significant. Result: the addition of rosiglitazone was able to induce apoptosis in ARH77 cells (Apoptotic cells 48%±9,3 vs 35%±7 of control). Moreover we found that, besides the increase of PPAR (30 folds), the addition of rosiglitazone induced expression of HIP1 (120 folds), HIPPI (650 folds), caspase 9 (6000 folds), DEDD (21 folds) and FAS-ligand (12 folds) compared to control. Conclusion: This study shows that the antidiabetic drug rosiglitazone, a PPAR activator, is able to induce apoptosis in multiple myeloma cells and its possible mechanisms of action is the activation of genes of the intrinsic apoptosis through HIPPI-HIP1 pathway.

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LONGITUDINAL FUNCTION OF LEFT VENTRICULAR BASAL SEGMENTS DISTINGUISHES CARDIAC AMYLOIDOSIS (CA) AT PRESENTATION FROM HYPERTENSIVE HYPERTROPHY: A SPECKLE TRACKING ECHOCARDIOGRAPHY STUDY

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Amyloidosis is a sistemic disease characterised by extracellular deposition of protein-derived fibrils in various organs including the heart. Accurate evaluation of heart involvment is crucial to plan treatment strategy, particularly in patients who are candidate to AutoSCT. The aim of our study was to evaluate differences of left ventricular (LV) global and regional function between CA and hypertensive LV hypertrophy (LVH). Methods: 20 patients with CA at presentation, 13 newly diagnosed, never treated hypertensives with LVH and 12 healthy controls underwent Doppler-echo examination including pulsed Tissue Doppler of the mitral annulus. Automated function Imaging (AFI), a software which utilizes Speckle Tracking Echocardiography (STE) was also performed. Peak longitudinal regional strain was measured at the 18 LV segments obtained in the 3 apical views and global longitudinal strain (GLS) calculated as the average of measurements. Strain of LV basal segments (average of 6 basal segments, BLS), middle segments (MLS) and apical segments (ALS) were also determined. Ten patients with CA also performed N-terminal pro natriuretic peptide type B (NT-proBNP) and Cardiac Troponin I (cTnI) determination. Results: The ratio between transmitral E velocity and early diastolic velocity of the mitral annulus (average of septal and lateral sites) (E/e' ratio) was higher in both CA (p<0.001) and LVH (p<0.01) than in controls, without significant difference between AC and LVH. GLS was lower in CA than in the other 2 groups. MLS and ALS did not differ among the 3 groups but BLS was lower in CA (-11.7±5.5%) than in LVH (- 16.3±3.6%, p<0.02) and controls (-18.7±3.2%, p<0.001). Among the 6 basal segments BLS reduction of CA was significant in the anterior (p<0.002), lateral (p<0.05) and infero-lateral walls (p<0.001). In the pooled population E/e' ratio was significantly related with BLS (r = -0.43, p<0.005 but not with GLS, MLS and ALS. NT-proBNP and cTnI mean value were 1187 pg/mL (range 265-25302) and 0.085 ng/ml (range 0.01-0.63) respectively. No correlation was observed between BLS and NT-proBNP (p=0.313) or cTnI (p=0.462). Conclusions: Even though NT-proBNP and cTnI are reliable and useful markers of cardiac involvement in Amyloidosis patients; STE-derived AFI analysis is a useful adjunctive tool to identify cardiac involvement. The impairment of BLS is associated with LV filling pressure degree.

CHRONIC LYMPHOCYTIC LEUKEMIA II

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LOW DOSE ALEMTUZUMAB IN FLUDARABINE-REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: Refractoriness to fludarabine-based chemo-immunotherapy is associated to poor prognosis in chronic lymphocytic leukemia (CLL). Alemtuzumab has been shown to be effective in this category of patients (pts) although associated with relevant toxicity in terms of infections. Aim: We evaluated efficacy and safety of alemtuzumab subcutaneously administered at lower dose in a cohort of fludarabine-refractory CLL pts. Methods: Thirty-nine fludarabine-refractory pts have been enrolled at our center and treated with 10 mg subcutaneous alemtuzumab three times weekly for 18 weeks. In 18 randomly selected pts, after obtaining lymphocyte count reduction of 1 Log, the antibody was then administered once weekly at the dose of 30 mg. Biological prognostic markers were tested in 33 pts, including CD38 and ZAP70 expression by flow-cytometry and FISH panel; IGHV mutational status was available in 10 pts. Results: Median age was 64 years (range 48–82) with 31% of the pts older than 70 years. The patient population was characterized by high-risk biologic disease profile as shown by the incidence of del(17p) (18%), del(11q) (27%), unmutated IGHV (80%), CD38+ (48%) and ZAP70+ (55%). Median previous therapy lines were 3 (1-6); twenty-one were pre-treated with FC(R). Low-dose alemtuzumab yielded a 44% (95%CI 23.0-64.2%) overall response rate (ORR) whereas complete remission (CR) was obtained in 3 pts (8%; 95%CI 0-21.1%). Two of the three pts in CR resulted MRD negative. Median overall survival (OS) and progression free survival (PFS) were 29.1 months (95%CI 21.7-39.0) and 10.3 months (95%CI 8.3-16.2), respectively. Both PFS (p<.0001) and OS (p=.04) were significantly longer in responding versus non-responding pts. Treatment was well tolerated: all and 3-4 grade non-CMV infection were 46% and 7%, respectively. CMV reactivation occurred in 27% of the pts, showing only one case of disease. Moreover, no death occurred during therapy. No significant difference both in terms of safety and efficacy was observed in elderly (>70 years) pts and between the two different schedules evaluated. Conclusion: Our data indicate that low dose alemtuzumab in fludarabine refractory CLL appears to be equally effective and better tolerated than the standard dose. This data questions the use of standard dose alemtuzumab in frail or elderly pts and provide evidence for an equally effective and more practical one-weekly alemtuzumab schedule.

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RITUXIMAB, IFOSFAMIDE AND FLUDARABINE (R-IFLU) IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: A PILOT STUDY

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Background: Fludarabine given with cyclophosphamide and rituximab is considered the cornerstone of chronic lymphocytic leukemia (CLL) treatment in fit patients(pts). While several purine-analogs have been evaluated in combinatory trial, no other oxazaphosphorine has been investigated in this setting. Ifosfamide has shown significant activity in non-Hodgkin lymphomas, but has been not yet evaluated in CLL. Aim: We conducted a pilot phase II study of ifosfamide given in combination with fludarabine and rituximab (R-IFLU) in relapsed/refractory CLL pts. Methods: Thirteen pts with relapsed/refractory CLL were enrolled. Therapy consisted of ifosfamide (750 mg/m2) and fludarabine (25 mg/m2) for three consecutive days, and rituximab (375 mg/m2) on day 3. Treatment was administered every 21 days up to 6 cycles. Response was assessed using the NCI-WG 1996 criteria. Maintenance with monthly rituximab (375 mg/m2) infusions was administered in responders for a total of 4 months. Results: Median patient age was 63 years (range 51-71). The median WBC count was 61.800/mmc (3.160-122.000), and 9 pts were in advanced Binet stage (5 B and 4 C). Six pts (46%) presented with bulky lymph nodes (≥5 cm). Seven pts (54%) showed an unfavorable cytogenetic profile, including 6 pts with del(11q) and one carrying del(17p). The median number of previous lines of therapy was 2 (1-4); all the pts were previously exposed to chlorambucil (69% refractory) and 29% to fludarabine (54% refractory). A median of 4 cycles of therapy was administered (2-6); the overall response rate was 69%, including 23% complete remission. After a median follow-up of 4 years all the pts progressed, with a median progression free survival of 20.5 months (95% CI 8.1-40.1). Median overall survival was 47.6 months (95% CI 33.8-86.6). Hematologic toxicity was the most common AE. Grade 3 or 4 neutropenia, thrombocytopenia, and anemia occurred in 8 (69%), 1 (8%), and 2 (15%) pts during therapy, respectively. Infection was the most common non-hematologic toxicity; it was reported in 8 pts (62%) during therapy, but a severe event was observed in only one patient, who experienced a grade 3 unknown fever. Infusion related toxicities occurred in 3 pts and were mild in all the cases. Conclusions: This pilot study shows that R-IFLU is feasible and effective in relapsed/refractory CLL. Further studies are needed to evaluate the potential role of ifosfamide as alternative oxazaphosphorine to cyclophosphamide.

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SALIVA IS A RELIABLE AND PRACTICAL SOURCE OF GERMLINE DNA FOR GENOME-WIDE STUDIES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Identification of tumor-specific somatic abnormalities by high-throughput genomics requires tumor DNA matched to germline DNA. In leukemias that do not achieve complete remission (CR) because of refractoriness or because are not candidate to treatment, peripheral blood (PB) cannot be utilized as a source of germline DNA due to tumor cell contamination. This study assessed the adequacy of saliva and urine as germline DNA sources for genome-wide studies in the context of chronic lymphocytic leukemia (CLL). One ml of saliva was collected from 82 CLL with overt disease and from 20 CLL in CR. A spot urine sample (50 ml) was collected from 30/82 CLL with overt disease and from 5/20 CLL in CR. Paired PB granulocytes were also collected. The median yield of DNA extracted from 1 ml of saliva was 7.4 g (range 0.3-176.2 g). Saliva DNA was of high molecular weight in 99/102 (97.0%) cases. Median percentage of non-human DNA from oral microflora or food remnants in saliva DNA was 7% (range 0-30%). Contamination of saliva DNA by tumor DNA was restricted to 13/102 (12.7%) cases, including 12/82 (14.6%) CLL with overt disease and 1/20 (5.0%) CLL in CR. DNA from saliva was amplifiable for the KRT1 control gene in all cases. PB granulocyte DNA tested for comparative purposes was contaminated by tumor DNA in all (82/82, 100%) CLL with overt disease and in 50% (10/20) collected during post-treatment CR. Overall, saliva DNA passed all quality controls for SNP-array in 77/102, (75.4%) cases and for next generation sequencing in 71/102 (69.6%). The median yield of spot urine DNA was 2.4 g (range 0.1-34.4 g). DNA from urine was of high molecular weight in 35/35 (100%) cases. Median percentage of non-human DNA in urine DNA was 3.6% (range 0-66%). Contamination of urine DNA by tumor DNA occurred in 5/35 (14.2%) cases, all with overt disease at the time of collection. Urine DNA was amplifiable for KRT1 in all cases. Urine DNA passed all quality controls for SNP-array in 29/35 (82.8%) cases, and for next generation sequencing in 13/35 (37.1%). DNAs from saliva (n=5) and urine (n=5) were successfully run on 6.0 SNP arrays, passed the quality control call rate thresholds. Our data suggest that DNA extracted from saliva and spot urine of CLL patients are superimposable in terms of quality. Given its higher yield, saliva is a useful source of germline DNA for genome-wide studies applied to leukemia patients.

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ABSENCE OF CD26 EXPRESSION ON T CD4 CELL LYMPHOCYTOSIS POSITIVE FOR ÙCLONAL TCRGAMMA GENE REARRANGEMENT IN PERIPHERAL BLOOD: DOES A MONOCLONAL T LYMPHOCYTOSIS EXIST LIKE MBL?

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Introduction: Flow cytometric (FC) immunophenotyping is a powerful modality in the caracterization of undetermined lymphocytosis. Patients referred to our hospital for persistent lymphocytosis were analyzed by FC to assess their phenotype . During 2010 we have analyzed a total of 107 samples with lymphocytes between 4500 and 10.000/mm3 and 15 (14%) showed abnormal levels of T cells antigen such as CD4 or CD8. We have further investigated T CD4 cells (CD4 count from 3000 to 5000 mm3) by using other T cell antibodies (like CD7 and CD26, whose absence is diagnostic for Sezary syndrome) to distinguish possible neoplastic from normal T CD4 lymphocytes. Methods: Cells from peripheral blood with T CD4 lymphocytosis were stained with a set of monoclonal antibodies and analyzed by FC to assess the expression of T cell antigens. Samples with phenotypic alterations were studied by molecular analysis and we have correlated the frequency of abnormalities in the expression in particular of CD7 and CD26 (but also CD3, CD5, CD8 and CD38) with TCR gamma gene clonal rearrangement. Results: All positive for clonal T rearrangement phenotypic alterations showed and abnormalities were seen in the expression of one or more T markers. In detail CD7 was absent/dim in 50% of samples and CD26 was undetectable in the entire 100% of patients. Conclusions: Since a CD4+CD26- profile appears restricted to monoclonal T CD4 lymphocytes, CD26 expression might represent a useful tool to distinguish clonal from normal T cells. Even if these CD4 proliferations might be defined as asintomatic lymphocytosis this group of patients should be monitored carefully, as represents a lymphocytic pattern with uncertain clinical outcome although presents the same immunophenotype of the Sezary syndrome

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LOSS OF THE INHIBITORY FACTOR SOCS3 IN T-CELL TYPE LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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T-cell large granular lymphocyte (T-LGL) leukemia is a chronic proliferation of clonal cytotoxic T lymphocytes (CTL). The long term survival LGLs is characterized by resistance to apoptosis through impaired survival signaling. Among the intracellular pathways found to be deregulated in LGL leukemia, JAK/STAT (Janus Kinase/signal transducer and transcription factor) signaling has been claimed to be associated with LGL transformation. In particular, neoplastic LGLs of patients display high levels and a persistent activation of STAT3 that, by inducing anti-apoptotic gene expression, contributes to the accumulation of the leukemic clones. In this regard no data are available concerning the role of the "suppressor of cytokine signaling 3 protein" (SOCS3), that is the specific negative regulator of STAT3 signalling. Physiologically, the activation of STAT3 induces the expression of SOCS3 and SOCS3 itself behaves as inhibitor of STAT3 activating kinase by a classic feedback loop. In this study, in 27 patients with T-LGL leukemia, using RT-PCR and Western Blotting analysis, we investigated the SOCS3 expression and its correlation with phospho-STAT3 and STAT3 expression, both at baseline and after cell stimulation. Our results showed that SOCS3 was down-expressed in patients with respect to controls (0.26 ± 0.11 vs 0.52 ± 0.25, $p<0.05$). In addition, when SOCS3/STAT3 axis was triggered with IL-6, that specifically induces SOCS3 transcription through STAT3 activation, we found increasing of SOCS3 only in normal cells, whereas SOCS3 low transcription levels remained unchanged in leukemic LGLs, despite further STAT3 activation. Finally, we evaluated whether SOCS3 gene silencing might be related to an epigenetic mechanism. Using the demethylating agent 5-aza-2'-deoxycytidine we demonstrated the increase of SOCS3 protein expression, suggesting that in pathologic cells an abnormal methylation of SOCS3 promoter is the responsible of SOCS3 protein down-expression. Overall, our results suggest that in T-LGL leukemia the loss of the inhibitory factor SOCS3 might contribute to the aberrant activation of STAT/JAK pathway in T-LGL leukemia.

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AN INCREASED NUMBER OF INDIVIDUALS WITH MONOCLONAL B-CELL LYMPHOCYTOSIS (MBL) CHARACTERIZE RECENT DATABASES OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) RAI STAGE 0: COMPARISON OF GISL O-CLL PROSPECTIVE STUDY AND GIMEMA COHORT

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The IWCLL 2008 revisions of minimal requirements for CLL diagnosis recommend using B-cell count rather than absolute lymphocyte count (ALC) for the diagnosis of CLL and suggest a B-cell threshold of 5.0 x 109/L to discriminate CLL from MBL. Distinguishing CLL from MBL, a pre-malignant condition, is relevant considering the low likelihood of developing full blown CLL in these individuals (i.e. 1-2% per year). However, information on the actual incidence of MBL in individuals evaluated for absolute lymphocytosis is lacking. Moreover, it is unclear whether recent changes in the definition of clinical features of CLL at presentation parallel the increased diagnosis of MBL. Thus, we performed a retrospective analysis of a large cohort of Rai stage 0 CLL patients enrolled in two observational multicenter studies: the GIMEMA database recruited patients diagnosed in the period 1990-1999 and the GISL O-CLL trial prospectively observed patients in the period 2005-2010. ALC was at least 5.0x109/L at onset, fulfilling previous NCI-WG criteria for CLL. Flow cytometry analysis, available for all patients, confirmed diagnosis (CD5+/SmIg weak, and CD5/CD19/CD23 co-expression). Re-classification of previously classified Rai stage 0 patients enrolled in the GIMEMA and the GISL O-CLL trials according to the 2008 IWCLL criteria identified a higher number of individuals diagnosed with MBL in the O-CLL cohort: 124/818 (15.1%) (GIMEMA cohort) and 124/298 (41.6%) (O-CLL cohort), $P<0.0001$). The median absolute number of CLL cells was lower for individuals with MBL in the O-CLL cohort (median 3.1 x 109/L; range 0.4-4.9) compared to individuals registered in the GIMEMA series (median 3.9 x 109/L; range 1.0-4.9; $P<0.0001$), while there were no gender differences [F/M ratio 1.0 (O-CLL series) vs 1.2 (GIMEMA cohort) (P=ns)]. The significantly younger age observed among MBL individuals included in the O-CLL trial was due to the upper age limit (70 years) set by the enrollment criteria (median 48 years; range 40-71) compared to those enrolled in the GIMEMA cohort (median 65 years; range 33-100; $P<0.0001$). In conclusion, although biased by the age inclusion criteria and greater attention to regular check-ups in the last decade, the present study shows a significant increase in the incidence of MBL cases diagnosed in the last few years. This observation, if confirmed, raises issues related to appropriate patient monitoring and follow-up policy for this new category of individuals exhibiting a pre-malignant condition.

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NOT PUBLISHED

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A CASE OF HAIRY CELL LEUKEMIA DURING PREGNANCY

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A 30-years-old women received diagnosis of hairy cell leukemia in 9 week of pregnancy. Hairy cell leukemia is a rare lymphoproliferative

disorder that usually presents with pancytopenia, splenomegaly and bone marrow infiltration; its occurrence during pregnancy is an extremely rare event and data provided by litterature for management of these patients are very limited. The diagnosis is based on the demonstration of mononuclear cells with cytoplasmic projections in a blood smear, the typical bone marrow infiltration pattern and the immunophenotipic profile by flow citometry. Our patient presented, on her initial blood count, hemoglobin level 8,7 g/dl, white cell count 3.540/mm3 and platelet count 50.000/mm3. The spleen measured, approximately, 7 cm at the physical examination and 19 cm on abdominal sonography. Bone marrow examination by flow citometry showed a 0,9% of a B-cells population CD19+ CD103+ CD11c++. The patient decided to continue pregnancy and started therapy with Interferon-alpha. The treatment was initiated in 12° week of gestation with Interferon-alpha at dose 3 x 106 U s.c. 3 times per week and deltacortene 10 mg/die. The therapy was well tolerated and tranfusional support was required until the 23 ° week of gestation. The course of pregnancy was normal. At week 36 ° the patient delivered a healthy infant with a natural delivery. Post-partum course was uncomplicated. She stopped therapy the day of delivery. The revaluation of disease at the end of gestation showed a complete hematologic remission. The pathological B-cells population in peripheral blood, analized by flow citometry, was 0,02%. The spleen measured 14 cm on abdominal songraphy while wasn't palpable at the physical examination. Actually, the patient is followed in our department an remains in complete remission of disease. Seven month after the end of therapy she has a normal blood count and not requires other chemiotherapy.

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SPONTANEOUS HBV REACTIVATION IN TWO PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA BEFORE STARTING OF CHEMOTHERAPY

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Background Hepatitis B virus (HBV) reactivation is a serious complication in haematological patients; assessment of baseline HBV status is very important in these patients: firstly, the prevalence of HBV infection is increased compared to the general population; secondly, chemoimmunotherapy may trigger or exacerbate hepatitis in HBsAg carriers. We describe two cases of spontaneous HBV reactivation in patients with chronic lymphocytic leukaemia (CLL) before starting chemotherapy. PATIENTS A 79-year-old man presented with fever, weight loss, splenomegaly and leukocytosis with absolute lymphocytosis (170,000/ L). Immunophenotype analysis was compatible with diagnosis of CLL. Moreover the patient, seronegative for HBV in a test made five years before, showed a rise in serum ALT and he was positive for both HbsAg and anti-HBc; HBV-DNA was detectable at 170.000.000 UI /ml (1 UI = 5,82 cp). The patient began therapy with Entecavir (0,5 mg daily), obtaining normalization of ALT in one month and a progressive reduction and negativization of HBV-DNA. After one month from HBV-DNA negativization, considering age and comorbidity, he started low dose chemotherapy with chlorambucil (6 mg weekly); Entecavir has been continued and ALT and HBV-DNA are still normal after four months from chemotherapy beginning. A 64-year-old man, with a diagnosis of CLL made two years before at another Institution, consulted our Department about an increase of lymphocytosis (90,000/ L), lymphadenopathies and splenomegaly. At the diagnosis of LLC he was seronegative for HbsAg and seropositive for anti-HBc. After two years from diagnosis and with progression of disease, he developed a hepatitis B flare with HbsAg positivization and HBV-DNA and ALT fast rising. HBV reactivation was treated with Tenofovir (245 mg daily).HBV-DNA and serum ALT became normal after one month. After six months the patient started treatment with a FCR chemotherapy regimen. Evaluation of disease after three cycles showed a complete remission and HBV-DNA has been persistently negative with normal values of ALT. Conclusions HBV reactivation is a frequent complication in haematological patients undergoing chemotherapy, but it is rare before treatment starting. Reactivation is probably caused by lack of immunological control due to the disease itself. In our experience an early antiviral treatment allowed us to control hepatitis reactivation and to perform therapy of underlying disease without complications

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ROLE OF PET/CT IN THE DIAGNOSTIC WORK-UP OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND CLINICAL SIGNS OF DISEASE PROGRESSION/RICHTER'S SYNDROME

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Chronic lymphocytic Leukemia (CLL) represents an heterogeneous disease that may be complicated in 5-10% of cases, by the occurrence of a disease transformation such as an aggressive diffuse large B-cell lymphoma (DLBCL) or a Hodgkin's lymphoma (HL) defined as Richter's syndrome (RS). Since the presence of enlarged "bulky nodes" can lead to the suspicion of a CLL progression as well as to the possibility of a disease transformation, histology is the only appropriate approach for a correct diagnosis. Bruzzi et al previously demonstrated how positron emission tomography/computed tomography (PET/CT) has a high predictive value in identifying RS. With the aim of evaluating the role of PET/CT in discriminating a RS from a CLL progression, this exam was performed in CLL patients with a clinical picture suggestive of the occurrence of a RS or a CLL progression. Between May 2008 and April 2011, 11 consecutive CLL patients were included. "Major" eligibility criteria were: a significantly enlarged node or a pathological tissue and/or progressive cytopenia and at least one of the following "minor" parameters: increased serum LDH or beta2 microglobulin levels; presence of B symptoms. All patients underwent a 18F-FDG PET/CT. Sites of abnormal 18F-FDG uptake with a maximum standardized uptake value (SUVmax) of 5 or greater were considered highly suggestive for a disease transformation. In all cases, the biopsy of the largest node or of the pathologic tissue, or of the bone marrow, in the case of cytopenia, were performed. The median age of patients was 61 years (range: 47-75). Systemic symptoms were reported by 3 patients, a Binet C stage was observed in 4 cases and increased LDH and/or beta2 microglobulin level was observed in 6. The median value of the maximum diameter of the nodal disease evaluated at the CT scan was 4.1 cm (range: 2-14 cm). The median SUVmax value recorded at the PET examination was 6.3 (range: 1.9–26.4). A node biopsy was performed in 7 patients, a fine needle biopsy in 2 (1 abdominal node; 1 thyroid infiltration), a bone marrow biopsy in 1. The histology revealed the presence of a RS in 5 cases (3 HL; 2 DLBCL), CLL in 5 cases, thyroid carcinoma in 1 case. The only parameter significantly associated with the diagnosis of RS was a SUVmax value of 5 or greater (p greater than 0.01). In conclusion, our data confirm the predictive value of 18F-FDG PET in suggesting the presence of a RS. This exam could help to identify patients with CLL who warrant a biopsy.

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EFFICACY OF LENALIDOMIDE AS SINGLE AGENT TO CONTROL MINIMAL RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA IN FIRST COMPLETE REMISSION

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Introduction. Recent studies have shown that Lenalidomide is a promising therapeutic option in Chronic Lymphocytic Leukemia (CLL). Most studies have been carried out in patients with refractory or relapsed CLL. A pilot study has been started at our Institution to verify safety and efficacy of Lenalidomide as single agent in patients with CLL in 1st Complete Remission (CR). Patients and methods. Four CLL patients (three males) in 1st CR and persistence of minimal residual disease (MRD) have been evaluated. Their median age was 61 yrs (45-70), the induction treatments were mainly Fludarabine combinations plus Rituximab; they were off-therapy in spite of persistence of MRD, identified as circulating CD19+/CD5+ve cells. Indeed, all patients showed a slow though consistent increase of their clonal B-cell population and therefore they were placed under Lenalidomide, given at 15 mg/day for 3 weeks/month, until disease progression or discontinuation for severe toxicity. Aspirin

at 100 mg daily was added for thromboembolic prophylaxis. Lenalidomide administration was approved by the Italian National Agency of drugs (AIFA). Results. Presently, all patients are under Lenalidomide, after a median of 21 cycles (7-22). No severe toxicities, in particular neither tumor flare nor symptoms of tumor lysis syndrome, have been recorded. So far, no patients showed clinical signs of disease progression. Circulating CD19+/CD5+ve clonal B-cells progressively decreased under Lenalidomide in 3 patients, with the following changes comparing 6 to 12 mos before vs. 6 to 18 mos after Lenalidomide: case #1 - from 5 to 66 cells/μL before vs. no more detectable cells; case #2 - from 840 to 6,411 cells/μL before vs. reduction to 2008 cells/μL; case#3 - from 69 to 189 cells/μL before vs. reduction to 5 cells/μL; in case #4 (un-mutated CLL) clonal B-cells are still growing (from 67 to 116/μL before vs. up to 522/μL), however the patient is still in her continuous 1st CR at 18 mos of Lenalidomide. A significant increase of activated T-cells (CD3+/DR+) has been observed in all patients following Lenalidomide. Conclusions: i. Lenalidomide can be administered at 15 mg/day, 21 days/month, for up to 22 mos in CLL patients in 1st CR, with reduced risks of early severe side effects; ii. the schedule is effective to control MRD; iii. further studies with prolonged follow up should verify both the efficacy and the possible late effects of Lenalidomide in CLL patients at risk of disease recurrence.

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IDENTIFICATION AND CHARACTERIZATION OF A PUTATIVE REGULATORY B CELL SUBSET (B REGS) IN CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) PATIENTS

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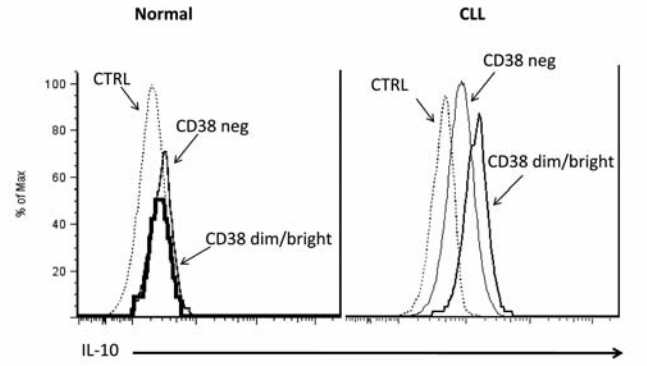
Università Magna Graecia, Catanzaro, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO S Francesco di Paola, Paola, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, AO di Cosenza, Cosenza, Università Magna Graecia, Catanzaro,AO di Cosenza, Cosenza

Immune system dysregulation in CLL patients is primarily due to the abnormal amount of blood regulatory T-cells and the elevated serum levels of IL-10, associated with the malfunctioning of circulating dendritic cells. Recently, a specific B-cell subset showing regulatory functions and able to suppress immune responses (B-reg) has been recognized. B-reg has been first described in mouse models of autoimmunity such as experimental allergic encephalomyelitis, inflammatory bowel diseases and collagen induced arthritis. Mouse B-reg cells show the common CD19+/CD5+/CD1d+ phenotype resembling an immature or primarily mature B-cell also producing IL-10 and TGF beta, through which most of immune suppressive effects can be exerted. In humans, emerging data show that a B-cell subset with B-reg functions exist in health and autoimmune setting. In this study, we sought to establish whether a B cell subset with a B-reg like immunophenotype could be identified in the context of CLL B-cell clones. We analyzed CD19+/CD5+ B-cells and checked the expression of specific surface markers (CD1d and CD38) in 23 CLL cases. Similarly, 10 healthy subjects were studied as controls. According to mean fluorescence intensity (MFI), we identified 3 distinguishable CD38 subsets in each analyzed sample: CD38neg (MFI log<10e2), CD38dim (MFI between log>10e2 and <10e3 and CD38bright (MFI log>10e3). Considering these first 23 CLL samples, the mean value of CD1d expression was higher among CD38dim (59±18% mean±sd) and CD38bright (51±20%) B-cell populations, while its percentage significantly lowered in CD38neg subset (29±20%). Notably, the amount of CD1d antigen was equally distributed among CD38neg/dim/bright subsets in healthy subjects. Furthermore, the magnitude of cytoplasmic IL-10 basal levels, detected in CD5+/CD19+ CLL population, largely fall within CD1d+/CD38dim/bright subsets (Fig.1). Again, no difference in IL-10 expression was observed among CD38 subsets in normal controls. In conclusion, our preliminary data suggest the presence of a putative CD5+/CD19+/CD1d+ B-reg cell population associated to neoplastic CLL clone, likely able to produce constitutively IL-10 among CD38bright subclones. This population resembles that described in mice and humans. Future studies are warranted for better immunophenotyping this B-cell subset and hopefully proving its regulatory function.



P256

CHLORAMBUCIL PLUS RITUXIMAB IN ELDERLY AND/OR UNFIT PATIENTS WITH PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA. A SINGLE CENTER EXPERIENCE

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Despite the increasing use of combination therapy with rituximab (R), fludarabine and cyclophosphamide for chronic lymphocytic leukemia (CLL), a significant proportion of patients (pts) are not suitable or eligible for such intensive chemotherapy due to co-morbidity and/or age. In those pts unfit for fludarabine based regimen, chlorambucil (CHL) remains a widely used first-line therapy. Its combination with monoclonal antibodies such as R seems to increase the overall responses rates with good tolerability. At our institution, starting from February 2008, elderly or unfit pts requiring therapy according to iwCLL criteria received CHL (days 1–10; 10mg/day p.o.) plus R (day 1-3 125 mg/m2 i.v. cycle 1, 500 mg/m2 cycles 2–6) every 28 days for 6 cycles. At study entry, FISH analysis, IgVH mutational status, expression of Zap-70 and CD38 were evaluated. The primary endpoint was the overall response rate, secondary endpoints included the adverse event profile, time to retreatment, progression-free and overall survival. A total 17 pts who had completed the treatment were included in this analysis; median age was 68 years (range 61–81). Four pts were Binet stage A, 8 stage B and 5 stage C; 4 were defined unfit for CIRS. Biological profile was characterized by good FISH profile (del13q in 9 pts, normal karyotype in 5 pts, trisomy 12 in 2 pts and del11q in 1 pts). Also 7 pts were CD38+ and 5 were Zap-70+, 50% of IgVH resulted mutated. The overall response rate was 82%; a CR was detected in 5 pts (29%) and a PR in 9 pts (53%). A progressive disease was recorded in 1 patient and a stable disease in 2. Now a day 6 pts (35%) showed progressive disease at a median of 19 months and 4 required therapy; 3 pts died (2 for secondary solid neoplasia and 1 patient for progressive disease) after 17, 31 and 23 months respectively. None of 17 enrolled patients required dose reduction of chemoimmunotherapy or need of hospitalization. Three patients experienced haematological toxicity (2 grade IV neutropenia requiring G-CSF and 1 haemolytic anemia) and four patients extra-haematological toxicity consisting of tumor lyses syndrome in 3 pts and CMV reactivation in 1 patient. These preliminary data confirm that in previously untreated CLL pts who are unable to tolerate a more intensive fludarabine-based chemotherapy regimen, the combination of R .plus CHL is an efficacious therapy with an acceptable tolerability profile resulting in a good ORR.

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LOW RISK CLINICO-BIOLOGICAL PROGNOSTIC PROFILE IN PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA AND A CONCOMITANT MYELOPROLIFERATIVE NEOPLASM. A RETROSPECTIVE MULTICENTRIC GIMEMA STUDY

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A greater incidence of secondary hematological or solid neoplasms compared to the general population is associated to chronic lymphocytic leukemia (CLL). The coexistence of CLL and myeloproliferative neoplasms (MPN) has been reported in the literature only as single cases: overall, 25 cases of CLL/chronic myeloid leukemia (CML), 18 of CLL/Polycythemia vera (PV), 12 of CLL/essential thrombocythemia (ET) and only 1 case of CLL/primary myelofibrosis (PMF). We retrospectively investigated the clinic-biological characteristics of CLL/monoclonal B-cell lymphocytosis (MBL) in 46 cases of CLL/MBL and a concomitant MPN, diagnosed between August 1985 and May 2010, from the CLL archives of 15 Italian centers on behalf of the GIMEMA chronic lymphoproliferative disorders Working Party. Thirty patients were males, 16 females; the median age at CLL/MBL diagnosis was 71 years. We present data from the largest series of concomitant CLL/MBL and MPN so far reported. Eight patients were classified as MBL; the remaining 38 were affected by CLL. Among MBL, a diagnosis of MPN (2 ET, 2 PV, 2 PMF, 1 CML, 1 MPN/MDS) was made simultaneous in 5 patients and later during the follow-up in 3 patients. None have so far progressed to an overt CLL; one patient died of an extra-hematological cause. All 38 CLL were diagnosed in Binet stage A. The diagnosis of MPN (16 ET, 8 PV, 8 CML, 4 PMF and 2 MPN/MDS) preceded that of CLL in 15 cases, was simultaneous in 7 and was subsequent in 16. Six patients have so far received chemotherapy for progressive CLL (2 before, 1 simultaneous and 3 subsequent to the MPN). Among stage A CLL, 6 patients have died after a median follow-up of 64.5 months; none of them experienced a progression of CLL requiring chemotherapy. The causes of death were never related to the lymphoproliferative disorders nor to the MPN. The biological prognostic parameters as IGHV (70% mutated), CD38 (78.5% negative), Zap-70 (71% negative) and del 17p or del11q (positive in only 7% of cases) confirmed the clinical data indicative of low risk CLL patients. None of the MBL patients have evolved into an overt CLL; only 6 stage A patients have experienced progressive disease requiring chemotherapy. Overall, 7 patients have died for causes not related to the lymphoproliferative disease nor to the MPN. Patients with a concomitant CLL/MBL and MPN usually have an indolent lymphoproliferative disorder with good clinic-biological prognostic features.

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MONOCENTRIC EXPERIENCE WITH BENDAMUSTINE IN ELDERLY PATIENTS WITH RELAPSED OR REFRACTORY LYMPHOPROLIFERATIVE DISORDERS

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Bendamustine is a bifunctional alkylating drug with demonstrated efficacy in the treatment of chronic and aggressive relapsed and refractory lymphoproliferative disorders. In our center, from April 2007 to March 2011, we treated with Bendamustine 25 patients, 8 females and 17 males, aged between 60 and 85 years, with relapsing or refractory lymphoproliferative disorders. Of these, 3 were suffering from aggressive lymphoma, 4 grade III Follicular Lymphoma (FCL), 9 Chronic Lymphocytic Leukemia (CLL), 5 Mantle Cell Lymphoma (MCL), 2 Marginal Lymphoma, one Indolent Lymphoma and one Peripheral T-Cell Lymphoma (PTCL). Twenty-three patients were treated with the combination of Bendamustine-Rituximab at a dose of Rituximab 375 mg/m2 on day 1 and Bendamustine 90 mg/m2 on days 1-2 every 21 days for a maximum of 6 cycles and the remaining 2 patients (one "frail" suffering from CLL and the other affected by PTCL) were treated with Bendamustine alone at a dose of 70 mg/m2 and 90 mg/m2, respectively. The median number of previous treatments received by patients was 2. The overall response rate (ORR) was 60%; the complete response rate (CR) was 28%; partial responses (PR) accounted for 32%, while the status of stable disease (SD) was observed in 12% of cases. In particular CRs were observed in 3 out of 5 cases of MCL (60%); in 3 out of 4 cases of FCL (75%) and in the case of aggressive Lymphoma (T-Cell Reach). The median overall survival (OS) from the time of diagnosis until the last follow-up is 64.5 months. The median follow-up for these patients from the end of treatment with Bendamustine is 10.1 months. The median progression free survival (PFS) is 7.9 months and the median of the time to next treatment (TTNT) was 9.7 months for patients who experienced disease progression. The toxicity profile showed high prevalence of haematologic toxicity, especially on the leukocyte lineage (grade III in 32% of cases, grade IV neutropenia in 20%). At now there were two deaths, one for fatal sickle cell crisis and the other one for grade IV infectious toxicity.

P259

TARGETING THE MEVALONATE PATHWAY AND DOWNSTREAM SIGNALING PATHWAYS AS A STRATEGY TO CIRCUMVENT MULTIDRUG-RESISTANCE IN CLL CELLS

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The mutational status of the tumor immunoglobulin heavy chain variable region (IGHV) is a very reliable prognosticator in chronic lymphocytic leukemia (CLL): patients with unmutated (UM) IGHV have a worse prognosis than patients with mutated (M) IGHV. Signals from the tumor microenvironment support the survival and protect CLL cells from the cytotoxic effects of chemotherapy. Multi drug resistance (MDR) is mediated by the over-expression of membrane transporters, like P-glycoprotein (Pgp), which actively extrudes several anticancer drugs. The Pgp protein is the product of the mdr1 gene whose regulation is under the positive control of the mevalonate (Mev) pathway, the Ras/Rho dependent signaling and the transcription factor Hypoxia-Inducible-Factor-1-alfa (HIF-1). The aim of this study was to investigate the role of metabolic and signaling pathways involved in basal and environment-mediated MDR in M and UM CLL cells, in order to identify specific targets of therapeutic intervention. Our results show that UM CLL cells have a significantly higher activity of the Mev pathway compared to M CLL cells. The higher levels of activity of the Mev pathway are associated with higher activity of Ras/Akt and Rho/Rho kinase signaling pathways and activation of HIF-1 . HIF-1 activation promotes a lower intracellular Doxo accumulation in UM CLL cells by positively regulating the expression of mdr1 mRNA. The murine stromal cell line M2-10B4 exerts a protective effect by upregulating the Mev pathway and activating the HIF-1 /mdr1/PgP axis, and reducing the accumulation of intracellular Doxo in UM CLL cells. Targeting the Mev pathway with Zoledronic acid (ZA) and/or statins, and targeting ERK-1/2 and HIF-1 with specific inhibitors (PD85 and YC1-10, respectively) significantly reduces basal and stromal-induced activity of HIF-1 /mdr1/PgP axis and significantly increases intracellular Doxo levels in UM CLL cells. These data indicate that targeting the HIF-1 /mdr1/PgP axis may be a strategy to circumvent basal and environment-mediated chemoresistance especially in UM CLL cells.

CHRONIC MYELOID LEUKEMIA II

P260

METHYLATION OF E-CADHERIN PROMOTER IN CHRONIC MYELOID LEUKEMIA CELLS

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The E-cadherin gene (CDH1), located on chromosome 16q22.1, encodes a protein product important in the maintenance of the epithelial phenotype mediated by Ca-dependent, homotypic cell-cell adhesion. The gene has been termed "metastasis suppressor" gene because the E-cadherin protein can suppress tumor cell invasion and metastasis. CDH1 gene expression is reduced or silenced in carcinomas of breast, liver, colon, stomach and prostate. Its inactivation occurs in undifferentiated solid tumors by both genetic and epigenetic mechanisms. However the role of E-cadherin in haematological malignancies is just starting to be recognized. In normal bone marrow, E-cadherin is expressed on erythroid progenitors, CD34+ stem cells and stromal cells, where it likely contributes to intercellular interactions during hemopoiesis. On the other hand, a reduction or lack of E-cadherin expression has been detected in leukemic cells (ALL and AML). In a previous study from our laboratory we have correlated the over expression of the zinc-finger transcription factor Slug to a reduction of the expression of E-cadherin protein in CD34+ chronic myeloid leukaemia (CML) cells. In this study we evaluated the methylation patterns of CpG island associated with the CDH1 gene promoter in normal peripheral blood and CML by bisulphite DNA treatment and subsequent methylation specific PCR (MSP). CDH1 promoter is essentially unmethylated in all normal control samples whereas CML samples show high level of hypermethylation of the CDH1 promoter, which can concur to the down regulation of E-cadherin protein in leukemia cells.

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SECOND MALIGNANCIES IN 559 CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH IMATINIB – A SURVEY BY THE GIMEMA CML WP

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Introduction. Imatinib (IM) is the standard of care for CML in early chronic phase, and, until now, remains a life-saving drug to be taken chronically. Long term side effects, including the incidence of 2nd malignancies, represent a potential relevant issue. Roy *et al.* (Leukemia 2005) reported an unexpected incidence of 2nd neoplasms in patients treated with IM after interferon; moreover, according to some in vitro studies, IM may enhance the malignant behavior of some type of carcinoma cell-lines. In contrast, 2 analysis, the first by Novartis Pharma (Pilot et al, Leukemia 2006) on 9518 patients, the second on 1083 patients in Czech Republic and Slovakia (Voglova et al, Neoplasma 2011), did not provided evidence for an increased overall incidence of 2nd malignancies. According to epidemiologic data (Registro Tumori) in Italy, the annual incidence of malignancies varies from 1%, in the range of age between 50 and 69 years, to 3% for patients over 70 years. AIM. To evaluate the incidence of 2nd neoplasms in CML patients treated with IM frontline. METHODS. Overall, 559 patients have been enrolled in 3 concurrent clinical studies of the GIMEMA CML Working Party: CML/021, IM 800 mg in intermediate Sokal risk patients; CML/022, IM 400 mg vs 800 mg in high Sokal risk patients; CML/023, observational, IM 400 mg. To better define the incidence of 2nd malignancies, in addition to the cases notified as severe adverse events, a specific query was sent to all participating GIMEMA Clinical Centers. Results. The median age at the diagnosis of CML was 52 (extr.18 – 84) years. The median follow-up is currently 60 months; cumulative patient years at risk are more than 2600. Twenty-two patients (3.9%) developed a 2nd neoplasm at a median time of 24.5 months (extremes 2-62) from the start of IM therapy; 7 of these malignancies were diagnosed within 1 year. Twenty out of 22 patients were older than 50 years at the diagnosis of the 2nd malignancy (median 64, extremes 37-77). Two patients had a relapse of a previously diagnosed neoplasm (in complete remission) and one patient progressed to Multiple Myeloma from a MGUS. Overall, 15 out of the 559 (2.7%) patients died due to 2nd neoplasm progression. Conclusions. In this multicentre nation-wide experience of CML patients treated with IM frontline, the incidence of secondary malignancies seems not to be superior to the observed incidence of neoplasm in the Italian national population.

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SESQUITERPENE OIL α-BISABOLOL, WHICH BINDS TO BID IN LIPID RAFTS, INDUCES APOPTOSIS IN BCR/ABL+ CELL LINES THROUGH MEMBRANE DEPOLARIZATION

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We showed that the plant-derived α-bisabolol is cytotoxic against human malignant non-hematological and leukemic cells. It enters cells via lipid rafts, binds to the pro-apoptotic Bcl-2 family protein BID, and induces apoptosis, with a preferential toxicity for malignant cells due to a higher content of lipid rafts in their plasma membrane. Here we demonstrate that the mechanism of action of α-bisabolol in BCR/ABL+ cells involves both cellular and mitochondrial membrane depolarization. To investigate the cytoplasmic membrane, BCR/ABL+ cell lines K562, LAMA86 and CML-T1 were treated with 160 M α-bisabolol for 30 minutes, 1 and 3 hours, and then incubated with TO-PRO-3 iodide, a cell membrane-impermeant nucleic acid stain, and evaluated by flow cytometry. The time-dependent increase of TO-PRO-3 fluorescence was detectable after 30 minutes and indicated the loss of cellular membrane integrity. When cells were loaded with the Ca2+ indicator Fluo-4 AM, flow cytometry revealed an increase of Ca2+ influx already after 15 minutes. These early events may trigger the apoptosis cascade induced by α-bisabolol. To disclose mitochondrial involvement, we assessed the mitochondrial transmembrane potential (m) with the fluorochrome JC-1. Microscopy and flow cytometry revealed that after 3 and 5-hour incubation with 80 μM α-bisabolol Δψm was completely dissipated in leukemic cells, thus indicating the occurrence of apoptosis, while normal T-lymphocytes, used as control, stayed vital. To investigate the mitochondrial target of α-bisabolol we evaluated the function of the mitochondrial permeability transition pore (mPTP), by loading cells with the calcein AM dye and adding CoCl2 to distinguish between intact and damaged mitochondria. Flow cytometry after 1-hour incubation with 160 μM α-bisabolol revealed that the function of mPTP was disturbed in BCR/ABL+ cell lines but not in normal controls. To evaluate the effect of α-bisabolol on mitochondrial respiration, which is strictly connected to Δψm, permeabilized leukemic cells were assayed for oxygen consumption in the presence of 3 μM α-bisabolol. We observed a significant decrease of NADH-supported state 3 respiration and oxygen consumption in leukemic cells as compared to untreated controls (140.0±70.5 vs 280.7±11.9 pmol O_2/min/10^6 cells; $p<.05$). Our data indicate that α-bisabolol toxicity is related both to its colocalization with membrane lipid rafts/BID and to its action on membrane integrity, thus triggering apoptosis.

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BCR/ABL TYPE OF TRANSCRIPT AND LACK OF MAJOR MOLECULAR RESPONSE ACHIEVEMENT ARE RELATED TO IMATINIB FAILURE AND WORSE PROGRESSION FREE SURVIVAL (PFS) IN CHRONIC PHASE CML PATIENTS

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Imatinib is approved as front line treatment for Ph+ chronic phase CML. In a series of 85 patients with these characteristics, we retrospectively evaluated if type of bcr/abl transcripts and/or type of molecular response to imatinib are correlated to frequency of loss of complete cytogenetic response (CcyR)(treatment failure) and/or to PFS, defined as time to loss of CcyR. Molecular diagnosis and identification of type of transcript (b3a2 or b2a2) were performed by RT-PCR analysis on peripheral blood (PB) and bone marrow (BM) samples leukocytes. Minimal residual disease was monitored through QT RT-PCR analysis every 4 weeks on PB, while on BM it was assessed, together with cytogenetic response, at 3, 6, 12 and 18 months from diagnosis and then once a year after CCyR achievement. Kaplan-Meier, Mann-Whitney and chi square tests were used for statistical analysis. Eighty-five chronic phase CML patients (median age 49 years; range, 23-78; M/F 51:34) who achieved CCyR with imatinib front line therapy were included in this study. All received imatinib 400 mg daily standard dose. Median treatment duration was 54,7 months (range 5-108) and median follow-up was 62,5 months (range 6,3-118,5). Sixty-three patients (74,7%) expressed b3a2 and 22 (25,3%) b2a2 transcript. Treatment failure occured in 13/63 (20,6%) b3a2, and in 9/22 (40,9%) b2a2 transcript expressing patients, with borderline statistical significance (p-value =0,054). Nevertheless, the group of b2a2 patients had worse PFS than that expressing b3a2 one (p value=0,014). As far as molecular response to imatinib is concerned, we considered the lack of MMolR achievement and correlated it with treatment failure (loss of CCyR). MMolR was obtained by 61/82 (74.4%) evaluable patients, while 21/82 (25.6%) never achieved it. In patients achieving MMolR, loss of CCyR was documented in 11/61 (18%) and in those not achieving MMolR loss of CCyR was documented in 10/21 (47.6%) patients, respectively (p= 0.017). Furthermore, worse PFS was found within the group of patients who never achieved MMolR during therapy (K-M, p value < 0,0001). Our preliminary findings suggest that in CML chronic phase patients, b2a2 transcript expression and lack of MMolR achievement are associated with a higher rate of imatinib treatment failure and a worse PFS.

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DASATINIB PLUS VINCRISTINE AS TREATMENT FOR CML PATIENTS IN UNPREDICTABLE LYMPHOID BLASTIC CRISIS

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Despite the dramatic improvement of therapeutic results in patients with chronic myeloid leukemia (CML), a minority of them still progress to blast crisis (BC) and die from their disease. Here we describe 3 patients in unpredictable lymphoid BC treated with a combination of dasatinib (D) plus vincristine (V) in order to achieve second chronic phase prior to allogeneic stem cell transplantation (SCT).The patients were aged 52, 41 and 25 years, respectively. All were in chronic phase at diagnosis and achieved complete cytogenetic remission (CCyR) at 4, 9, 6 months from the start of imatinib; one patient did also achieve major molecular response (MMolR). Lymphoid BC was diagnosed at 13, 20 and 31 months from diagnosis; cytogenetic analysis revealed complex karyotype along with Ph chromosome. Mutations S265C and H336R were detected in two patients. Treatment at BC consisted of dasatinib 140 mg/d, prednisone 50 mg daily for 30 days and vincristine 1.4 mg/m2 i.v. given as 4 weekly doses. Complete hematological response (CHR), was achieved in 2 patients at day 25 and 30 of treatment, in whom CCyR was also demonstrated. One of them did also achieve MMolR. One patient experienced marked bone marrow blast reduction (from 75 to 7%) and achieved CHR with subsequent therapy with Nilotinib for 40 days. During treatment for BC all patients experienced non-hematological side effects consisting of peripheral neuropathy (Grade 1-2) and constipation (Grade 2); none of them developed pleural effusion due to dasatinib. Hematologic toxicity was limited to thrombocytopenia and neutropenia (Grade 1) in all patients. One patient developed WHO Grade 3 anemia requiring transfusion of 4 units of blood. No platelet transfusions were administered and no patient needed hospitalization. All patients underwent SCT in second chronic phase, receiving TKI until the transplant. None of them remained on TKI post-alloSCT. None of the patients experienced acute graft versus host disease at any time from transplant. One patient died 4 months after SCT from a pulmonary sepsis, one is still alive in MMolR at the last follow-up of April 2011 and the last experienced meningeal relapse 5 months post-SCT and died in the setting of progressive disease. Our data demonstrate that the association DV is feasible and effective for patients with lymphoid BC; toxicity is manageable and does not jeopardize the possibility of subsequent SCT. Results seem to favorably compare with single TKI therapy.

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THE OLIGOMERIZATION AND DC2 DOMAINS OF THE BCR PROTEIN MODULATE ITS SUBCELLULAR LOCALIZATION AND REGULATE BCR-ABL TRANSFORMING ACTIVITY

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The Breakpoint Cluster Region (BCR) protein is implicated in the pathogenesis of Chronic Myeloid Leukemia (CML) as its N-terminus portion is fused in frame with most of the ABL sequence, generating the BCR-ABL chimaeric oncoprotein. To date, the intracellular localization of BCR is still controversial. We therefore wanted to investigate the mechanisms regulating the subcellular localization of BCR and to determine if and how they modulated the localization and function of BCR-ABL. Computational analyses recognized two putative Nuclear Localization Signals (NLS) and a Nuclear Export Signal (NES) in BCR. Both NLSs directed GFP in the nuclear compartment, while the NES favored its cytoplasmic localization. The latter effect was abolished after treatment with the exportin-1 inhibitor Leptomycin B (LMB). Despite these findings, wild-type BCR was an exclusively cytoplasmic protein because of a lack of nuclear import. However, removal of the N-terminus oligomerization domain (OD), of the C-terminus Protein Kinase C conserved region 2 (DC2) and of the Rho-GAP domain generated a construct displaying strong nuclear staining that was further increased by LMB. As the OD and part of the DC2 are preserved in BCR-ABL, we wanted to establish if these domains contributed to the subcellular localization of the oncoprotein. To this end, we generated a ∅DO-∅DC2 BCR-ABL mutant and its kinase-proficient counterpart ∅DO-∅DC2 BCR-ABLP1124L. Each construct was transiently expressed both in HeLa and in BaF3 cells but failed to relocalize to the nucleus. Interestingly, both mutants were devoid of transforming activity as confirmed by expression in CD34-positive progenitors. Our results have identified two putative NLS and one potential NES in the BCR sequence. The NLSs are functionally/structurally inhibited by the OD, DC2 and Rho-GAP domains of BCR, causing its cytoplasmic localization. Deletion of the OD and DC2 regions in BCR-ABL fails to relocalize the oncoprotein to the nuclear compartment, regardless of its catalytic activity. However, a kinase-proficient ∅DO-∅DC2 BCR-ABL does not display transforming potential, suggesting that the DC2 domain plays a pivotal role for BCR-ABL-dependent oncogenic activity.

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LOW-DOSE IMATINIB IN VERY ELDERLY (> 75 YEARS) CML PATIENTS: IS IT ENOUGH?

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In the "real world" of clinical practice, many very elderly CML patients have been treated with imatinib (IM) at lower dose than standard 400 mg/day, but there are no data on the results. To highlight this issue, we retrospectively revised 44 CML patients in chronic phase treated with low-dose IM according to physician decision when aged > 75 years in 26 haematological Italian Institutions; there were 25 males and 19 females, median age at IM start was 80.7 years (IR 77.8 – 85.6), Sokal Risk at diagnosis was intermediate in 24 patients, high in 16 and not evaluable in 4. Two or more concomitant diseases requiring specific treatments were present in 32/44 patients (72.7%), with 35 patients (79.5%) assuming 3 or more concomitant drugs. Ten patients (22.7%) were in late chronic phase (≥ 12 months from diagnosis) and pretreated (8 with HU and 2 with IFN) before starting IM; on the whole, median time from diagnosis to IM was 2.0 months (IR 0.7 – 6.1). Starting dose of IM was 300 mg/day in 29 patients (65.9%) and < 300 mg/day in 15 patients (34.1%); overall, 10 patients (22.7%) needed a dose reduction and 9 (20.4%) discontinued IM for toxicity (early toxicity in 7 and late toxicity in 2). Excluding the 7 patients who discontinued IM due to early toxicity, maximum tolerated daily dose during treatment was 300 mg in 20 patients and < 300 mg in 17 patients. According to CTC-AE, grade 3 – 4 hematological and extra-hematological toxicities were observed in 8 (18.1%) and 11 (25.0%) patients, respectively; 4 patients (9.1%) presented a pleural effusion during IM treatment. After a median treatment period of 29.4 months (IR 7.9 – 54.4), 5 patients are still too early (< 6 months of treatment) and 39 are evaluable for response. Of them, 7 (17.9%) discontinued IM due to early toxicity and 1 (2.5%) was resistant: the remaining 31 patients (79.6%) achieved a complete hematological response (CHR). Among these 31 patients in CHR, 4 refused any other karyotipic or molecular evaluation (2 are still alive in CHR and 2 died in CHR from unrelated causes), 4 achieved CHR only and 23 (58.9% of all 39 evaluable patients) achieved a cytogenetic response (CyR), which was major in 4 patients and complete (CCyR) in 19 (48.7% of all 39 evaluable patients). In addition, among the 19 patients in CCyR, 11 (28.2% of all 39 evaluable patients) achieved a molecular response (major molecular response in 5 patients and complete molecular response with undetectable BCR/ABL hybrid gene at qualitative nested PCR in 6 patients). After a median follow-up of 17.5 months (IR 8.4 – 49.5), 8 patients have died (1 from disease progression and 7 from unrelated causes), 2 patients were lost to follow-up and 34 are still alive: 2-year and 5-year overall survival were 85.1% (CI95% 71.5 - 98.7) and 67.3% (CI95% 45.9 - 88.5), respectively. In conclusion, in very elderly CML patients also a reduced dose of Imatinib appears to be safe and effective enough to achieve sustained cytogenetic and molecular responses and prolonged overall survival; thus, no upper age limit should be given and also very elderly (and with concomitant severe diseases) patients should have this chance of cure.

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SHEPHERDIN-DEPENDENT SUPPRESSION OF SURVIVIN INDUCED BY A BCR-ABL/JAK2/STAT3 PATHWAY SENSITIZES IMATINIB RESISTANT CML CELLS TO DIFFERENT DRUGS

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The BCR-ABL oncoprotein of Chronic Myelogenous Leukemia (CML) displays constitutive tyrosine kinase activity leading to the activation of different pathways that favour cell proliferation and survival. Treatment of CML cells with Imatinib Mesylate (IM) suppresses BCR-ABL catalytic activity reverting the anti-apoptotic phenotype of the leukemic cells. Survivin (SVV) is a member of the inhibitor of apoptosis proteins that is highly expressed in most forms of human cancer causing reduced sensibility to multiple death stimuli. Previous evidence has suggested that BCR-ABL kinase activity induces SVV expression thereby reducing the sensitivity of the leukemic clone to multiple apoptotic stimuli. We wanted to ascertain the signaling pathway(s) triggered by the BCR-ABL kinase to induce SVV expression and determine whether reducing SVV levels increased the anti-proliferative activity of different cytotoxic compounds on IM-resistant CML cells. We found that inactivation of BCR-ABL catalytic activity decreased SVV levels confirming that, in CML cells, SVV expression relies on BCR-ABL constitutive tyrosine kinase activity. BCR-ABL-mediated up-regulation of SVV involved the JAK2/STAT3 pathway since silencing of either JAK2 or STAT3 caused a consistent reduction of SVV. In cells unresponsive to IM, SVV silencing failed to restore sensitivity to the drug, indicating that SVV is not directly involved in the development of IM resistance. Cell lines unresponsive to IM because of point mutations in the BCR-ABL kinase domain were highly responsive to HU after down-regulation of SVV. However, this was not the case in cells failing IM because of BCR-ABL gene amplification. Finally, incubation of IM-resistant cells with cell-permeable Shepherdin, a peptidomimetic compound that down-regulates SVV expression by preventing its interaction with heat shock protein 90, enhanced cell death induced by IM, HU, Ara-C and Doxorubicin. BCR-ABL kinase activity induces SVV expression in CML cells via the JAK2/STAT3 signaling pathway. Suppression of SVV fails to resensitize resistant cells to IM. However, the combination of Shepherdin with different cytotoxic drugs significantly increases the activity of these compounds on cells unresponsive to IM because of point mutations (including T315I) in the BCR-ABL catalytic domain.

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HIGH BCR-ABLIS EXPRESSION LEVELS AT DIAGNOSIS AND AFTER 3 AND 6 MONTHS OF TREATMENT ARE ASSOCIATED WITH AN UNFAVORABLE RESPONSE TO IMATINIB

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Imatinib mesylate (IM) has shown remarkable efficacy for the treatment of Chronic Myeloid Leukemia (CML) patients (pts) in the chronic phase of the disease. However, a growing number of pts either fail IM or develop intolerance to the drug. We wanted to identify molecular parameters precociously associated with IM response. We therefore examined the outcomes of the first 192 newly diagnosed CML pts accrued to the observational SCREEN multicenter study, receiving IM 400 mg daily. Median follow-up was 37 months (range 12-72). Responses were rated according to the European Leukemia Net 2009 guidelines. Peripheral blood samples were used for BCR-ABL determination by RQ-PCR according to the International Scale (IS) using either GUS (at diagnosis) or ABL (at every other time-point) as reference genes. According to the ELN criteria, 105 pts (54.7%) achieved an optimal response; 46 pts (23.9%) had a suboptimal response; 35 pts (18.2%) failed IM because of either primary (22 pts) or secondary (13 pts) resistance. 6 pts (3.2%) were intolerant to IM. Kaplan-Meyer estimates at 5 years for overall, progression-free (PFS), event-free (EFS) and failure-free survival (FFS) were 93.3%, 92.2%, 80.5% and 72% with incidences of complete cytogenetic response (CCyR) and major molecular response (MMR) 89.5% and 64.7%, respectively. When we clustered all subjects in optimal responders (ORs) and suboptimal/resistant (S/R) pts, we found that high BCR-ABLIS/GUS transcripts at diagnosis were associated with an unfavorable response to IM (p=6.33e-13). Moreover, high BCR-ABLIS/GUS transcripts at diagnosis significantly correlated with a lower probability of obtaining a CCyR (p=0.017) and lower rates of EFS (p=7.98e-7) and FFS (p=9.29e-10). As WBC counts were not significantly different between ORs and S/R pts, increased amounts of BCR-ABLIS/GUS transcripts were probably representative of the aggressiveness of the leukemic clone. We also observed that pts displaying >10% BCR-ABLIS after 3 months of IM or >1% BCR-ABLIS after 6 months of therapy had a significantly lower probability of achieving an optimal response (p=3.88e-9 and p=2.63e-16, respectively). We conclude that high levels

of BCR-ABLIS/GUS at diagnosis allow the rapid identification of CML pts that are unlikely to benefit from IM. Furthermore, failing to achieve BCR-ABLIS transcript levels <10% after 3 months or <1% after 6 months of IM significantly reduces the probability of subsequently obtaining an optimal response.

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EFFICACY OF TKI SECOND GENERATION IN CML-ADVANCED PHASE AS BRIDGE TO STEM CELL TRANSPLANTATION

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Pts presenting CML-Advanced phases (AdP) have a survival of 3-6 months and scarce response to imatinib. The main indication of SCT for CML is the advanced phases of disease. 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. We report here 10 pts affected from CML-AP who received Dasatinib prior to alloSCT. Donors were matched siblings (4), matched unrelated (4) or blood cord unit (2). 8 were male and 2 female with a median age of 36,4 (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 5 patients, Y253 and E255K in 4 patients, and a non codified mutation in 1 patient. Dasatinib induced complete hematological response (CHR) in 4 pts, and complete (n=2) and partial cytogenetic response (PCyR) (n=1) prior to SCT. 3 patients did not achieved a complete haematological response 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=7) or micofenolate association until +30 (n=3). Pts received a mobilized peripheral blood stem cell graft with 3,52–11,04x 10^6 CD34+ cells/kg (n=6), with bone marrow cells (1,7x106/Kg CD34 cells)(=1) and cord blood unit with 0,1x10^6 CD34+ cells/kg (n=3). Dasatinib was stopped 6 days before transplant procedure. 7/10 pts successfully engrafted reaching ANC > 0.5x109/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. 10/10 patients presented chimerism was 97–100%. Transplant related toxicities were grade I/II. No pts developed hyperbilirubinemia or VOD. Hyperacute extensive GVHD (Gr III) was observed in 2 pts at +9 and +26. Eight patients are alive, all of them in complete molecular response with a median follow-up of 14.8 (12–19) months, 2 died of aGVHD, 1 for infection. We may conclude 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. T315I positive patients are most alive in CHR. Dasatinib represents an efficient bridge to transplant to improve the outcome of this subset of patients.

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THE ROLE OF SPARC IN CHRONIC MYELOID LEUKEMIA AND IT COMBINATION WITH IM IN VITRO

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Background. Imatinib (IM) is the elective drug for chronic myeloid leukaemia (CML). However, a small percentage of patients may develop resistance to the drug. A recent report has demonstrated that an intracellular increase of SPARC (secreted protein acid and rich in cysteine) may be associated to resistance to IM in CML cells. However, SPARC is a multi function protein with different effect on different disease. It has been reported that in haematological malignancies, SPARC may have both a tumor suppressor (5q¬-MDS and AML) or a protumoral (multiple myeloma) function. Methods and Results. We measured SPARC in peripheral blood of CML patients and found that, at diagnosis, patients have low level of SPARC in the peripheral blood in respect to healthy controls ($p<0.001$ as mRNA and $p<0.05$ as protein) but, once treated with IM, they show a progressive increase of SPARC mRNA and protein, reaching level higher than normal controls after 12 months of therapy ($p<0.01$ as mRNA and $p<0.001$ as protein). At this time, all analyzed patients were in cytogenetic remission. In these conditions, CML cells are virtually absent in the peripheral blood of CML patients. Therefore, the SPARC mRNA that we have measured at 12 months is produced by normal cells, most likely monocytes, and not by CML cells. We next exposed K562 to human recombinant SPARC for 2 days and then to IM for 24 h. After 72 h SPARC and IM alone induced a mortality respectively of 18±3,2% and 29±1,6% compared to untreated cells. Their association resulted in additive effect with a mortality of 37,5±3,7%. After 96 h the combination SPARC/IM resulted more effective than IM alone with an increase of mortality of 16,5±3% ($p<0.01$ vs IM). Flow cytometry analysis evidenced an accumulation of K562 cells in G0/G1 after 24 h exposure to IM alone (15±1,7% vs untreated cells; $p<0.01$) or SPARC alone (14,5±4,1%; $p<0.01$). SPARC/IM combination showed an additive effect (26,5±3,3%; $p<0.001$). Conclusion. SPARC is reduced in CML cells at diagnosis. After IM treatment, SPARC is produced in large quantity by normal cells and it might contribute to the response because, in vitro, SPARC induces a G0/G1 cell cycle arrest, reduces growth rate of K562 cells and increases their sensitivity to IM.

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VERY DEEP MOLECULAR RESPONSES IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH NILOTINIB 800 MG DAILY: 3-YEARS RESULTS OF A A GIMEMA CML WP STUDY

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Background: Nilotinib (NIL) is a potent BCR-ABL inhibitor, approved by FDA and EMEA for the frontline treatment of Ph+ Chronic Myeloid Leukemia (CML). The ENESTnd trial showed superior efficacy of NIL vs imatinib (IM), with higher and faster molecular responses: by 24 months, 44%, 36% and 20% of patients treated with NIL 600, NIL 800 and IM 400 achieved a complete molecular response (CMR4: BCR-ABL/ABL ratio <0.01IS). Despite in vitro data support the resistance of Ph+ leukemia stem cells to TKI, some clinic experiences seem to suggest that a selected cohort of CML patients in CMR could be considered for drug discontinuation. Aims: To describe the dynamics of molecular response, included CMR, and to evaluate the 3-years outcome of CML patients treated frontline with NIL 800mg. Methods: A multicentre phase 2 trial was conducted by the GIMEMA CML WP (ClinicalTrials.gov.NCT00481052). Minimum follow-up: 36 months. Definitions: Major Molecular Response (MMR): BCR-ABL/ABL ratio < 0,1%IS; CMR: undetectable BCR-ABL transcript levels with ≥10,000 ABL copies; failures: ELN definitions; events: discontinuation for any reason. All the analysis has been made according to the intention-to-treat principle. Results: 73 patients enrolled; median age 51 years; 45% low, 41% intermediate and 14% high Sokal risk. The CCgR rate by 12 months was 100%. The overall estimated probability of MMR was 97%, while the rates of MMR at 12 and 24 months were 85% and 82%, respectively. The overall estimated probability of CMR was 79%, while the rates of CMR at 12 and 24 months were 12% and 27%, respectively. No patient achieving a MMR progressed to AP. Only one patient progressed at 6 months to ABP and subsequently died (high Sokal risk, T315I mutation). Five additional patients had an event: 3 patients, recur-

rent episodes of amylase and/or lipase increase (no pancreatitis); 1 patient, atrial fibrillation unrelated to study drug; 1 patient died for causes unrelated to study drug. With a median follow-up of 39 months, the estimated probability of overall survival, progression-free survival and failure-free survival was 97%, the estimated probability of event-free survival was 91%. Conclusions: The proportion of patients achieving a CMR is increasing. The high rates of responses are being translated into optimal outcome for most of patients. These findings promise for further improvements in CML care. *Acknowledgements: European LeukemiaNet, COFIN, Bologna University, BolognAIL*

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APPLICATION OF FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH) ON BONE MARROW AND PERIPHERAL-BLOOD SAMPLES FOR THE EVALUATION OF CYTOGENETIC RESPONSE TO IMATINIB IN CHRONIC MYELOID LEUKEMIA

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Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder induced by the fusion of the ABL gene with the BCR gene. The hallmark of CML is the translocation (9;22)(q34;q11). The translocation is observed in up to 100% of metaphase cells at diagnosis. After the introduction of the tirosyn kinase inhibitor imatinib mesylate, it has become more relevant to monitor cytogenetically the response to treatment. On the basis of chromosome banding analysis (CBA) of marrow cell metaphases, the cytogenetic response (CgR) is classified as none, minimal, minor, partial, or complete according to the percentage of Ph-metaphases (95%, 95-66%, 65-36%, 35-1%, and none). The achievement and the maintenance of a complete cytogenetic response (CCgR) is of particular importance because a CCgR is the most solid marker of progression-free survival and overall survival. The cytogenetic response by CBA requires marrow cells, which cannot be always sampled, and an adequate number of banded metaphases, which cannot be always obtained. Finally, cytogenetic analysis requires bone marrow harvesting. Fluorescence in situ hybridization (FISH) enables a rapid detection of chromosomal rearrangements, even on interphase cells, and thus, avoids the requirement of metaphase obtention and is applicable to a variety of cytological samples, including peripheral-blood samples (PB). Thus, FISH is used, with increasing frequency, as a substitute for CBA. Given the need for frequent monitoring in patients with CML, interest in non-invasive methods has increased over years. In this study, we investigated the usefulness and accuracy of FISH on peripheral-blood specimens for the evaluation of cytogenetic response in CML. We performed the comparison of cytogenetic analysis by CBA, by interphase FISH on bone marrow specimens and by interphase FISH on peripheral blood samples, simultaneously collected paired sets, from CML patients. The analysis was performed on 50 samples obtained from patients that were representative of the different cytogenetic response groups. Cytogenetic analysis by CBA were highly correlated with FISH analysis results on bone marrow and on peripheral blood samples.Thus, cytogenetic response might reliably be monitored by analyzing interphase peripheral-blood cells. This study confirms the results of other reports that suggested the feasibility of interphase FISH on peripheral blood specimens to monitor cytogenetic response.

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THE OCCURENCE OF MULTIPLE MYELOMA(MM)IN A PATIENT WITH CHRONIC MYELOID LEUKEMIA (CML) AND VON WILLEBRAND'S DISEASE (VWD): A CASE REPORT

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A 62-year old white male with VWD presented with a diagnosis of CML (Low Sokal Risk) and monoclonal gammopathy in January 2003. At the time of the diagnosis, bone marrow (BM) biopsy showed high cellularity with myeloid hyperplasia, 2% mature plasma cells (PC); cytogenetic analysis revealed the Ph chromosome in all of 20 metaphases examined [46,XY,t(9;22)(q34;q11)]; RT-PCR showed fusion of bcr and abl genes (b3a2). Immunofixation showed monoclonal IgG k (0.37 mg/dl) in serum and absence in urine.The patient was started on Imatinib at the standard dose of 400 mg/day, achieving a MMR at 24 months after treatment initiation. In April 2009 the BM aspiration revealed 19% PC infiltration and BM biopsy revealed 35% PC infiltration.These findings indicated the coexistence of CML and asymptomatic MM. His CML remained in MMR and normal karyotype (46,XY[20]) was found on cytogenetic evaluation. On January 2010 he developed low back pain and MRI was performed which showed the presence of pathological tissue at level D12-L1. At the same time, monoclonal gammopathy of the IgGk type was found at 0.7 mg/dl and BM biopsy revealed 50%pleomorphic PC infiltration. These findings indicated the progression to symptomatic MM. His CML remained in MMR and the chromosomal abnormality detected in our patient was a complex karyotype (52,XY, add(1p), +der(3),del(4q),?der(6),-7,-8,+9,+11,+14, +18, +19, +21, +m). He started treatment with a Bortezomib based regimen-VD-(thalidomide was contraindicated in view of the VWD) and Imatinib treatment was suspended. After 4 cycles of therapy Imatinib treatment was resumed and after 8 cycles of therapy the patient underwent stem-cell harvest and then front-line high dose intensity autologous stem cell transplantation. The CML remained in MMR and the MM showed a very good partial response. Discussion. Our patient developed MM after 75 months of treatment with imatinib mesylate. The association of MM and CML Ph+ has been considered to be mostly coincidental (an extremely uncommon event reported only in 12 cases), so an etiological correlation of the two disease entities (from a common pluripotent stem cell) could not be justified. Moreover there is no documented evidence that the administration of Imatinib can promote exclusive PC expansion resulting in MM. Observed interactions of Imatinib with novel agents used for the treatment of MM, such as immunomodulatory drugs or proteasome inhibitors, should be analyzed to improve the management of this rare disease concomitance.

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IMATINIB LONG-TERM EFFECTS STUDY: GLOBAL INDEPENDENT ASSESSMENT OF IMATINIB IN CHRONIC MYELOID LEUKEMIA: RESULTS AT 7 YEARS

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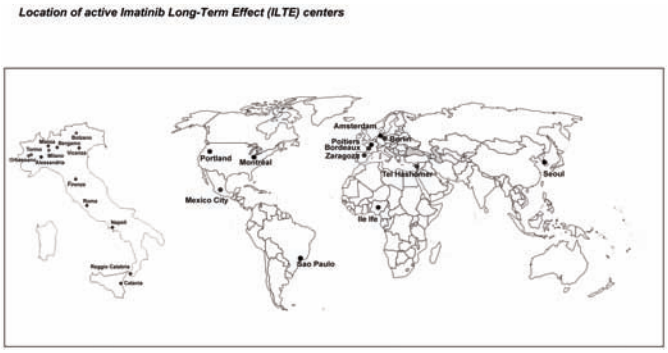
Imatinib slows development of chronic myeloid leukemia (CML). However, available information on morbidity and mortality is largely based on sponsored trials whereas independent long-term field studies are lacking. The independent, multicenter Imatinib Long Term Effects (ILTE)study assessed overall survival, loss of complete cytogenetic remission (CCyR), attainment of negative Philadelphia chromosome hematopoiesis assessed with quantitative polymerase chain reaction (PCR) , serious adverse events (SAE), and toxicities not qualifying as SAE (NSAE) but judged by treating physicians as substantially affecting quality of life. Consecutive CML patients who started imatinib before 2005 and who were in CCyR after two years were eligible. Overall survival, incidence of the first adverse events, and loss of CCyR were estimated according to the Kaplan-Meier method and compared with the

standard log-rank test. Cumulative incidence of death was broken down into incidence related or unrelated to CML, accounting for competing risks. Standardized incidence ratio were calculated based on population rates specific for gender and age classes. The results at Dec. 31st 2008 are as follows: a total of 832 patients were enrolled with a median treatment duration of 5.8 years. A comparison of the observed mortality rate in CML patients with the rate in the general Italian population showed no excess mortality. Twenty deaths were observed (6 CML-related), with a 4.8% mortality incidence rate (standardized incidence ratio = 0.7; 95% CI = 0.40 to 1.10). There were 139 recorded SAE, of which 19.4% were related to imatinib. Among the 830 NSAEs (which developed in 53% of patients), the most frequent ones were muscle cramps, asthenia (weakness), edema, skin fragility, diarrhea, conjunctival hemorrhages, osteoarticular pain and tendon or ligament lesions (68% were imatinib-related). Nineteen patients (2.3%) discontinued imatinib because of drug-related toxicities. Forty-five patients lost CCyR, corresponding to a rate of 1.4 per 100 person years. Durable (> 1 year) negative Philadelphia chromosome hematopoiesis, as evaluated by PCR was attained by 179 patients. In conclusion, CML-related deaths are uncommon in CML patients who are in CCyR two years after starting imatinib. Survival is not statistically significantly different from that of the general population. Side effects are present but generally not serious. Updated results at Dec. 31st 2009 will be presented.



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HEAT SHOCK PROTEIN 90 (HSP90) REGULATES THE EXPRESSION OF PREFERENTIALLY EXPRESSED ANTIGEN OF MELANOMA (PRAME) IN CELL LINES DERIVED FROM PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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PRAME is a tumor antigen overexpressed in various malignant tumors including solid tumors and many hematologic malignancies, but is absent on normal tissues, including hematopoietic progenitor cells. PRAME expression was found to correlate with disease progression in CML, since we demonstrated its increasing expression in blast crisis as compared to chronic phase CML patients. Moreover, several groups demonstrated that PRAME may significantly contribute to maintaining the tumor phenotype, since it is a strong inhibitor of all-trans retinoic acid (ATRA) receptor RAR , a crucial pathway for differentiation of both normal and malignant hematopoietic cells. We demonstrated that several CML and AML patients have high level of PRAME protein detected by Western Blot analysis, in spite of low PRAME mRNA expression, as assessed by Real-Time PCR. Thus, we speculate that PRAME protein might be protected from degradation by heat shock protein complex (Hsp90 and Hsp70). In particular, the Hsp90 is a molecular chaperone that play a critical role in the folding, maturation and conformational stabilization of several oncogenic proteins. We demonstrated that Ab-PRAME co-immunoprecipates Hsp90 protein and that Hsp70 cooperates with the chaperone protein Hsp90 to bind PRAME. These data were further confirmed by confocal immunofluorescence (CIF) microscopy that showed colocalization between PRAME, HSP70 and Hsp90. The importance of heat shock protein to stabilize PRAME protein was corroborated by data on the exposure of K562 cell line to 10μM of Hsp90 inhibitors, 17-AAG [17-(allylamino)-17-demethoxygeldanamycin] for 36h, that significantly reduced the level of PRAME. Interestingly, 17-AAG treatment significantly reduced leukemia cell line proliferation. In conclusion, our data seems to identify PRAME as a novel Hsp90 client and suggest that PRAME–Hsp90 interaction may have a crucial role to support leukemia cell survival. These findings may have significant implications for developing combinatory and effective therapies for leukemia patients and for offering a strategy to inhibit the oncogenic functions of PRAME by clinically available Hsp90 inhibitors.

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MESENCHYMAL STEM CELLS (MSC) DERIVED FROM BONE MARROW (BM) OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA PROTECT PH+ LEUKEMIC CELLS FROM TKI INDUCED APOPTOSIS.

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CML can be effectively treated with Imatinib, a drug rationally designed to turn off the tyrosine kinase activity of oncogenic BCR-ABL protein. In the 6-year follow-up of the International Randomized Study of Interferon vs. Imatinib (IRIS), the cumulative best complete cytogenetic response rate was 82% and the estimated event free survival at 8 years was 85%. Indeed, several in vitro data have confirmed that Ph+ CD34+ progenitor cells crammed in BM niches are resistant to TKI treatments. Thus, we cultured Ph+ cell lines (human chronic myeloid leukemia K562 and human B cell precursor BV173 cell lines) in the presence of TKI on a monolayer of MSC expanded from BM aspirates of 10 CML untreated patients, to assess the role of BM niche in the regulation of TKI responsiveness. We demonstrated that MSCs significantly protect Ph+ cells from TKI-induced apoptosis. Indeed, we detected in average 55%, 47% and 46% of vital K562 cells and 53%, 61% and 50% of vital BV173 cells after 1μM Imatinib, 100nM Nilotinib and 50 nM Dasatinib treatment for 72hr, respectively. In contrast, less than 5% of vital Ph+ cell lines could be detected after 72hrs of TKI exposure. Moreover, TKI treatment increased the expression of CXCR4 receptor (evaluated by flow cytometry analysis) in K562 cell lines only when cell line were exposed to MSC (23% of CXCR4+ K562 cells by Imatinib, 21% of CXCR4+ K562 cells by Nilotinib and 27% of CXCR4+ K562 cells by Dasatinib treatment for 72hrs). Notably, the SDF-1 and its cognate receptor CXCR4 (which expression is regulated by BCR-ABL) have emerged as critical mediators of stromal/leukemic cell interactions. Thus, we evaluate if the two major stromal-derived cytokines, i.e. SDF-1 and SCF, might be responsible for the CXCR4 expression regulation in K562 cell line treated with TKI and exposed to MSC. We demonstrated that TKI did not significantly modify CXCR4 expression in K562 cell line exposed to 100ng/ml of SDF1 and 5 ng/ml of SCF. Furthermore, both cytokines did not protect Ph+ cell lines from apoptosis induced by TKI exposure. Taken together, these findings indicate that CML BM-derived MSC produce a strong effect on the regulation of TKI responsiveness in Ph+ cells, and that it is not related to SDF-1 or SCF chemokines.

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MONITORING IN PAEDIATRIC PATIENTS WITH CHRONIC MYELOID LEUKAEMIA; EVALUATION OF THE LOW LEVELS OF QUANTITATIVE BCR-ABL TRANSCRIPT USING THE CEPHEID XPERT BCR-ABL MONITOR ASSAY™

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Background Molecular monitoring of BCR-ABL mRNA has become an important criteria for evaluating the response to treatment in chronic myeloid leukaemia (CML). The real-time quantitative polymerase chain

reaction (RQ-PCR) techniques require optimization for measurements of the BCR-ABL transcript. The assays differ among laboratories because several protocols with many intrinsic variables exist. For this reason, values can vary widely among molecular laboratories. Many European organizations have proposed recommendations to overcome comparability difficulties of results between centres. In addition, manual procedures are time-consuming and about three weeks is the time for reporting results to clinicians. That turnaround time could be desirable shorter. Patients and Methods Peripheral blood and/or bone marrow aspirate specimens were obtained from 5 paediatric CML patients on TKI therapy for an overall of 20 evaluations of molecular monitoring. We used the Cepheid Xpert BCR-ABL Monitor assay™ (Cepheid, Sunnyvale, CA) to assess the BCR-ABL and ABL (endogenous control) transcripts. The GeneXpert evaluation was performed comparing the quantitative BCR-ABL transcript levels determined from the Italian reference laboratory of Turin, in a duplicate sample of our patients. Results The mean value of BCR-ABL transcripts was 0,09 ± 0,89 according to the reference method and 0,12 ± 0,11 with the Cepheid Xpert BCR-ABL Monitor assay™ . The differences between methods were evaluated by Bland Altman statistical method. No significant bias was found = -0,022 (95% CI from -0,050 to 0,005). Statistical analysis, performed by the Passing Bablock method, showed an intercept = -0,006 (95% CI from -0,022 to 0,010), slope= 1,305 (95% CI from 0,917 to 1,764), and the Cusum test for linearity showed no significant deviation from linearity (P>0.10). The final results showed a good correlation with those obtained by using the reference method, converted to IS (Figure 1). Conclusions The Xpert BCR-ABL Monitor assay™ covers a lower concentration range. The method seems reliable for detecting the BCR-ABL transcript in CLM molecular monitoring. The short assay time could facilitate clinical decision for the TKI treatment.

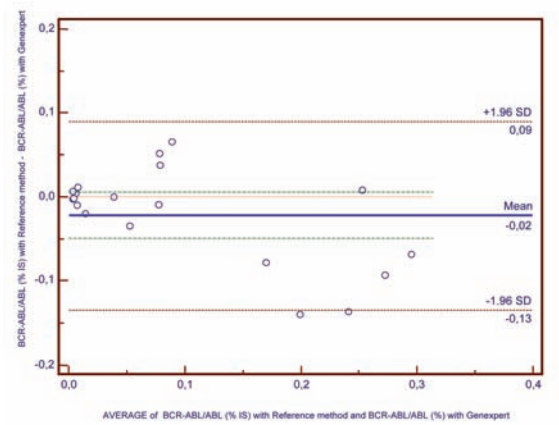

Figure 1. Comparison of the GeneXpert and the reference method for the quantification of relative ratios of BCR-ABL and ABL gene transcripts from clinical samples.

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DELETION AND INSERTION EVENTS IN TYROSINE KYNASE DOMAIN OF BCR-ABL IS A COMMON PHENOMENON IN CHRONIC MYELOID LEUKEMIA.

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Resistance to TKI therapy has been frequently associated with BCR-ABL TK domain point mutations in patients with CML. In the last years several cases of patients expressing ABL deletion/insertion mutants have been reported. A total of 830 patients affected by CML were treated with TKI and were classified as suboptimal/failure responders according the ENL recommendations. The ABL sequences of oncogenic Bcr-Abl gene in 230 out of 830 were analyzed by DHPLC and Sequencing. We detected ABL mutations in 118 out of 230 (51%) tested patients: 80% of them report single nucleotide modifications in ABL TK domain, whereas the remaining patients show ex7-9 ABL deletion (19%) or insertion (1%). Among these two kinds of patients, 16 and 6 patients were in early and late chronic phase, respectively. Deletions and insertions of ABL and confirmed by ARMS-PCR. Based on wt BCR-ABL crystallography, mutated sequences were analyzed by homology modeling both to generate 3D structures and to predict TKI bindings. Homology modeling showed that 15 deletion and one insertion events caused frameshift and early truncation of ABL with loss of 3 out 5 Imatinib and all two Dasatinib H-bonds. The same phenotype was observed in two patients with a complex BCR-ABL rearrangement (ex4-9 ABL deletion and ex13 BCR insertion). Noteworthy, we observed ABL D363-R386del in 4 patients and homology modeling reveals that the entire activation loop have been lost, without modification in Dasatinib H-bonds. Moreover, we identified some mutations that do not modify TKI interactions in ABL: one patient reported ABL t1143del causing a C-Terminal truncated ABL protein; another had simultaneously insertion of ABL 293-1P and Lys294Gln exchange. In this last case, the insertion seems to attenuate the Imatinib- bond power. Indeed, the ins293-1P clone became predominant during Imatinib therapy, whereas it disappears during Dasatinib treatment. Notably, the TK domain is significantly altered in the majority of the detected ABL deletion/insertion alterations, suggesting that ABL oncogenic activity is strongly compromised, and that Ph+ mutated cells are prone to add further alterations giving survival advantages to the leukemic clone. In conclusion, screening for deletion/insertion ABL mutation associated with homology modeling analysis to predict TKI binding, may be an experimental tool to help tailoring therapy for patients with CML.

CELL THERAPY AND ALLOGENEIC TRANSPLANTATION II

P279

PREDICTING CHRONIC GRAFT VERSUS HOST DISEASE ON DAY +100 AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background and aim of the study : We have previously shown that chronic graft versus host disease (cGvHD) and transplant related mortality (TRM) can be predicted by three laboratory values on day +100: platelets count (Plt), serum cholinesterase (CHE), gamma-glutamyltransferase (g-GT). The aim of this study was to confirm this result in a larger number of patients. Patients and methods: The patient population consisted of 1008 consecutive patients with hematologic disorders allografted between 1990 and 2008, alive on day +100 and free of cGvHD. The donor was an HLA identical sibling (n=740) or an alternative donor (n=268). The median follow-up was 1288 days (range 100-7237). The 3 previously described indicators (Plt, CHE, g-GT) remained significant. Additional indicators were serum albumin and total iron binding capacity (TIBC). Median levels at day +100 were used as cut off: Plt 100x10^9/L, CHE 3500 IU/ml, g-GT 50 IU/L, TIBC 205 mg/dl, and albumin 3.9 gr/dl. Two prognostic groups were identified: low risk (0-2 negative predictors), and high risk (3-5 negative predictors). Results: The overall risk of developing moderate/severe cGVHD was 29% and the risk of TRM beyond day +100 was 19%. The two risk groups identified patients at different risk of cGvHD (24% vs 35%,p=0.0002) and of TRM (11% vs 28%, p <0.0001, see table1). The actuarial survival of the 2 groups at 10 years was 66% vs 43% (p <0.0001). In multivariate analysis, including donor/recipient data, phase of disease, donor type, and year of transplant, the RR for patients with high day+100 score was: RR 1.97 for overall mortality, 2.1 for cGvHD and 3.0 for TRM. Other predictors were year of transplant, donor age, disease phase, recipient gender and donor type. Conclusions: The use of 5 laboratory tests on day +100 (Plt, CHE, g-GT, Albumin and TIBC) predicts cGvHD and mortality, with a greater accuracy as compared with our previous day +100 score. Strategies to prevent cGvHD and delayed mortality may include immune-modulation with/wo intensified infection prophylaxis in high risk patients.

	low risk	high risk	p	RR
TRM	11%	28%	<0.0001	3.0
cGVHD	24%	35%	0.0002	2.1
Overall survival	66%	43%	<0.0001	
Relapse related death	20%	23%	ns	
TRM (cGVHD +/- infections)	8%	21%	<0.001	
Overall mortality				1.97

Table 1: comparison between high risk and low risk patients according to the score

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EPSTEIN-BARR VIRUS (EBV)-DNA MONITORING STRATEGY AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: RISK FACTORS AND OUTCOME OF EBV REACTIVATION.

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Objectives: We analysed risk factors and outcome of Epstein-Barr virus (EBV) reactivation and EBV-associated post-transplant lymphoproliferative disorder (PTLD) in 100 patients with hematologic malignancies who underwent allogeneic hematopoietic stem cell transplantation (HSCT) and that were prospectively monitored for EBV-DNA between January 2008 and December 2010. Patients and methods: Whole-blood EBV-DNA level was measured weekly in the first month after HSCT and at 2-week intervals for at least 6 months. Table 1 summarizes patients' and transplant' main characteristics. Pre-emptive rituximab was administered in cases with viral load > 10000 cp/ml. Results: After a median observation time of 7 months (range 2-32), 39 patients (39%) experienced EBV reactivation at a median of 60 days after transplant (range: 20-826) with a median viral load of 3244 cp/ml (range 280-1432127); viral load was >10000 cp/ml in 16 cases. Transplants from HLA-mismatched donors and T-cell depletion with antithymocyte globulin (ATG) were significantly associated with EBV reactivation (p=0.005 and p=0.016, respectively). Patients receiving low-dose ATG (ATG-Fresenius 10 mg/kg) had significantly lower EBV reactivation than those who received standard-dose ATG (ATG-Fresenius 30 mg/kg or Thymoglobulin Sangstat/Genzyme 7.5 mg/kg) (p<0.001). Finally, EBV positive patients had a significantly lower CD4+ count at day +30 (p=0.001). Thirty-one out of 39 (79%) EBV-positive patients never had symptoms or signs of PTLD. Of them, 22 underwent reduction of immunosuppression, the other 9 received also pre-emptive therapy with weekly rituximab (median, 2 doses); none of them developed PTLD and all achieved EBV-DNA negativity. Eight patients (21%) had clinical evidence (4 cases) or biopsy-proven (4 cases) PTLD at the time of EBV detection. Three out of 8 died from PTLD, while the other 5 achieved long-term remission after 1 or more rituximab ± chemotherapy (vincristine, CHOP). An EBV viral load > 10000 cp/ml was predictive of PTLD development (p=0.007). Overall, EBV reactivation did not significantly affect survival nor transplant-related mortality. Conclusions: ATG use, mismatched graft and lower CD4+ count at day +30 were significantly associated with EBV reactivation. Despite of a strict EBV-DNA monitoring strategy, 8% of our patients developed PTLD, suggesting the opportunity of rituximab administration as prophylaxis in patients at high-risk of EBV reactivation.

EBV reactivation **N° of patients**	**NO** **(n=61)**	**YES** **(n=39)**
Age (y), median (range)	50 (22-70)	51 (20-68)
Sex (M/F)	34/27 (56/44%)	26/13 (67/33%)
Diagnosis		
Acute leukemia	28 (46%)	18 (29%)
Lymphoma /chronic leukemia	18 (46%)	12 (31%)
Multiple myeloma	9 (15%)	6 (10%)
other	6 (15%)	3 (8%)
Disease status at transplant		
Complete response	26 (43%)	23 (59%)
Partial response	20 (33%)	8 (20,5%)
Resistance/progression	15 (24%)	8 (20,5%)
Donor		
HLA-identical sibling	23 (38%)	9 (23%)
Matched unrelated	18 (29%)	6 (15%)
Mismatched unrelated	20 (33%)	24 (62%)
Stem cell source		
Bone marrow	14 (23%)	7 (18%)
Peripheral blood	47 (77%)	32 (82%)
Conditioning		
Myeloablative	22 (36%)	11 (28%)
Reduced-intensity	39 (64%)	28 (72%)

ATG	36 (59%)	32 (82%)	
High-dose ATG	21 (34%)	29 (74%)	
ATG-F		25 (41%)	15 (38%)
ATG-S		11 (18%)	17 (44%)
CMV reactivation	25 (41%)	21 (54%)	
Acute GvHD - grading			
Gr. 0-I	40 (66%)	23 (59%)	
Gr. II	17 (28%)	6 (15%)	
Gr. III	2 (3%)	8 (21%)	
Gr. IV	2 (3%)	2 (5%)	
Total lymphocytes count			
+30 days	436	367	
+90 days	626	480	
CD4+ lymphocytes count			
+30 days	84	30	
+90 days	131	62	
Chronic GvHD - grading			
Mild	10 (16%)	6 (16%)	
Extensive	9 (15%)	9 (23%)	

Table 1. Patients' and transplant' characteristics.

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SEQUENTIAL MOLECULAR MONITORING OF CHIMAERISM STATUS IN HAPLOIDENTICAL, UNMANIPULATED BONE MARROW TRANSPLANTATION FOR HAEMATOLOGICAL MALIGNANCIES

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Background: The unmanipulated haploidentical-BMT represents a new and very promising transplant procedure for patients lacking a HLA-matched donor and on urgency for high-risk haematologic malignancy. Here, we describe the sequential molecular monitoring of chimaerism status in 44 patients undergoing such haploidentical transplant. Materials and Methods: 44 patients (median age 38 yrs,15-61) with high-risk haematologic disorders underwent unmanipulated, haploidentical, G-CSF primed BMT: for AML(n=25), ALL (n=7), CML (n=2), NHL (n=2) HL, (n=4), plasmacell leukaemia (n=3) and severe aplasia (n= 1) Thirty-two out of 44 patients received a myeloablative regimen (MAC) and 12 a reduced intensity conditioning (RIC). All 44 patients received an identical GvHD prophylaxis. Donors were primed with G-CSF at dose of 4 mcg/Kg/d for 7 consecutive days and BM was infused unmanipulated at day 0. Molecular monitoring of chimaerism was performed by "STR fingerprint" analysis both at early stages and during the follow up. Results: median dose of total nucleated, CD34+ and CD3+ cells was 7x10e8/Kg (1.01-28.7), 4.3x10e6/Kg (1.17-11.48) and 47.3x10e6/Kg (2.5-97.8), respectively. The cumulative incidence (CI) of neutrophil engraftment was 90% at 30 days. At day 15, the chimaerism status was complete (CC) in 20 patients, while the remaining 11 showed a transient mixed chimaerism (MC). At day 30, 27 out of 31 evaluable patients showed a CC status. Two of the remaining 4 patients converted to CC at day 60 , while 2 with persisting MC died of transplant-related mortality (TRM) at days 160 and 365, respectively. No difference of molecular chimaerism was detected between recipients of MAC and RIC regimens. All 12 patients transplanted after RIC regimen showed a CC status at day 30. Acute GvHD was grade 0-1 in 28 patients (70%), grade 2 in 9 (22%) and grade ≥3 in 4. In 24 evaluable patients, chronic GvHD was limited in 2 (9%) and extensive in 2 (9%). With a median follow-up of 18 months (range2-48), 12 of 17 (75%) patients transplanted in early disease stage and 11 of 27 (40%) in advanced phase are surviving in haematological remission with persistent complete molecular chimaerism. Conclusion: high engraftment rate with full donor hematopoietic reconstitution, low incidence of grade 2-4 acute GvHD and an acceptable TRM suggest that G-CSF-primed, haploidentical, unmanipulated bone marrow transplant represents an excellent alternative for high-risk patients who lack a HLA matched donor.

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ALLOGENEIC STEM CELL TRANSPLANTATION AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION RELAPSE IN DIFFUSE LARGE B CELL LYMPHOMA PATIENTS: A RETROSPECTIVE GITMO STUDY

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Autologous hematopoietic stem cell transplantation (AHSCT) has been shown to be an effective therapy for patients (pts) with aggressive lymphoma. Patients who relapse after an autologous hematopoietic stem cell transplantation have a very poor prognosis. We have retrospectively analyzed diffuse large B-cell lymphoma patients in the GITMO database who performed an allo-SCT after an auto-SCT relapse. Analyzing GITMO data-base, from 1995 to 2008 in 93 Italian transplant Unit, 3449 patients were treated with autologous stem cell transplantation. After a median observation period of 12 months 293 patients were lost to the follow-up and 884 patients relapsed or progressed after autologous transplant . Considering these 884 patients, 165 patients (19%) were treated with allo-transplant. This 165 patients were considered and the last follow-up was July 2009. The median interval between auto-HSCT and allo-SCT was 13 months (range 3-128 months). One hundred and eight patients (65%) received transplants from an HLA-identical sibling and 57 (35%) from an unrelated donor. A reduced intensity conditioning regimen was used in 116. After allo-SCT 72 patients (43%) obtained a CR and 9 a PR with an ORR of 49%; 84 patients (51%) showed a rapid progression of disease. Ninety-one patients died, 45 due to disease and 46 to treatment related mortality. Acute graft versus host disease was recorded in 57 patients and a chronic GvHD in 38 patients. With a median follow-up period of 24 months (2-144) from allo, the Overall Survival (OS) was 39% and after a median period of 21 months (2-138) from allo the Progression Free Survival (PFS) was 32%. In a multivariate analysis, the only factor which affected OS was the status at allo-SCT and for PFS status at allo-SCT and stem cell donor. In conclusion it is apparent from the present study that about one fourth of patients with DLBCL who experience relapse after autologous transplantation may be treated with an allogeneic transplantation. This retrospective analysis confirms that the only one parameter affecting either OS or PFS was the response status at the moment of all-HSCT and does not confirm that RIC could permit to obtain better results in aggressive lymphoma. Probably a myeloablative conditioning should be reconsidered in pts with aggressive disease because of the slow-acting graft versus lymphoma effect is overriden by the rapid growth of the tumor.

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PERSISTENCE OF THE BCR/ABL GENE IN GREATER THAN 10-YEAR SURVIVORS OF MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA

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Allogeneic hematopoietic stem cell transplantation (HSCT) was the only curative therapy for patients with chronic myeloid leukemia (CML) prior to the advent of tyrosine kinase inhibitors. Even if studies on the long-term outcome of HSCT for CML have shown very rare relapses after 10 years from transplant, a late molecular assessment of the disease has never been evaluated. With the aim of verifying whether patients who survive for many years after a myeloablative HSCT from an HLA identical sibling, apparently free of CML, may have residual leukemic cells, we performed a molecular assessment of the BCR-ABL1 gene, by quantitative real-time polymerase chain reaction (RQ-PCR) analysis, from peripheral blood, in all surviving patients transplanted at our Institution between 1984 and 1999. Overall, out of 51 patients, 8 refused to participate in the study, 3 were not traceable and 4 patients had developed an hematological relapse. The RQ-PCR analysis was thus performed on 36 patients, with a median follow-up from transplant of 220 months (range 127-327), and in continuous hematological remission lasting more 10 years with a negative cytogenetic analysis monitored during the first 5 years after transplant. RQ-PCR resulted positive in 7 patients (19%) with a BCR-ABL/ABLIS % ranging between 0.008 and 62 (median 0.2). Six patients were in remission at a median of 18.5 years after transplant (range 12-21), while 1 patient had had an hematological relapse 24 months after transplant but had obtained a second 16 year cytogenetic remission following donor lymphocyte infusion. At 21 years after transplant the patient, who showed a BCR-ABL/ABLIS % of 62, concomitantly presented a previously undetected increase in the number of platelets and white blood cells (CML hematological relapse). The 6 patients with a low number of copies and a normal blood cell count were monitored with qRT-PCR every 3 months: molecular evidence of disease persisted in 3 cases and disappeared in 2; 1 patient developed a CML hematological relapse (12 years after transplant). Both patients with CML hematological relapse received imatinib obtaining a prompt molecular remission. In conclusion, our study shows that residual leukemic cells, identified by molecular analysis, may persist in CML patients long after an allogeneic HSCT. This phenomenon is of unknown prognostic significance, but molecular surveillance in long-term survivors may answer questions regarding patterns of late relapse and possible therapeutic strategies.

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ALLOGENEIC STEM CELL TRANSPLANTATION IN 45 ELDERLY PATIENTS WITH POOR PROGNOSIS ACUTE MYELOID LEUKAEMIA OR MYELODYSPLASTIC SYNDROME: RESULTS OF REDUCED-TOXICITY CONDITIONING REGIMEN.

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Background: allogeneic (allo) stem cell transplantation (SCT) from a matched related (MRD), matched unrelated (MUD), mismatched related (MMRD) or cord blood (CB) donor, is the only curative strategy for poor prognosis acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). Patients (pts) older than 60 are not routinely offered an alloSCT as the procedure is considered highly toxic. Aim: evaluation of feasibility and efficacy of alloSCT in elderly AML and MDS pts, after reduced-toxicity treosulfan-based conditioning protocols. Methods: from 10/2002 to 11/2010, 45 pts, median age 64 (60-72). Diagnosis: de novoAML 16, sAML 13, therapy-related AML/MDS 4, MDS 12. Donors: MRD 10, MUD 9, MMRD 24, CB 2. Disease status at SCT: CR1 12, CR2 9, upfront 7, refractory 8, relapsed 9. Conditioning regimens contained fludarabine in 44 cases, treosulfan in 40, melphalan in 3, thiotepa in 2, cyclophosphamide and busulfan in 1 case, ATG in 34 cases. GvHD prophylaxis: T-cell depletion in 11 cases, CSA/MTX in 24, rapamycin/MMF in 10. All pts received SCT from peripheral blood. Results: 38 of 40 pts (95%) evaluable at day +30 were in CR, included 21 out of 26 (81%) not in CR at SCT. Mortality (TRM) within day +100 was 20%. Relapses: 12 (31.5%). With a median FU of 617 (30-1659) days 16 pts (36%) are alive in CR. Median EFS from SCT of all pts is 227 (3-2289) days. Of 14 pts older than 65, 4 (28.5%) are alive in CR, median EFS is 159 (14-2289) days. Conclusions: alloSCT is feasible in elderly pts with poor prognosis AML/MDS. Mortality rates are largely inferior to the advantage provided by allogeneic immunotherapy in leukemia free-survival, as compared to recent chemotherapy-only treatment protocols (Lowenberg B et al, NEJM 2009). Age should not be per se a limiting factor in patients candidate to alloSCT.

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SEQUENTIAL CONDITIONING FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLOHSCT) IN PATIENTS WITH PRIMARY INDUCTION FAILURE (PIF), REFRACTORY OR RELAPSED ACUTE LEUKEMIA. A SYSTEMATIC REVIEW

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AlloHSCT of a PIF Acute Leukemia patients is associated with poor outcome and currently in Italy a MUD search is not allowed if CR is not achieved. Schmidt et al. (JCO 2005, Blood 2006) reported 2 large series of relapsed or refractory pts transplanted after a SC. SC for alloHSCT is a conditioning during the cytopenic phase induced by a cytoreductive chemotherapy for refractory hematological malignancies in order to reduce tumor burden and to perform early the transplant. Aim of the review is to collect literature data to support early donor search for PIF patients. A systematic review was performed using an highly sensitive bibliographic search performed by 2 independent reviewers. From each paper were extracted the type of study, participants, disease status, type of SC, immune-suppressive regimen, type of donor and stem cell source, planned donor lymphocyte infusions. The following outcomes were evaluated: engraftment, toxicities, disease response, mortality, OS, DFS, aGVHD and cGVHD. Ten studies including 589 enrolled patients were found. No RCT were found. Inclusion criteria were PIF or relapsed or refractory leukemia. Median age was between 30 and 55. SC consisted in different combinations in the cytoreductive phase (fludarabine, cytarabine and amsacrine, high dose melphalan, daurorubicin and cytarabine or the sperimental drug CPX381) and different subsequent conditioning regimens (mainly non myeloablative). In 6 studies TBI was part of the SC. MUD and PB stem cells were used in the majority of cases. Prophylactic DLI were planned in 3 studies. Engraftment and full chimerism was obtained in more than 90% of the patients. Grade III-IV toxicity was reported between 13 and 62%. SC was able to induce CR in 65%-100% of patients and relapse rate was 20-47%. TRM at 100 days was between 18 and 35%. OS was reported between 85% at 100 days and 45% at 7 years and ranged between 22 and 67% at 2 years. Acute GVHD was diagnosed more than the half of patients and chronic GVHD was diagnosed in 33 to 54% of patients. Median follow-up was 4.6 to 41 months. Better OS was associated with GVHD, non secondary AML, higher CD34+, PIF and use of a MUD. Despite the absence of controlled studies and several biases, SC is a valid option for PIF and high risk relapsed or refractory acute leukemias. Donor search should be performed as soon as possible, even if complete remission is not achieved and outcome of early transplantation with SC in PIF is promising.

Study	Patients	Toxicity (III-IV) (%)	Disease Response (%)	Relapse Rate (%)	TRM (%)	OS	AGVHD	CGVHD	F-UP (mo)
Qi Fa BBMT 2009	51	13	94	27	35	58 (1y) 45 (7y)	50	50	41
Kreiji EBMT 2008	50	N.A.	91	37	24 (1y)	44 (9m)	62	54	21
Huyn EBMT 2010	74	N.A.	N.A.	47	21	22 (2y)	39	19	...
Von Bonin EHA 2009	26	62	65.4	N.A.	N.A.	52 (1y)	N.A.	N.A.	8.2
Schmid JCO 2005	75	35	92	15 pts	20 (100d) 33 (1y)	42 (3y)	61	45	31
Schmid Blood 2006	103	52	91	35 pts	11 (100d) 17 (1y)	32 (4y)	63	33	25
Steckel EBMT 2009	50	N.A.	98	N.A.	34 (2y)	67 (2y)	N.A.	N.A.	N.A.
Kobbe EBMT 2009	116	N.A.	N.A.	42	N.A.	41 (2y)	N.A.	N.A.	23
Tsitsikas ASH 2010	33	N.A.	N.A.	N.A.	18	45	4 pts	N.A.	13
Gergis ASH 2010	11	2 pts	N.A.	N.A.	N.A.	85 (100d)	3 pts	N.A.	7.5

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THE INFUSION OF SUICIDE GENE-MODIFIED DONOR T CELLS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION PROMPTS THYMIC RENEWAL IN ADULT PATIENTS BY AN IL-7 DEPENDENT MECHANISM

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In haploidentical Hematopoietic Stem Cell Transplantation (HSCT), the infusion of donor lymphocytes genetically modified to express the Herpes Simplex Virus Thymidine kinase (HSV-Tk) suicide gene allows GvHD control, while rapidly providing an effective and polyclonal T cell repertoire against pathogens and underlying malignancies (Ciceri and Bonini et al., Lancet Oncology, 2009). In the TK007 phase I/II clinical trial 28 adult patients with hematologic malignancies received purified HSV-Tk-transduced cells after T cell-depleted HSCT, and 22/28 experienced a rapid and stable T cell immune reconstitution. Even though their engraftment is necessary to achieve these effects, HSV-Tk+ cells represent the minority of lymphocytes circulating in treated patients. Therefore, we hypothesized an indirect role of HSV-Tk+ cells in prompting T cell development from graft progenitors by a thymus-dependent pathway. T cell reconstitution of treated patients demonstrated recovery of naive T cells, mostly negative for the HSV-Tk transgene. The newly reconstituted CD4+ naive T cells were almost entirely comprised by CD31+ recent thymic emigrants. Accordingly, CT scans documented an increase in thymic volume and single joint T cell Receptor Excision Circles counts rose following HSV-Tk cell add-backs. Comparison with a cohort of patients subject to T-cell replete HSCT further suggested a unique direct role of HSV-Tk+ cells in promoting thymopoiesis. The newly generated HSV-Tkneg T cells granted full immune competence against infectious agents, which was not compromised in those patients in whom the suicide gene was activated to control GvHD. Interestingly, serum levels of IL-7 markedly rose after Tk-cell add-backs, suggesting that the genetically manipulated T cells may mediate the release of this stromal cytokine, in turn supporting the generation and maturation of T cells. Notably, in the absence of HSV-Tk cell engraftment, no increase in IL-7 serum levels was observed and patients did non achieve the immune reconstitution. These data show that the infusion of suicide gene-modified T cells induces IL-7 release, boosts the function of the adult thymus and prompts the recovery of a polyclonal, fully competent, T cell repertoire. A phase III clinical trial (TK008 study) to assess the efficacy of HSV-Tk+ cells in the context of haploidentical HSCT for leukemia started in 2010 in Italy, and is currently expanding to multiple centers throughout Europe.

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PROTEOMIC PATTERNS IN SALIVA OF PATIENTS SUBMITTED TO ALLOGENEIC STEM CELL TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCIES

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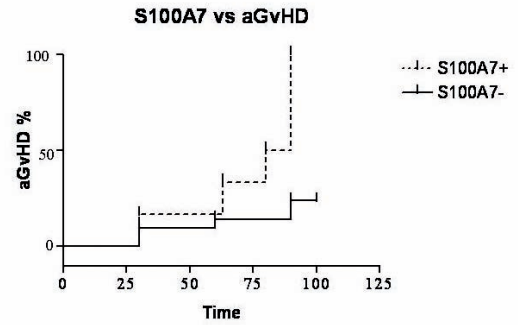
Graft versus Host Disease (GvHD) is one of the major life threatening complication after allogeneic stem cell transplantation (SCT), which develops in 30-80% of all SCT despite immunosuppressive prophylaxis. Diagnosis of GVHD is mainly based on clinical features. Recently the application of proteomic tools, allowing screening for differentially expressed or excreted proteins in body fluids, could allow detecting specific biomarkers and whole saliva is highly attractive for the non-invasive specimen collection. In this aim we collected salivary specimens of 27 patients (pts) submitted to alloHSCT between December 2005 to September 2010 in our Institution. They were: 13M/14F, median age 43 years (range 16-61). Underlying diseases were: 14 AML, 4 ALL, 3 MM, 1 LLC, 3 LNH, 2 MDS. Graft was obtained from sibling donor and MUD in 16 and 11 pts respectively. GvHD prophylaxis was performed with CSA-MTX in 13 pts, CSA-MMF in 10 pts; CSA and Campath in 1 pt and 3 pts received no GVHD prophylaxis. Samples of whole saliva were mixed immediately in a 1:1 (v/v) ratio with aqueous 0.2% trifluoroacetic acid solution and centrifuged at 8,000 g at 4°C for 5 min. The obtained solutions were analyzed by High-Performance Liquid Chromatography (HPLC) coupled to ElectroSpray-Ionization Mass Spectrometry (ESI-MS). Eleven out of 27 pts (40%) developed aGVHD, involving oral mucosa. The chromatograms of the acidic soluble fraction of salivary proteins of pts with aGVHD were compared to the asymptomatic ones. Different expressions of S100A8 (calgranulin A, M average 10834 Da; elution time 37.0 min) and S100A7 (psoriasin; E27 D variant, M average 11368 Da; elution time 40.4 min) were observed: 6 pts out of 11 with GVHD showed the presence of S100A8, while the protein was detectable in the HPLC-ESI MS profile of only one pt without GVHD (p 0.04). S100A7 was absent in pts without GVHD and detectable in 6 pts out 11 with GVHD (p 0.009). S100A8, together with S100A9 (calgranulin B), is part of an etero-dimeric leukocyte-derived protein called calprotectin, which is over-expressed in oral mucosal inflammation. S100A7 is highly expressed in keratinocytes derived from psoriatic skin and it is chemotactic for CD4+ T cells. We found a statistically significant association between the presence of these proteins and the development of aGVHD, but further studies should clarify if these proteins could be considered either a marker of GVHD or an index of mucosal inflammation.



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INCREASED SERUM CONCENTRATION OF THE INFLAMMATORY CHEMOKINES IL-8, MIP1-ALPHA AND IP-10 AT 3 MONTHS AFTER ALLOGENEIC HSCT CORRELATE WITH THE DEVELOPMENT OF CHRONIC GVHD.

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Background: Although chronic GVHD (cGVHD) is the major cause of long term morbidity after allogeneic HSCT, no biomarkers have been identified to predict its development. We recently observed that patients with chronic GVHD have increased numbers of activated donor Antigen Presenting Cells (APC) in the PB and BM (Arpinati et al. Transplantation 2008; 85: 1826-32). The aim of the study was to detect whether the measurement of APC number and function early (i.e. at 3 months) after transplant may predict the development of cGVHD. Patients and methods: 27 consecutive patients transplanted between January 2007 and July 2009 were enrolled at 3 months after transplant, provided they were in complete remission and free of chronic GVHD. Their PB was assayed for: APC (CD14+ and CD16+ monocytes, myeloid and plasmacytoid and CD16+DC) and lymphocyte (B, NK, CD4 and CD8+ T cell) numbers; expression of costimulatory (CD86), and adhesion (CD49d and CD11a) molecules and chemokine receptors (CXCR4, CCR2 and CCR5) on the surface of monocyte subsets; phagocytic activity and production of the inflammatory cytokines TNFalpha and IL-12 by purified CD14+ monocytes; and serum concentration of chemokines implicated in APC trafficking (IL-8, IP-10, MIP1-alpha and 1-beta, MCP-1 and RANTES). Results: 8 patients developed chronic GVHD at a median of 24 days (range 7-339) after enrollement, resulting in an actuarial probability of 36%. Patients who did not develop chronic GVHD (n=19) were analysed as controls. Demographic and transplant-related variables were similar in the two groups. Chronic GVHD patients had increased serum concentration of IL-8 (median 149 vs 33 pg/ml, p=0.04), IP-10 (1.58 vs 0.35 ng/ml) and MIP1-alpha (detected in 6/8 vs 6/19 patients, respectively) while the concentration of MIP1-beta, MCP-1 and RANTES was sim-

ilar. Monocyte numbers, phenotype and function were similar in the two groups. Interestingly, cGVHD patients had decreased numbers of circulating mDC (p=0.02), pDC (p=0.005) and NK cells (p=0.009). Conclusion: the serum concentration of certain inflammatory chemokines may be increased before the clinical diagnosis of chronic GVHD. Further studies in a larger cohort will determine the accuracy of chemokine levels as biomarkers of chronic GVHD.

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LONG TERM STUDY OF THE IMPACT OF QUANTITATIVE MOLECULAR MONITORING OF BCR-ABL TRANSCRIPTS ON THE RISK OF RELAPSE OF CML AFTER ALLOGENEIC HSCT.

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Background: Monitoring of minimal residual disease through RT-PCR analysis of bcr-abl transcripts allows early detection of CML relapse after allogeneic HSCT. However, the introduction of more sensitive techniques, such as quantitative PCR, may result in decreased specificity, leading to false positive results. Patients and methods: In this study we reviewed the results of molecular analysis of bcr-abl transcripts in all patients with p210+ CML who underwent allogeneic HSCT from 1983 through 2007. Q-PCR analysis was started in 2002. Out of 189 patients, 87 patients had available Q-PCR data; of these, 63 patients with at least three separate Q-PCR data were included in the study. Median time to the 1st Q-PCR analysis was 2196 days (35-7823). Median age was 36 years (13-56), 62%/38% received transplant from a related/unrelated donor, 62% with BM. 32% were in accelerated phase (AP). Patients with at least one positive Q-PCR value (measured as a ratio of bcr-abl to abl of > 0.001) were classified as Major Molecular Remission (MMR) patients. Each event was defined as one or more consecutive positive results. Results: 60/63 patients are alive after a median follow up of 3693 days (898-9405). 6 have relapsed 2142 (1419-3746) days after transplant. 52 (83%) patients had at least one positive result (28 with a value of >0.01, 24 with a value of <0.01), whereas 11 (17%) had persistent undetectable transcripts. In MMR patients, 94 events occurred. 29 patients had only one event, while 6 had >3 events. In 10 patients, the event occurred within 1 year after transplant, whereas in 28 it occurred after >5 years. 6/52 MMR patients relapsed, as compared to 0/11 with persistent undetectable transcripts (p=0.19). Relapse did not correlate with the Q-PCR value, the number of events or the time to the event. Finally, of 46 MMR patients who did not relapse, 35 had undetectable transcripts at last follow up. Positive Q-PCR had low specificity (19%) and positive predictive value (12%) in predicting relapse after allogeneic HSC transplantation. Conclusion: Our data confirm that the detection of low levels of bcr-abl transcripts (as based on Q-PCR) has a poor accuracy in predicting relapse, and it should not be considered as the sole indication to start treatment. It appears that fluctuation of bcr-abl transcript levels is common as late as > 10 yrs after transplant, possibly suggesting the long term persistence of CML stem cells.

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FOLLOW-UP OF WT1 GENE EXPRESSION AS MONITORING OF MINIMAL RESIDUAL DISEASE IN 50 PATIENTS WITH ACUTE MYELOID LEUKEMIA FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION.

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Introduction.. Wilms Tumor gene (WT1) overexpression is described in several oncological diseases including acute leukemias. The majority of acute myeloid leukemia (AML) patients don't have a suitable specific molecular marker for monitoring minimal residual disease (MRD). Quantification of WT1 in bone marrow samples can be useful as a marker of MRD and can predict AML relapse. METHODS and Results. We have evaluated, sequentially and using a quantitative RT-PCR technique (Leukemia-NET method), WT1 expression in 50 consecutive AML patients that overexpressed WT1 at diagnosis and that underwent allogeneic stem cell transplantation (SCT) at our centre. The cDNA level of WT-1 was detected in bone marrow samples at diagnosis at the time of transplant and after the allogeneic SCT. Samples of diagnosis showed high WT1 expression levels in all cases with a mean of 5570 (SD 4055) copies of WT1/10000 Abl, median 4600 (range 658-23913) copies WT1/10000 Abl. At transplant 34 pts (68%) were in complete cytologic remission (CcR) and 16 (32%) had refractory or relapsed AML. Bone marrow samples from pts in CcR at BMT showed significantly lower WT1 expression levels (mean 88±130), compared to the samples from pts with relapsed or refractory disease (mean 5727±4265) (P<0,001). After BMT a rapid decline of WT1 expression levels was observed in all pts that achieved and/or maintained a condition of CcR, especially in those that were in CcR at SCT. After a median follow up of 11 mths from transplant, 10 out 50 pts relapsed (20 %) and all of them had high expression levels of WT1 before the cytological relapse. Three of these pts were successfully reinduced with DLI ± chemotherapy with a rapid reduction of WT1 levels. Conclusions 1)In our experience there is a complete concordance between WT1 expression levels (measured by quantitative RT-PCR) and status of AML before and after SCT. 2)Our study confirms that longitudinal quantitative evaluation of WT1 after SCT may be useful as a non-specific leukemia marker (NSLM) for monitoring MRD and as a predictor of AML relapse. 3)Based on these results cases with an increase of WT1 levels after SCT and without GVHD should be candidate to DLI and/or discontinuation of immunosuppressive therapy.

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CHRONIC GRAFT-VERSUS-HOST DISEASE AND RECONSTITUTION OF B-CELL COMPARTMENT AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Objectives of the study. We evaluated recovery and engraftment of B lymphocytes during the first 6 months after allogeneic stem cell transplantation (SCT) in patients with or without chronic Graft versus Host Disease (cGvHD). The final objective was to identify a possible correlation between the development of cGvHD and the B lymphocytes, in terms of B cell recovery, B cell chimerism and circulating levels of a B cell activating factor cytokine (BAFF). Patients and methods. We analysed peripheral blood samples from 82 consecutive patients, mean age 48 years (range 20-69), who received allogeneic SCT between 2008 and 2010. The hematopoietic chimerism was studied at day 90, 120 and 180 on whole PB, on immunomagnetically sorted CD19+ cells and on granulocyte fraction. We evaluated B, T and NK cells in PB by immunophenotyping at day 30, 90, 120 and 180. Moreover, we analysed serum levels of BAFF before transplant and then at day 90, 120 and 180. Results. Forty-three out of 82 patients developed cGvHD at a median of 5 months after SCT, with at least 2 organs involved and a median score of 3 according the Organ Scoring System. Chronic GvHD progressed from a preexistent acute GvHD in 25 cases. We could not analyzed B cell chimerism at day 90 in more of 80% of cases, due to the low count of CD19+ cells, but at day 120 we found full donor chimerism (FDC) on CD19+ cell fraction of all patients who had reached FDC in the whole blood and in the granulocyte fraction.We did not observed any significant difference in the kinetic of B cell recovery between patients with cGvHD (n=43) and without cGvHD (n=39). At day 30, mean B cell counts were 12/µl in cGvHD patients and 6/µl in cGvHD negative patients (p=0.3); they were 57/µl in cGvHD group and 17/µl in cGvHD negative group at day 120 (p=0.1) and 92/µl in cGvHD group and 65/µl in cGvHD negative group at day 180 (p=0.4). The mean BAFF levels had a peak at day 90 (7936 pg/ml) in cGvHD negative patients and in cGvHD patients (8132pg/ml) and then returned to values similar to those before transplantation in both groups. Conclusions. The B cell compartment had stable engraftment of donor origin ad day 120 in all patients. Severe B lymphocytopenia persisted through the first 180 days after SCT in all patients, without any significant difference between cGvHD positive and negative patients. BAFF serum levels had a peak at day 90, but this increase could not be correlated with the subsequent development of chronic GvHD.

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ALLOGENEIC STEM CELL TRANSPLANTATION (HSCT) FOR ADULTS WITH MYELODYSPLASTIC SYNDROMES (MDS): RELEVANCE OF PRE-TRANSPLANT DISEASE STATUS

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Background: allogeneic HSCT is currently the only potential curative therapy for MDS. Approximately 40% of patients may be cured with HSCT, but this strategy is limited to a selected minority of them. Aim of the study was to investigate the outcome of adult patients with MDS who received an allogeneic HSCT in two Italian centers. Methods: We retrospectively analyzed 77 adult MDS patients (median age 53) receiving an allogeneic HSCT between January 1995 and September 2010. At the time of diagnosis, 3 patients (4%) had IPSS low-risk disease, 19 (25%) intermediate-1, 25 (33%) intermediate-2 and 9 (12%) high-risk disease. At the time of HSCT, 22 patients (29%) were in complete remission, 12 (16%) were untreated, and 43 (56%) had relapsed/refractory disease; 49 patients (64%) received active treatment before HSCT. Peripheral blood stem cells was the graft source in 68 cases (88%), bone marrow in 8 (10%) and umbilical cord blood in 1 (2%); 50 patients (65%) received grafts from an HLA-identical sibling, 24 (31%) from a matched unrelated donor and 3 (4%) from a partially matched related donor. Forty-three patients (56%) received myeloablative preparative regimens and 34 (44%) received reduced intensity conditioning. Results: primary neutrophil engraftment was achieved in 71 patients (92%) at a median of 16 days (range 11-30) after HSCT. The cumulative incidence of acute graft-versus-host disease (aGVHD) and chronic GVHD by 1 year (yr) were 30% (19%-41%, 95% CI) and 57% (45%-68%, 95% CI) respectively. The cumulative incidence of transplant-related mortality (TRM) at 100 days and 1 yr was 13% (5%-21%, 95% CI) and 20% (11%-29%, 95% CI) respectively. The 2-yr progression free survival (PFS) and overall survival (OS) were 41% (30%-52%, 95% CI) and 48% (36%-59%, 95% CI) respectively. In multivariate analysis, advanced disease status at the time of HSCT was the major independent unfavorable risk factor associated with an inferior 2-yr PFS (HR 4.48, 2.13-9.45 95% CI, p<0.001) and OS (HR 4.11, 1.94-8.70 95% CI), while the use of a donor other than an HLA-identical sibling was the independent variable associated with higher TRM (HR 2.81, 1.04-7.63, 95% CI, p 0.04). Conclusions: our data suggest that disease status at the time of transplant have a significant impact on survival and treatments required to reach this goal may have value in leading to improved outcome.

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REDUCED-INTENSITY CONDITIONING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADVANCED STAGE MYCOSIS FUNGOIDES AND SÉZARY SYNDROME: LONG-TERM RESULTS FROM A SINGLE-INSTITUTION PHASE II STUDY

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents the only curative strategy for advanced stage mycosis fungoides (MF) and Sézary syndrome (SS), which are characterized by very poor prognosis with conventional therapy. However, high transplant-related mortality (TRM) counterbalances survival benefit, particularly because patients with MF/SS are often elderly and in poor conditions. Reduced-intensity conditioning (RIC) regimens significantly decrease TRM, allowing gradual establishment of full donor chimerism and concomitant acquisition of clinical remission. In our Institution, since 09/2000, 17 patients underwent allo-HSCT following a RIC regimen that included fludarabine/CTX/TBI200 (up to 2001, 3 patients) or pentostatin/TBI200 (from 2002 to present, 11 patients) for HLA-identical sibling, pentostatin/TBI200 for UCB (1 patient), Melphalan/CP1H/Fluda/TBI200 (up to 2003, 1 patient) and pentostatin/TBI200/ATG (from 2004 to present, 1 patient) for MUD transplants. GvHD prophylaxis included cyclosporin A and mycophenolate mofetil for all patients. At the time of transplant all patients (12 males and 5 females; median age 49 years, range 37-66) had stage III/IV refractory MF (n=11) or refractory SS (n=6). Median time from diagnosis to HSCT was 48 months (range 13-252). Source of stem cells was peripheral blood in 15 patients, cord blood in 1 patient and BM in 1 patient (MUD). Full donor chimerism was achieved in 14 patients (median time 6 months, range 1 to 12), while graft failure was observed in 2 patient (1 MUD and 1 UCB). Acute GvHD occurred in 7 patients (6 grade I-II and 1 grade IV), whereas chronic GvHD was observed in 8 (extensive in 3). Clinical complete remission (CR) was obtained in 12 patients. Six patients died, 2 in CR (1 for sepsis; 1 for aGvHD) and 4 from progressive disease. With a median follow-up of 59 months (range 2-122), 10 patients are alive with a clinical CR maintained in 9. Molecular remission (mCR) was documented in all evaluable pts (8 out of 10), often following the withdrawal of immunosuppression. With a median follow-up of 78 months (range 2-122), 8 patients maintained a mCR. Overall, we noticed an association between chronic GvHD and long-term mCR. We conclude that RIC allo-HSCT is feasible in patients with advanced stage refractory MF/SS and may represent an effective strategy of cure, suggesting a crucial role of the immunomediated GvL effect in inducing and maintaining remissions.

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THE NATIONAL INSTITUTE OF HEALTH (NIH) CONSENSUS CRITERIA IDENTIFY A SUBGROUP OF PATIENTS ("LATE-ACUTE GVHD") PREVIOUSLY DIAGNOSED WITH CHRONIC GVHD, CHARACTERIZED BY AGGRESSIVE DISEASE AND POOR OUTCOME

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Chronic GVHD (cGVHD) is the most common long-term complication of allogeneic hematopoietic stem cell transplant (HSCT), and a leading cause of late non-relapse mortality (NRM). The Seattle's classification defined as cGVHD any manifestation persisting or developing beyond 100 days from HSCT. The use of non-myeloablative conditioning regimens and the introduction of different HSC sources have pushed the limits of this classification. The NIH proposed in 2005 new criteria for the diagnosis and classification of GVHD, based on clinical, laboratoristical and histopathological findings instead of the time of onset. Accordingly, 2 categories for GVHD were distinguished (acute and chronic), each with 2 subcategories (classic acute/late-acute vs classic chronic/overlap syndrome). Aim of our study was to assess the feasibility of NIH classification and its impact on overall survival (OS), relapse mortality (RM) and NRM. From 1993 to 2009, 193 consecutive patients with hematological malignancies underwent related or unrelated HSCT at our Institution. Of these, 159 (82.4%) patients surviving at least 100 days after HSCT and not receiving additional HSC, a second HSCT or donor lymphocyte infusion, were considered. According to the Seattle's classification 83 patients had cGVHD. Of these, 74 could be reclassified according to NIH criteria as: late-acute GVHD (19, 26%), overlap syndrome (11, 15%), classic cGVHD (44, 59%). After a median follow-up of 96 months (range 18-206), OS was significantly higher in the group of 74 patients with any type of GVHD as compared to those without (p=0.001). Among the first, patients with NIH-defined cGVHD (overlap syndrome and classic cGVHD) had a significantly better OS than those with late-acute GVHD (p=0.003). Overall, RM was significantly lower in the 74 patients with GVHD as compared to those without (p=0.002), while it did not significantly differ among GVHD subgroups. NRM was higher in the 74 patients with GVHD as compared to those without, being significantly higher in late-acute GVHD as compared to NIH-defined cGVHD (p=0.02). At multivariate analysis, NIH-defined cGVHD showed an independently significant protective effect on RM (HR 0.325, 95% Conf. Int. 0.15- 0.70; P=0.004) whereas its effect on NRM was not significant (HR 0.493, 95% Conf. Int. 0.24-1.01; P=0.055). NIH classification is feasible and plays an important role in identifying a previously undistinguished subgroup of patients (late-acute GVHD) with a poor outcome

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6-SULFO LACNAC DENDRITIC CELLS (SLAN-DC): CHARACTERIZATION IN HEMATOPOIETIC STEM CELLS SOURCES AND RECONSTITUTION FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT

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Dendritic cells (DC) are specialized antigen presenting cells, which coordinate the components of the innate and adaptive immune response. Human peripheral blood DC can be divided into two main populations, named plasmacytoid (pDC) and myeloid (mDC), displaying different phenotypic and functional characteristics. 6- Sulfo LacNAc+ DC (Slan-DC) are a newly characterized subpopulation of human blood DC, showing a pro-inflammatory activity. In particular, by releasing TNF-alpha and IL-12 upon stimulation with LPS, Slan-DC might play a pivotal role in the development of GVHD. Aim of this study was to characterize Slan-DC in the hematopoietic stem cell (HSC) sources and to evaluate their reconstitution and origin (donor vs. recipient) following allogeneic hematopoietic stem cell transplant (HSCT). We analyzed 12 peripheral blood (PB) and bone marrow (BM) samples, 10 G-CSF- stimulated apheresis products (AP) and 12 cord blood (CB) samples from healthy donors and PB and BM samples from 28 transplanted patients throughout 1 year- follow-up. Samples were obtained after informed consent with the local ethical committee approval. Slan-DC, pDC and mDC were analyzed in parallel and identified by flow-cytometry analysis using MDC-8, CD123 and CD1c respectively, in combination with lineage-negative markers and HLA-DR. CD83 was used for maturation assessment. The origin of reconstituting Slan-DC was established by FISH analysis of sexual chromosomes after isolating by cell-sorting Slan-DC from PB of recipients of sex-mismatched HSCT (at day +21-30). The median absolute number (x106/L) and the frequency (on CD45+ cells) of Slan- DC were [PB 12,28 (2,68%); BM 1,67 (0,15%); AP 1828 (3,16%); CB 2,6 (0,43%)], of pDC [PB 4,89 (1,47); BM 48 (4,8%); AP 898 (1,85%); CB 8,46 (1,46%)], of mDC [PB 11,98 (3,64%); BM 9 (0,93%), AP 651 (1,13%); CB 13,26 (1,77%)]. Slan-DC reconstituted early after HSCT, reaching the median number observed in PB from healthy donor at day +21 (Fig. 1). As compared to pDC and mDC the median number of Slan-DC (with the exception of day +50) was similar or higher of that of donors throughout the follow-up. Reconstituting Slan-DC did not express CD83 as those from donors. In 6/6 cases FISH analysis demonstrated that Slan-DC derived from donor. Our data show that Slan-DC represent a consistent part of DC contained in HSC sources and that they reconstitute early after HSCT. Further studies are ongoing in order to assess their role in GVHD.

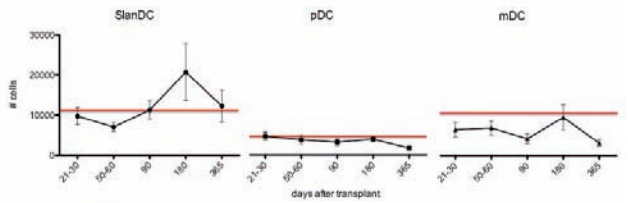

Figure. 1
Kinetic of reconstitution of SlanDC, pDC and mDC in PB samples from patients at different time-points after transplant (absolute number of cells/ml). The red lines indicate the median value of SlanDC, pDC and mDC in PB from healthy donors.

MYELODYSPLASTIC SYNDROMES

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PATIENTS WITH MYELODYSPLASTIC SYNDROMES SHOW REDUCED FREQUENCIES OF CD4+CD8+ DOUBLE POSITIVE T-CELLS

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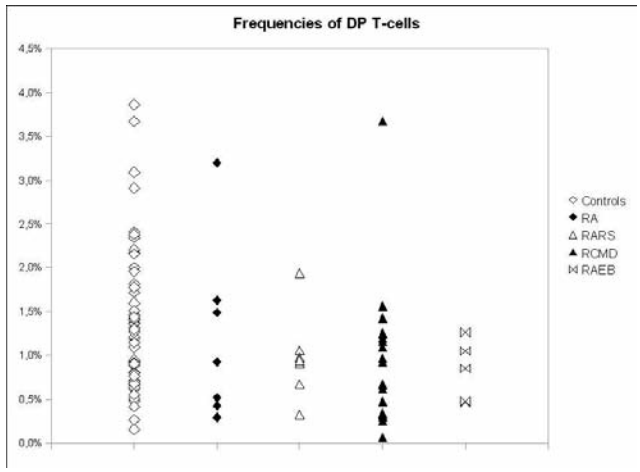
Even though the expression of CD4 and CD8 on thymocytes is considered mutually exclusive, CD4+CD8+ double positive T-cells (DP) represent a small subset of T-lymphocytes which have been described in the peripheral blood of normal individuals as well as in some pathological conditions. From the functional point of view DP are able to act as differentiated effector memory cells with specific antiviral functions. Although a DP cell population has been described in the lymph nodes of patients with nodular lymphocyte predominant Hodgkin's Lymphoma, the relative representation of this cell subset has never been analyzed in patients with myeloid malignancies. As myelodysplastic syndromes (MDS) are a group of clonal disorders characterized by a marked immune dysregulation specifically involving the T-cell compartment, we evaluated the frequency of DP in the peripheral blood of 41 patients with MDS and 40 age-matched normal controls by using flow cytometry. We showed that MDS patients when compared with normal controls had reduced frequencies of DP (0,99% ± 0,72% vs 1,38% ± 0,80% calculated on total lymphocytes; $p<0,05$). We then looked at the possible impact on DP frequencies of several patient- and disease-related factors but, after stratifying patients by WHO adapted Prognostic Scoring System (WPSS), cytogenetics, hemoglobin levels, neutrophil and platelet counts, transfusion dependence and coexistence of autoimmune phenomena, we could not detect any statistically significant difference. However by comparing DP frequencies in patients belonging to different WHO subclasses, we demonstrated a further reduced frequency of DP in patients with refractory cytopenia and multilineage dysplasia (CRDM) than in patients belonging to the other WHO subclasses (0,98% ± 0,43% vs 1,00% ± 0,68%; $p<0,005$). Our data further suggest that an abnormal activation of the T-cell compartment may be deeply involved in the pathophysiology of MDS, especially in subtypes such as RCMD which are more likely characterized by the functional inhibition of hematopoietic precursors mediated by auto-aggressive T-lymphocytes described in these disorders. The very recent evidence that Myb is a fundamental promoter of DP survival, along with the demonstration that this gene is typically down-regulated in MDS patients due to the abnormal expression of specific micro-RNA, could well explain the reduced frequency of DP we observed in our patients.



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IRON OVERLOAD IN LOW-RISK MYELODISPLASTIC SYNDROMES (MDS): A MULTICENTRIC STUDY

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Background. Blood transfusion is the only supportive therapeutic chance in MDS patients refratory to other treatments. Several studies have demonstrated that patients with LOW-RISK MDS (IPSS: LOW, INT-1) have an elevated morbidity and mortality risk after the transfusion of more than 100 units of blood red cells. The use of iron chelators could reduce or prevent the iron overload damage. Deferasirox is a once-daily oral iron chelator that has demonstrated good efficacy and acceptable safety profile. Aims. We investigated the effectiveness and the safety of deferasirox therapy in reducing the iron overload in polytrasfused LOW-RISK MDS patients. Methods. We have treated 82 patients affected by LOW-RISK MDS refractory to any treatment modality and blood transfusion dependent form at least 1 year. All patients (44 male and 38 female, median age 73 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 8g/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. Results. After 6 months from the beginning of the therapy with deferasirox all the patients showed a reduction of ferritinemia (an about 60% decrease, r: 58-65%). Interestingly, after 6 months from the beginning of deferasirox therapy, a reduction of the transfusion request (50%) was recorded in 20 patients and one patient was transfusion-independent. Until to-day (24 months after the beginning of the therapy) we have not recorded either toxicity or adverse events. Conclusions. Our results confirm the effectiveness of the therapy with deferasirox in reducing the iron overload in polytrasfused LOW-RISK MDS patients with acceptable toxicity profile. Moreover, our results show a significant reduction of the transfusion request in about one third of patients. The positive effect on haemopoiesis of iron-chelation is already known and is due to the reduction of toxicity caused on haemopoietic precursors by an excess of free-iron in the bone marrow. Moreover, recent studies 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 improvement of the Hgb level.

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EFFECT OF LENALIDOMIDE ON INOSITIDE-DEPENDENT SIGNAL TRANSDUCTION PATHWAYS

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Lenalidomide (Len) has proven effectiveness in 70-80% of low-risk MDS cases with del(5q), resulting in transfusion-independence, and inducing a rise hemoglobin levels, suppression of the 5q clone and improvement of bone marrow morphologic features. In del(5q) MDS, Len might suppress the dysplastic clone, while in non-del(5q) it may promote effective erythropoiesis, via activation of EPO signalling, which in turn is associated with PI-PLCgamma1 pathway. However, the exact molecular mechanisms underlying the effect of Len in MDS cells are still unclear. Interestingly, Len targets the phosphatase PP2A, whose gene is located in the common deleted region and which usually targets Akt. Indeed, Akt-dependent pathways are critical in low-risk MDS, which display a marked apoptosis and a low proliferation rate. Recently, our group showed that inositide signalling pathways are involved in the MDS progression to AML. In particular, we demonstrated not only that MDS can show alterations on PI-PLCbeta1 and Akt pathways, but also that Akt is inversely correlated with PI-PLCbeta1, therefore affecting MDS cell survival and differentiation. Here, we report on a patient affected by MDS who was successfully treated with Len. The patient, a 58-year old female, was diagnosed with Refractory Anemia (IPSS: Low) and was given only supportive care before undergoing Len treatment. Shortly after the beginning of the therapy, in 2009, the patient showed a clinical favorable response to Len, and subsequently achieved complete hematologic and cytogenetic remission. To assess the molecular effects of Len on inositide signalling pathways, we analyzed the expression of critical genes involved in cell proliferation and differentiation, i.e. PI-PLCbeta1 and its downstream target Cyclin D3, as well as PI-PLCgamma1, which is linked with EPO signalling and Akt activation. That is why ongoing analyses are also trying to examine the effect of Len on Akt, and the correlation between Len and the expression of other genes specifically associated with erythropoiesis, like Globin genes. So far, our results indicate that both PI-PLCbeta1 and Cyclin D3 are not significantly affected by Len, whereas PI-PLCgamma1 is specifically induced. Consequently, these findings hint at a specific activation of PI-PLCgamma1 signalling following Len treatment, and possibly pave the way to further investigations aiming to better understand the role of these pathways in the mechanism of action of Len in del(5q) MDS.

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5-AZACITIDINE FEASIBILITY AND SAFETY IN ELDERLY MYELODYSPLASTIC PATIENTS

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Azacitidine has been shown to improve prognosis in elderly myelodysplastic syndrome patients: a 2-year overall survival was reported to be 55% vs 15% of conventional care regimen, with good tolerability. We present our experience on the treatment of elderly MDS patients (> 65 years) outside of clinical trials. Overall, 27 out of 45 patients consecutively treated with azacitidine for intermediate-2/high risk MDS, were elderly, with a median age of 70.4 (range 65.4-82.3). There were 2 females and 25 males; according to WHO criteria, 2 patients were classified as refractory cytopenia with multilineage dysplasia (RCMD), 7 patients as refractory anemia with excess of blasts (RAEB) type 1, 14 patients as RAEB type 2 and 4 patients as chronic myelomonocytic leukemia type 2 (CMML). Morphologic evaluation revealed trilinear dysplasia in 11 patients. Cytogenetic analysis was performed in all patients: 15/27 had abnormal karyotype, with the most common alteration being monosomy 7. According to IPSS stratification, 19 patients were intermediate-2 and 8 patients were high risk. Nineteen patients had transfusion need before azacitidine, which was started in a median time from diagnosis of 5 months. Performance status according to ECOG was 0 in 3 patients, 1 in 17 patients and 2 in 7 patients. All patients performed a median of 4 cycles of azacitine (range 2-19) with a schedule 5+2+2 (median azacitidine treatment cycle length 28 days); the first evaluation of response was carried out after the fourth cycle. According to IWG 2006 criteria, we detected complete remission in 7 patients (CR, 28%), improvement of bone marrow dysplasia and blast percentage in 4 patients (partial response, 16%), stable disease in 9 patients (36%) and progression of disease to acute leukaemia in 4 patients (16%). CR was detected in 3 patients with normal karyotype (2 RAEB-2 and 1 CMML), in 2 patients with chromosome 9 alterations (1 CMML and 1 RAEB-2), in 1 patient with complex karyotype (RAEB-2) and in 1 patient with del5q (RAEB-2). Transfusion independence, defined as transfusion-free period of > 2 consecutive months, was reached in 8 patients (30%). All patients who received at least 1 dose of azacitidine were evaluated for safety. We observed that the frequency of adverse events decreased after the first two cycles. Overall, during the first four cycles we detected grade 1-2 nausea in 18% of patients, grade 1-2 pruritus in 14% of patients, grade 2-3 infections due to neutropenia after azacitidine administration in 29% of patients (requiring hospitalization in 4). As to hematologic toxicity, 25% of patients experienced grade 3-4 thrombocytopenia and 30% of patients experienced grade 3-

4 neutropenia. In conclusion, our data showed that advanced age should not preclude effective treatment with azacitidine in MDS patients. Adverse events resulted not particularly serious and easily manageable.

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PROGNOSTIC ROLE OF FLOW CYTOMETRY IN MYELODYSPLASTIC SYNDROMES (MDS): THE PIEDMONT MDS REGISTRY ANALYSIS

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Prognosis in myelodysplastic (MDS) syndromes is currently defined by morphology and cytogenetic, however these two parameters have some important limits. Flow-cytometry has been recently included in the diagnostic panel for MDS, and its prognostic significance is under evaluation. Piedmont MDS Registry data were analyzed to evaluate the impact of immunophenotypic features of bone marrow cells on overall survival (OS) and leukemia-free survival (LFS) in a large cohort of MDS patients. Marrow aspirates from 424 MDS patients were analyzed by flow cytometry. The immature compartment of myeloblasts was analyzed by the quantitative expression of CD34 (<3% vs. >3%), CD117, and CD11b /CD66b (<5% vs. >5%); myeloid maturation was analyzed by the expression of CD11b+/CD66b++ (<15% vs. >15%) and CD11b+/CD66b+ (<25% vs. >25%). In univariate analysis, the expression of immaturity markers (CD34+, CD117+, CD11b /CD66b) was associated with shorter LFS and OS ($p<0.0001$); higher expression of differentiation markers (CD11b+/CD66b++ and CD11b+/CD66b+) was associated with longer LFS ($p<0.0001$ and $p=0.0002$, respectively) and OS ($p<0.0001$). In multivariate analysis, expression of CD34+ ($p=0.007$), CD117+ ($p=0.013$), and CD11b+/CD66b++ ($p=0.023$) retained independent prognostic value for OS, while only the expression of CD34+ was a prognostic factor for LFS ($p=0.0003$). Two different risk groups were defined according to the presence of 0–1 or > 2 of these factors with significant different LFS and OS ($p<0.0001$). This score showed prognostic value in predicting survival even in subanalysis according to IPSS and WHO subgroups. In conclusion flow cytometric analysis in MDS may provide meaningful prognostic information. Blast percentage expressed as CD117+ or CD34+ cells and the quantitative assessment of myeloid maturation showed high prognostic value for survival, even in IPSS subgroups that included patients with heterogeneous clinical behavior.

P301

MANAGEMENT OF IRON OVERLOAD IN MYELODYSPLASTIC SYNDROMES (MDS): COMBINATION OF DEFERASIROX AND DEFEROXAMINE IN A HEPATOPATIC PATIENT

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Iron overload is the consequence of a long term transfusion therapy, a life-saving treatment for patients with chronic anaemia in MDS, thus it is necessary to prevent this complication by applying a correct iron chelation therapy. Deferasirox is a well tolerated oral iron chelator drug that produces relevant benefits but because of its potential hepatotoxicity it is not recommended for patient with preexisting hepatic diseases. Here we report the case of a 62-year-old man affected by HCV positive cirrhosis and MDS (Refractory Anaemia, IPSS 0.5). Recombinant Erythropoietin therapy was ineffective and a RBC transfusion program was started (2 blood package pro month). At a ferritin serum concentration near 700 ng/mL iron chelation therapy with deferoxamine was proposed in consideration of patient hepatic disease: compliance to subcutaneous injection was very bad, transfusion need increased exponentially until to 2 blood package pro week and serum ferritin concentration reached, in 12 months, the level of 6198 ng/mL. Since high levels of ferritin correlate with a very dangerous condition for hepatic cells, therapy with deferasirox was started but at reduced dosage (10 mg/kg/die). Before treatment start an accurate study of hepatic, renal and cardiac functions was performed. After three months serum ferritin concentration was not modified as well as other biochemical parameters, then deferasirox dosage was gradually increased reaching 30 mg/kg/die after two months and no liver damage was observed. After five months of iron chelation therapy with deferasirox at full dosage serum ferritin concentration remained very high (5098 ng/mL). Then, considering all risks related to transfusion dependent secondary hemochromatosis, with patient informed consent, a combined iron chelation therapy with deferasirox (30 mg/kg/die) and deferoxamine (2 g/day for 5 days/week) was established and after 3 months serum ferritin concentration lowered to 3000 ng/mL. At the present time, the patient receives 2 RBC package pro week and, after two years of combined iron chelation therapy, serum ferritin concentration is at a stable level nearby under 3000 ng/mL. No serious adverse event has been observed. In conclusion we suggest that combined therapy with deferasirox and deferoxamine could be considered a safe and almost useful therapeutic choice in the management of critical transfusion dependent iron overload in old MDS patients with hepatic disease.

P302

ERYTHROPOIETIN PLUS DANAZOLE, PREDNISONE, B12 AND FOLATE IN REFRACTORY CYTOPENIA WITH MULTILINEAGE DYSPLASIA. MONOCENTRIC PROSPECTIVE STUDY

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Background RCMD is a well defined entity in MDS. The overall median survival is about 30 months. The appropriate treatment of RCMD is still unclear. Aims To verify if immunosuppressive treatment with danazole and prednisone plus erythropoietins and vitamins is safe and feasible in RCMD treatment. Methods This is a monocentric, prospective, randomized study, regarding the period from July 2008 to December 2010. 30 patients with RCMD were randomized to receive erythropoietin alpha 40000 UI sc/weekly or erythropoietin beta 30000 UI sc/weekly, B12 400 mg/day and folate 5 mg/day (15 patients - group A) or erythropoietin alpha or beta plus B12 and folate at the same dose and danazole 400 mg/day for 1 year and prednisone 50 mg/day for 1 month, then gradually suspended in the following month (15 patients - group B). Median follow-up was 15 months (R 5-29 months). In the group A median age was 67 years (R58-73). M/F was 9/6. 2 patients presented a karyotype with +8 , and 1 with +9. In the group B median age was 65 years (R55-70). M/F was 8/7. No patient had an abnormal karyotype. In the group A all patients received erythropoietin beta. In the group B 8 patient received erythropoietin alpha and 7 beta. In group A at diagnosis median Hb level was 9 g/dl (R7-10), PLT 90000 (R70000-98000), ANC 750 (R250-1000). In group B at diagnosis median Hb level was 8 g/dl (R7-9), PLT 45000 (R30000-70000), ANC 450 (R250-900). Results In group A all patients after therapy achieved a better Hb level with a median increase of Hb of 1.5 g/dl after a median of 2.5 month (R1-5). No improvement was observed in neutrophil and platelets count. In group B all patients achieved a normal Hb (>10 g/dl), PLT (>100000/mcl)and absolute neu-

trophil count (ANC > 1000/mcl) after a median of 1 month of treatment (R1-3 month). The 8 patients of group B treated with erythropoietin alpha achieved a normal level of Hb (>10 g/dl) with a median of 1 month sooner than the 7 patients of group B and all patients of group A treated with erythropoietin beta (median: 1 month in group with epo alpha, 2 monthin group with epo beta) . Summary/Conclusion In RCMD treatment with erythropoietin, danazol an prednisone with B12 and folate support seems to be safe feasible and effective.

P303

PURE RED CELL APLASIA (PRCA) AND MYELODYSPLASTIC SYNDROMES (MDS) WITH DEL(5Q)

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MDS associated with PRCA is a rare condition characterized by severe anemia, transfusion dependence, reticulocytopenia, reduction of erythroid precursors and multilineage dysplasia. In PRCA erythroid precursors are nearly absent, while megakaryocytes and granulocytic precursors are usually present at normal levels. Damage to erythroid progenitors appears to be immune mediated and in about 10% of cases acute myeloid leukemia represents the latest evolution. Conventional immunotherapy is ineffective while alemtuzumab combined with CyA seems to be a valid choice. Nearby 25 cases of MDS with PRCA have been described until today and 5 of them were associated with del(5)(q14q34). 2008 WHO classification defined MDS with isolated del(5q) as a syndrome characterized by bone marrow blast count < 5%, isolated del(5q) and absence of Auer rods. Here we report 3 cases of severe transfusion dependent macrocytic anaemia in which del(5)(q14q34) was associated with erythroblastopenia and myelodysplasia. (M/61 y.o.) with a transfusion dependence of 4 units/month, received diagnosis of PRCA and underwent 12 cycles of alemtuzumab + CyA during 3 years: transient remissions from transfusion dependence were followed by relapses; after 3 years del(5q)was evident in a bone marrow that appeared dysplastic: lenalidomide treatment was started, after few months AML merged with fatal evolution. (F/35 y.o.) received diagnosis of PRCA after 1 year treatment with steroids and transfusions (2 units /month). She underwent three courses of CyA and alemtuzumab with short transient periods of transfusion independence: a second bone marrow investigation, performed after one year, showed del(5q) and lenalidomide therapy was started: transfusion independence was obtained after 2 months. (M/65 y.o.) with a transfusion dependence of 4 units/month, received diagnosis of PRCA and was treated with a single course of alemtuzumab and CyA without any result, cytogenetic revision of bone marrow highlighted the presence of del(5q) and treatment with lenalidomide was started 3 months after diagnosis. No hematological improvement was observed and after 9 courses therapy was stopped. Nowadays patient is transfusion-dependent after 21 months from diagnosis. Here we stress the difficulty of diagnosing PRCA within unilineage myelodysplastic syndromes and focus on the relationship among MDS with erytrhoid aplasia and del(5q) in order to speculate on the role that lenalidomide could play in such entities.

P304

FLOW CYTOMETRY INDICATORS IN MYELODYSPLASTIC SYNDROMES (MDS) AND MYELOPROLIFERATIVE DISORDERS (MPD): A COMPARATIVE ANALYSIS.

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MDS are a heterogeneous group of hematologic neoplasms characterized by cytopenias, dysplastic features of bone marrow, cytogenetic alterations and enhanced risk to evolve towards acute myeloid leukaemia. This evolution has been assessed by an index (IPSS) that categorizes MDS in four classes of risk (Low, Int1, Int2 and High). Diagnostic criteria are based on cytomorphology, histopathology and cytogenetic alterations. In the last years flow cytometry (FCM) analysis of bone marrow (BM) has been introduced as an important co-criterion to improve diagnosis of MDS, especially those lacking cytogenetic abnormalities and minor risk of leukemic evolution, to distinguish them from idiopathic cytopenias of undetermined significance (ICUS). Recently, a lot of immunophenotypic indicators have been suggested to describe MDS BM, but they are still required to be validated while needing further standardization in multicenter studies. In our work, we investigated by FCM BM immunophenotypes of 68 MDS (20 Low and 48 Int1) classified according to WHO criteria (25 RA, 5 RARS, 25 RCMD, 9 5q–, 3 RP, 1 RN, in comparison to 20 MPD (14 TE, 4 PV, 2 MF). We focused on 8 cytometric parameters: 1) % of CD34+ cells; 2) % of CD34+/CD19+ B cell precursors in CD34+ gate; 3) % of hematogones (CD34-/CD19+/CD45dim); 4) % of CD11b-/CD16- cells in granuloid gate; 5) % of monocytes (CD14+); 6) % of CD14+/CD56+ atypical monocytes; 7) CD11b/CD16 granuloid maturation profile abnormality; 8) CD71/FSC erythroid maturation profile abnormality. Comparison between data obtained from MDS and MPD showed significant increase in percentage of CD34+ cells and CD11b-/CD16- granulocytic precursors in MDS cohort (p< 0,0001), without differences while considering the two IPSS subgroups or unilineage versus multilineage dysplasia. In both diseases we found an abnormal expression profile for granulocytic and erythroid maturation investigated by CD11b/CD16 and CD 71 and a significantly reduced percentage of B cell precursors and hematogones. No differences were found in monocyte compartment when comparing the 2 groups of patients. In conclusion, the majority of immunophenotypic abnormalities and maturational blockades within different compartments of BM cells observed among patients with MDS or MPD syndromes seem to be expression of abnormal BM proliferation/differentiation without disease specificity, while increase of CD34+ cells and CD11b-/CD16- granulocytic precursors represents a good marker for MDS.

P305

EVALUATION OF MYELO-ERYTHROID DIFFERENTIATION IN PATIENTS AFFECTED BY MYELODYSPLASTIC/MYELOPROLIFERATIVE SYNDROMES IN TREATMENT WITH 5-AZA: FLOW CYTOMETRY ROLE.

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Background: The multi-color multiparameter flow cytometry (MCPFC) has a role in the diagnosis of myelodysplastic/myeloproliferative process studying the distribution of antigens CD11b, CD13, CD16, HLA-DR, CD66b, CD10, CD64, CD14 to identify the abnormal differentiation of myeloid cells in the bone marrow and the prognostic assessment risk by assigning a score based on the number of cytometric abnormalities . In addition, the CD34+ CD117+ count in bone marrow aspirate and in peripheral blood, both, with MCPFC provides additional data on the evolution of MDS.The use of hypomethylating agents such as 5-AZA, which acts differentiating myeloid population, makes it necessary the use of MCPFC to better understand the phenotypic abnormalities of myeloid populations and if the number of CD34+ and CD117+ cells is changed. Materials and methods. We studied 5 patients with MCPFC including 2 with RAEB-II, 1 RCMD, 1 AML with multilineage dysplasia, 1 CMMoL treated with 5-AZA-7 75 mg/mq d1 every 28 days.Were also evaluated peripheral CD34+ and CD117+ cells at diagnosis and after every two cycles of therapy. Results: in peripheral blood and bone marrow after 6 cycles of therapy there was a reduction in the proportion of blasts (CD45/SSC), in the number of CD34+ and CD117+ and in cytometric alterations of granulocyte and monocyte populations. In addition, in peripheral blood, after every two cycles of therapy, there was a gradual and progressive decrease in the number of CD34+ and CD117+. Finally, in the bone marrow of two patients with RAEB-II after 6 cycles of therapy there was an increase in erythroblasts.Conclusion: The MCPFC diagnosis and during treatment has allowed the assessment of the effects of therapy with 5-AZA on myeloid and erythroid maturation through an analysis of the patterns of distribution of antigen expression. In addition, the CD34+ cell counts in peripheral blood has proven very useful as a tool for monitoring the disease during treatment with 5-AZA.

P306

TREATMENT OF MYELODYSPLASTIC SYNDROME PATIENTS WITH ERYTHROPOIETIN, 13-CIS-RETINOIC ACID AND DIHYDROXYLATED VITAMIN D3: RESULTS OF A RETROSPECTIVE STUDY WITH LONG TERM FOLLOW UP

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In our previous paper (Ferrero et al, BJH 2009) we reported the treatment of 63 MDS patients (16 RAEB1, 47 non- RAEB; median age 75,) with a combination of recombinant erythropoietin (alfa or beta epoetin, median 65000 U/week), 13-cis-retinoic acid (20 mg day) and dihydroxylated vitamin D3 (1 ug/day). Eleven of the 16 RAEB1 patients also received intermittent, low dose of 6-thioguanine. In spite of adverse prognostic factors for response to EPO (all patients with Hb < 9.5 g/dl, 70% transfusion dependent, 51% IPSS intermediate 1 or 2), 64% of non-RAEB and 50% of RAEB1 displayed an erythroid response according to Cheson et al (Blood 2006). At a previous evaluation (41 months of follow-up) a survival advantage was evident for non- RAEB patients with erythroid response. Now we updated the casistic after 3 more years. Median follow up is now 76 months (46-160). Median duration of erythroid response has increased to 22 (2-99+) months for non- RAEB and 6 (2.5-34.5+) months for RAEB1, with 21% of responses in non- RAEB patients lasting more than 3 years. The response duration has not been affected by IPSS or WPSS. Thirty-three of 46 non- RAEB and 14/16 RAEB1 patients died, with a median survival of 55 and 15 months, respectively. Evolution to acute myeloid leukemia occurred in 11 patients (5 RAEB1: 36% and 6 non- RAEB: 13%). In non- RAEB patients overall survival (OS) was significantly affected by IPSS score (p: 0.02), WPSS score (p: 0.01) and transfusion dependence (p: 0.006), as expected. These parameters did not significantly modify OS in RAEB1 patients, possibly due to the low patient number. Although erythroid response did not correlate with known risk factors such as IPSS score, caryotype and transfusion requirement, it confirmed its positive prognostic role for OS in non- RAEB patients (p: 0.01, HR 2.289), with a median OS of 71.5 months (12-156+) for responders and 30.6 months (5-149) for non-responders. A trend towards a better OS for responders was also observed among RAEB1 patients (median 17 months versus 10 months for non- responders), however, due to the low numbers of patients in this group, the difference was not statistically significant, (p 0.07). In conclusion, long- term follow-up confirmed the positive role for response duration and OS of our combined treatment in a group of non- RAEB patients, most of them with unfavorable prognostic features, compared to literature data on EPO alone treatment.

P307

DIFFERENTIAL DIAGNOSIS IN PATIENTS WITH CYTOPENIA AND PHENOTYPIC DYSPLASIA: A SYSTEMATIC FLOW CYTOMETRY APPROACH ALLOWS DISTINCTION OF PNH AND VITAMIN B12 DEFICIENCY FROM MDS

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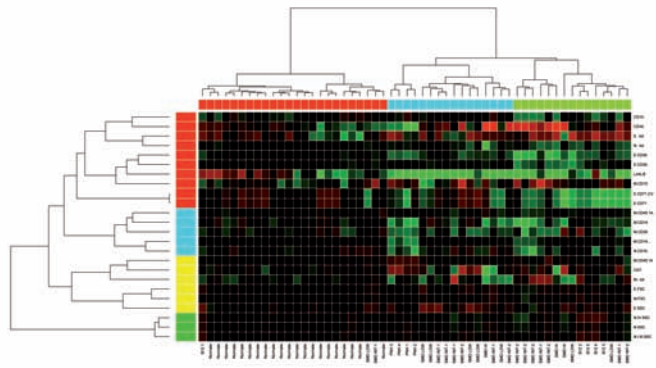
The diagnosis of myelodysplastic syndromes (MDS) relies on documentation of morphologic dysplasia in bone marrow (BM). Many reports propose flow cytometry (FC) as an useful integrating technique. Nonetheless, "dysplasia" is not specific of MDS and it can be found also in some other disorders, causing cytopenia as well. We carried out a systematic analysis by FC of BM cells from 25 patients with MDS and with dysplasia-related disorders, i.e. Paroxysmal Nocturnal Hemoglobinuria (PNH,n=4) and vitamin B12 deficiency (B12 def,n=5). Our aim was to search for any phenotypic profile specifically associated with different diseases. Methods. 2x106 BM cells were stained with quadruple combinations of monoclonal antibodies; 50000 BM cells per tube were acquired. Data acquisition was performed using FACSCalibur (Becton Dickinson). For data analysis, we used Infinicyt (Cytognos) software. Some major BM compartments were identified on the basis of FSC and SSC properties and reactivity for CD45/CD34. Twenty-four phenotypic parameters were expressed as percentage of positive cells within a compartment and/or mean fluorescence intensity (MFI; arbitrary relative linear units). Each individual value was normalized with respect to the mean value obtained for each parameter in normal samples and a log2 transformation was then applied to normalized values. Ward's hierarchical clustering analysis of the resulting normalized log2 ratios was performed to identify subgroups of individuals. Results. The clustering analysis effectively grouped the several disease categories (Figure 1). Some nodal parameters emerged as being able to attribute "phenotypic dysplasia" to specific diseases. As an example, SSC signal on neutrophils in B12 def was significantly higher than what revealed in controls and in MDS. Regarding PNH, some alterations (reduced CD14 on monocytes and CD16 on neutrophils) were consistent with the lack of GPI but the analysis on BM expose data to misinterpretation since these antigens are acquired with maturation. The systematic approach of our analysis aided to solve between MDS and PNH (i.e. CD14-neg monocytes in PNH showed higher levels of CD45 than in normal BM or MDS). In conclusion, through a systematic FC approach, we highlighted some aberrancies specific for B12 def and PNH and defined useful profiles to avoid attributing phenotypic dysplasia to MDS.



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(IN)EFFICIENCY OF 1ST LINE EPO APPROACH IN MYELODYSPLASTIC PATIENTS WITH DEL 5Q-

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Efficiency and safety of Epo in patients with myelodysplastic syndromes (MDS) have been demonstrated by various studies. On the other hand, MDS patients with 5q deletion and transfusional requirement can achieve high response rates with Lenalidomide, but this drug has shown severe hematological and extra-hematological toxicities. Thus, it could be advisable to evaluate the efficacy of EPO treatment as 1st therapeutic choice in this subset of patients. To address this issue, 24 patients with MDS and del 5q- (M/F 2/22, median age at onset 71.9 yrs, IR 62.9 – 78.6) who received EPO treatment at our Institution from 6/2000 to 7/2010 were retrospectively evaluated. According to WHO 2008, 13 patients were classified as del5q-syndrome, 2 as refractory anemia, 5 as refractory citopenia with multilineage dysplasia and 4 as refractory anemia with excess of blasts-1: according to IPSS risk assessment, 7 patients were low-risk, 15 intermediate-1 and 2 intermediate-2. As to karyotype, 17 patients had an isolate del5q-, while in the remaining 7 patients there were other co-existing cytogenetic abnormalities. Median age at EPO start was 72.6 yrs (IR 63.2 - 79.3), with a median interval from diagnosis of 9.5 months (IR 2.0 – 20.6). Median Hb level at baseline was 8.6 g/dl (IR 7.8 – 9.5) and 16 patients (66.6%) were transfusion dependent. Median EPO level at baseline was 214 mU/ml (IR 128 – 420). Eighteen patients received high-dose EPO (80,000 UI weekly) and 6 patients standard dose (40,000 UI weekly). On the whole, only 3/24 patients (12.5%) (1/16 with transfusional requirement and 2/8 without transfusional requirement at baseline) achieved an erythroid response according to IWG 2006 criteria, with increment of Hb level > 2 g/dl in 2 patients and disappearance of transfusional need in 1 patient: these responses were lost after 8.0, 14.5 and 16.5 months, respectively. In the remaining 21 patients, EPO treatment was discontinued after a median period of 4.0 months (IR 2.0 – 5.9) due to lack of response. This retrospective evaluation showed a sub-

stantial inefficacy of EPO treatment in patients with del 5q-, thus supporting the choice of lenalidomide as 1st line approach in such subset of MDS with transfusional requirement.

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IRON OVERLOAD AND IRON CHELATION THERAPY IN MYELODYSPLASTIC SYNDROMES AND IN OTHER TRANSFUSION-DEPENDENT CHRONIC ANEMIAS. RETROSPECTIVE STUDY OF 45 PATIENTS

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To date, several guidelines and consensus conferences recommend to start iron chelation therapy (ICT) to treat iron overload in transfusion-dependent patients (pts) affected by myelodysplastic syndromes (MDS) with a longer life expectancy. However, several barriers may limit the initiation or the continuance of ICT in MDS pts: older age, comorbidities, poor compliance, renal health concerns and poor gastrointestinal tolerance (for deferasirox, ICL), difficulty of prolonged subcutaneous administration (for deferoxamine, DFO). Moreover, recently published data from the large prospective EPIC study (Gattermann, 2010) show a high discontinuation rate for ICT in MDS. Therefore, with the aim of assessing the feasibility of ICT in the daily clinical practice, we retrospectively analyzed our single-center experience on ICT in MDS and other chronic anemias. From July 2003, in our Institution, 45 pts (25 males), median age: 74 (39-91) yrs, with transfusion-dependent anemia, were considered for ICT, because of a diagnosis of iron overload, i.e. both a transfusion history of at least 20 units of RBC and a serum ferritin higher than 1000 ng/ml. 28 pts (62.2%) were affected by lower-risk MDS (IPSS risk: low or intermediate-1), while 13 pts (28.8%) showed a higher-risk MDS (IPSS risk: high or intermediate-2) but were considered for ICT because of responsiveness to hypomethylating therapy and/or elegibility for allogeneic SCT. 4 pts (8.8%) were affected by other diseases (idiopathic myelofibrosis: 2 pts; aplastic anemia: 2 pts). 10 pts (22.2%) didn't start ICT because of the presence of one or more contra-indications (renal failure, poor clinical condition, lack of compliance, burden of other concomitant treatments). 35 pts underwent ICT: 22 pts (62.8%) received ICL, 10 pts (28.5%) received DFO and subsequently ICL, and 3 pts (8.5%) received DFO. Median time from diagnosis to assessment of iron overload: 15 months; from diagnosis to the start of ICT: 21 months. Median number of RBC transfusions pre-ICT: 40 (from diagnosis), and 12 (in the last 12 weeks). Median serum ferritin (SF) level pre-ICT: 2510 ng/ml; median SF after ICT: 1813 ng/ml; median duration of ICT: 16 months. Adverse events occurred in 14 pts (40%): renal (transient increase of serum creatinine): 8 pts; gastrointestinal: 4 pts; cutaneous: 2 pts. Permanent discontinuation of ICT: 2 pts. In conclusion, in our experience ICT appears feasible even in a population of elderly pts, if carefully selected.

P310

ASSESSEMENT OF RESPONSE TO 5-AZACYTIDINE IN THERAPY RELATED MYELOID NEOPLASMS

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Introduction: Therapy-related myeloid neoplasms (t-MN), including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), are characterized by unfavorable prognosis, mainly due to previous exposure to chemo- and/or radiotherapy, poor patients' clinical conditions and multiple genetic and epigenetic alterations at the onset of disease. Similarly to high-risk de novo MDS, these patients are expected to respond to treatment with demethylating agents, particularly in the setting of anomalies of chromosome 7. Methods: We aimed at evaluating the response rate to 5-Azacytidine (5-AZA, CelgeneTM) in t-MN, and the safety of treatment. In a multicentric retrospective study, we analyzed biological and clinical characteristics of 29 patients diagnosed with t-MN in 8 Italian Centres and treated with 5-AZA. Results: All patients had received chemotherapy (18), radiotherapy (5), or both (6) for the primary malignancy. Primary neoplasms were hematological malignancies in 15 cases (2 AML, 9 lymphoproliferative diseases and 4 multiple myeloma), 2 had a previous breast cancer, 3 a genito-urinary tumor and 3 other tumors. According to morphology, there were 7 AML and 22 MDS (1 RA, 9 RCMD, 4 RAEB-I and 8 RAEB-II). Cytogenetic risk group was predictive of poor prognosis in 13 patients, intermediate in 3 and good in 10. WPSS in t-MDS patients was low in 2 patients, high in 15 and very high in 5 patients. Ten patients had received a previous treatment for their t-MN (4 with chemotherapy and 6 with growth factors). Azacitidine was administered at a median dose of 75 mg/sqm/day (range 50-75 mg/sqm) for 7 days, every 4 weeks for a median of 4 cycles (range 2-15 cycles). Response was assessed according to the modified International Working Group (IWG-2006) criteria. Treatment response was evaluated in 27 patients after a median of 4 cycles (median 2-15): there were 3 complete response (CR), 1 partial response (PR), 3 hematological improvement (HI), resulting into an overall response rate of 26%, 10 stable disease (SD) and 10 progressive disease. Only one t-AML patients achieved CR. No adverse events have been reported. At a median follow up of 9.3 months, 9 patients died and 18 are still alive: 2 in CR, 2 with HI and 4 with SD. Conclusions: t-MN patients had poor response rate to hypomethylating agents, different from that reported for high risk MDS. Given the poor biological profile of t-MN, new drug associations might improve these results.

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DECITABINE ON HIGH RISK MDS IS EFFECTIVE AND FEASIBLE WITHOUT HOSPITALIZATION

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Aberrant DNA methylation is a common feature in MDS. The hypomethylating agents 5-azacytidine (Vidaza) and 5-aza-2'-deoxycytidine (Decitabine, DAC) are active in different MDS subtypes. Decitabine is a demetilating agent approved for the high risk MDS from FDA but not yet from EMEA. Patients affected by High Risk Myelodysplastic syndrome (MDS) have a poor prognosis with a median survival of 12-14 months. We treated 10 patients with HR-MDS with with decitabine 20mg/m2 x 5 days as single agent in Day Hospital regimen. 4 patients had diagnosis of AREB-1, 2 AREB-2, 4 LMMC-2. IPSS score was INT-2 for 6 patients and HIGH for 4 patients. Methods: Medical records of 10 patients who received decitabine-based MDS therapy were reviewed and survival was calculated by the Kaplan Meir method and differences in survival calculated by the log-rank test using SPSS software. Results: 4 patients received decitabine therapy as first line, 6 patients as second line after azacitidine failure. Mean age of patients was 67.9 years with a range of 45 -83 years. Median overall survival of all patients was 219 days, range 27-371 days. Overall response rate (CR+PR+HI) was 70% with median duration of response was 3.5 months. Mean number of cycles was 4 (range 1-9). The response rate for 4 patients in first line was 78% at 5 months of follow up, the rate for second line therapy was 42% at 9 months of follow up. 9 patients were treated on Day Hospital regimen and only 2 required a long hospitalization (42, 77 days). Conclusions: Decitabine-based treatment for high risk MDS is feasible and well tolerated. This preliminar study suggests that further investigation of decitabine-based for MDS is warranted.

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REFRACTORY ANEMIA WITH RING SIDEROBLASTS ASSOCIATED WITH MARKED THROMBOCYTOSIS (RARS-T). PATIENTS CHARACTERISTICS AND INCIDENCE IN A SINGLE CENTRE SERIES

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In the WHO 2008 classification, the refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) rappresents a provisional entity within the category of MDS/MPD unclassifiable, defined by platelet count > 450 x 109/l, proliferation of megakaryocites and blasts <5% in bone marrow, and ring sideroblasts >15% of nucle-

ated erythropoiesis. From 2000 to 2010 in our centre we observed 84 consecutive cases of MDS and MDS/MPD: 11 RA, 8 RARS, 18 RCMD, 11 RAEB-1, 8 RAEB-2, 8 MDS-U, 11 CMML, 3 atypical CML, 1 5q- syndrome and 5 cases classified as RARS-T. All the patients were analyzed for blood parameters, bone marrow morphology (May Gruenwald Giemsa and Perls iron staining), and cytogenetic analysis; PCR for JAK-2V617F was done in all the cases with thrombocytosis. The 5 patients classified as RARS-T had these characteristics at the diagnosis: sex 3 male/2 female; median age 79 (range 76-83); median WBC count 8.6 x109/l (range 5.2-10.9); median Hb level 9.1 g/dl (range 8-10.6); median platelet count 700 x 109/l (range 300-900). The median ring sideroblast count was 30% (range 20-60). In 2 patients the platelet count at the diagnosis was <450 x109/l, but the megakariocytes in the bone marrow had already increased, while the platelet count increased also in peripheral blood within a few months and the diagnosis was revised from RARS to RARS-T. The karyotype was normal while the JAK-2V612F was mutated in all 5 patients. All patients were treated with erythropoietin at the front line, with the addition of hydroxyurea when the platelet count was above 600 x109/l. 2 patients had a haematological change: the first has evolved into an overt myelofibrosis and died 3 years after diagnosis, the second has evolved into RAEB-1 4 years after diagnosis and is still living. 2 patients died due to comorbidity (the first for vascular disease 2 years after diagnosis, the second of a biliary tract cancer diagnosed 6 months after the haematological disease). The last patient is still alive and clinically stable. In our limited series, the incidence of RARS-T is particularly high (6% of all the MDS and MDS / MPD) than usually reported by other authors. Equally unusual is the presence of JAK-2V612F mutation in all cases. The appearance of thrombocytosis after the diagnosis of RARS in 2 out of 5 cases represents a confirmation of the hypothesis that the RARS-T can evolve from a preexisting RARS due to the occurrence of new mutations (eg. JAK-2V612F).

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AZACITIDINE FOR HIGH RISK MYELODYSPLASTIC SYNDROMES. RETROSPECTIVE EVALUATION OF TWO DIFFERENT DOSING SCHEDULES

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Azacitidine (AZA) has proven effective (response rate: 60-80%) in myelodysplastic syndromes (MDS). The currently approved AZA regimen (AZA 7) is 75 mg/sqm/die subcutaneously (SC) for 7 days every 28 days. Recently a different AZA dosing schedule (AZA 5-2-5: 50 mg/m2/d subcutaneously for 5 days, followed by 2 days no treatment, then 50 mg/m2/d for 5 days), which avoids week-end dosing, has shown to induce therapeutic responses consistent with the currently approved schedule, in a population of mainly low risk pts (Lyons, 2009). These data prompted us to investigate the therapeutic effect of the AZA 5-2-5 regimen in high risk MDS pts (IPSS risk: high or intermediate-2). From September 2004, in our Institution, 28 high risk MDS pts. were treated with 2 different AZA dosing schedules. Group 1 (9 pts, 8 males, median age: 68, range 60-84 yrs) received the AZA 7 regimen, while group 2 (19 pts, 13 males, median age: 69, range 37-81 yrs) received the 5-2-5 AZA regimen. Moreover, as our group (Follo, 2009) demonstrated that phosphoinositide-phospholipase C (PI-PLC) beta1 may represent a target for AZA, we quantified the degree of PI-PLCbeta1 methylation and gene expression before and during AZA administration in both groups. Pts of group 1 received a median number of 12 (1-59) AZA cycles. Among the 8 evaluable pts (i.e.: at least 6 cycles) 5 (62.5%) showed a favourable response, following IWG criteria (Cheson, 2006): 1 Complete Remission (CR), 1 Partial Remission (PR) and 3 Hematologic Improvement (HI). 3 pts died because of evolution into Acute Myeloid Leukemia (AML), and 4 pts for other causes. One pts, still alive and under imatinib therapy, developed Ph1+ Chronic Myeloid Leukemia (CML) after 59 courses of AZA. Mean follow-up of group 1 pts: 31 (13-79) months. Pts of group 2 received a median number of 8 (1-17) AZA cycles. Among the 15 evaluable pts, 13 (86.6%) showed a favourable response: 5 CR (33.3%) and 8 HI. 3 pts died because of evolution into AML, and 2 for other causes. 2 pts underwent allogeneic stem cell transplantation, both after achieving CR. 12 pts are alive, 9 of them still under AZA treatment. Mean follow-up of group 2 pts: 14 (1-35) months. PI-PLCbeta1 methylation and gene expression appeared to be related to the therapeutic response, but not to the dose schedule. Our results, although larger studies are required, seem to confirm the effectiveness of the more convenient AZA 5-2-5 regimen, even in high risk MDS.

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HEMATOLOGIC RESPONSE IN MYELODYSPLASTIC SYNDROMES (MDS) RECEIVING DEFERASIROX AS IRON CHELATION THERAPY: UPDATED SYSTEMATIC REVIEW OF THE PUBLISHED CASES, INCLUDING PERSONAL EXPERIENCE

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Chelation therapy, employed to avoid organ damage due to iron accumulation in transfused MDS, may also induce, in some patients, hematologic improvements that lead to a significant reduction (or even abolition) of blood transfusion requirement. These effects are generally not related to the decrease of ferritin levels. We performed a systematic review of the literature in order to evaluate this phenomenon in MDS treated with the new oral once-a-day iron chelator deferasirox. Until April, 2011, 23 MDS patients in whom deferasirox treatment was associated with a significant improvement in hematologic values were reported. According to WHO classification, there were 3 RA, 7 RCMD, 1 RAEB-1, while in 12 cases morphological subtype was not specified. Fifteen patients had low or intermediate-1 and 1 patient high IPSS risk. In 7 patients IPSS was not specified. According to IWG criteria applied,twelve patients had major and 7 minor erythroid responses. A major platelet response was observed in 7 patients, while a major response in neutrophil count occurred in 2 patients. Six patients had a combined (erythroid + platelet or neutrophil) improvement. Response were both early (within 4-6 weeks) or delayed (after more than 3 months of chelating treatment). The duration of hematologic response was generally longer than one year and in one case reached 38 months. In two cases, response was lost after interruption of the drug and was gained again when deferasirox was resumed. It is not clear which is the true incidence of hematologic response in MDS patients receiving deferasirox: larger series indicate a possible percentage of 5 to 10%. Several mechanisms have been also suggested. Iron chelators promote iron release from storage sites, facilitating its usage for hematopoietic tissue; furthermore, the reduction of iron store seems to up-regulate erythropoietin response. In MDS deferasirox significantly reduces reactive oxygen species, membrane lipid peroxidation and toxic plasmatic, as well as intracellular labile iron pool, concomitantly increasing reduced glutathione, the major cellular antioxidant, in blood cells. Finally, deferasirox is a potent inhibitor of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), which is abnormally activated in MDS blast cells. An update of this review, including new personal clinical and biological findings, will be presented at the meeting.

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FREQUENCY OF H63D MUTATION IN THE HFE GENE IN ADULT PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Most patients with Myelodisplastic syndromes (MDS) present with transfusion dependent anemia leading to iron overload. The hereditary hemochromatosis gene (HFE) plays a pivotal role in iron homeostasis. An increased incidence of HFE gene mutations has been described in acute leukemias and lymphoproliferative disorders, while few data are available in patients with MDS, in whom HFE mutations could have major clinical relevance, because of iron overload. Among general population, the allelic frequencies of H63D, the more frequent mutations of HFE gene in Italy is 0.16. To investigate the allelic frequency of HFE gene mutations in 72 adult patients with MDS diagnosed according to the current WHO criteria; H63D were selected on the basis of results from previous studies addressing the geographical distributions of HFE mutations in Italy. As control group, 230 healthy blood donors referring to the

same hospital district was adopted. HFE genotyping was performed by a polymerase chain reaction (PCR) method using sequence-specific-primers, and the products were analyzed on agarose gel and by Reverse Dot Blot. Statistical differences between the prevalence of HFE genotypes in the patients and controls as well as between different diseases and subtype of disease were assessed using the chi square test. P values were considered as statistically significant at a value < 0.05. In addition, for any difference Odd's ratio and confidence intervals (CI) were also calculated. The median age was 75 years for MDS patients (range 24-92) and 40 years for blood healthy donors (19-55). The allelic frequency of H63D mutation was 23.6 % in MDS patients and 25.2% in the healthy controls (P = 0.87; odds ratio = 0.88; 95% CI = 0.13 to 1.18). Furthermore, neither difference was found between poor risk and high risk MDS (with or without blast excess); finally, serum ferritin levels at diagnosis did not differ between patients with and without mutation. Our data demonstrate the absence of correlation between the presence of H63D mutation and the occurrence of MDS as opposed to normal population. In addition, no difference exists in the allelic frequency of the mutation between different type of MDS.

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FISH TECHNIQUE IS A GOOD TOOL FOR IMPROVING DIAGNOSIS OF PRIMARY MYELODYPLASTIC SYNDROMES (MDS)

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Myelodysplastic syndromes are a heterogeneous group of clonal haematological diseases characterized by bone marrow failure with abnormal maturation and differentiation of myeloid cells, peripheral cytopenias , dysplastic features in one or more myeloid lineages and enhanced risk of transforming in acute myeloblastic leukaemia (AML). Several efforts have been performed to classify those syndromes with a prognostic evaluation of the leukemic transformation risk (WHO classification, IPSS score); the evidence of cytogenetic abnormalities is one of most important parameter to consider in combination with cytopenias, dysplasia and numbers of bone marrow blast cells. The aims of this work is to evaluate the contribute of fluorescent in situ hybridization (FISH) to improve the cytogenetic analysis of MDS to better characterize the patients providing relevant information in cases with unsuccessful conventional cytogenetic study or in cases of MDS patients with normal karyotype or ipodiploid karyotype. Fluorescent *in situ* hybridization (FISH) is more sensitive than CCA allowing for the detection of minor clones and of submicroscopic lesions. We have studied 147 patients suspected to be affected by primary MDS. The conventional cytogenetic analysis has been performed on bone marrow samples; in 97 of them (66%) we have obtained 20 metaphases for each; of these, 34 (35%) exhibited a normal karyotype, while 63 (65%) showed chromosomal abnormalities or ipodiploid karyotype; the remaining 50 cases showed insufficient number of metaphases or with any metaphases. FISH analysis, using the following probes: LSI CSF1R/D5S23, D5S721 Dual Color Probe for 5q31-35 region , LSI D7S486/CEP 7 Dual Color Probe for chromosome 7 , 8p11.1-q11.1. 8 and LSI D20S108 for 20q12 region(VYSIS), was performed in cases with normal karyotype or ipodiploid karyotype and in cases with unsuccessfull conventional cytogenetic analysis. The FISH technique applied ,as control, on positive or negative samples by conventional cytogenetic analysis showed no differences. The aims of this work is to evaluate the contribute of FISH in cases of MDS patients with uninformative kariotype , in order to improve the results to better characterize the MDS patients, not only for their differential diagnosis, but also for employing targeted drugs in well defined cytogenetic sub groups.

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CLINICAL RESPONSE TO AZA IN PTS WITH MDS/AML: A SINGLE CENTER EXPERIENCE

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5-Azacytidine (AZA) is an hypomethylating agent that is efficacy to prolong survival in higher risk patient with MDS and AML. In our institution, from January 2006, we have treated with AZA 38 pts with MDS or AML diagnosis: 21 had a MDS Low/INT-1 (9 pts with High WPSS), 8 had INT-2/High IPSS risk score and 9 AML. Median age was 73 yrs (range: 55-85, with 22 pts >70 yrs). According to WHO classification, there were 2 RA (5.2%), 3 RARS (7.9%), 11 RCMD (28.9%), 8 RAEB-1 (21%), 5 RAEB-2 (13.1%) and 9 AML pts (23%). At baseline, transfusion dependence was present in 33 pts (86.8%), all for erythrocytes and 6 pts also for platelets. Karyotype was good in 12 pts (41.3%), intermediate in 4 (13.8%), poor in 3 pts (10.3%) and missing in 10 pts (34.5%). AZA was administered at dose of 75 mg/sqm/d s.c., for 7 days each 28. Median number of cycles received was 6 (range: 1-44), a dose reduction was necessary in 13 pts (34.2%) (median number of cycle with AZA dose reduction: 4 cycle). All pts with transfusion dependent received AZA plus EPO until transfusion independency. Concomitant medication were iron-chelation in 18.7% of pts. On 26 evaluable pts, the ORR, according to IWG2006 criteria, was 58% (15 pts).4 pts achieved a complete remission (CR) (15%), a partial response (PR) was observed in 2 pts (7.7%), while a haematological improvements (HI) was observed in 9 pts (34.6%). On 19 pts receiving more than 6 cycles, the ORR, according to IWG2006 criteria, was 73.7% (14 pts); CR in 4 pts (21%), PR in 2 pts (10%) and HI in 8 pts (42%), in particular the ORR in evaluable pts with Low/Int-1 risk was 69.2%. The ORR in pts with AML/AMLs was 33.3%. Median duration of best response, in all responder pts, was 5 months (range 1-35). The median OS in 35 pts evaluable was 10 months, in responder pts (n=13) 32 months and in non-responder (n=10) was only 6 months (32 vs 6 months p-value=0.0017) with clear survival benefit for responding pts. In conclusion, the use of 5-AZA in real life, in an unselected group, offers good benefit in terms of ORR and OSS.

ACUTE LYMPHOBLASTIC LEUKEMIA

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MOLECULAR EVALUATION OF MINIMAL RESIDUAL DISEASE (MRD) IN BOTH B AND T LINEAGE ADULT ACUTE LYMPHOBTLASTIC LEUKEMIA (ALL) IS MORE INFORMATIVE ON PARALLEL SAMPLING OF BONE MARROW (BM) AND PERIPHERAL BLOOD (PB)

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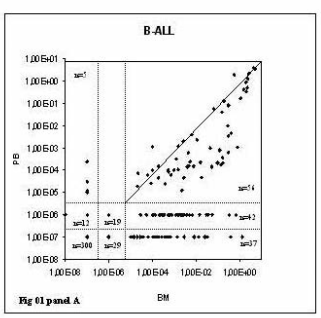
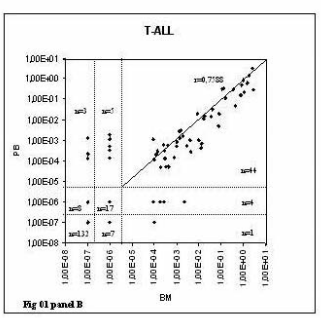
Background. Detection of MRD has become a crucial tool to evaluate prognosis and therapeutic strategies both in childhood and adult patients with ALL. Repeated BM sampling during the clinical course can be problematic for many patients. Preliminary results obtained in children showed that PB sampling cannot replace BM in B precursor ALL, but could be considered for T-ALL. Here we report our comparative analysis in a large prospective study performed in adult B and T ALL. Material and methods. A paired BM/PB MRD analysis was conducted by real-time quantitative PCR (RQ-PCR) on 721 paired samples (500 from B and 221 from T-ALL) derived from 107 adult patients (73 B-precursor ALL and 34 T-ALL) enrolled in 2 consecutive clinical trials launched by NILG. Two informative Ig or TCR derived molecular probes (with a sensitivity ranging from 10^{-3} to 10^{-5}) were used in 77 patients (51 B-precursor and 26 T-ALL) while only one probe was available for 30 (22 B-precursor and 8 T-ALL). Results. In B precursor ALL 200 out of 500 paired samples showed a measurable MRD level in BM and/or PB (figure 1 panel A). In 45-paired samples (22.5%) the amount of MRD was similar in BM and PB while in 72 (36%) MRD was significantly higher (up to 3 log difference) in the BM compared to PB; in only one case (0.5%) a higher MRD level was detected in PB. In 66 paired samples (33%) MRD was detectable only in BM and in several of these cases the amount of residual disease could be as high as 10-2. In 16 pairs (8%) only the PB proved positive but at very low levels (usually ≤ 10-4). In T-ALL 132 out of 221 paired samples proved negative both in the BM and PB whereas a positive MRD was found in at least one sample of the remaining 89-paired analyses (figure 1 panel B). Among these latter samples, a positive MRD was detected in both BM and PB with comparable levels in 48 paired samples (54%), while the amount of detectable disease was remarkable different (from 1 to 2 log) in 22 pairs (25%), being higher in BM in 15 cases. In 8 paired samples (9%) MRD proved positive only in the BM while in 11 (12%) only in the PB. Conclusion. MRD detection on BM samples is more sensitive compared to PB, in both B and T lineage ALL. However, in some T-ALL cases, the PB may provide discordant and informative results. Our results suggest that either in B precursor ALL as well as in T-ALL, an MRD evaluation must be always performed on BM but preferably, PB samples should be tested in parallel.





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HYPERKINETIC T-ALL WITH T(8;14)(Q24;Q11): T-LINEAGE COUNTERPART TO BURKITT LEUKEMIA AND A CASE FOR A SHIFT TO BURKITT-TYPE CHEMOTHERAPY?

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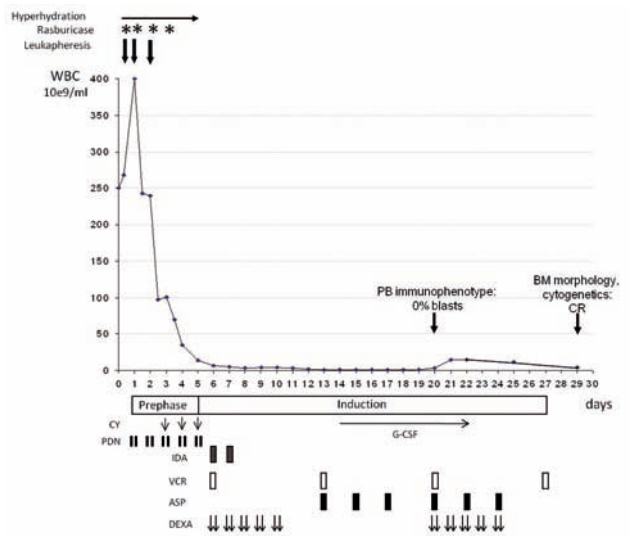
Sporadic cases of T-ALL (acute lymphoblastic leukemia) with t(8;14) display an extremely aggressive behavior with poor response to standard chemotherapy. This rare entity was identified in children while only anecdotal cases are known in adults. Translocation (8;14)(q24;q21) differs from t(8;14)(q24;q32) of Burkitt B-ALL for the chromosome 14 breakpoint fusing the MYC oncogene to TCR alpha/delta genes. The highly malignant course of t(8;14) T-ALL, reminiscent of B-ALL, suggests that it should be treated with alternative regimens like those effective in B-ALL rather than standard ALL therapy. We observed a 44 year-old patient suffering from backache and malaise since a few days before referral, who presented with WBC 251 (x10e9/L) (100% lymphoblasts), Hb 16,4, platelet 159, marked splenomegaly and mediastinal widening. Flow cytometry revealed T-lymphoblasts with cortical phenotype (cyCD3 94%, CD1a 82%) but low TdT expression (10%), and cytogenetics showed t(8;14)(q24;q11). The patient underwent immediate leukapheresis and received hyperhydration along with rasburicase. On admission day the WBC increased from 268 to 400 over 15 hours, yielding an extrapolated doubling time of 29 hours. Then additional leukaphereses were performed and prephase treatment started (prednisone 20 mg/m2 BID on days 1-5, cyclophosphamide 300 mg/m2 QD on days 3-5), reducing the leukemic burden (Figure). Standard induction led to blast clearance on day 20 (0% CD1a+), and complete hematologic and cytogenetic remission on day 29. The molecular analysis of minimal residual disease was performed and will be presented together with the early postremissional course. T-ALL carrying t(8;14) is extremely rare (<1%). The case described documents the uniqueness of this syndrome, clinically akin to B-ALL (high proliferation rate, lymphomatous presentation, low/negative TdT expression despite hyperproliferative status, poor response to standard anti-ALL therapy), except for the MYC rearrangement involving TCR and not IgH genes. Because occasional adult T-ALL cases (<5%) are consistently TdT negative and/or exhibit an explosive growth rate, performing an ultra-rapid cytogenetic diagnosis (supplemented by FISH) is necessary in hyperkinetic T-ALL in order to identify t(8;14) and modulate treatment accordingly, perhaps favoring fractionated/rotational drug regimes like those active in B-ALL. A collaborative inter-group registry would improve knowledge on this ALL variant and its treatment.



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EFFICACY AND TOXICITY OF LIPOSOMAL CYTARABINE – DEPOCYTE® – ADMINISTRATION IN ADULT PATIENTS WITH ACUTE LEUKEMIA OR NON-HODGKIN LYMPHOMA

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Central nervous system (CNS) involvement at diagnosis or at relapse in Acute Lymphoblastic Leukemia (ALL) and in Non-Hodgkin Lymphomas (NHL) may be observed in 3-8% of patients, and is considered an adverse prognostic factor. Conventional systemic cytotoxic drugs do not cross the blood-brain barrier and are generally ineffective to control CNS disease. Intrathecal (i.t.) administration of methotrexate, or cytarabine +/- steroids are currently used as prophylaxis or treatment in patients with CNS leukemic or lymphomatous disease at diagnosis or at relapse. A liposomal formulation of cytarabine – Depocyte® (Mundipharma, Cambridge, UK) - has been approved for the treatment of lymphomatous meningitis. Depocyte® has a prolonged half-life in cerebrospinal fluid (CSF), allowing a sustained cytotoxic drug level over 14 days. This pharmacokinetic property allows to reduce lumbar punctures leading to a better compliance for patients, expecially in childhood setting. We treated 16 patients (6 ALL; 6 NHL; 3 PCNS-NHL; 1 AML), median age 42 yrs, by a median of 4 i.t. doses (range, 2-5 doses) (50 mg/dose, once every two weeks) of Depocyte®. Meningosis was observed in 4 patients at diagnosis and in 10 patients at relapse, while two patients with PCNS-NHL did not show meningeal disease. Median CSF cell count before starting treatment was 105/uL (range, 0-2000/uL). Meningeal disease was confirmed by cytology as well as by cytofluorimetric analysis. Cell clearance was obtained in 12/14 patients in which CSF cell count quickly decreased after the first administration in all but 2 patients in which CSF blast cells disappeared after 2 doses. CSF remission was confirmed by FACS analysis in 10/12 cases. I.t. administration was interrupted because of death for disease progression in 6 patients, while in 4 patients the treatment was completed. No patient showed toxicity or adverse events related to Depocyte®, with the exception of 2 patients who developed a grade III neurotoxicity: 1 patient with a profound somnolence, while the other patient with a severe sensitive and autonomic peripheral neuropathy. In both patients treatment was interrupted. Depocyte® is highly effective and well tolerated in the treatment of meningeal leukemic or lymphomatous disease, while no activity was observed in patients with PCNS-NHL. Treatment should be soon discontinued when a severe adverse event occurs.

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EFFICACY AND FEASIBILITY OF TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA WITH HYPER-CVAD PROGRAM: RESULTS FROM AN ITALIAN CENTRE.

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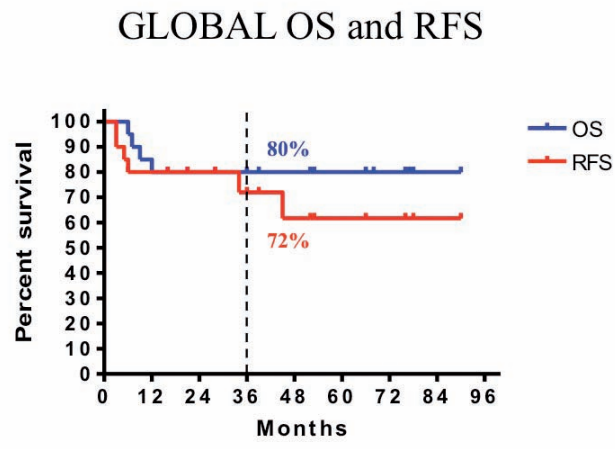
Background: Modern intensive chemotherapy regimens have improved the prognosis of patients with adult acute lymphoblastic leukemia (ALL), however the optimal initial therapy for the treatment of adult ALL is yet to be defined. Hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD) alternating with high dose methotrexate and cytosine arabinoside (HD-MTX–Ara-C) program has become a widely used treatment for adult ALL, although publication of outcomes is largely limited to American single-centre experiences. We performed a retrospective analysis of patients treated at our Institution. METHODS: We performed a retrospective analysis of 20 ALL patients treated with Hyper-CVAD/HD-MTX–Ara-C as first line treatment between 2003 and 2010 with a mean follow-up time of 41 (range 6-90) months. Male/female ratio was 13/7. Patients' mean age was 40 years (range 18-65), and 3 patients (15%) were > 60 years. The incidence of Philadelphia chromosome (Ph)-positive, T-cell ALL and Burkitt leukaemia were respectively 15%, 25% and 20%. Nine patients (45%) were high risk for hyperleukocytosis, cytogenetic or refractoriness to steroid pre-treatment. Ph-positive ALL were treated with association of Imatinib, while 5 of 6 CD20-positive ALL received Rituximab. Results: A complete response (CR) was achieved in 95% of patients. The induction mortality rate was 5% (one case). Ten patients (50%) completed the planned treatment, and median recycling time was 33 days. With a mean follow-up time of 41 months, the 3-year overall survival (OS) rate was 80% and the 3-year relapse free survival (RFS) was 72% (Figure 1). Sixty-eight non-haematological severe adverse events were reported in 122 administrated cycles (55.7%), in particular 27 infections (22.1%, one death). We observed 4 relapse during treatment (3 high-risk patients) and 2 during follow up (all high-risk patients). Allogenic stem cells transplantation was performed in 3 high-risk patients at first complete remission and in 3 relapsed patients. High risk-status, lineage, CD20-positive ALL or age > 35 years were not predictive of poor OS. We observed a significant shorter RFS in high risk status patients (34 months vs not reached, p 0.02), no difference for lineage, CD20-positive status or age > 35 years. Conclusions: Hyper-CVAD is an effective and tolerable induction strategy for adult ALL. Although time density is not easily reproducible, treatment is not detrimental on patients' outcome.



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NEXT GENERATION PAIRED END TRANSCRIPTOMIC RE-SEQUENCING TECHNOLOGY REVEALS NOVEL COMMON GENETIC ALTERATIONS IN THE HEDGEHOG PATHWAY IN ADULT BCR-ABL1 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Nowadays high throughput "Next Generation Sequencing" technologies are revolutionizing genomics and transcriptomics by providing a cost-efficient and single base resolution tool for a unified deep analysis of tumor complexity. Taking advantage from this tool, we used the Illumina/Solexa platform to define in a single procedure and at high sensitivity the full repertoire of leukemia-related mutations in 3 adult BCR-ABL1+ ALL cases treated with tyrosine kinase inhibitors. Patient median age was 55.3 years (range, 40-70); no additional chromosome abnormalities were detected except for one case. All the selected cases had previously been profiled by high resolution SNP (Affymetrix SNP6.0) and gene expression (Affymetrix Human Exon 1ST Array) arrays, as well as candidate gene re-sequencing (IKZF1, PAX5, JAK2, CDKN2A, CDKN2B, IDH1, IDH2), lacking missense point mutations. Two cases harbored the deletion of IKZF1, and in one case PAX5 and CDKN2A/B losses were also found. Poly(A) RNA from blast cells was used to prepare Illumina cDNA libraries according to the manufacturer's recommendations. Sequencing by synthesis was performed on an Illumina Genome Analyzer IIx platform, with standard sequencing kits and nucleotide incorporation cycles, generating 75 base pairs (bp) paired end sequence reads. A total of 57, 51 and 9 million reads were obtained from the 3 samples

and high quality sequence reads were mapped to the reference sequence of the human genome (UCSC hg19) using the Maq software, finding out 58,205, 48,913 and 136,937 putative new single nucleotide variants (SNVs) in the CDS/EXON regions not reported in the dbSNP build 130. Of these, 874 distributed on 290 genes, affected both samples. A functional analysis was carried out on common mutated genes using the GeneGo software (www.genego.com) and in the list of the most significant GeneGo Pathway Maps we found the "Development Hedgehog Signaling" (p value = 1.400e-4), including genes such as casein kinase 1, alpha 1-like (CSNK1A1L), catenin (cadherin-associated protein) beta 1 (CTNNB1) and heat shock protein HSP 90-beta (HSP90AB1). In conclusion, this study provided, for the first time, a comprehensive overview of a BCR-ABL1+ ALL transcriptome, identifying novel mutations potentially involved in ALL. Supported by: European LeukemiaNet, AIL, AIRC, Fondazione Del Monte di Bologna e Ravenna, FIRB 2006, Ateneo RFO grants, Project of integreted program (PIO), Programma di Ricerca Regione–Università 2007–2009.

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A CASE OF ATYPICAL BCR-ABL MRNA TRANSCRIPT IN ADULT B-ACUTE LYMPHOBLASTIC LEUKEMIA

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Alternative breakpoints mapping outside typical M-bcr and m-bcr regions are rare and controversial information have been reported about the correlation between different BCR-ABL chimeric genes and their clinical outcome. BCR-ABL fusion gene is found in around 25% of adult ALL cases. Several atypical BCR-ABL fusion genes have also been observed almost exclusively in chronic myeloid leukaemia(CML) and little is known about atypical transcripts and clinical outcome in ALL, where one of the atypical transcripts in adult is e1a3. We report a case a of a 37-year old female Philadelphia(Ph) positive acute B lymphoblastic leukemia(ALL) with this uncommon e1a3 fusion transcript. She was admitted to our hospital for leukocytosis, anemia, thrombocytopenia and splenomegaly(14 cm). Cytofluorimetric analysis revealed a common B-ALL phenotype (positive for CD10, CD19, CD20 CD34, HLADR and TdT). Conventional cytogenetic showed 46,XX t(9;22)(q34,q11) karyotype. RNA from bone marrow sample was subjected to molecular analysis by RT-PCR following a conventional two-step PCR procedure (Leukemia 1999; 13:1901-1928) to assess the presences of Major/minor BCR-ABL rearrangement. E1a3(347bp) amplification product was obtained with m-BCR-ABL primers and further comfirmed by direct sequencing. After imatinib and hyper-cvad rituximab protocol, complete remission was obtained and she received sibling bone marrow transplantation (BMT). During the follow-up BCR-ABL transcript has been monitored monthly by nested PCR without the possibility of a quantitative analysis as specific primers were not available. To date, 16 months from the onset and 9 months after BMT, the patient is still in partial remission with PCR RT nested positivity, 2 % of donor chimerism (by using STR) and with a moderate grade of GVHD. To our knowledge, only 7 adult B-ALL patients with this atypical BCR-ABL transcript have been described and 4 of them died by 18 months after diagnosis despite BMT. The identification of this e1a3 transcript by using only nested PCR does not permit its quantification and its specific MRD monitoring. In the contest of CML, this e1a3 transcript is associated with a non-aggressive phenotype. In conclusion this case seems to confirm the good clinical outcome and a less aggressive treatment might be also considered.

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TRATTAMENTO CON CLOFARABINA E CICLOFOSFAMIDE NEI PAZIENTI AFFETTI DA LEUCEMIA ACUTA LINFOBLASTICA IN RECIDIVA E/O RESISTENTI.

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Introduction: Promising activity with DNA damage and apoptosis in both AML and ALL blasts from the peripheral blood and the marrow of clofarabine in combination with cyclophosphamide and has been reported (Karp JE et al, Blood 2007). We present a series of eight cases in which clofarabine was combined with cyclophosphamide in adult patients with relapsed or refractory acute leukemia. Methods: Patients aged 23–57 years with refractory/relapsed ALL were treated at the dose of clofarabine 10mg/m2 + cyclophosphamide 400g/m2 on days 1-3 and 8-10. We evaluated the overall remission rate (ORR), duration of remission (DOR) and overall survival (OS). Minimal residual disease (MRD) by molecular targeting was considered in all patients. Results: Seven patients received clofarabine 10mg/m2 + cyclophosphamide 400mg/m2, both on Days 1–5 and 8-10;one patient received only one cycle. All patients had relapsed/refractory lymphoblastic leukemia and had received multiple priors tharapies. Six had pre-B cell ALL, 2 pts had T cell ALL; two pts had received a prior hematopoietic stem cell transplant (HSCT). Three patients achieved a morphologic complete remission (CR); two patients went on to receive allogeneic transplants after clofarabine/cyclophosphamide salvage. The median of Overal survival (OS) for all the patients was 104 days, the media was 189 days. The overall remission rate (ORR) was 42%, and we estimated a duration of remission (DOR) as 288,67 days in media (we calculated from the first day of salvage therapy). Treatment was complicated by neutropenic fever (n=4), grade III-IV mucositis (n=2), prolonged aplasia >30 days (n=2). One patient died of sepsis before completing the regimen. Conclusion: Combination treatment with clofarabine and cyclophosphamide in adults pts with refractory or relapsed ALL resulted in an ORR of 43%, two pts including 3 of 8 responders proceeded to HSCT. The safety profile is acceptable in this relapsed/refractory population. More studies with this combination in adults are warranted.

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PHARMACOGENETICS IN PEDIATRIC ONCOEMATOLOGY: IMPACT OF ABCC2, MTHFR C677T, MTHFR A1298C AND TYMS POLYMORPHISMS ON METHOTREXATE TOXICITY.

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Methotrexate is one of the most important drugs used in Pediatric Oncology. Children vary in their response and pattern of toxicities, probably because of polymorphisms of genes encoding enzymes involved in the drug metabolism. The aim of this study is to investigate the association of selected genetic variations and clinical features with the toxicity of methotrexate treatment. The study included 44 children with acute lymphoblastic leukaemia (68%), lymphoma (18%), osteosarcoma (7%) or medulloblastoma (7%). The genetic analysis was performed by real-time polymerase chain reaction, using DNA extracted from subjects' peripheral blood. The ABCC2 (ATP-binding cassette, sub-family C - CFTR/MRP- member 2) polymorphic allele frequencies were 25% and 5% for CT and TT genotypes, respectively. The MTHFR (methylenetetrahydrofolatereductase) C667T polymorphic allele frequencies were 50% and 30% for CT and TT genotypes. The MTHFR A1298C polymorphic allele frequencies were 37% and 6% for AC and CC genotypes. The TYMS (thymidylatesynthase) polymorphic allele frequencies were 54% and 32% for 2/3 and 3/3 genotypes, respectively. Of all the patients, 70,5% had high serum methotrexate levels or toxicity: in this group the frequency of homozygosity or eterozigosity polimorphisms of MTHFR C677T and TYMS was elevated (50 and 63,6%, respectively). No significant relation was found between ABCC2 and MTHFR A1298C polymorphisms and adverse events. High serum methotrexate levels were more frequent in children younger than 6 years, treated with 2 g/m2 of methotrexate; toxicities of kidney, liver, skin and central nervous system were more common in children older than 9 years, who received 5 g/m2 of methotrexate. In conclusion, the present study suggests that MTHFR C677T and TYMS are significantly related with methotrexate toxicity: genotyping these genes and considering patients' clinical features might be useful to personalize chemotherapy and avoid unpleasant adverse events.

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USE OF UNICEL DXH 800 CELL POPULATION DATA AS HELPING TOOL IN ALL-L1 DIAGNOSIS: CASE REPORT

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Introduction and methods. Our lab gives service for both laboratory hematology and clinical laboratory test using UniCel DxH 800 and Cytomics FC500 devices. DxH800 performs leukocytes differential with the Flow Cytometric Digital Morphology (FCDM) technology, that gets information on cells in native state measuring Volume (V), Conductivity (C) and 5-angle Scatter light laser (MALS, UMALS, LMALS, LALS, AL2). Mean (M) and standard deviation (sd) of each measurements are collected in 56 Cell Population Data (CPD). CPD changes compared to normal values are well know to correlate to morphological alteration of the cells. In this report we describe some highlights of a case report of ALL-L1 patient that came in our lab for routine purposes. Results. DxH800 CBC/Diff/Ret test of the blood sample reported mild leukocytosis with high monocytosis (WBC=16227/µl, MO#=11.754), anaemia (Hgb=8.22 g/dl) and thrombocytopenia (Plt=42.100/µl), NRBC=1.68/100 WBC. Immature Granulocyte and Blast alarms were present, and differential scatterplot showed the clear presence of lymphocytes (LY), monocytes (MO) and lymphomonocytoid elements. LY-M-C was 124 (a.u.), higher than the current cut-off of 117. Based on this value, the analyser triggered a specific comment for the presence of abnormal and atypical lymphocytes. All these data, taken together, drove us to slide review that confirmed us the huge presence of lymphoblastic cells with morphological features of immaturity. Peripheral blood immunophenotype revealed CD38+ (80%), CD3c+ (5.0%), TdTc+ (75.0%) CD34+ (75.0%) CD79Ac+ (77.0%) CD19+ (80.0%) HLA-DR+ (73.0%) MPOc-, CD7+ (7.0%) CD22-, CD13+ (5.0%), CD14-, CD10-, CD45+(99%). Bone marrow immunophenotype revealed CD38+ (90%), TdTc+ (92.0%), CD34+ (90.0%), HLA-DR+ (89%), CD19+ (90%), CD45+ (95%), CD117-, CD22-, CD3c+ (2.0%), CD79Ac+ (89.0%). The diagnostic outcome was L1-B-ALL; the patient was directed to chemotherapy and now we are following the outcome of the therapy. Conclusion. This case report is an example of our validation process that permits us to address the diagnostic route of suspected samples. This process is based on DxH 800 diagnostic thorough information, integrated by the diagnostic suspect, evaluation of the blood smear and flow cytometry data. DxH 800 information consist in analytic parameters, histogram/scatterplot evaluation and validation messages based on CPD. This process is continuously updated on our experience and on literature data.

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NELARABINE IS SAFE AND EFFECTIVE IN ADULT RELAPSED OR REFRACTORY T CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) AND LYMPHOBLASTIC LYMPHOMA (T-LBL): THE BOLOGNA EXPERIENCE

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Background. Nelarabine (N) is approved for the treatment of T-ALL and T-LBL that have not responded to or has relapsed after treatment with at least 2 chemotherapy regimens. Aim. To evaluate safety profile and efficacy of N as savage therapy in 16 adult relapsed or refractory T-ALL or T-LBL. Methods. After obtaining an informed consent, 16 patients (median age 33 years, range 19-45, M/F= 13/3) affected by T-ALL (n=10) and T-LBL (n=6) received savage therapy with N (median cycle=1, range 1-3), administered at standard adult dosage (1500 mg/sqm on days 1, 3 and 5, every 21). Four patients were primary resistant to induction treatment, 7 patients were relapsed after two previous chemotherapy regimens (including allogeneic BMT in 4 cases and autologous SCT in 1 case); the remaining 6 patients had a molecular relapsed disease (MRD positive). Molecular characterization was performed, including NOTCH and WT-1 genes mutational status. GEP analysis, according to Ferrando A. stratification (Cancer Cell 2002), is still ongoing. Results. Currently, 12 out of 16 patients are evaluable, due to a too short follow up in the other 4 cases. Seven out of 12 patients obtained a complete remission (CR) (5 T-ALL 2 T-LBL);a partial remission (PR) was documented in 2 cases, with an overall response rate (ORR) of 75%. Median duration of CR was 10 weeks (range 2.8-54+). Among these, 2 out of 4 patients in molecular relapse reached a molecular CR and underwent an allogenic BMT (currently in CR after a median follow up of 12 months). Extra- hematological toxicity, not clearly related to the drug, occurred in 3 cases, determining, a complete and irreversible paraplegia, a condition of mental confusion, and a peripheral neuropathy, respectively. Conclusions. N showed a strong efficacy also in cases with low levels of residual disease, in addition to a good safety profile. Neurological toxicity needs to be strictly monitored. *Acknowledgments. European LeukemiaNet, AIL, AIRC, Fondazione Del Monte di Bologna e Ravenna, FIRB 2006, PRIN 2008, Ateneo RFO grants, Project of integrated program (PIO), Programma di Ricerca Regione – Università 2007 – 2009.*

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PEDIATRIC-LIKE INTENSIFIED THERAPY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background. Acute lymphoblastic leukemia (ALL) shows different outcome in children and adults, with event-free-survival rates of 70-80% and 30-40% at 5 years, respectively. Results improved in young adults/adolescents with de novo ALL, if treated with pediatric regimens. Clinical studies are ongoing in older patients, toxicity related-therapy seeming the limiting issue. Aims We report a single centre experience on adult ALL patients treated with an intensive pediatric-like schedule, aiming to assess its tolerability and efficacy. Methods From 11/07 to 03/11 we treated 24 ALL patients (M/F=17/7) according to modified AIEOP-LAL2000 regimen. Treatment consisted of 7 days steroid pre- treatment, and 4 drugs 78-days induction (phase IA+IB) after which high risk patients were treated with 3 polychemotherapy blocks, while intermediate and standard risk patients went on 8- weeks consolidation and subsequent intensification. A 2 cycle consolidation therapy with nelarabine was planned for T-ALL patients. Patients with HLA-matched donor underwent allo-SCT; 2- years maintenance therapy was given to the others. Median age was 30 years (17-47). Results 22/24 patients completed phase IA, 2 being out for grade IV toxicity (intestinal occlusion and sepsis). 17 (71%) obtained complete remission (CR), 5 (21%) were refractory. One of the resistant patients achieved CR after polychemotherapy blocks, 2 after phase IB. Median induction duration (IA+IB) was 95 days (82-136); delays were mostly experienced during phase IB due to hematologic toxicity. After induction, 3/19 CR patients received consolidation therapy, then 2/3 underwent allo-SCT. 5 received blocks: 3 undewent allo-SCT, 2 dropped out for reversible grade III renal toxicity. 5 patients were treated with nelarabine, then 2 underwent allo-SCT. 3/19 directly underwent allo-SCT. 1 patient completed the whole therapeutic program due to lack of suitable donors for allo-SCT. With a median follow up of 12 months (3-50), 14/24 (58%) patients are alive, 9 in CR (5 underwent allo-SCT). 10 patients died, 5 for relapsed/refractory disease, 5 in CR (3 after allo-SCT). Median CR duration was 12 months (3+-46). Conclusions Pediatric inspired therapy demonstrated significant hematologic toxicity in adult ALL patients, this causing lack in dose-intensity maintenance. The overall outcome is similar to the one reached in other studies conducted on larger series: longer follow-up and larger population are needed to draw definitive conclusions.

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A PRELIMINARY EXPERIENCE OF DETECTION OF MINIMAL RESIDUAL DISEASE (MRD) IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL).

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Minimal residual disease (MRD) is the most powerful prognosticator in childhood acute lymphoblastic leukemia (ALL) and there is strong

evidence supporting its relevance even in adults. The aim of our study was to correlate MRD with clinical features and outcome in a series of 38 patients (22 males and 16 females) affected with ALL. Median age at diagnosis was 37 years (17-63), 23 (60%) patients had ALL of B-origin and 15 (40%) of T-origin. Eleven (29%) of 38 patients had a white blood cell count (WBCc) ≥100x109/L and 10 (26%) had extra-hematologic localization. Ten (26%) and 2 (5%) of 31 patients with a successful karyotypic analysis carried BCR/ABL and 11q23 rearrangement, respectively. Eighteen patients were treated within the LAL0904 clinical trial and 20 received 2 courses of HDARAC and HD mitoxantrone (HAM program). Overall, 17 patients were submitted to allogeneic stem cell transplantation after consolidation. MRD presence was investigated in BM samples after completion of induction (MRDind) and consolidation therapy (MRDcons), using a 3-5 colours flow cytometric assay. The threshold of positivity was established at 3x10e-4 residual leukemic cells, a level that corresponded to the median MRD value measured after induction and consolidation. MRD assessment was available for all of 34 patients (89%) who achieved complete remission after induction and for 32 (84%) after consolidation. Twenty-one (62%) of 34 patients were MRDind positive and 16 (50%) of 32 MRDcons positive. We found a correlation between MRD positivity and B-lineage: 13 of 20 B-ALL (65%) were MRDind positive and 12 of 18 (66%) MRDcons positive (p=0.03). Of 19 patients with abnormal karyotype, 12 (63%) were MRDind positive and of 18 evaluable after consolidation 9 (50%) were MRDcons positive. Of 15 evaluable patients treated within LAL0904 protocol, 9 (60%) were MRDind positive and 5 (38%) of 13 MRDcons positive. Of 19 patients treated within HAM program, 12 (63%) and 8 (42%) were MRDind and MRDcons positive, respectively. A statistically significant difference was observed in DFS between MRDcons negative vs MRDcons positive patients (57% vs 42% at 2 years, p=0.03). Although still based on a few numbers, our results support the need to implement studies of flow cytometry MRD detection in adult with ALL with the final goal to stratify risk of relapse on individual basis and personalize therapeutic approach.

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PROPHYLAXIS OF LEUKEMIC MENINGOSIS IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: LIPOSOMAL CYTARABINE (LIP ARAC, A NEW APPROACH

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Patients with Acute lymphoblastic leukaemia (ALL) can present central nervous system (CNS) progression or recurrence during the course of their malignancy. The Leukemic Meningitis (LM) is profoundly morbid and often fatal, so that prevention of CNS relapse is increasingly a goal of primary therapy for patients with ALL. All patients are at risk and receive prophylaxis. The use of direct intrathecal instillation of anticancer drugs (MTX, AraC) is the approach that has successfully been used in both the treatment and prevention of leptomeningeal involvement to circumvent the pharmacologic sanctuary resulting from the blood-brain barrier. However, the use of MTX or AraC necessitates frequent and prolonged courses of lumbar punctures. Some patients receive radiotherapy as a part of their prophylactic program. Lip Ara-C (Depocyte), an encapsulated microvesicular liposome preparation, has shown significant activity in the treatment of neoplastic meningitis, with a significant pharmacokinetic advantage in comparison with free araC. On the other hand there are limited data for prophylaxis. Our retrospective study, among Italian Hematology Centres, aims to evaluate the safety and tolerability of lip araC in the CNS prophylaxis of ALL meningeal recurrences. Eighteen patients aged 27-81 years (median 50) have been preventively treated with 2-10 doses of lip araC 50 mg. Diagnosis consisted on 13 B-ALL, 4 T-ALL, 1 hybrid cell. All patients were treated according with GIMEMA, NILG and BMF protocols. All patients received lip araC 50 mg every 2 or 3 weeks and had corticosteroids for prevention of chemical aracnoiditis. The toxicity were G1 and G2 headache, G1 nausea/vomiting, localized or diffuse bone pain, 1 transitory sfincterial incontinence. None of the patients developed unexpected long term neurological side effects. IT lip araC therapy with concomitant corticosteroids appears to be feasible and well tolerated in the prophylactic setting. Considerable concerns about the safety of this drug arose from recent observations that lip AraC might contribute to neurologic side effects when given too closely to high-dose systemic chemotherapy known to penetrate the brain-blood barrier.Careful adherence with preventive measures might help physicians to minimize side effects possibly related to the administration of lip Ara C. Superior efficacy of lip ara-C compared with standard IT therapy in the LM prophylaxis should be confirmed in prospective clinical trials

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CD20+ PROGNOSTIC SIGNIFICANCE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background. The prognostic significance of CD20 expression in Acute Lymphoblastic Leukemia (ALL) blasts is still a matter of debate in adult ALL patients. These patients' outcome has been considered up to now to be variously affected both by non homogeneous chemotherapy approaches and by different biological parameters. Aim. Aim of our study was to evaluate the prognostic impact of CD20 expression in 147 adult ALL patients (<60 y.rs), diagnosed according to the FAB/WHO classification and homogeneously treated between 1996 and 2010. Patients and Methods. Cut-off for CD20+ expression was 20%. All patients were treated according to the 0904 Gimema Protocol (Prednisone, Vincristine, Daunoblastine, Asparaginase). Patients' median age was 31 years (range: 15-60). Results. The median age of patients expressing CD20 (62 pts, 42%) was higher than of CD20- patients (40 vs 26, p=0.039), while there were no differences regarding white blood cells and platelets counts, percentage of peripheral and bone marrow blasts cells, recurrent genetic abnormalities as t(9;22), t(4;11), t(8;14). CD20+ patients showed a lower incidence of myeloid antigen expression (CD13 and/or CD33, p.0.04), but this was not confirmed in CD20+ Ph- patients. There was no correlation between Ph+ (41 pts) and CD20+ and the outcome was independent of CD20 expression. In the Ph- group (106 pts), Disease free survival (DFS, p=0.9) and Overall survival (OS, p=0.24) did not seem to be affected by CD20 expression. Conclusions. Taken together, our data seem to suggest that CD20+ ALL adult patients are older and have a lower expression of myeloid antigens, but these data are uncorrelated with OS and DFS. Although our patients sample was a homogeneous study cohort for age and treatment, further large case series are needed to evaluate the true prognostic impact of CD20 expression in adult ALL patients and the role of CD20 antibodies therapy.

ACUTE MYELOID LEUKEMIA

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WT1 (WILM'S TUMOR 1) AND FLT3-ITD (INTERNAL TANDEM DUPLICATION) A RETROSPECTIVE DIAGNOSTIC STUDY IN ACUTE MYELOID LEUKEMIA (AML) AND ACUTE PROMYELOCYTIC LEUKEMIA (APL)

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Introduction: Acute Myeloid Leukemia (AML) is a clonal disorder linked to abnormal myeloid stem cells production involving granulocyte, erythrocyte and platelet cell lines. Several genetics factors are involved in development of this hematopoietic disorder characterized by abnormal production of immature cells. Among them, FLT3 (fms-related tyrosine kinase 3) and WT1 (Wilm's Tumor-1) genes which encode for important tyrosin kinase-related proteins. The presence of mutation or expression variation in these genes are linked to increased risk of disease relapse. Material and methods: RNA extracted from peripheral or bone marrow samples were obtained from 136 AML patients afferent to Hematology department at the Cancer Hospital of Cagliari, from January 2004 to August 2010. 22 of them (16%) were affected of Acute Promyelocitic Leukemia (APL), a severe form of AML caused by the interruption of promyelocitic differentiation. Average age of patients was 56 years, with the main frequency ranged between 61–70 years. FLT3 PCR reactions were done using 20 pmol of specific primers starting from 3ul of cDNA obtained from each RNA. WT1 Real-Time PCR reactions were carried out using specific primers and probe (Ipsogen, Inc.). Results: 13% of cases (18) in onset were positive for FLT3 gene internal tandem duplication (ITD). Among them, 13 patients (72,2%) were AML and 5 (22,8%) were APL. Regarding WT1 gene expression analysis, 12 cases of patients followed from onset and during follow-up have shown normalized expression levels even if 3 patients incurred however in molecular and morphological relapse. Furthermore, one more patient had only over-expression of WT1 without indication of morphological relapse. Interestingly, in the 4 cases we were able to analyzed for both FLT3 and WT1 markers from onset during follow-up (15-19 month), we have found an association of response: the presence of FLT3-ITD mutation correlating with WT1 up-regulation at onset and, in remission phase, the ITD disappearance together with normalization of WT1 expression level. Conclusions: Molecular monitoring of WT1 gene transcripts and FLT3-ITD mutation represents an important prognostic factor in context of AML. The evaluation of these markers at diagnosis and in particular during the follow-up, provides additional information on the likelihood of progression and risk of recurrence. In the present study we have described the molecular characterization of FLT3-ITD mutation and WT1 gene expression in order to observe as these markers together correlates with disease remission or progression phases. Unfortunately, we could monitor only 26% (35) of patients studied for both gene variation as in onset as during follow-up phases. We have demonstrated the concomitant presence (at onset) and reduction (remission) of FLT3-ITD and WT1 RNA expression levels in four cases of AML patients indicating in advance few cases of disease relapse prior to any morphological evidences. The results obtained in this study on the possible association between FLT3-ITD and WT1 gene regulation, should be take in account as preliminary data considering the limited number of complete data analyzed for these markers.

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INTERNAL TANDEM DUPLICATION (ITD) MUTATIONS OF THE FLT3 RECEPTOR AND CXCR4 EXPRESSION IN DE NOVO ADULT ACUTE MYELOID LEUKEMIAS (AML)

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Introduction. Internal tandem duplication (ITD) mutations of the FLT3 receptor are associated with a high incidence of relapse in acute myeloid leukemia (AML). It is also known that the complete immunophenotypic characterization contributes to define the prognosis of de novo adult AML. 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. Methods. We investigated the expression of the chemokine receptor CXCR4 on bone marrow blast cells in a group of adult de novo AML with FLT3-ITD. We have observed 40 young adult de novo AML patients (median age: 44 years, r: 22-60) in the last 4 years, who presented FLT3-ITD-positive AML blasts in bone marrow blood. On the basis of FAB classification the patients were considered LMA-M5 (15 pts.), LMA-M2 (10 pts.), LMA-M4 (6 pts.), LMA-M1 (6 pts.) and LMA-M0 (3 pt.). 12 patients showed complex chromosomal anomalies; 19 patients presented hyperleukocytosis (WBC> 40x10(e)9/L). The clinical outcome was that one of a "high risk" AML; at the present only two patients are still alive in CR (+36 months and +40 months). Results. We found at the diagnosis, in all cases, an high CXCR4 expression on leukemic blasts, as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5. Conclusions. Several studies have shown the prognostic significance of the expression of differentiation myeloid markers at the diagnosis of adult de novo AML. However, specific immunophenotype expression patterns associated with internal tandem duplication (ITD) mutations of the FLT3 receptor are still unknown. Further studies are warranted to confirm the correlation between FLT3-ITD and "CXCR4 over-expression" immunophenotypic pattern.

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ACUTE MYELOID LEUKEMIA WITH CONCURRENT MYELOID SARCOMA: CLINICAL AND BIOLOGICAL FINDINGS, OUTCOME

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The aim of the study was to describe clinical pictures and outcome of AML with concurrent Myeloid Sarcoma (MS) uniformly treated in our institution from January 1995 to March 2011. We found 24 patients, 8 male and 16 female, median age 51 years (range 15-71), median follow-up 13.8 months (range 2.0-164.6) and median follow-up of survival patients 30.5 months (range 5.7-164.6). In our population the incidence of MS was 6,1% of all AML patients treated in our department during the same time. The anatomical sites involved were: skin (n=13), lymph nodes (n=4), spleen (n=1), central nervous system (CNS, n=2), female genital tract (n=2), parotid gland (n=1), kidneys (n=1), paraspinal masses (n=2) and lungs (n=1). Cytologic or histologic diagnosis was obtained in 19/24 pts; the remaining 5 pts were diagnosed by radiological picture. Median of white blood cells count (WBC) at presentation was 47.5 x109/L (r: 1.1-238) and median of lactate dehydrogenase level was 814 U/L (r: 233-2924). According to FAB classification we found 3 M0,5 M1,2 M2,7 M4,6 M5,1 M6. Cytogenetic analyses were available in 22 patients and we divided them according to Medical Research Council criteria. Most of the patients fell into intermediate risk group (16 patients with normal karyotype and 2 with abnormality 11q23/MLL), 3 pts into favourable risk and 1 pts in high risk group. Data regarding FLT3-ITD mutation were available in 19 patients: 4 patients were mutated. Complete remission (CR) was obtained in 14/24 patients (58.3%). Relapse Free Survival (RFS) was 23.7 months and Overall Survival (OS) was 17.9 months (OS 64.5% at 1 yr). In univariate analysis, CR was statistically associated with better OS (p=0,0066, HR 3,84). We also observed that patients undergoing AlloHSCT in 1st CR had a significant better outcome (p=0,017, HR 7,65). Age, sex, WBC count, FAB subtype, karyotype, presence of skin involvement had no influence on OS. In a Cox multivariate analysis, OS and RFS were influenced only by CR, even when adjusted by age, sex, WBC and cytogenetic-molecular risk (p=0,019). In our experience obtaining a CR was a significant indicator for better OS. The 4 pts who underwent allogeneic procedures in first remission are up to now alive and well.

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RISK-ORIENTED TREATMENT IN ACUTE MYELOID LEUKAEMIA (AML). A PILOT STUDY IN A SINGLE INSTITUTION

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The recent WHO classification reflects the fact that an increasing number of AML can be categorized based upon their underlying cytogenetic or molecular abnormalities, and that these genetic changes form clinico-pathologic-genetic entities.The prognosis of these entities is variable and the therapeutic approach (allogeneic BMT vs autologous BMT or multiple chemotherapy consolidations) would be different.During the last three years, we examined a cohort of young patients affected by AML, using a diagnostic approach to assess a prognostic categorization and to guide the therapy. 35 patients, median age 50 yrs (range 16-62), M/F 15/20, were studied.1 patient died before the start of induction therapy. The other 34 were treated with Idarubicine 10 mg/mg dd 1-3-5, Ara-c 3 gr/mq /12 h dd 1-3-5-7 and Etoposide 50 mg/mq dd1->5.The post remissional therapy,after 1 consolidation cycle,was addressed by the prognostic clinical-biologic categorization.The patients were defined as low risk AML if they were CBF positive or NPM1-A+/FLT3- and CR was achieved with the first induction therapy and WT1 after first consolidation was normal; high risk if FLT3+ or with bad prognosis karyotype or if CR was achieved with a double induction therapy, or WT1 post first consolidation was high.3 patients were considered in an intermediate risk group because of normal karyotipe without any other abnormalities;1 of these patient relapsed and died; the other 2 are actually in first CR after HD Ara-C consolidation.CR was achieved in 30 (4 after a second cycle) (CR 88%), 2 patients died during induction and two were resistant. 10 patients were allocated in the good prognosis group and were treated with autologous BMT (2 pts) or multiple high doses Ara-C chemotherapy (8 pts). 2 patients relapsed, the first at 10 months with a CBF+ cKIT mutated AML, and the other with a NPM1-A+/FLT3- with abnormal karyotype.14 patients were allocated in the high risk group: 3 relapsed, and subsequently died, while they were checking for an unrelated donor (1 was transplanted in 2 CR and another with autologous in 2nd CR),1 is still waiting for transplant and 11 were transplanted (7 from a identical sibling, 1 cord and 3 from an unrelated donor).1 died for sepsis after transplant and the other 10 are actually in CR.Our results suggest the validity of a novel risk-adapted therapeutic strategy.The allotransplant approach limited to high-risk group reduces overall toxicity and mortality. However,further studies are warranted.

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OUTCOME OF NORMAL KARYOTYPE ADULT/ELDERLY ACUTE MYELOID LEUKEMIA (NK AML) CARRYNG FLT3-ITD/NPM1 MUTATION: A SINGLE CENTRE ANALYSIS

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Background: NK AML are classified as intermediate cytogenetic prognostic group, but they can be placed into a high risk group if based on the presence of FLT3-ITD and absence of mutation in NPM1 gene. In adults/elderly NK AML, FLT3-ITD aberration is detected in 20-30% of pts, having an impact on disease free survival (DFS) and overall survival (OS) but not on complete remission (CR); assessment of cytogenetic as well as molecular status, should be used to chose therapeutic approach and transplant options. Aim: we have retrospectively evaluated outcome of our cohort of NK AML FLT3-ITD+ve pts concerning DFS, OS and relationship with age <60 y(Group A) vs >60 y(Group B) and intensive vs conservative therapy. Patients and Methods: between January 2007 and April 2011, 118 consecutive adult/elderly AML pts were diagnosed at our Center. Sixteen were NK/FLT3-ITD+ve (13%), while NPM1 was positive in 6 evaluable patients (37%). Group A: 9 pts, 2 M, median age 33y (r 19-60), 3 NPM1+ve, median WBC 56x10^9/L (r 4,6-300). Seven were treated according to standard intensive chemotherapy, 1 differentiating conservative therapy (retinoic acid+LoDAC); one was not evaluable for early death. Group B: 7 pts, 2 M, median age 70y (r 62–80), 3 NMP1+ve, median WBC 42x109/L (r 6,5– 221).One received standard intensive therapy, 5 differentiating conservative therapy, 1 supportive therapy. Results: in group A 4 pts obtained CR (44 %) after intensive chemotherapy, 3 NPM1-ve; one was refractory, 3 died during induction. Median DFS and OS were 9 and 11month respectively. Up to now three pts (NPM1-ve) are alive in 1stCR (+13,+10, +1m) and 1(NPM1+ve) died for fungal infection at + 2 months. In group B 3 pts (42%) achieved CR, 2 with differentiating conservative therapy, 2 NPM1-ve, but relapsed at +2, +14 and +12m; one is in 2nd CR at +2 months, 3 (1 NPM1+ve) were resistant, 1 died early. Median duration of DFS and OS were 12 and 18 m respectively. Comments: in our small series of high risk NK AML we obtained a similar CR in group A and group B (44 vs 42%), independently from age, intensive chemotherapy and NPM1+ve status, while differentiating treatment allowed to obtain better DFS (12 vs 9m) and OS (18 vs 11m) in older pts. If confirmed in larger series, this observation may suggest that non intensive treatment associated with target agents may represent a valid therapeutic option in NK high risk older pts or in younger unfit not eligible for intensive approach.

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THE ADDITION OF LOW DOSE GEMTUZUMAB OZOGAMICIN TO INDUCTION, CONSOLIDATION AND MAINTENANCE THERAPY OF ELDERLY PATIENTS WITH NON M3 AML: UPDATE OF GENOA EXPERIENCE AND ANALYSIS OF PROGNOSTIC FACTORS

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Background and aims. We updated our experience with low dose gemtuzumab ozogamicin (GO) added to fludarabine, Ara-C and anthracycline (MY-FLAI3)(Clavio et al Br J Haematol, 2007)and reviewed the prognostic impact of cytogenetics and molecular biology (mutations of FLT3 and NPM genes and expression of WT1 and BAALC genes) at diagnosis. Patients and methods. Eighty-five consecutive CD33+ AML patients (60 years of age or older) were treated. The median age was 68 years (60-82), the karyotype was unfavourable in 21 patients (25%), intermediate in 63 (74%) and favourable in 1 (1%). Twenty-eight patients had secondary AML (33%). The induction therapy consisted of Fludarabine 25 mg /sqm, Ara-C 1 g /sqm, idarubicin 5 mg / sqm, all for 3 days, followed by GO 3 mg / sqm at day 4. Complete responders received the same regimen as consolidation therapy and started a twelve-month maintenance therapy consisting in alternating GO (3 mg/sqm) with Ara-C (1 g /sqm every 12 hours for three times), every 3 months. Results. Neutrophil (N > 0.5 x 109 / l) and platelet (Plt > 25 x 109 / l) recovery required a median of 15 days (range 10-26) and 16 days (range 7-30) from the end of therapy, respectively. There were four early deaths during induction (5%). Twenty-six major infectious complications were recorded (sepsis in 15 patients, pneumonia in 3, aspergillosis in 5). Non-haematological toxicity was very mild (no grade 3 or 4 hepatic toxicity). Forty-seven patients (55%) achieved a complete remission (CR).Complete remission rate was 64% and 28% in good-intermediate /poor karyotype patients, respectively (p 0.03). Median duration of CR and OS were 9 months (range 1-70) and 12 months (range 1-72), respectively; 36-month projected EFS and OS were 12% and 18%, respectively. Cox regression analysis disclosed that good-intermediate karyotype patients had a better EFS (p 0.006) and OS (p 0.002) compared to those with poor risk cytogenetics. Patients with denovo AML had a better EFS compared to patients with sAML (p 0.029). Among molecular markers only BAALC expression < 1000 affected OS, although with borderline statistical significance (p 0.05. Conclusions. The addition of low dose GO to induction, consolidation and maintenance therapy is well tolerated and associated with a good antileukemic activity. In elderly AML patients classical prognostic factors such as karyotype and clinical history (de novo vs secondary disease) appear to be more important than molecular markers.

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WT1 OVEREXPRESSION AT DIAGNOSIS IS THE BEST INDEPENDENT PREDICTOR OF FAVORABLE OUTCOME IN DE NOVO NON-M3 AML PATIENTS.

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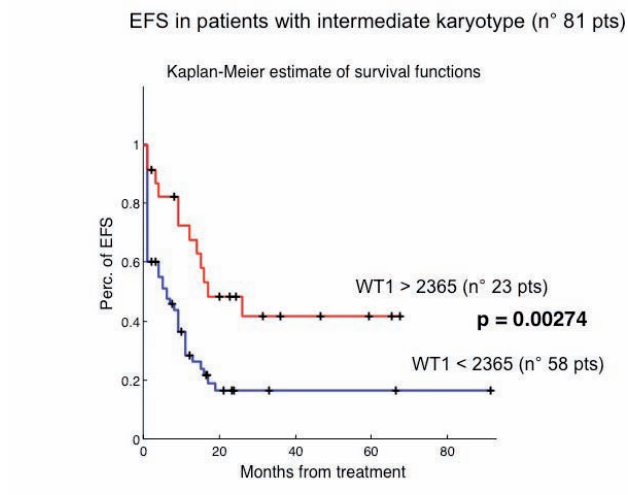
Patients and methods. A cytogenetic and molecular profile was performed in 100 consecutive untreated non M3 denovo AML patients treated with conventional induction and consolidation chemotherapy, with the aim of predicting CR rate and long term outcome. Expression levels were obtained by Real-Time-PCR upon normalization on Abl expression. Fifty-five patients were younger than 60 years (median age 46) and 45 were older (median age 69). Intermediate or favourable karyotype was detected in 81 patients (81%), unfavorable karyotype in 19 patiens (19%). Results: FLT3: 21 patients (21%) had ITD and 7 (7%) had exon 20 mutations. WT1: in 26 patients (26%) expression was > 2365 (>75th percentile); in 74 < 2365 (74%); NPM (A and B): the genes were mutated in 37 patients (37%) and non mutated in 63 (60%); BAALC: in 49 patients expression was < 1000 (49%), in 51 > 1000 (51%). Molecular profile was similar in the younger and in the older subgroups of patients. In the whole cohort of patients CR rate was influenced by age (p .0002), cytogenetics (p .002), WT1 expression (CRs: 21/26 in pts with WT1 > 2365; 41/74 in pts with WT1 < 2365, respectively, p 0.02) NPM gene mutations (p 0.009), BAALC expression (CRs: 36/49 pts with BAALC < 1000; 26/51 in pts with BAALC > 1000, respectively, p 0.02) The presence of FLT3 ITD did not affect CR rate. In pts with not unfavourable karyotype both WT1 overexpression and NPM mutations retained a favourable impact on CRrate. In the whole cohort WT1 overexpression, younger age and not unfavourable karyotype had a significant positive impact on EFS and OS both in univariate and multivariate analysis. In the subgroup of younger patients with not unfavourable karyotype WT1 expression retained its predictive value on EFS and OS whereas FLT3 ITD showed a significant impact on EFS (p 0.034) but only in the univariate analysis. In elderly patients only karyotype significantly influenced OS (p 0.001). Conclusions. In the subgroup of patients with not unfavourable karyotype high WT1 expression and NPM gene mutations were associated with higher CR rate. OS and EFS were affected by age, cytogenetics and WT1 expression at diagnosis. In the subgroup of younger patients with not unfavourable karyotype WT1 overexpression was the best predictor of favourable outcome.



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COMPUTER AIDED IDENTIFICATION OF SMALL MOLECULES INTERFERING WITH THE FUNCTIONAL INTERACTION OF THE UROKINASE RECEPTOR WITH FMLP RECEPTORS IN ACUTE MYELOID LEUKAEMIA CELLS.

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The urokinase receptor (uPAR) is a GPI-anchored cell-surface receptor involved in cell migration and tissue invasion. uPAR functionally interacts with fMLP receptors (fMLP-Rs) through a specific domain in the D1-D2 linker region, the SRSRY sequence (aminoacids 88-92). In fact, uPAR expression is required for fMLP-dependent migration in monocytes and basophils; on the other hand, fMLP-R expression is required for uPA-induced cell migration. We demonstrated that a soluble cleaved form of uPAR could contribute to hematopoietic stem cell mobilization by promoting their fMLP-R-mediated migration and by CXCR4 desensitization. Moreover, increased levels of intact and cleaved uPAR have been detected in acute myeloid leukemia (AML) cells as well as in plasma, in urine, and especially in the plasma compartment of bone marrow aspirates of patients with AML. Because proteolytic cleavage of uPAR exposes the SRSRY chemotactic sequence, uPAR may play a role in the pathophysiology of acute leukemia through its interaction with fMLP-Rs. All these considerations prompted us to search for small molecules targeting uPAR interaction with fMLP-Rs. To this aim, a "structure-based" virtual screening of a diversity library of small molecules was conducted using the recently solved uPAR crystal structure, focusing on residues implicated in the functional interaction with fMLP-Rs. This analysis generated a list of 31 lead compounds predicted to inhibit fMLP-R interaction with uPAR. Two compounds were selected for their ability to impair migration toward uPA and fMLP of KG1 leukemic cells, which express both uPAR and fMLP-Rs. Identified compounds also impaired KG1 cell adhesion to various extracellular matrix components and specifically inhibited their serum-induced proliferation. Since AML cells show high level of uPAR expression, as compared to their normal counterpart, selected inhibitors are expected to be leukaemia specific. Identified compounds could be useful both as analytical tools to better elucidate uPAR role in intracellular signalling and as leads for pharmaceuticals.

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67 KDA LAMININ RECEPTOR INVOLVEMENT IN THE TRAFFICKING OF NORMAL AND LEUKEMIC STEM CELLS; COMPUTER AIDED IDENTIFICATION OF A SMALL INHIBITORY MOLECULE.

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The 67 kDa laminin receptor (67LR) is a cell-surface receptor with high affinity for laminin (LM), which plays a key role in tumour progression. 67LR is also involved in normal human T lymphocytes homing and in acute myeloid leukaemia (AML), lymphoma and myeloma cell adhesion and migration. We demonstrated 67LR involvement in granulocyte colony-stimulating factor (G-CSF)-induced mobilization of CD34+ hematopoietic stem cells (HSCs), in human healthy donors. 67LR has also been shown to contribute to HSC homing to BM after transplantation, in mice. We now investigated the role of 67LR in the trafficking of leukemic CD34+ HSCs and identified a 67LR inhibitory small molecule. Flow cytometric analysis showed a strong upregulation of 67LR expression in BM as well as in peripheral blood (PB) CD34+ cells from acute myeloid leukemia (AML) patients, as compared to normal BM and PB CD34+ cells. Then, we investigated whether 67LR upregulation could modulate CD34+ leukemic cell adhesion and migration to LM and stromal derived factor 1 (SDF1), the key chemokine in HSC trafficking. 67LR-transfected CD34+ cells from the KG1 cell line showed increased migration toward LM and SDF1, as compared to untransfect-

ed cells; on the contrary, 67LR overexpression did not increase CD34+ KG1 cell adhesion to LM. 67LR activation by LM determined increased phosphorylation of p38 MAP Kinase, protein Kinase C and calcium/calmodulin-dependent protein kinase II. Using the high resolution crystal structure of 67LR (PDB code: 3BCH), we identified 46 compounds that were predicted to interact with a previously identified LM-binding site on 67LR. One out of these 46 chemical hits, specifically inhibited 67LR-mediated CD34+ KG1 cell adhesion, migration and proliferation to LM. Leukemic cells undergo changes in adhesive properties that allow their migration into the circulation, leading to the development of extramedullary disease. Our data show that 67LR overexpression is associated with a migratory phenotype both in cytokine-stimulated normal CD34+ HSCs and in leukemic CD34+ HSCs. Moreover, we demonstrated that 67LR function can be specifically blocked by a newly-identified small molecule, making 67LR a promising therapeutical target in myeloid leukemia.

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IMPACT OF A FLUDARABINE-BASED INDUCTION THERAPY ON THE OUTCOME OF CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA (CN-AML) ACCORDING TO FLT3-ITD AND NPM1 MUTATIONAL STATUS

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Introduction Cytogenetically normal acute myeloid leukemia (CN-AML) defines a clinical entity with an heterogeneous outcome, as 5-year survival ranges from 24 to 42%. In this patients, accounting for about 45% of AML cases, many important prognostic factors have been identified in the last years. Among these, mutations of FLT3 and NPM1 genes are the most widely studied. Fludarabine-based induction therapy displayed interesting results in AML, with high rates of complete remission (CR). However, no specific data are available about fludarabine effect on the outcome of CN-AML patients according to specific molecular "signature". We analyzed 137 patients treated with a fludarabine-based induction therapy, evaluated response according to internal tandem duplication (ITD) in FLT3 gene and/or NPM1 mutation. Methods 137 patients with CN-AML and assessed for FLT3-ITD and NPM1 mutations were included in the study. All patients received fludarabine as a part of induction course, according to institutional protocols. Results Median age of patients was 54 years (range: 20-79), with 61 (45%) aged >55. FLT3-ITD was detected in 44/137 (32%) patients. No association was found between FLT3 status and age, FAB subtype or CD34 expression, but FLT3+ patients had significantly higher WBC count (p=0.03). NPM1 mutation was studied in 100/137 (73%) cases and detected in 41/100 (41%) patients. NPM1 mutation was found in 17/32 (53%) FLT3+ patients and in 24/68 (35%) FLT3- ones (p=ns). CR was achieved in 100/137 (73%) cases, and was affected only by age and CD34 positivity, while no correlation was found with WBC, FLT3 and NPM1 mutations. Leukemia free survival (LFS) was significantly shorter in FLT3-ITD+ patients compared to un-mutated ones (11vs28 months, p=0.05), and in CD34 positive cases (p=0.02). No differences were found considering NPM1 status. Overall survival (OS) was worse in elderly patients (p=0.001) and CD34+ cases (p=0.002), while FLT3 and NPM1 mutations did not have a significant impact. Considering survival by the combination of FLT3 and NPM1, LFS was similar in the four (FLT3+/NPM+, FLT3+/NPM-, FLT3-/NPM+, FLT3-/NPM-) groups, while a trend for longer OS was observed in FLT3-/NPM1+ patients compared to all other groups (5-yrs OS 60%vs30%), even if difference didn't reach statistical significance. Conclusions Our data suggest that fludarabine was able to improve LFS and OS in FLT3-ITD+ patients, but did not completely abrogate the advantage of NPM1 mutation.

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NPM1- A QUANTIFICATION AND FLT3- ITD ASSOCIATION IN ACUTE MYELOID LEUKEMIA

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Introduction: The mutations of exon 12 in Nucleophosmin 1 (NPM1-A) gene is one of the most frequent genetic alteration detectable in adult and pediatric acute myeloid leukemia. These mutations are observed in 25% of de novo AML and in 45-53% of AML with normal karyotype. Forty different type of mutation have been described but the main mutation is Mutation A (about 72%) B and D. These mutations in patients without concomitant FLT3-ITD (internal tandem duplications) confer improved prognosis, with good response at induction treatment and OS. In addition NPM1 quantification can be utilized as suitable target for minimal residual disease (MRD) study. Aims: The aim of the study is to characterize the molecular alterations and mutational status in acute myeloid leukemia and to apply a new prognostic molecular target useful for MRD study. Materials and methods: From 2009 to 2011 in "A. Businco" Cancer Hospital, Hematology Department in Cagliari (Sardinia) we have studied 18 cases of AML in onset and during follow-up. On 18 cases we performed NMP1-A expression study and FLT3 ITD analysis. NPM1 quantification was performed with RT-PCR real time procedure for quantitative determination by using ABI Prism 7000 instrument and standardized Ipsogen diagnostics Kit. While FLT3 ITD were evaluated with RT-PCR procedure, NPM1 -A results were normalized over ABL copy numbers. All polymerase chain reactions were evaluated on 2% agars gel electrophoresis. Five cases of AML NPM1 results have been validated in other Haematology Department. Results: Among 18 patients examined, 17 were AML and only one APL (Acute Promyelocytic Leukemia). All samples analyzed for FLT3-ITD were negatives except one case that resulted invaluable. In 44.4% of cases NMP1-A detection was positive. The mean value of NMP1-A copies/ABL was 471 (0-9737copies). Furthermore, 11.1% were invaluable for a high CT (Cycle Threshold) of ABL control gene 44.4% of samples were negatives while the remaining were negatives. Conclusions: Our results emphasize how NPM1 quantitative evaluation in association with negative FLT3-ITD analysis might be an important prognostic marker in AML onset and follow-up. The monitoring of this quantitative molecular marker could be helpful also in MRD study in normal Karyotype AML patients. The present few data are only indicative for a more complete study that should be done.

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ROLE OF PROTEIN KINASE CK2 IN THE SURVIVAL AND CHEMORESISTANCE OF ACUTE MYELOID LEUKEMIA CELLS.

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The critical involvement of protein kinase CK2 in the regulation of cellular apoptosis suggests that it may be involved in tumor cell resistance to conventional and unconventional therapies. We have studied the role of CK2 in acute myeloid leukemia (AML) cell survival and response to chemotherapeutic agents, using AnnexinV/Propidium Iodide staining, Western Blot analysis of Caspase3 and Parp cleavage and Real Time PCR of antiapoptotic gene expression. CK2 catalytic subunit expression level and activity were increased in AML cells as compared to normal CD34+ hematopoietic cells. CK2 inactivation with the synthetic chemical inhibitor K27 or RNA interference caused AML cell apoptosis of p53 wild-type but not of p53-null cells, suggesting that apoptosis triggered by CK2 inhibition needs the presence of functional p53. Inhibition of CK2 activity with either K27 or CX-4945, a new inhibitor used in phase I clinical trials, was associated with an increased sensitivity to the cytotoxic effects of doxorubicin and daunorubicin. Cells were also nucleofected with siRNA oligos directed against CK2 catalytic, regulatory or both subunits. Interestingly RNA interference of CK2 reduced cell via-

bility and enhanced apoptosis induced by daunorubicin, indicating a prominent role of this subunit in the resistance to chemotherapy. Since CK2 may regulate Stat3 signaling pathway we analyzed Ser727 Stat3 phosphorylation and its transcriptional capability upon daunorubicin exposure and CK2 blockade: we observed a decrease of Ser727 Stat3 phosphorylation that resulted in the reduction of the Stat3 target genes expression Socs3 and Mcl1. These data highlight the relevance of CK2 in AML cell survival: this kinase may influence the activation of anti-apoptotic pathways implicated in AML cell resistance to chemotherapy.

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CXCR4 EXPRESSION IN ACUTE MYELOID LEUKEMIA (AML)

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CXCR4 is a chemokine receptor involved in the homing and maintenance of hematopoietic and leukemia stem cells in the bone marrow microenvironment where is attracted by the gradients of its ligand CXCL-12. The role of CXCR4/CXCL12 axis could explain the existence of leukemic clones resistant to cytotoxic chemotherapy and eventually the persistence of minimal residual disease. We evaluated by flow cytometry the expression of CXCR4 on leukemic cells from 71 AML patients with a median age of 68 years. A complete panel of antigens for the diagnosis of Acute Leukemia also including CXCR4 was used. To correlate CXCR4 expression with clinical characteristics and survival, the patients were divided into two groups according to CXCR4 % of positive cells: ≤10% - group A; >10% - group B, which was considered positive for the expression of CXCR4. The group A (CXCR4:≤10%) enclosed 44/71 patients with a median age of 65 years; most of the leukemias (50%) were M4. In this group complete remission was achieved in 20/44 patients (45%). The group B (CXCR4>10%) enclosed 27/71 (38%) patients with a median age of 71 years, most of the leukemias (41%) were M1. In this second group complete remission was achieved in 14/27 patients (51.8%). CXCR4 expression ranged from 11% to 92% (median value 24%). Statystical analysis was performed to correlate CXCR4 with continue as well as discontinue variables, including surface antigens, laboratory parameters, clinical characteristics and survival by Spearman, Mann-Whitney and 2 tests. Overall survival (OS) was 13 and 10 months, while disease free survival (DFS) was 7 and 5 months in group A and group B respectively. Survival curves by Kaplan-Meier analyzed by log-rank test showed no statistical difference between the two groups. We found an inverse correlation between CXCR4 and CD34 (p≤0.0004; r=-0.4082), CD13 (p≤0.0002; r=-0.4347) and CD117 (p≤0.0209; r=-0.2737) while a direct correlation was found with CD45R0 (p≤0.0278; r=0.2631). These results were also confirmed with contingency tables by 2 test. Our results demonstrate an unexpected direct correlation between CXCR4 and a maturation-associated specificity (CD45R0) with a concomitant inverse link of CXCR4 with antigens associated with cellular immaturity, such as CD34 and CD117. These findings induce to reconsider the role of this chemokine receptor which could have a different fate in normal and leukemic hemopoiesis.

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AGE-SPECIFIC INCIDENCE OF CYTOGENETIC SUBGROUPS OF ACUTE MYELOID LEUKEMIA IN ROMAGNA

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It is well known that the different cytogenetic subgroups of acute myeloid leukemia (AML) show different age-specific frequencies. We tested this fact in a series of patients with AML observed and followed in 2 Hematology Departments in Romagna from 1995 to 2010. We divided the patients according to the 3 major cytogenetic prognostic subgroups (1) and calculated the distribution into the population of patients. Moreover, we calculated the survival of the different prognostic groups, according to age. Within a 16-year period, 292 patients with AML were admitted in the Hematology wards of Ravenna and Rimini. 155 were males and 137 females. Their age varied between 20 and 90 years, median 64 years. 107 patients showed a normal karyotype. 33 patients showing a t(8;21), inv (16) or t(15;17), were included in the favourable group. 78 patients who had a complex karyotype, or missing/deletion of chromosomes 5 or 7 were included in the unfavourable group. Other abnormalities and normal karyotypes were observed in 181 patients and were included in the intermediate group. With a cut-off at 60 years, of the 90 (31%) young patients, 17 were in the favourable group (19%), 57 in the intermediate (63%) and 16 in the unfavourable (18%)one, while of the 202 old patients 16 were in the favourable group (8%), 124 in the intermediate (61%) and 62 in the unfavourable one (31%), respectively. Survival curves were calculated on 142 patients with available information, M3 excluded. Figure 1 shows the survival curves of the patients divided according to prognostic group and age. In the subgroup with favourable cytogenetics old patients were 4 with a median survival of 92 mo, and young ones were 6 all alive at 12 years. In the intermediate group, old patients were 67 with a median survival of 15 mo, and young ones were 25 with a median survival of 19 mo. In the unfavourable group, old patients were 30 with a median survival of 4 mo. and young ones were 10 with a median survival of 10 mo. We confirm previous observations regarding the higher incidence of complex/unfavourable karyotypes in older patients (2). Moreover, in our series, cytogenetics plays a strong prognostic role, negatively influenced by age. *Acknowledgemnts: Ravenna AIL and Network Ematologico Regione Emilia-Romagna NERER.* *References. 1) Farag SS, Blood 2006,108: 1677-83. 2) Bacher U, Haematologica 2005, 90: 1502-10.*

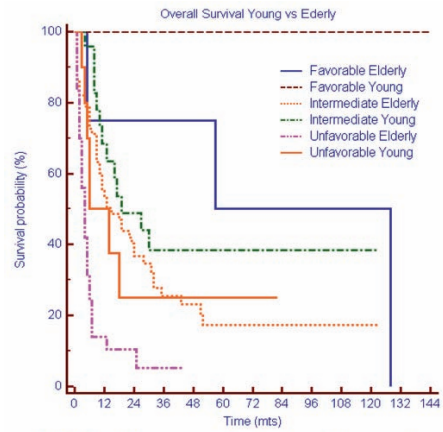

Fig. 1 - Survival curves according to cytogenetic prognostic classes and age.

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AZACITIDINE IN A COHORT OF ELDERLY PATIENTS AFFECTED BY SECONDARY ACUTE MYELOID LEUKEMIA

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Secondary acute myeloid leukemia (AML) is associated with a poor prognosis. Conventional intensive chemotherapy followed by allogeneic transplant represent the standard of treatment in younger patients but this approach is often unavailable in elderly patients. Prognosis is therefore usually dismal in this setting due both to the biological characteristics and intrinsic chemoresistance of the disease and also to the frailty of the patients who are often already immunodepressed and colonized. Azacitidine is a DNA methyl transferase inhibitor acting through the inhibition and the re-expression of previously silenced genes. Fenaux et al recently showed a survival advantage in low blast count AML treated with Azacitidine when compared to Conventional care regimen. Between april 2009 and august 2010 we have therefore enrolled 10 patients affected by secondary AML, aged more than 65 years (median:

72, range: 65-82 years) to azacitidine treatment. One patient had a previous diagnosis of breast cancer (normal karyotype), 1 received autologous transplant for NHL, 3 evolved from CMML (2 normal karyotype and 1 X trisomy), 5 had a previous history of MDS or cytopenias and macrocytosis (2 normal karyotype, 1 with 7q-, 1 with chromosome 11 abnormalities and 1 complex karyotype). Average BM blasts, LDH and WBC count before therapy were 30% (20-45%), 485 U/l (277-1066 U/l) and 9500/mcl (997-47100/mcl) respectively. Patients received 102 Azacitidine courses consisting in a 75 mg/sm daily s.c. administration for 7 (5+2) every 28 days. Eight patients received treatment as first line choice while 2 patients were treated after relapse post intensive chemotherapy. Grade III-IV haematological toxicity consisted in 28 neutropenias, 14 thrombocytopenias, 11 anemias; two patients need hospital admission for 2 infectious complications (1 pneumonitis and 1 sepsis). We observed 4 hematological CR, 3 Cytogenetic complete remission, 2 PR and 1 stable disease with haematological improvement. Nine patients are still alive and 6 of them are mantaining response with a median duration of 11 months (range 4-16) after a median follow up of 14 months (range: 6-20). One year OS and EFS were 85% and 45% respectively. These results show the feasibility of Azacitidine treatment in a setting of elderly secondary AML patients with intriguing results wich suggest a possible role of this drug in the treatment of patients not eligible to allogeneic transplantation.

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OUTPATIENT MANAGEMENT OF ARSENIC TRIOXIDE TREATMENT FOR PATIENTS WITH RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA

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Arsenic trioxide (ATO) represents the treatment of choice for patients with relapsed acute promyelocytic leukemia (APL). Moderate to severe hematological and extra-hematological toxicities, including the so-called "differentiation syndrome", are a common finding and either at diagnosis or relapse, patients receiving ATO are hospitalized. In our previous experience with ATO for APL patients in relapse, grade >1 WHO toxicity were unusual and limited to mild EKG abnormalities and/or leukocytosis with variable respiratory distress. Accordingly, we investigated the feasibility of single agent ATO therapy in an outpatient regimen. Here we report data on 4 patients with APL in first or subsequent relapse, managed with ATO in absence of hospitalization. The aim of this study was of evaluating the feasibility and safety of such an approach. Four patients with APL in molecular (n=1) or hematological (n=3) relapse were treated in an outpatient setting. Main characteristics of the patients and previous therapies for APL are summarized in table I. ATO was given at the dose of 0.15 mg/Kg IV for 30 consecutive days including Sunday or holidays at day-hospital. In all patient routine CBC was performed daily, while main biochemistry values were assessed twice weekly for the first week and then weekly for the remaining period. EKG for the evaluation of any abnormality including QT prolongation was performed once weekly. All patients completed the programmed cycle without any delay. No patient needed hospitalization during or after treatment. Neither WHO grade >1 hematological or extra-hematological toxicity was observed, nor any intravenous antibiotic therapy was required. One patient showed a mild prolongation of QT interval, not clinically relevant. All patient obtained complete remission at the end of first cycle and received their consolidation treatment as programmed. In those patients (n=2) in whom subsequent consolidation with ATO was programmed, the drug was as well administered in the outpatient setting. In spite of the limited number of patients, our data demonstrate that therapy with ATO in patients with relapsed APL is feasible and safe, provided that careful evaluation of blood and biochemical parameters, as well as daily clinical examination is assured.

TABLE I

#	Age at relapse (years)	Sex	Type of Relapse	N° of relapse	Previous therapy	OS at relapse
1	72	M	Hematologic	2nd	IDA+ATRAx2; GO	24
2	28	M	Hematologic	1st	AIDA	31
3	47	M	Hematologic	1st	AIDA	16
4	29	M	Molecular	1st	AIDA	18

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MONITORING MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA (AML) BY REAL TIME WT1 EVALUATION: 3 YEARS REPORT IN 61 PATIENTS

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Background: WT1 is an oncosuppressor gene overexpressed in bone marrow (BM) and peripheral blood (PB) in about 80% of AML at onset, being low or undetectable in healthy BM and PB. Therefore WT1 could be a useful marker to evaluate minimal residual disease (MRD) especially in AML patients (pts) without specific molecular markers. Patients and Methods: 210 BM (57 onset,153 follow up,F.U.) and 68 PB (11 onset,57 F.U.) from 61 AML pts, 8 healthy BM and 9 aphaeretic products from non-AML pts were analyzed by RQ PCR (Cepheid, smart cycler). WT1 expression was normalized by evaluating the expression of the housekeeping gene Abl, using the following calculation: WT1/ABL *104. According to Cilloni et al. values >250 were considered pathological. 8 pts had a favorable karyotype, 25 intermediate (+8 or normal karyotype), 8 unfavourable (complex karyotype) and 20 unevaluable; 21 had a favourable molecular profile, 29 intermediate(no molecular markers),10 unfavorable (FLT3 ITD) and 1 not evaluable. Results: Median WT1 levels were 8,6 (range1,1–25,7) in 8 healthy BM. At onset 50 pts (82%) had overexpressed WT1: median 1794 (range 273.5–9176) in BM and 1970,9 (range 64,5-10693) in PB; 11 pts (18%) had low WT1 in BM (median:115,8-range 52.5-226-). With a median F.U. of 9 months (range1-29) 3 patterns have been identified: a) complete remission(CR) with reduction of WT1 (23pts); b) refractory AML with persisting high levels of WT1 (5 pts) ;c) reduction of WT1 at response to treatment and a subsequent increase predicting relapse from 30 to 119 days before morphological relapse (4 pts). In the 9 aphaeretic products the median WT1 value was 3.2 (range1.15-15.2) although the high count of CD34+ cells (median 2550/ul, range 597-13650), suggesting that WT1 expression does not correlate with normal immature progenitors. In 90% of cases we observed a strong correlation between WT1 values in BM and PB (r2=0.97). Conclusions: WT1 is over-expressed in high percentage (82%) of AML and in these pts WT1 monitoring predicts the clinical outcome. In pts with normal levels at onset, the role of this marker in F.U. needs to be refined: preliminary data suggest that also in this subset WT1 variations are related to the course of disease. WT1 could be useful for MRD evaluation also in the aphaeretic products and in PB, but larger series of patients are needed to confirm this point.

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HYPERLEUKOCYTIC ACUTE LEUKEMIAS: INCIDENCE AND TREATMENT. REPORT FROM A SINGLE CENTER.

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Up to 30% of adult patients with acute leukemia present at diagnosis with hyperleukocytosis and symptoms of leukostasis. The aim of this study was to analyse the incidence and the treatment of this clinical presentation. A retrospective analysis on 30 patients (pts) with hyperleukocytic leukemia observed at our Institution from January 2005 to December 2010 was performed. For each patient, clinical information including age, sex, subtype of leukemia, hemoglobine value, platelets and WBC count was reported in a database. Therapeutic approaches to hyperleukecytosis were considered, in order to establish safety and prognosis improvement of leukapheresis compared to standard chemotherapy only. Among 283 consecutive pts with AL treated at our institution during the study period, we found 30 pts (11%) with an initial WBC count > 100x109/L; median WBC count at presentation was 155x109/L (100- 409 x109/L). Twenty-eight pts were affected by acute myeloid leukemia (AML), 1 by Ph1+ acute lymphoblastic leukemia (ALL), and 1 by biphenotypic leukemia. The main clinical manifestations were: pulmonary (5 reporting dyspnea and 2 respiratory failure) and neurological (1 affected by confusion and 1 by headache). Twenty pts were treated with daily leukapheresis (median 2 courses, 1-3) until WBC count was <50x109/L or the patient's clinical status improved enough to allow initiation of chemotherapy. No adverse events related to procedure were reported. In the remaining 10 pts, leukapheresis was avoided due to the

older age (8 cases), or diagnosis of APL and ALL (2 cases); 4 pts assumed only hydroxyurea as cytoreductive therapy. Standard chemotherapy was begun within 24 hours of admission, based on age and performance status. Nine pts are still alive and in complete remission, 6 after allogeneic transplantation, with a median follow up 21 months (5-70); 17 pts died in spite of chemotherapy assumption, after a median follow up of 7 months (1-30). Early death occurred only in 1 patient, 12 hours after admission. The overall survival (OS) at 6 months was 56.6% (17 pts), of which 12 (70.5%) had undergone leukapheresis. Hyperleucocytosis is still a challenge for clinicians, and a favourable outcome is achieved only in a little percentage of pts, prevalently of younger age, as confirmed in our series. Leukapheresis seems to be a safe procedure, although its OS impact is not clear, probably due to the little population examined.

	Patients (n 30)
Sex: M/F	15/15
Age: < 60/> 60	17/13
Type: AML/ALL	28/1*
Therapy related AL	5
Leukapheresis	
(course)	
one	8
two	11
three	1
OS (6 months)	
Alive	17
Dead	12

Table 1

* 1 was bifenotipic AL

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ACUTE PROMYELOCYTIC LEUKEMIA (APL) IN PATIENTS AGED > 70 YEARS

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Acute Promyelocytic Leukemia (APL) is a rare subtype of Acute Myeloid Leukemia (AML) more common in younger adults, with a median age of 45 – 50 years at onset. The use of All-trans Retinoic Acid (ATRA) as tailored treatment has made APL a very curable disease also in patients aged > 60 years; however, there are only few case reports in very elderly APL patients. To address this issue, we revised clinical data and treatment results in 12 patients aged > 70 years with newly diagnosed APL followed at our Institution from 1/91 to 12/2008. Clinical characteristics at onset were as follows: M/F 7/5, median age 74.7 years (range 70.0 – 80.8), M3/M3v 11/1, median WBC 1.3 x 109/l (range 1.0 – 7.4), median PLTS 53 x 109/l (range 12 – 302), BCR1/BCR3 6/6. According to Sanz risk score, 7 patients were at low-risk and 5 at intermediate-risk; 6/12 patients had arterial hypertension, 4/12 a concomitant cardiologic disease, 3/12 a cerebro-vascular disease and 2/12 a previous neoplasia. Induction therapy consisted of ATRA + Idarubicin in 8 patients (2/8 with reduced Idarubicin dosage) and ATRA alone in 4 patients; in this latter group, however, 2/4 needed to add chemotherapy (CHT) based on Mitoxantrone + AraC due to hyperleukocytosis during ATRA treatment. All patients achieved both morphological and molecular Complete Remission (CR) after a median time of 50 (range 29 – 65) and 105 (range 51 – 239) days, respectively. Infective complications were observed in 10/12 patients (4 episodes of FUO, 6 sepsis, 2 cystitis and 1 oral abscess) while ATRA syndrome occurred in 2/12 patients; in addition, there were 3 episodes of cardiac ischemia and 3 episodes of paroxystic atrial fibrillation. All but one patient received consolidation therapy (based on CHT alone in 7 patients, CHT + ATRA in 3 patients and ATRA alone in 1 patient), followed by maintenance treatment in 8 patients. Four patients had a relapse (hematological in 3 cases and molecular in 1 case) after 8, 11, 35 and 56 months respectively. At present, 6 patients are still alive, 4 died due to disease progression (3) or senectus while in CR (1) and 2 were lost to follow-up while in CR: mean event-free survival and overall survival were 89.2 months (95%CI 52.6 – 125.8) and 99.9 months (95%CI 65.0 – 134.7), respectively. In conclusion, ATRA-based treatment of APL is safe and effective also in very elderly patients, with long-lasting disease-free and overall survival.

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ANALISI E SIGNIFICATO PROGNOSTICO DELLO STUDIO DELLA MALATTIA RESIDUA MINIMA IN 32 PAZIENTI AFFETTI DA LEUCEMIA ACUTA MIELOIDE NON M3.

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The prognostic role and the impact of Minimal residual disease (MRD) on treatment decision in acute myeloid leukemia (AML) patients has not been clearly confirmed so far. In our center, AML MRD study is performed using multiparameter flow cytometry (MPFC) analysis of abnormal leukemia associated immunophenotypes (LAIP) and RT-PCR analysis of WT1 (Wilms tumor gene 1) expression. Our study aims to analyse and compare the sensitivity, specificity and the prognostic significance of the two techniques. Thirty-two AML patients were enrolled in the MRD protocol between May 2008 and September 2010. A total of 138 bone marrow samples (28 post induction, 25 after consolidation, 14 post-transplant, 71 at follow-up) were analysed, in 85 of these a comparison of the two techniques was feasible. The comparison between the two methods showed a 77.6% concordance rate. The molecular test had a 0.87 positive predictive value (PPV) and 0.97 negative predictive value (NPV). MPFC had a 0.67 PPV and a 1 NPV. The median levels of MRD were significantly higher in relapsing patients, compared with those in CCR, expecially after induction and at follow-up. The incidence of relapse was significantly higher after induction in WT1 positive patients (p=0.01) and at follow-up in WT1 (p=0.0001) and LAIP positive patients (p= 0.0001). At induction check point all 5 WT1 positive patients relapsed. WT1 clearance at induction also helped to identify false LAIP positive patients, since 5/10 MPFC positive MRD after induction are still in CCR. All 8 WT1 positive patients at follow-up relapsed vs 13% (3/23) of WT1 negative patients. The 3 WT1 negative patients had a positive MRD at MPFC suggesting the possibility to lose WT1 expression at relapse. The multivariate analysis confirmed an higher incidence of relapse in WT1 positive patients post induction (RR 9.9) (p = 0.005) and at follow-up (RR 10.6) (p = 0.005). The study of the MRD with MPFC anticipated hematological relapse of 4 months (range 1-6.5) in 7 patients, while WT1 positivity advanced relapse of 3 monhs in 5 patients. Our study confirm the prognostic role of WT1 positivity after induction. MPFC MRD also plays an important role in anticipating diagnosis of relapse expecially in those patients who lose WT1 at recurrence. Larger comparative studies are needed to establish the real predictive power of the two methods and resolve some technical issues as the adjustment of cut off and the choice of the more strategic checkpoint analysis.

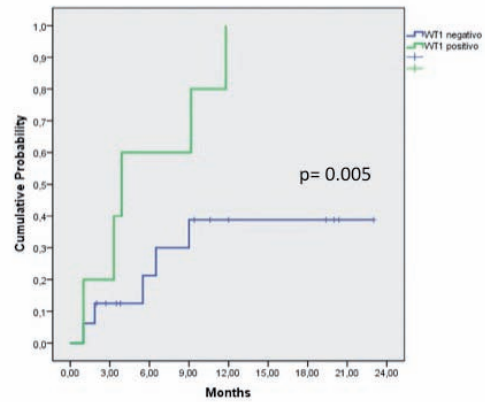

Figure 1: Incidence of Relapse and WT1 expression after induction.

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CLINICAL CHARACTERISTICS AND THERAPEUTIC RESULTS IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND LOW BONE MARROW (BM) BLAST COUNT (20-30%) TREATED WITH CONTINUOUS SEQUENTIAL INFUSION OF FLUDARABINE AND CYTARABINE

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The presence in the BM of 20 % or more blast cell is required for diagnosis of AML. Notwithstanding, the 20% blast threshold is not a mandate to treat patients with aggressive chemotherapy and, mainly in the elderly, therapeutic decision can be difficult. In this phase II trial we investigated the efficacy and toxicity of a regimen including fludarabine (F) and ARA-C (CI-FLA) given as continuous sequential infusion in a series of 40 untreated patients aged more than 60 years with 20-30% BM blast count. A comparison with 100 patients with higher BM blast percentage uniformly treated in the same period is also presented. Between June 2001 and October 2010, 40 out of 140 patients (28%) were found with 20-30% BM blast count. Median age was 67 years (61-81). Cytogenetic analysis was successful in 38 patients (95%) and showed normal karyotype (intermediate) in 24 (63%), while 14 (37%) were classified as unfavourable. Patients achieving CR were programmed to receive a reduced course of CI-FLA, followed by G-CSF from day 15 to mobilize CD34+ cells. Overall, 27 patients (67%) achieved CR. There were 5 induction deaths (12%), while 8 patients (20%) were refractory to induction treatment. The median number of days to neutrophil >0.5x10E9/l and platelet >20x10E9/l was 19 (7-34) and 20 (9-38), respectively. Documented infections occurred in 5 cases (12%). Twenty-two patients (81% of remitters) were eligible for consolidation and mobilization of CD34+ cells, collection being successful in 15 of them (68%). Median number of CD34+ cells/kg collected was 6.8x10E6 (2.5-40.3), median number of apheresis being 2 (1-2). Thirteen patients (32% of the whole population) received autologous stem cell transplantation (ASCT). Median disease free survival (DFS) and overall survival were 9 and 10 months, respectively. Survival at 5 years is projected to 23%. The only parameter significantly related to DFS duration was the presence of unfavorable cytogenetics. In particular, DFS was 29 months for patients with diploid karyotype as opposed to 7 months for those with adverse one (p:0.001). Finally no difference was found with patients with > 30 % BM blast count as to CR achievement and duration, toxicity and overall survival. CI-FLA is effective and well-tolerated in elderly patients with low blast count AML. Therapeutic results are encouraging as to CR achievement and ASCT feasibility; however best results are achievable in the subgroup of patients with diploid karyotype.

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FOUR DRUGS COMBINATION (FLUDARABINE, CYTARABINE, IDARUBICIN, ETOPOSIDE) AS INDUCTION THERAPY FOR NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA PATIENTS YOUNGER THAN 65 YS: RESPONSE AND FOLLOW-UP OF 127 PATIENTS.

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Standard regimen of induction chemotherapy for Acute Myeloid Leukaemia (AML) is represented today by association of cytarabine and antracyclin, with Complete Remission (CR) rate of about 60-65%. The main goal of adding further drugs to induction therapy is to increase the CR rate and the possibility to approach transplant procedures. One hundred and twenty-seven consecutive AML patients were included between 2002 and 2010 in three haematological centres. All patients were younger than 65 with a median age of 45 years (range 18-65), they were 59 males and 68 females. According with cytogenetic characteristics, white blood count (WBC › 30.000/mmc) and secondarity, we considered 95/127 (75%) of patients had high risk AML. Twelve patients had CBF-AML. No molecular studies are reported. The induction regimen (FLAIE) included Fludarabine (25 mg/sqm), Ara-C (2 g/sqm), Etoposide 100 mg/sqm on days 1-5, Idarubicin (6 mg/sqm) on days 1, 3, and 5. Patients were evaluated for response rate and treatment-related adverse events. After induction with FLAIE, CR occurred in 69% of patients (88 of 127 cases); twelve out of 34 resistant patients (9%) achieved CR after the second course of therapy. There were three cases of death during induction (DDI 2 %). Four patients were lost to follow up after induction. The toxicity of FLAIE was acceptable, with grade III/IV of hematological and extra-hematological adverse events comparable with standard induction therapy. Post remission therapy was addressed to perform HSCT according to risk stratification and donor availability. Eighty-two patients (64% of the 123 evaluable patients) underwent transplant procedures when in first CR (28/123 autologous HSCT and 54/123 allogeneic HSCT), five patients had allogeneic HSCT in second CR, after early relapse. Three patients had related allogeneic transplant when not in CR, but they died. Thirty-three patients were treated only with chemotherapy, for resistant disease or lack of donors and the harvest of autologous stem cells failed. After a median follow-up of 54 months (range 1-96), 49% (60 of 123 evaluable patients) are alive (59/60 in CR), with a median Overall Survival (OS) of 54 months and median Disease Free Survival (DFS) of 20 months. Relapse rate is 35% at 4 ys. The probability of 4-year OS and RFS were 36 and 30%. These results suggest that four drugs induction chemotherapy can slightly improve efficacy of standard induction therapy and it is safe. In our series we can report lower relapse rate that what reported by others, perhaps for the good rate of accessibility to transplant procedure we have.

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CRYPTIC CHROMOSOMAL REARRANGEMENT IN A PATIENT WITH ACUTE MYELOID LEUKEMIA (AML)

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Most patients with Acute Myeloid Leukemia (AML) at presentation show clonal cytogenetic aberrations , some of which having a diagnostic or prognostic significance. The fusion gene AML1-ETO deriving from the translocation (8;21) is associated with a peculiar leukaemic morphology, being mostly present in M2 but also in M4 and M1 FAB subtypes. This rearrangement is associated with a good prognosis if no mutations occur in FLT3 gene Cryptic or complex translocations involving more than chromosomes 8 and 21 are rare, regarding about 3% of LMA patients. Uncertain is their prognostic significance. S.A., a 28 years old female patient, at presentation on August 2010, showed: Hb 8gr/dl, WBC 3100/µl, PLT 78000/µl, myeloid blasts in peripheral blood (40%) and in bone marrow (60%). Immunophenotype: CD13+,CD33-,CD34+,CD56+,CD117+,HLA-DR+,CD19+. FLT3 was negative. Diagnosis of AML-M4 was made. Standard cytogenetic study (RBA and RHG banding) showed a translocation (8;17). FISH analysis with LSI AML1/ETO DC, DF probes showed the presence of the AML1-ETO fusion gene, then the involvement of the chromosome 21. The FISH with painting probes for chromosomes 8, 17 and 3 revealed a small chromosome 17 region on the long arm of a chromosome3. The karyotype was: 45,X,-X,t(8;17;3;21)(q22;q24;q27;q22) . The patient died of Pseudomonas aeruginosa infection at the 20th day after induction chemotherapy (GIMEMA /EORTIC AML 10 protocol). When complex rearrangements are present in neoplastic patients we can hypothesize mutations in genes located at breakpoints and/or new fusion genes forming, vanishing the favourable prognostic value of a known aberration. In the case here reported, besides the AML1-ETO fusion gene forming, we can speculate on the presence of genes – on chromosomes 3q27 and 17q24 – as MAP3K13 and MAP2K6 coding for tyrosinkinases which play important roles in signal transduction and apoptosis.

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SERUM FERRITIN AS A PROGNOSTIC MARKER IN YOUNG ADULT ACUTE MYELOID LEUKEMIA

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Background. Different studies have demonstrated an iron overload contribution to post-transplantation liver toxicity, infectious events and poor survival in patients undergoing hematopoietic stem cell transplantation for haematological malignancies. Aims. So far, in the clinical setting of adult acute myeloid leukaemia (AML) there is no evidence of the possible role of iron in response and survival rates. We studied the role as a prognostic factor of pre-treatment serum ferritin in young adult AML. Methods.The study sample included 60 consecutive adult de novo AML patients (24 males and 36 females, median age 44 years, range 16 to 60 yrs). The serum ferritin level was determined at onset of the disease in each case. According to the FAB criteria the subtypes were: 6 M0, 39 M2, 9 M4, 5 M5, 1 M6. M3 subtypes were excluded from the analysis. NPM, FLT3 and cytogenetic evaluation was performed for all cases. The NPM mutation was present in 25 patients(42%) and 24 (40%) harboured the FLT3 alteration (ITD: 15 (25%); D835: 9 (15%)). Twenty (33%) patients were in the unfavourable cytogenetic group, 11 (18%) in the favourable group and 29 (48%) presented a normal karyotype (NK). The patients were subdivided into two groups according to serum ferritin values (< 800 versus > 800 ng/ml). Student's t-test or the Mann-Whitney test was performed for comparisons of means. Two-tailed Fisher's exact test was used to compare categories. Overall survival (OS) was measured from the time of diagnosis to death or last follow-up visit and was calculated using the Kaplan-Meier method; the log-rank test was used to compare survival curves. Logistic regression was performed for multivariate analysis. Only p values <0.05 were considered statistically significant. Results. Twenty-four (40%) patients showed a ferritin serum value > 800 ng/ml. Compared with the < 800 ng/ml group, patients with serum ferritin > 800 ng/ml were more frequently non responders to chemotherapy (33 vs 76%, p= 0.003) and they had a shorter OS (218 vs 661 days, p =0.005). Moreover, patients with serum ferritin > 800 ng/ml showed a higher frequency of documented infections during induction treatment (31% vs 3%, p= 0.001). At multivariate analysis, FLT3, NPM, Cytogenetic and Ferritin value (<o>800 ng/ml) all showed a statistical correlation with the response rate (p=0.02; p=0.02; p=0.03; p=0.02, respectively). Conclusions. The results of our study suggest a link between serum ferritin and AML prognosis. Further studies are needed to confirm the utility of serum ferritin as a prognostic marker in the adult acute myeloid leukaemia setting.

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GENTUZUMAB OZOGAMICIN AS SINGLE AGENT TO CONTROL MOLECULAR PROGRESSION AND HEMATOLOGICAL RELAPSE OF NPM1-MUTATED AML: A CASE REPORT

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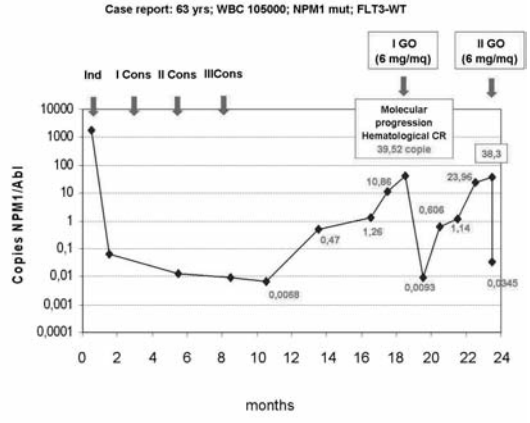
Acute myeloid leukemia (AML) with nucleophosmin (NPM1) gene mutations displays distinctive clinical and biological features which lead to its inclusion as a new provisional entity in the 2008-WHO classification of myeloid neoplasms. Although generally associated with favourable prognosis (in absence of FLT3-ITD mutation), about 50% of patients experience disease relapse. Thus, new therapeutic strategies are warranted. A rather consistent finding in NPM1-mutated AML is the high intensity expression of the CD33 molecule on the surface of leukemic cells, including the small fraction of CD34+ cells with a leukemic stem cell phenotype. This makes CD33 an attractive target for therapy with Gemtuzumab ozogamicin (GO), a chemo-immunotherapy agent consisting of a monoclonal antibody against CD33 conjugated to calicheamicin which triggers apoptosis when hydrolyzed in leukemic blasts. Interestingly, GO has been reported to exert significant activity in refractory NPM1-mutated AML. Other potential applications include the use of this immunoconjugate for eradicating minimal residual disease (MRD). Given the proven time stability of NPM1 mutation and the possibility to monitor MRD by quantitative assessment of NPM1 mutated transcript copies, and the evidence that increasing MRD levels after achieving complete remission (CR) are nearly uniformly followed by rapid overt AML relapse, prevention of overt relapse by MRD-guided therapeutic interventions, the so-called pre-emptive therapy, seems to be a reasonable approach. We tested the potentiality of GO as single agent in the control of MRD in a 65-year old patient with NPM1 mutated AML which in I CR after induction and 3-consolidation chemotherapy experienced molecular relapse and progression of the disease at 9 months of off-therapy. In particular, numbers of NPM1 mutated transcript copies showed a progressive increase up to 39,51 when GO was administered at the dose of 6 mg/sqm. This therapy allowed rapid molecular response (Figure 1). However, in absence of further therapy we observed again a progressive increase of MRD in the next 4 months up to 38,33. A second dose of GO allowed again marked reduction of MRD down to 0,034 copies (Figure 1). At the moment the patient received the third dose of GO and is alive in CR.



STEM CELLS AND AUTOLOGOUS TRANSPLANTATION

P357

TLR3-ACTIVATED HUMAN MESENCHYMAL STROMAL CELLS OF DIFFERENT TISSUE ORIGIN SIGNIFICANTLY PROLONG THE SURVIVAL AND FUNCTION OF NEUTROPHILS

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Introduction: bone marrow-derived mesenchymal stromal cells (BM-MSC) are stromal precursors endowed with extensive immunomodulative properties. In this study, we aimed to assess whether Toll-like receptor(TLR)3- and TLR4-activated BM-MSC influence human neutrophil responses under coculture conditions. Methods: MSC were expanded and characterized from bone marrow, thymus, spleen and subcutaneous lipoaspirates. PMN were immediately cocultured after isolation. Apoptosis was tested using Annexin-V / propidium-iodide and FACS. Respiratory burst was assessed by superoxide anion (O2-) release estimated by the cytochrome C reduction assay. Results: TLR3 triggering by poly(I:C) dramatically amplifies, in a more significant manner than TLR4 triggering by LPS, the antiapoptotic effects that resting BM-MSC constitutively exert on neutrophils under coculture conditions, preserving a significant fraction of viable and functional neutrophils up to 72 hours. In addition, TLR3- and TLR4-activated BM-MSC enhance respiratory burst ability and CD11b expression by neutrophils. The coculture in absence of cell contact and the incubation of neutrophils in supernatants harvested from TLR3- and TLR4-activated BM-MSC yield comparable results in terms of increased survival and immunophenotypic changes, thus suggesting the involvement of endogenous soluble factors. Neutralizing experiments reveal that the biological effects exerted on neutrophils by TLR3-activated BM-MSC are mediated by the combined action of IL-6, IFN- and GM-CSF, while those exerted by TLR4-activated BM-MSC mostly depend on GM-CSF. MSC isolated from thymus, spleen and subcutaneous adipose tissue behave similarly. Finally, the effects exerted by TLR3- or TLR4-stimulated BM-MSC on neutrophils are conserved even after the previous priming of BM-MSC with IFN- and TNF- . Conclusions: our data highlight a novel mechanism by which MSC sustain and amplify the functions of neutrophils in response to TLR3- and TLR4-triggering and may consequently contribute to inflammatory disorders.

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NOTCH-3 AND NOTCH-4 SIGNALING PROMOTES THE RESISTANCE TO STEROIDS OF HUMAN B-ALL CELLS IN PRESENCE OF MESENCHYMAL STROMAL CELLS

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Background. Notch signaling plays an important role in promoting self-renewal of hematopoietic stem cells and in the development of T-ALL. Its role in B-ALL pathogenesis is widely unknown. Aim. To evaluate the role of the Notch pathway in the development and chemo-resistance of B-ALL cells in culture with bone marrow mesenchymal stromal cells (BM-MSCs). Methods. MSCs were expanded in vitro from BM samples of healthy donors and B-ALL patients. B-ALL cells were obtained by density gradient centrifugation from BM samples of 10 newly- diagnosed patients with high blast count (median: 89.5%; range: 69-96). B-ALL cells and MSCs were studied for the expression of all Notch receptors and ligands. B-ALL cells were then co-cultured with BM-MSCs at 10/1 ratio for 3 days in absence or presence of hydrocortisone, by adding either anti-Notch-3, - 4 or GSI XII for 3 days period. Apoptosis of B-ALL cells was evaluated by Annexin-V/7-AAD staining, while proliferation and cell cycle analysis were assessed by flow cytometry with the CFSE and Propidium methods, respectively. The expression of active Caspase-3 and Bcl-2 were assessed by flow cytometry. Results. We found that hydrocortisone promoted apoptosis of B-ALL cells in culture alone, but a consistent rescue from apoptosis of B-ALL cells was observed when co-cultured with MSCs. The blockade of Notch-3 and -4 or all Notch signaling by GSI XII in presence of hydrocortisone dramatically lowered the number of overall live B-ALL cells even in co-culture with MSCs. Accordingly, active Caspase-3 was over-expressed and Bcl-2 was weakly expressed in B-ALL cells in culture alone in presence of hydrocortisone. Active Caspase-3 down- regulation and Bcl-2 over-expression in B-ALL cells were observed following co- culture of B-ALL cells with BM-MSCs in presence of hydrocortisone. These features were reverted by adding either anti-Notch-3 + anti- Notch-4 antibodies or GSI XII in presence of hydrocortisone. Conclusions. Notch-3 and -4 signaling confer resistance to hydrocortisone by up-regulating Bcl-2 in B-ALL cells in culture with MSCs.

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GENE EXPRESSION ANALYSIS IN CORD BLOOD HEMATOPOIETIC STEM CELLS: C34+KDR+ VERSUS CD34+KDR-

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The process by which a multipotent stem cell maintains its identity and in the same time can generate lineage restricted committed progenitor cells (PCs) is one of the most challenging subject of investigation in the last decade. The most well studied model in this scenario is the hematopoietic system. Distinct FACS (fluorescence-activated cell sorting) markers were used to characterize and define a certain population to isolate the hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs). One of the marker used to isolate a population enriched for HPCs/HSCs is the CD34 surface antigen. Among the CD34+ cells, KDR has been considered as a new marker of primitive HSC capable of hematopoiesis both in vitro and in vivo. The human CD34+KDR+ (0.1–0.5% of the CD34+) have been functionally characterized from different groups (Pelosi and references therein[1]). These cells have been described as the so called hemangioblast, bipotent for the hematopoietic and the endothelial lineage [1]. The understanding of the molecular mechanism of hematopoietic stem and progenitor cells maintenance and expansion is crucial to increase the current therapeutic applications and uncover its neoplastic deregulation. Although many gene expression profiling studies have been conducted on hematopoietic cell population enriched for different surface antigens in attempt to identify stemness genes, none has been performed to compare the population CD34+KDR+ and CD34+KDR-. For this reason we compared the gene expression in CD34+KDR+ (HSC) versus the CD34+KDR- (HPCs) cell population, highly enriched for less primitive hematopoietic (lineage-committed, isolated from normal human neonatal placental/umbilical cord blood). After SMART (Clontech) amplification technique [2] we performed a cDNA microarrays analysis, finding a panel of genes differentially expressed between CD34+KDR+ and CD34+KDR- cells. Among them we found transcripts, preferentially expressed in HSCs, comprising transcription factors (like LHX2-LIM/HOMEOBOX PROTEIN LHX2) or DNA binding proteins (such as Id1 -INHIBITOR OF DIFFERENTIATION 1-), and signaling molecules that might play roles in key functions (e.g., self-renewal/differentiation i.e. Dvl3 dishevelled 3). Moreover, we found in HPCs a marked overexpression of molecules involved in adhesion/migration such as CD164 and CD49d [3]. This increased expression could be important for the differentiative process in the hematopoietic renewal. Different expression of unknown function transcripts were also detected by microarray analysis. Gene expression analysis of HSC and HPC populations represents a step toward a better comprehension of the transcript balance of normal human hematopoiesis and may be useful to identify genes involved in leukemogenesis and cancer stem cells. 1. E Pelosi, M Valtieri, S Coppola et al. Identification of the hemangioblast in postnatal life. Blood 2002; 100(9):3203-8. 2. G Iannolo, MR Sciuto, C La Rosa, C Conticello. MARCH-I expression in cord blood CD34+KDR+ cells. Clin Biochem. Mar 6. [Epub ahead of print] 3. S Forde, BJ Tye, SE Newey, et al. Endolyn (CD164) modulates the CXCL12-mediated migration of umbilical cord blood CD133+ cells. Blood. 2007 Mar 1;109(5):1825-33. Epub 2006 Oct 31. Abbreviation HSC -Hematopoietic Stem Cell KDR/Flk1/VEGFR2- vascular endothelial growth factor receptor-2 SMART - (Switching Mechanism At 5' end of RNA Transcript) Keyword Gene array, KDR, Stem cell, Hematopoiesis

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HUMAN CULTURED MATURE ADIPOSE CELLS CHANGE BACK INTO FUNCTIONAL MESENCHYMAL STEM CELLS

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Mature adipocytes with a single, large lipid droplet were generally considered to be in the terminal stage of differentiation and stationary, having lost their proliferative ability. When mature adipocytes were subjected to ceiling culture, first lost their lipid droplets, then were capable to change their morphology into fibroblast-like cells and they could successfully be maintained in culture. In this study, we characterized the mature adipocyte isolated from human omental and subcutaneous fat tissues (n=12, 53-81 years old) in the floating top layer by collagenase digestion and filtration. In culture, mature adipocytes lost mature adipocyte markers (Adiponectin and aP2 level expression decrease) and acquired typical features of bone marrow-derived (BM) mesenchymal stem cells. These cells were able to differentiated into multiple mesenchymal cell lineages and to redifferentiate into mature adipocytes, indicating that these cells show characteristics of adipogenic progenitors. Flow cytometric analysis revealed that dedifferentiated cells comprised a highly homogeneous cell population, rapidly reverted to mesenchymal immunophenotype in vitro. Indeed, they stained positive for CD90, CD105, CD73, CD44, CD29 and negative for CD34, CD133, CD45 and CD117. Embryonic stem cell genes, required for self-renewal and pluripotency, including Nanog, Oct4, Sox17, Gata4, Tbx1 were expressed at high levels. Moreover, mature adipocytes expressed similar molecular levels of CD45 and CD34, transcripts that code for proteins typically expressed in stem cells, to fat stromal vascular cells, that represented a well known source of stem cells. The morphological change of mature adipocytes observed in culture was associated with functional properties. Indeed, dedifferentiated cells were able to inhibit the proliferation of stimulated lymphocytes in co-culture, while mature non-dedifferentiated adipocytes stimulated their growth. Furthermore, like MSCs, dedifferentiated cells, after trypsinization and reseeding, were able to proliferate in vitro, making a feeder layer able to maintain the survival and complete differentiation of hematopoietic stem cells. In addition to bringing new insight into the biology of adipose tissue, these findings provide informations on functionality and potential role of these cells and a better understanding of the mechanism of dedifferentiation may lead to benefits in regenerative medicine and cell therapy.

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TELOMERE SHORTENING IS AN EARLY AGING SIGN FOLLOWING EXPOSURE TO CYTOTOXIC DRUGS: ASSESSMENT BOTH IN VITRO ON CULTURED MESENCHYMAL STEM CELLS AND IN VIVO ON CELLS FROM PATIENTS UNDERGOING CHEMOTHERAPY

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Introduction. A good indicator of the degree of cell ageing is the length of telomeres (TL). A variety of cellular stresses, including chemotherapeutic drugs, have been shown to induce TL shortening and promote accelerated senescence. In the present study, human mesenchymal stem cells (MSCs) from normal adults have been grown in culture and exposed to sub-lethal doses of cytotoxic drugs. Next, TL has been assessed in cells from lymphoma patients undergoing chemotherapy. Main aim of the study has been to investigate the initiation of the aging process by chemotherapy. Patients and Methods. Cultured MSCs, identified for immunophenotype, and for growth and differentiation properties, were exposed for 2-hr/week to 10 nM doxorubicin (Doxo) and 500 ng/ml etoposide (Eto). Peripheral blood (PB) cells were obtained from five lymphoma patients undergoing chemotherapy (2 CHOP, 1 CVP, 1 MINE and 1 HDS). TL was assessed on neutrophils and mononuclear cells (MNC) before and after each chemotherapy course. TL was assessed by flow-fluorescence in situ hybridization and Southern blotting. Results. A marked TL shortening occurred in MSC already at day 7 after exposure to sub-lethal doses of Doxo and Eto. Indeed, a minimum of 5 days in culture was required to identify the earliest signs of telomere loss. Further TL shortening was observed later in culture. A single 2-hr exposure to Doxo or Eto induced a TL loss, that was maintained unchanged at long-term in culture. Similarly, a consistent reduction in TL was detected in neutrphils from patients receiving chemotherapy, while no significant variations could be documented in MNC. TL shortening was detectable already after the first chemotherapy course in two patients and at the second in a third patients. Conclusions. i. human cultured MSCs exposed to chemotherapeutic drugs offer a simple and reliable means to investigate the correlation between DNA damage and cell ageing; ii. telomere loss is clearly detectable in MSCs already after 7 days following exposure to Doxo and Eto and remains detectable at long-term in culture as a permanent signature of the previous DNA damage; iii. the in vitro observation seems to recapitulate the phenomenon of TL shortening that occur in vivo in patients receiving chemotherapy treatments; iv. further studies in chemotherapy-treated cells may define the main cellular and molecular mechanisms responsible for telomere loss and premature aging.

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PRE-OPERATIVE BONE MARROW-DERIVED CELL MOBILIZATION BY G-CSF (GRANULOCYTE-COLONY STIMULATING FACTOR)ENHANCES OSSEOINTEGRATION OF BONE SUBSTITUTE IN PATIENTS UNDERGOING SURGERY WITH HIGH TIBIAL VALGUS OSTEOTOMY

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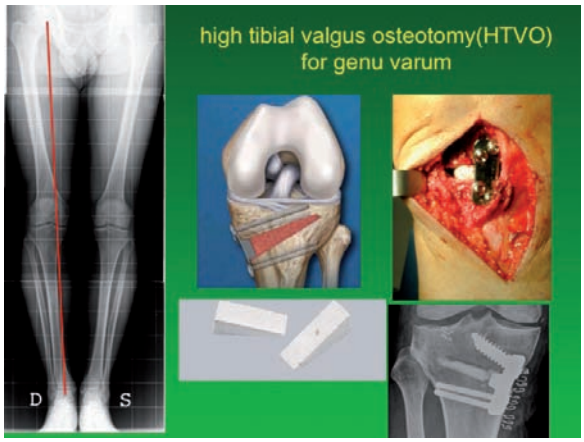
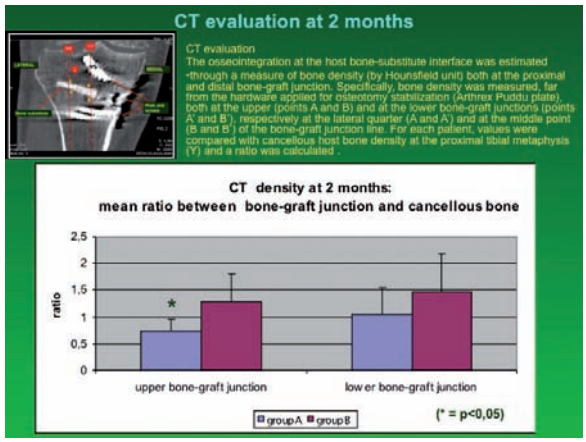
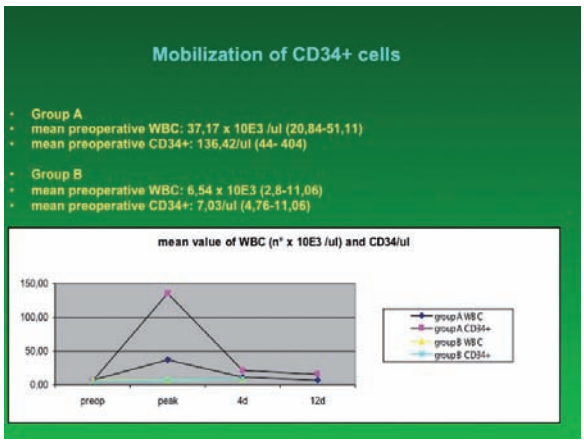
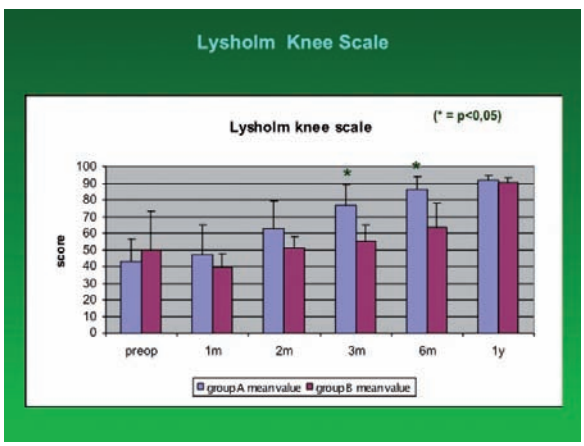
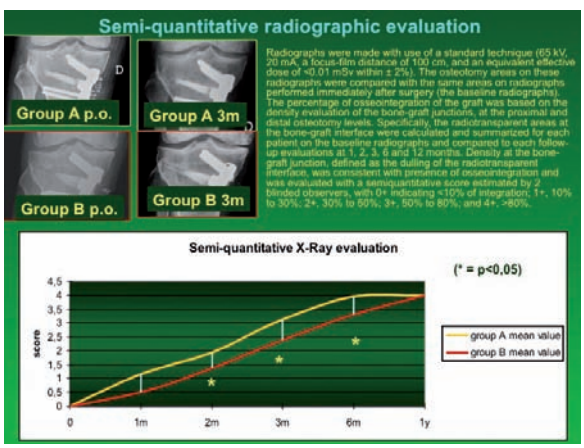
Introduction. Osseointegration is a slow process that starts at bone-implant interface. It might benefit from the addition of bone marrow-derived cells (BMC). Aim of the study was to verify feasibility, safety and efficacy of preoperative BMC-mobilization by G-CSF in patients undergoing high tibial valgus osteotomy(HTVO) for genu varum. Methods. 24 patients were enrolled in a prospective phase II trial. The osteotomy gap was filled by hydroxyapatite-tricalciumphosphate substitute. Two consecutive cohorts of 12 patients were assigned to receive(GROUP A) or not receive(GROUP B) a daily dose of 10µg/kg of G-CSF for 3 consecutive days, with an additional dose 4 hours before surgery. BMCmobilization was monitored by WBC count and flow cytometry analysis of circulating CD34+ cells. All patients underwent clinical score (Lysholm and SF-36) X-ray evaluation preop.,at 1,2, 3 and 6 months after surgery to compare the percentage of integration at the interface between host bone and bone substitute CT scan of the host bone-substitute interface at 2 months to estimate the osseointegration through a semiquantitative score and a measure of bone density. Results. All patients of both groups completed the study. The most common adverse events in Group A were mild to moderate bone pain and muscle discomfort. There were no severe adverse events. mean preoperative WBC and CD34+ values were 39,09 x 103 /µl (21,57-51,11) and 131,58/µl (29.1 - 404) in Group A and 6,77 x 103 (2,8-12-06) and 7,67/µl (5,4-12) in Group B, respectively. Patients of Group A displayed a slight increase in overall performance at 3 and 6 months compared to Group B($p< 0,05$) Semiquantitative X-ray evaluation: higher rate of bone substitute osseointegration in Group A than in Group B at 2, 3 and 6 months post-surgery($p< 0,05$) CT scan: bone density at the host bone-substitute interface (Hounsfield unit) was lower in Group A at the interfaces compared to Group B, accordingly with advanced stage of bone remodelling($p< 0,05$) Conclusions. G-CSF administration induces pre-operative mobilization of bone marrow-derived cells, is feasible in orthopaedic surgery and allows the circulation of high numbers of CD34+ve cells. It may hasten bone graft substitute integration as suggested by clinical, X-ray and CT evaluations, as a result of a direct activity of G-CSF or of a cellular effect mediated by either hematopoietic or endothelial progenitors mobilized by G-CSF or by the combination of all these factors.











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RISK FACTORS AND DIAGNOSTIC STRATEGIES FOR CYTOMEGALOVIRUS INFECTION IN PATIENTS WITH LYMPHOMA UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION: RETROSPECTIVE ANALYSIS FROM THE ROME TRANSPLANT NETWORK

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Background Current data on cytomegalovirus (CMV) infection and disease following autologous hematopoietic stem cell transplantation (ASCT) for malignant lymphoma are limited. Patients and Methods We performed a retrospective cohort study on 128 adult patients with lymphoma (81 B-cell, 11 T/NK-cell and 36 Hodgkin's lymphoma) consecutively undergoing non-selected peripheral blood ASCT and tested for CMV infection by two different diagnostic strategies [clinically driven (n=80) vs weekly surveillance plasma quantitative PCR (n=48)]. Specific antiviral therapy started only in presence of a proven diagnosis of clinically relevant CMV infection. AIM To determine the incidence of and risk factors for CMV symptomatic infection or end-organ disease, defined according to published recommendations, and to compare the two different diagnostic strategies. Results Sixteen patients (12.5%) [11 with CMV symptomatic infection and 5 with end-organ disease (4 pneumonias and 1 enteritis)] required specific antiviral therapy and 4/16 died; TRM was significantly influenced (7-fold risen, p=0.001). In univariate analysis, HBcIgG seropositivity, 90Y-Ibritumomab Tiuxetan-containing conditioning, mean of Rituximab administration (8 vs 4) and mean age at transplant (52 years vs 44) were factors significantly (p<0.05) associated with the development of CMV symptomatic infection or end-organ disease. In multivariate analysis, only a pre-transplant HBcIgG seropositivity (p=0.014) and conditioning regimens containing 90Y-Ibritumomab Tiuxetan (p=0.022) proved to be independent predictors of clinically relevant CMV infection. Between the two diagnostic strategies, PCR testing cost was significantly higher in the surveillance arm (p<0.001), whereas no statistically significant difference was observed concerning CMV symptomatic infection and/or end-organ disease incidence, diagnostic and treatment timing, TRM. Conclusions The incidence of CMV infection and disease seems not to be significantly increased over the last 15 years. Clinicians must be aware of this potentially life-threatening complication. HBcIgG seropositivity and 90Y-Ibritumomab Tiuxetan-containing conditioning should be considered as predictors of clinically relevant CMV infection. The role of Rituximab remains doubtful. Clinically driven diagnostic strategy proved to be the standard, but a randomized trial only for high-risk patients with routine monitoring and preemptive therapy in the experimental arm is warranted.

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PREVENTION OF MUCOSITIS AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL SUPPORT: A PHASE II OPEN LABEL, RANDOMIZED STUDY WITH AMIFOSTINE.

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Purpose The aim of this phase II open-label randomized study was to demonstrate the efficacy of intravenous amifostine on preventing mucositis in patients receiving high-dose chemotherapy (HDC) and autologous peripheral stem cell support (PBSC). Patients and methods. From September 2001 to February 2008, 181 patients with hematological malignancies and solid tumors were enrolled in the study. Conditioning regimens were melphalan 200 mg/m or thiotepa 600 mg/m plus melphalan 160 mg/m according to different diseases. Autologous PBSC were reinfused at day 0. Ninety patients were randomly assigned to receive intravenously amifostine at the dose of 730 mg/m , (infusion of 15 minutes) 30 minutes before melphalan (day-1) and amifostina 340 mg/m2 before thiotepa infusion (day -3 and -1) . Ninety-one patients didn't receive any prophylaxis. The primary endpoint was the efficacy of amifostine in preventing oral mucositis. Secondary end point was the tolerability of the drug. Oral mucositis were prospectively evaluated according to University of Nebraska Oral Assessment Score (Rapaport, JCO 2000). Results. No statistically significant differences of mucositis were documented between the two groups in terms of incidence (G1 68.9% vs 76.9% ;G2 51.1% vs 49.5%;G3 6.7% vs 8.8%); duration (G1 6.2 vs 6.3 days; G2 4.4 vs 4.8 days; G3 2 vs 3.1days); maximum score (10.3 vs 11.1); incidence of analgesic therapy (60.8 % vs 59.1%); need for parenteral nutrition (19% vs 19%); and time to discharge (14.5 vs 11.4 days). No differences were observed in haematological reconstitution ,i.e. time to reach an ANC more than 1.0 x 10^9/L (11.2 vs 11.8 days) ; time to reach a platelets count more than 20 x 10^9/L (8.3 vs 8.6); incidence of fever (58.2% vs 69%); duration of fever (3.7 vs 3.2 days) ; and number of documented infections. Amifostine infusion was well tolerated and only 3 patients manifested nausea, 4 patients a transient hypotension , one patient rhinorrea and one patients sweating. Conclusions. This phase II open label randomized study shows that intravenous amifostine before HDC is well tolerated. However, amifostina infusion does not reduce the incidence and the severity of mucositis.

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INTENSIVE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELLS TRANSPLANTATION IS A POTENTIAL CURATIVE THERAPY FOR ADULT PATIENTS WITH T(4;11)/MLL-AF4+ ACUTE LYMPHOBLASTIC LEUKEMIA.

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Allogeneic stem cells transplant [Allo-SCT] remains the best option for adults affected by acute lymphoblastic leukemia [ALL] with translocation t(4;11)/MLL-AF4; however, when this procedure is not possible, autologous stem cell transplant [Auto-SCT] could represent an alternative if an undetectable transcript level is achieved. Here, we report our experience on three patients with MLL-AF4+ ALL successfully treated with Auto-SCT following intensive chemotherapy. The clinical and biological characteristics of the patients at diagnosis are reported in Table 1. They received induction and consolidation chemotherapy according to the GIMEMA "LAL 2000" and "LAL 0904" protocols, reaching undetectable MLL-AF4 levels at molecular analysis. Peripheral blood stem cells were mobilized and collected after consolidation, resulting negative for minimal residual disease [MRD]. Patients then received a conditioning regimen with busulfan and etoposide, followed by stem cells infusion, with no life-threatening complications. All patients reached hematological recovery between day +19 and day +25, and then started a conventional maintenance treatment for at least one year. Bone marrow aspirates for morphologic and MRD analyses were performed during and after the maintenance therapy. All patients are still in continuous complete remission after 114, 99 and 32 months, respectively, with persistent absence of the MLL-AF4 transcript. Prognosis in adult patients with MLL-AF4+ ALL is poor. Vey et al. compared the outcome of these patients assigned to Allo-SCT (with a sibling donor) or randomized to receive Auto-SCT or conventional chemotherapy; a significant better disease-free survival was observed in the group treated with Allo-SCT (66±13 vs. 11±10 vs. 20±17; p=0.02). However, the lack of a donor or a prolonged search time period makes this strategy not always applicable. Giebel *et al.* recently reported that patients with detectable MRD levels prior to the Auto-SCT have a worse outcome. Moreover, when trying to define the impact of SCT on MLL-AF4+ ALL patients, Cimino et al. suggested that the pre-transplant strategy is more important than the SCT per se. Our experience, as well as that of others, suggests that Auto-SCT might be a valid therapeutic option for MLL-AF4+ ALL patients provided that a molecular remission is achieved with intensive pre-transplant chemotherapy. Prospective trials on a larger number of patients are needed to confirm these promising results.

	Patient n°1	Patient n°2	Patient n°3
Age (years)	24	28	44
Sex	M	M	F
Organomegaly	splenomegaly	hepatosplenomegaly and lymphoadenomegaly	no
Signs and Symptoms	fever, headache and asthenia	cervical lymphoadenomegaly	deep vein thrombosis
White blood cells (/mm3)	300,00	60,15	326,2
Haemoglobin (mg/dl)	6,2	13,9	11,7
Platelets (/mm3)	11	154	81
Blasts on BM (%)	75	85	95
Blasts on PB (%)	90	85	93
Immunophenotype	CD19+;CD34+;+TdT+; HLA-DR+; CD10-	CD19+;CD34+;cyCD22+TdT+; HLA-DR+; CD10-	CD19+;CD34+;+TdT+; HLA-DR+; CD10-
Expression of CD13 and CD33	no	yes	no
Cytogenetic	46xy,t(4;11)(q21;q23), i(79)[17]/46xy[1]	48xy,+x,t(4;11)(q22;q23), +der(4)t(4;11)(q22;q23) [13]/46xy[2]	46,xx,t(4;11)(q21;q23) [8] /46,xx [2]
Molecular Biology	MLL-AF4	MLL-AF4	MLL-AF4

Table n°1: Clinical and biological characteristics of patients at diagnosis.

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POSSIBLE ROLE OF INHIBITORY KIR 2DL5 IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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Autologous hematopoietic stem cell transplantation (AHSCT) offers a potential cure for patients with active multiple myeloma. Unfortunately, not all patients achieve complete remission. About 40% of treated patients become resistant to chemotherapy and present with relapse following AHSCT. Patient response to chemotherapy is influenced by a variety of factors, capable of modifying the outcome of the transplant procedure. Immunogenetic factors may also be involved, particularly natural killer (NK) cell reactivity and polymorphisms of killer cell immunoglobulin-like receptor (KIR) genes. The aim of this study was to investigate the possibility of a relationship between KIRs and the outcome of AHSCT. Fifty-two patients received AHSCT for multiple myeloma in our Center from April 2003 to October 2010. Induction therapy consisted of thalidomide-dexamethasone (32 subjects) combined with bortezomib (20 subjects). KIRs and their respective HLA Class I ligands were studied in all recruited subjects. The KIR gene frequencies in our patients were similar to those found in the general population, which in our study was represented by 181 healthy individuals of the Sardinian Voluntary Bone Marrow Donor Registry. Twenty-eight patients (54%) achieved complete remission (CR) or a very good partial response (VGPR). The remaining 24 patients (46%) only achieved a partial response (PR) and/or relapsed (R). A statistically significant difference was observed between these two groups of patients for the 2DL5 inhibitory KIR gene, KIR 2DL5 [CR/VGPR = 35.7% vs PR/R = 83.3%, hazard risk (HR) = 0.24, 95% confidence interval (CI) 1.3 – 58.4, P =.02]. Patients carrying only 5 of the 7 possible inhibitory KIR genes had a sig-

nificantly lower risk for developing relapse (HR=0.24). Although our data need to be confirmed, this parameter may help identify patients at high risk for relapse who require careful monitoring during AHSCT.

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STUDY OF THE CORRELATION BETWEEN THE POLYMORPHISM OF IMMUNOGLOBULIN G FC RECEPTOR FC RIIIA AND THE COMPLICATIONS RELATED TO RITUXIMAB THERAPY AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS AFFECTED BY NON HODGKIN LYMPHOMA

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Rituximab is a well-known monoclonal antibody used in maintenance therapy after autologous hematopoietic stem cell transplantation (HCT) in patients affected by NHL. Previous studies demonstrated the linkage between clinical response to Rituximab and the polymorphism of immunoglobulin G Fc receptor related to its antibody-dependent cellular cytotoxicity (Fc RIIIA 158 V/F). We recruited 50 patients (pts) treated in our Division between April 2004 and June 2010, 25 of whom received Rituximab after HCT and 25 were used as control arm. Pts' characteristics are described in Table 1. Our aim was to study the impact of this polymorphism on outcomes, effects and complications related to the treatment. During the follow-up only 24% of pts showed neutropenia, without a significant correlation to the polymorphism so we did not prove a significant reduction of the neutrophil count induced by Rituximab. Considering the outcome variables we observed that PFS was linked to the disease status at transplant (p=0.001) and to the NHL histotype (p=0.003); considering the OS a correlation was also found with the age of pts (p=0.007) and the use of Rituximab (p=0.01) but not with the polymorphism. In the Rituximab group, we found that all the five cases of relapse occurred in the subgroup of pts heterozygous (V/F) for the polymorphism. In particular, none of homozygous pts (V/V) (0/8) did show any event, whereas 5/17 of heterozygous ones relapsed (29.4%) (p=ns). Then considering the ten pts who were in partial remission at transplant we found a significant difference (p=0.038) about the response after the treatment including Rituximab: five homozygous pts reached a complete response (100%), while only two of five heterozygous pts got to the same goal (40%). The other three pts remained in partial response or showed a progression of the disease. The polymorphism was finally correlated to PMN engraftment: homozygous pts reached a count of PMN>500/mmc (p=0.02) and PMN>1000/mmc (p=0.012) before heterozygous ones. Our experience demonstrates that Rituximab is useful in the mainteinance therapy after autologous HCT in pts affected by NHL and the polymorphism Fc RIIIA 158 V/F seems to be a predictive variable of response in the treatment with Rituximab: the cellular receptor presents a stronger binding to the monoclonal antibody when the allele V is present. Better remarks could be achieved after the study of a larger number of patients and a longer follow-up.

	RITUXIMAB ARM	CONTROL ARM
No patients	25	25
Gender (M/F)	16/9	14/11
Age, median (range)	50 (37-62)	48 (16-66)
Histology	17 follicolar LNH 8 mantellar LNH	14 DLBC LNH 4 mantellar LNH 7 other LNH
Disease status at transplant	13 CR 10 PR 1 VGPR 1 SD	8 CR 9 PR 2 VGPR 6 PD
Conditioning regimen	21 BuMel 2 BEAM 2 others	15 BuMel 4 BEAM 6 others
Follow-up	19 CR (76%) 5 PD (20%) 1 SD (4%)	11 CR (44%) 10 PD (40%) 4 SD (16%)
Deaths	1 (4%)	7 (28%)
Median OS (range)	34 mesi (6-74 mesi)	27 mesi (2-67 mesi)
Median PFS (range)	31 mesi (3-72 mesi)	24 mesi (1-67 mesi)
Episodes of neutropenia (N < 1.0 x 10^9/L)	6 (24%)	9 (36%)
Episodes of severe neutropenia (N < 0.5 x 10^9/L)	4 (16%)	5 (20%)
Engraftment		
N > 0.5 x 10^9/L	+11	+11
N > 1.0 x 10^9/L	+11	+11
L > 0.5 x 10^9/L	+26	+19
PLT > 20 x 10^9/L	+11	+10
PLT > 50 x 10^9/L	+14	+17
PLT > 100 x 10^9/L	+26	+26
Polymorphism	17 heterozygous V/F (68%) 8 homozygous V/V (32%)	16 heterozygous V/F (64%) 8 homozygous V/V (32%) 1 wild-type F/F (4%)

Table 1. Pts' characteristics

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BORTEZOMIB BASED TREATMENT FOLLOWED BY AUTOLOGOUS TRANSPLANT IN MULTIPLE MYELOMA. A SINGLE CENTRE EXPERIENCE.

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In the last years Bortezomib Treatment (BT) has demonstrated an high response rate in Multiple Myeloma, both in first-line and in relapsed/refractory patients (pts). We present a group of 42 pts who have been treated in the last 5 years with Bortezomib based regimens in first line (23 pts) or after failure of the first-line treatment to achieve a Complete Remission or Very Good Partial Remission (CR/VGPR) (19 pts). After BT, they have been submitted to single or double Autologous Hemopoietic Stem Cell Transplantation (AHSCT) as consolidation program. Pts who received BT at diagnosis had advanced disease or adverse prognostic factors. Aim of the study: Primary end points were the response rate after BT and after AHSCT, the Progression Free Survival (PFS) and the Overall Survivall (OS). Secondary end points were incidence of severe complication in AHSCT and the difference between pts treated in first and second line. Results: 28/42 (67%) pts obtained a CR/VGPR after BT, 13/42 (33%) pts had partial remission (PR) and only 1 pt had refractory disease. CR/VGPR was observed in 19/23 (83%) and 9/19 (47%) pts treated in first and in second line respectively. After AHSCT 36/42 pts had a CR/VGPR and only 6/42 pts remained in PR. 16/42 (38%) pts relapsed after AHSCT: 15 of them were in CR/VGPR and one in PR. Progression of disease after AHSCT was observed in 7/23 (30%) and 9/19 (47%) pts who received BT in first and in second line respectively. 2 pts have been submitted to Allogeneic Transplant: one relapsed and one with PR after AHSCT. 7/42 pts died, 6 for progression of disease and one for transplant related mortality (TRM) after allogeneic transplant. Median PFS from AHSCT is 24 months (median OS not yet reached) with a median follow-up of 22 months. TRM after AHSCT is 0/42, we had 2 cases of life threatening infections (pneumonia), three pts suffered CMV reactivations and no other severe complication. No significant differences appeared between pts treated in first or in second line with BT in terms of PFS and OS. Conclusion: BT seems to be very efficient in order to obtain a CR/VGPR also in pts refractory or relapsed after other treatment. HSCT can improve the response obtained after BT. Despite these results 15/36 (41%) pts who achieved a CR/VGPR had a progression of the disease. These data suggest the necessity to explore which is the role of the AHSCT as consolidation treatment and to evaluate the strategy to maintain a CR/VGPR after its obtaining.

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PROGNOSTIC VALUE OF PRETRANSPLANT POSITON EMISSION TOMOGRAPHY USING FLUORINE 18-FLUORODEOXYGLUCOSE IN PATIENTS WITH LYMPHOMA TREATED WITH HIGH-DOSE CHEMOTHERAPY AND STEM CELL TRANSPLANTATION

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Autologous stem cell transplantation (ASCT) is considered the standard salvage therapy for relapsed or refractory Hodgkin (HD) and non-Hodgkin lymphoma (NHL). 18F-fluoro-deoxyglucose positron emission tomography (FDG-PET) was largely used to explore the predictive value in early evaluation of treatment and at the end of therapy. We want to evaluate the role of FDG-PET performed before autologous stem cell

transplantation (ASCT). Between January 2005 and August 2010 in our centre were performed 122 autologous stem cell transplantation (ASCT) in patients with relapsed or refractory Hodgkin (HD) and non Hodgkin lymphoma (NHL). Eighty one out 122 transplanted patients are retrospectively evaluable to have performed an FDG-PET before ASCT. The median age was 42 years (range 19-67 years); patients received autografts for NHL (44 pts) and HD (37 pts). At time of transplant 48 pts (59%) were in complete remission (CR) , 5 pts (6%) in partial remission (PR) and 28 pts (35%) with refractory disease. The FDG-PET before ASCT was negative in 48 pts (59%) and positive in 33 pts (41%). After a median period of observation of 22 months (range 0-76 months) the overall survival (OS) was 93% in the FDG-PET-negative group and 45% in the FDG-PET positive group (p: 0.000). The progression free survival (PFS) was 94% and 51% (p : 0.000) respectively for pts with FDG-PET negative and positive after a median time of observation of 18 months (range 0-76 months). After three months form ASCT 73 out 81 patients performed FDG-PET for restaging, the FDG-PET was negative in 58 pts (79%) and positive in 15 pts (21%). The PFS was 93% in the FDG-PET-negative group and 27% in the FDG-PET positive group (p: 0.000). The OS was 93% and 35% respectively in FDG-PET negative and FDG-PET positive scans (p:0.000). Our results confirm that a negative pre-transplant FDG-PET is associated with a better OS and PFS. Half patients with a pre-transplant FDG-PET positivity could be recovered by high dose therapy and transplant. Moreover patients with a positive FDG-PET after transplantation is associated with a poor prognosis and should be considered for alternative treatments

P370

HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PRIMARY REFRACTORY OR RELAPSED HODGKIN OR NON HODGKIN LYMPHOMA: LONG TERM OUTCOME IN OVER 300 PATIENTS

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Autologous stem cell transplantation (ASCT) is considered the standard salvage therapy for relapsed or refractory Hodgkin (HD) and non Hodgkin lymphoma (NHL). We want to analyze clinical outcome and significant prognostic factors for overall (OS) in a group of 327 patients with HD and NHL undergoing ASCT. We report the results of a retrospective analysis in 327 consecutive patients given HD CT and autologous transplant as second-line therapy between October 1987 and November 2010 by our centre. Results: 180 pts were male, 147 female. Median age was 39 years (range, 14 to 67 years). Patients received autografts for NHL (217 pts) and HD (110 pts). At time of transplant, 164 were in complete remission, 68 have a chemo-sensitive disease and 95 a resistant or refractory disease. Regimens that included total-body irradiation (TBI) were used only in 5 patients. The remaining received chemotherapy-only high-dose regimens, the most frequent being BEAM (121 pts), ICE (121 pts), MITMEL (63 pts), other (17 pts). To proceed to ASCT, patients had to have adequate cardiac, pulmonary, hepatic, and renal function. The median time to engrafment was 11 (range 7-20) days for absolute neutrophil count (>500), 11 (range 5-40) days for platelets (20.000). Treatment was well tolerated, and therapy related mortality was only 0.6%. Sixthy patients were lost to follow-up and they are not considered in the statistical analysis, 165 patients are alive without evidence of disease and 102 died most of them for progressive disease. The overall survival after a median period of observation of 5 years (range 1 to 256 months) was 52%, according to histotype it was 54% for NHL and 50% for HD. The only one parameter significantly associated with a better OS was status at transplantation. Complete remission and partial remission patients showed an OS of 59% and refractory patients 40% (p: 0.000). This significant difference was observed either in NHL (p: 0.004) or in HD (p: 0.03). In conclusion autologous stem cell transplantation is a safe and effective salvage therapy in NHL and HD patients. Our data, consolidated by a long follow-up, confirm that high dose therapy could be effective also in about one third of patients with refractory disease to standard chemotherapy.

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ENGRAFTMENT IS NOT ASSOCIATED TO DURATION OF REINFUSION IN PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Autologous stem cell transplantation (ASCT) is nowadays performed mostly with DMSO-cryopreserved peripheral blood stem cells (PBSC), with different modalities of thawing and reinfusion. A correlation between engraftment and the time elapsing from PBSC thawing and the end of reinfusion could be hypothesized. We analysed the ASCT performed at our institution to evaluate the correlation between engraftment and timing of PBSC thawing and reinfusion. Data from 41 ASCT performed in 38 patients (pts) since February 2007 were retrospectively analysed. Primary disease was myeloma in 16 pts, non-Hodgkin's lymphoma in 13 pts, Hodgkin's lymphoma in 5 pts, and acute myelod leukemia, amyloidosis, chronic lymphocytic leukemia, and Evan's syndrome in 1 pt each. Median age at ASCT was 59 years (range 33-69), and 18 pts were male. Conditioning regimens were high-dose melphalan (140 or 200 mg/sqm) in 20 cases, BEAM in 19 cases, and BUCY2, and cyclophosphamide-ATG in 1 pt each. All pts received pegfilgrastim 6 mg on day +1. Thirty-six procedures were first ASCT, four were second ASCT, one was a third ASCT. Median number of infused CD34+ cells was 5.13x10^6/Kg (range 1.83-11.54); units of PBSC were one in 18 cases, two in 21 cases, three in 2 cases. Viability at cryopreservation was tested in 33 PBSC units, with a median value of 91% (range 70-99). Median duration of reinfusion was eight minutes (range: 3-21 minutes). Median time to engraftment was 10 days (range 9-18) for neutrophils (PMN) and 14.5 days (range 10-33) for platelets (PLT); one patient, now at day +210, did not engraft for PLT, and is presently not transfusion dependent. Engraftment at +100 was evaluable in all pts; at this time median PMN count was 2000/mmc (range 400-4100), and median PLT count was 130000/mmc (range 23000-244000). CMV reactivation was observed in 5 pts. Age at ASCT, number of previous lines of chemotherapy, number of CD34+ cells infused, viability, and CMV reactivation showed no correlation with time to engraftment and cell counts at +100. Considering a time of ten minutes between the end of thawing and the end of reinfusion as a threshold, both engraftment and cell counts at +100 did not correlate with duration of reinfusion. Our analysis does not suggest that duration of reinfusion might affect engraftment. However, considering the small number of pts and the retrospective nature of our study, a larger multicentric study may probably solve this question.

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POST-THAW NUMBER OF VIABLE CD34+ CELLS DOES NOT REPRESENT A BETTER PREDICTOR OF ENGRAFTMENT IN AUTOLOGOUS STEM CELLS TRANSPLANTATION

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High dose chemotherapy followed by autologous stem cells transplantation represents an important therapeutic option in numerous malignant diseases. The number of infused CD34+ cells is considered to be a predictor of hematopoietic engraftment. The aim of the study is to evaluate if CD34+ cells dose measured at the moment of infusion (post-thaw) may represent a better predictor of engraftment than CD34+ cells dose evaluated pre-freezing. Total and viable CD34+ cells were counted applying flow cytometric ISHAGE gating strategy in single platform before freezing and after thawing. Stem cells were cryopreserved in 10% dimethyl sulfoxide (DMSO). 84 consecutive autologous peripheral blood stem cells transplantations performed between November 2009 and March 2011 were analysed. 50 patients (pts) were affected by multiple myeloma, 20 by Non Hodgkin Lymphomas, 10 by Hodgkin Lymphoma and 4 by solid tumours. All pts received myeloablative conditioning regimen; G-CSF was administered at day +6 to all pts. The median number of infused CD34+ cells was 5,19x 106/kg (range 1,99-13,49) at pre-freezing evaluation and 2,66 x106/kg (range 0,79-8,91) at post-thaw evaluation. Only 4 out of 84 pts (5%) received less then 2,5x 106/kg CD34+ cells at pre-freezing evaluation, while 39 out of 84 pts (46%)

received less then 2,5x 106/kg CD34+ cells at post-thaw evaluation. Median neutrophils engraftment (> 0.5x109/l) was 11 days (range 9-15) and median platelets engraftment (> 20 x109/l) was 12 days (range 9-22). Number of CD34+ cells evaluated both at time of harvest (pre-freeze) and prior to infusion (post-thaw) significantly correlates with neutrophil (p< 0.01; p< 0.01) and platelets engraftment (p<0.01; p=0,013). This study did not show that a routine evaluation of viable CD34+ cells at the moment of infusion may be a better predictor of engraftment than viable CD34+ cells count performed before freezing.

P373

COMPARISON OF THE EFFECTS OF +3 DAY POST-TRANSPLANT DAILY FILGRASTIM DOSES VS A SINGLE PEGFILGRASTIM DOSE IN AUTOLOGOUS SCT

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Background The use of high-dose chemotherapy followed by autologous stem cell transplantation is an important treatment option for selected patients with hematological malignancies. However, the high chemotherapy dosage used for pre-transplantation preparation exposes patients to the risk of neutropenic complications, including bacterial and fungal infections, that in rare cases can be fatal. The post-transplant administration of filgrastim reduces the time to neutrophil recovery and has therefore become standard practice in many institutions. Alternatively, the long-acting filgrastim formulation, pegfilgrastim, can be administered as a single 6 mg dose and has a significantly increased half-life, partly due to a decreased renal clearance. Patients and Methods In this study, data on 72 consecutive adult patients who received an auto-SCT between January 2009 and December 2010 and filgrastim (36 pts) or pegfilgrastim (36 pts) after transplantation were retrospectively examined. Diagnoses were non Hodgkin lymphoma (27 pts), Hodgkin lymphoma (12 pts) and Multiple Myeloma (33 pts). Standard conditioning regimens (HD-Melphalan or BEAM) were used. The mean CD34+ stem cell doses infused were 4.1 and 4.9 x106/kg (p=ns) in the pegfilgrastim and filgrastim groups, respectively; the groups were matched for age, sex and underlying disease. Patients in the filgrastim group received daily subcutaneous injections of 5mg/Kg/day starting on day +3 post-transplantation until ANC>1 x109/L; patients in the pegfilgrastim group received a fixed dose of 6 mg subcutaneously on day +3 post-transplantation Results The median time to ANC 0.5 x109/L was 9 and 10 days (p=0.04), respectively, in the pegfilgrastim and filgrastim groups. There was no significant difference in platelet engraftment between the pegfilgrastim and filgrastim groups (11 vs 12 days, respectively,p=.0.09). The median number of days with febrile neutropenia in the pegfilgrastim group was 2 (range 1-5), versus 3 (range 1-6) in the filgrastim group(p=0.1). There was no difference in the incidence of documented infections (20% in the pegfilgrastim vs 25% in the filgrastim group, P=0.3). Median hospital stay (from day 0) was 15 days for the pegfilgrastim and 16 days for the filgrastim group (P=0.2); there were no significant differences in survival at day +100 or at 1 year . Conclusions We conclude that a single injection of pegfilgrastim administered on post-transplant day+3 shows comparable safety and efficacy profiles to day+3 daily injections of filgrastim.

QUALITY OF LIFE AND SUPPORT THERAPY

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LONG TERM EFFICACY OF PERCUTANEOUS VERTEBROPLASTY IN MULTIPLE MYELOMA (MM) PATIENTS: EXPERIENCE OF THE "GRUPPO EMATOLOGICO DI ROMAGNA" (GER)

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Vertebral compression fractures occur in approximately 60% of MM patients and can cause pain, persistent disability and dismail quality of life. Appropriate therapy of MM or radiotherapy can lead to improvement of symptoms in a significant percentage of patients, but these positive effects can take time to be perceived. Vertebral agumentation techniques have been recently proposed as suitable options to relieve bone pain from vertebral compression fractures in patients with benign osteoporosis or neoplastic diseases such as MM. Aim of this study was to analyze the clinical course and outcome of 35 consecutive MMpatients (19M, 16F, median age = 67.6yrs) treated in the Centers referring to GER, who underwent percutaneous vertebroplasty from 2006 to 2010. Fifteen patients (43%) were newly diagnosed while 20 patients were relapsed or refractory after 1-3 lines of therapy. All the patients were treated because of severe pain, the extent of vertebral fractures was assessed by nuclear magnetic resonance imaging. Fifty-five procedures were performed at C2-L5 levels, 60% of the patients were treated at a single level, a maximum of three levels were treated in 6 patients, 12 procedures (22%) were performed at L1 level. Thirty one patients (88%) experienced reduction of pain, with 60% (73% newly diagnosed and 55% relapsed/refractory) showing complete disapperance of symptoms, 2 patients (2.7%) reported no or little improvement. Responses were durable, after a median follow-up of 14 months no further collapse of the treated vertebrae was observed. After vertebroplasty, first line or salvage therapy was administered to 30 patients, 8 newly diagnosed patients were scheduled to receive autologous stem cell transplant, and peripheral blood stem cell collection was not affected by the procedure. In conclusion, percutaneous vertebroplasty appears to be useful in MM patients with painful vertebral fractures as it allows rapid and durable achievement of pain control, without interfering with further therapeutic programs Work supported in part by RiminiAIL

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HEALTH-RELATED QUALITY OF LIFE IN PEDIATRIC PATIENTS WITH BETA-THALASSEMIA ACCORDING TO CHILD AND PARENT REPORTS

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Introduction Despite the worldwide diffusion of beta-thalassemia and its high incidence in developing countries, health related quality of life (HRQoL) has been reported only rarely. We investigated HRQoL characteristics related to clinical and laboratory variables in thalassemia children from Middle Eastern countries. The level of agreement between child-self and parent-proxy ratings of HRQoL was also investigated. Material and methods From November 2007 to August 2008, 60 children from Kurdistan, Libya, Palestine, Syria and Iraq (34 males and 26 females; median age, 10 years; range, 5-17 years) diagnosed with thalassemia were studied. The PedsQL 4.0 generic Scale Score was used to measure the following HRQoL dimensions: physical, emotional, social and school functioning; both patients and their parents completed the questionnaire. Results Transfusion and iron-chelation therapy was irregular in 79% of patients; the median age at the start of transfusion and iron chelation therapy was 11.5 and 49 months, respectively; hepatomegaly

was present in 77%, chronic hepatitis in 65% and ferritin values above 1300 ng/ml in 78% of patients. The scores of parents were lower than those of their children for Emotional Functioning (mean 75 vs 85; p=0.002), Psychosocial Health Summary (mean 70.3 vs 79.1; p=0.015) and the Total Summary Score (mean 74.3 vs 77.7 p=0.047). HRQoL was not influenced by ferritin levels, hepatomegaly (yes vs. no) or frequency of transfusions and/or iron chelation therapy. Multivariate analysis showed that a delayed start of iron chelation had a negative impact on total PedsQL scores of both children (p=0.046) and their parents (p=0.007). Conclusion Delayed iron chelation was significantly associated with lower HRQoL. Comparisons between self and parent-proxy reports showed lower parental ratings for the psychosocial and emotional dimensions as well as the total summary score. According to previous studies in childhood illness, parental ratings of their child's HRQoL tend to be lower and possibly reflect a series of parental distress factors. Overall, PedsQL 4.0 represents a useful tool for the measurement of HRQoL in pediatric thalassemia patients. In order to improve HRQoL in these patients, care givers will need to provide more understanding and support to the parents.

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PSYCHOTHERAPY FOR PATIENTS WITH HAEMATOLOGICAL NEOPLASMS

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This study outlines the main psychological issues that emerged during psychotherapy and highlights the psychological path that was offered to the patients (pts) admitted to our institution from January 2008 to March 2011. Seventy pts, 27 males and 43 females, median age 50 years (range 19-68 years) were offered psychological support; 60% of them were affected by acute myeloid leukemia (42 pts) 10 pts by non-Hodgkin's Lymphoma, 9 pts by acute lymphoid leukemia and 9 by other haematological neoplasms. Each pt received a median of 13 psychotherapy sets (range 1-24); 90% of the pts who underwent transplant procedures continued psychotherapy during the transplant period. The main psychological traits that emerged are common among oncologic patients: the tendency to strengthen their defence mechanisms; the incapability to discern between the emotional arousal and flow and the sensation of being overwhelmed by it; the sense of lost and of lack of choices. Nevertheless, a specific trait could be highlighted among our hematological pts and it can be traced back to the protracted hospital stay required for the majority of the pts, i.e. acute leukemias. Being hospitalized for extended periods of time, with a routine of "admission/discharge/admission", makes the pts perceive themselves in an ambivalent and not defined condition. This situation causes a sense of time and life suspension that pts dislike, fear and manage with difficulties. Psychotherapy sessions covered three main issues: 1) the emotional issue (e.g. help pts to cope with emotions); 2) the psycho-educational issue (e.g. illustrate and teach strategies to cope with the psychological disturbances linked to both disease and hospitalization) 3) the resource issues (e.g. help pts to focus and to adequately use their inner resources). Psychotherapy was also supported by imaginative techniques. The main goal of the psychological support was to enhance the sense of self-efficacy and to stimulate an active coping. The majority of pts, at the end of the psychotherapy, showed - improved consciousness of defence mechanisms and ability to overcome limits; - ability to discern different emotions and sensations with containment of emotional distress - acceptance of the condition of "patient" resulting from awareness that their other Ego roles could coexist and be active. The last point is the source for pts to overcome the sense of "life suspension".

P377

INTERVENTIONS TO ENHANCE QUALITY OF LIFE: WHAT PRIORITIES IN A BONE MARROW TRANSPLANT CENTER?

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Introduction. Health-related quality of life (QoL) is a topic of increasing interest in the field of haematopoietic stem cell transplantation (HSCT). In this regard, several QoL items, also when they are taken into account as singular issues, may represent areas of possible intervention. Purpose. To identify an order of priorities among the different QoL items, with the secondary goal of streamlining resources allocation, in the framework of the daily activities of a HSCT unit. Materials and methods. We analyzed the ability of our HSCT unit, as perceived by health professionals, to manage QoL issues. With this aim, the operators (4 doctors, 10 nurses, 1 auxiliary and 2 volunteers) of our unit received a newly developed questionnaire, comprising two groups of questions: the first group (A) questions asked to assess the current capacity of HSCT operators to manage QoL issues, in terms of prevention, monitoring and treatment; the second group (B) questions asked to measure the benefit eventually generated by an improvement in the management of these objects; each answer was given by choosing a value on a scale from 1 to 4. Results. By December 2010, 17 HSCT professionals received the questionnaire; 2 weeks after delivery, 13 questionnaires were returned (9 nurses, 2 doctors, 1 psychologist, 1 auxiliary and 1 voluntary); the compilation rate was of 76%. So that, 1,260 responses were evaluable for the analysis. The average value attributed to each item among A-group questions (evaluating current management) allowed to rank: breathlessness, gastric symptoms (nausea-vomiting-anorexia) and pain (score >2.66) versus socio-economic aspects, familial aspects and memory disorders (score <1.79) in the first and in the last three places, respectively. Physical symptoms received a significantly higher score (average = 2.6), when compared with mental, social, and economic items (average = 1.8). Analyzing responses received for the B-group questions, the score range between individual items (3.0-3.4) resulted too small to discriminate a priority order, as for A-group questions. Conclusions. Social, familial, economic aspects and psychological symptoms are those to whom preferentially address resources; however, all aspects were found to be susceptible to a desirable management enhancement, in order to improve QoL in HSCT patients.

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DYSGEUSIA IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

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Introduction. Dysgeusia is an important issue in cancer patients, given its high frequency and potentially devastating effect on quality of life. Data regarding this symptom from the oncohematological setting are still lacking. Aim. In order to address this issue, we performed a cross-sectional study on a series of both inpatient and outpatient patients affected by haematological malignancies. Materials and methods. Dysgeusia was assessed by patient's interview and scored by a verbal rating scale as "absent", "mild", "moderate" and "severe". Causes of dysgeusia were also assessed. Results. From July 2010 to August 2010, 48 patients (27 males) were evaluated with a median age of 67 (range 25-93) years. Diagnosis was acute leukaemia, lymphomas, multiple myeloma, myelodisplastic/myeloproliferative disorders and other diagnosis in 14 (29%), 13 (27%), 9 (19%), 7 (15%) and 5 (10%) cases, respectively. Disease phase were: first diagnosis, remission, relapse, progression and stable disease in 14 (29%), 18 (37%), 8 (17%) and 8 (17%) cases, respectively. Thirty-five (73%) patients were on active therapies, 9 (19%) on supportive treatments, whereas the remaining 4 (8%) were receiving no treatment. We had 22 (46%) inpatient and 26 (54%) outpatient; out of the latter group, 12 patients were followed by a home care service and 14 were day hospital-managed. Dysgeusia was observed in 22/48 (46%) patients. Symptoms were scored as mild, moderate and severe in 10 (21%), 7 (15%) and 5 (10%), respectively. Dysgeusia was related to drugs, disease, mucositis and infection in 16 (34%), 2 (4%), 1 (2%) and 1 (2%) patients, respectively, while mechanism remained undetermined in 2 (4%) of them. Statistically significant difference on incidence of severe dysgeusia emerged between the patients on causal therapies compared to those undergoing other treatments. Discussion and conclusions. The results of our study outlines the need of a careful evaluation of the taste function by physicians curing patients with blood cancers in order to increase our knowledge about the profile of toxicity of antineoplastic treatments and to develop effective interventions to manage this quality of life impairing symptom.

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ACCIDENTAL FALLS IN HOME CARE HEMATOLOGICAL PATIENTS

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Introduction. Accidental falls are one of the most important concerns in patients (pts) suffering from both medical and surgical disease, on the one hand for the potential consequent complications (fractures, bleeding) often requiring hospitalization, on the other hand for the medicolegal implications arising from falls, particularly in protected environments, such as inpatient ward, nursing homes and home care (HC). HC hematological patients frequently represent an at-risk category, for both age, clinical aspects (anemia, fatigue, disability) and risk of complications development (hemostatic disorders, treatment induced / increased osteoporosis). Aim. To retrospectively evaluate frequency, risk factors and effects of falls in home care managed hematological patients. Materials. Clinical data of 177 pts registered in our home care service from January 2010 to March 2011 were examined retrospectively. Among these, 117 pts, with adequately recorded clinical data, were considered evaluable for further analysis. Fall episodes were extracted and analyzed; complications and outcome were explored. Results. Population data of the 117 evaluable pts were: 50 male, 67 female, median age 83 (20-98); diagnosis and disease phase are reported in table. 23 episode were identified, among 20 / 117 pts (17 %). Fall complications were observed in 20 / 23 events (87 %) and were classified as: non-fracturative trauma and fracture in 13 and 7 events, respectively. Fracture site was: femur, humerus rib and other in 5, 3, 2 and 2, respectively (multiple sites in 2 pts). Outcome was: healing in 20 and death in 3 episodes (as fracture complication). No statistically significant differences were noted for gender, diagnosis, disease status at entry and transfusions requirement. Conclusion. Accidental falls are frequent complications in pts with hematological disease during HC management, with potential severe consequences development. Larger studies are needed to identify risk factors, thus providing a tool for risk assessment and prevention, with the aim to reduce falls frequency and to improve outcome, quality of life and general management in home care pts.

Diagnosis	n	%
AL	16	14
MM	13	11
Lym	9	8
cMPD	17	15
MDS	37	32
Other	25	21
Disease status	**n**	**%**
Terminal	22	19
Advanced	32	27
Chronic	51	44
Active treatment	12	10

AL: acute leukemia; MM: multiple myeloma; Lym: Lymphoma; cMPD: chronic myeloproliferative disorders; MDS: myelodisplastic syndrome.

Table. Diagnosis and disease phase

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PATIENT-PHYSICIAN COMMUNICATION TRAINING IN HEMATOLOGY: RESULTS OF INTENSIVE COMMUNICATION WORKSHOP FOR ITALIAN HEMATOLOGISTS

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Background and aim: disclosing unfavourable medical informations to patients is a stressful situation for many doctors who lack the communication skills to deal with this problem. The primary aim of this study was to evaluate the feasibility and efficacy of a 3-day communication course model for senior Italian hematologists. Methods: the course, initially designed for US oncologists, was modified to address specific educational areas expected to be relevant to the targeted participants. Topics regarded communication challenge in critical situations such as diagnosis, relapse, chronicity and transition to palliative care for hematological malignancies. A three-day intensive communication workshop was held for 14 hematologists from different hematological center in Italy. The course included formal lectures, small group work, role play and interviews with "simulated patients" played by trained actors. Participants completed questionnaires before and after the 3-day workshop. Results: An improvement in self-efficacy, knowledge of communication skills, favourable changes in attitudes towards disclosure of medical information and assessing patients' concerns and fears were demonstrated at the end of the course. Knowledge of breaking bad news skills significantly ($p < .001$) increased on the three types of knowledge questions: ability to correctly identify the best choice to case scenarios, increased knowledge of breaking bad news skills on the true–false items and breaking bad news skills. Conclusions: The course was feasible and succeeded in improving parameters associated with effective communication behaviours. Such results may help clinicians acquire the interpersonal skills needed for honest and effective communications with patients.

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USE OF COMBINED ORAL ADMINISTRATION OF ANALGESIA AND ANXIOLYSIS FOR PAIN MANAGEMENT IN BONE MARROW BIOPSY.

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Introduction: Bone marrow aspiration and biopsy (BMAB) is an invasive procedure causing considerable pain and anxiety in adult patients. Nonetheless, there are no data on the factors that can modify the perception of pain. For adults the main type of analgesia for BMBA is the infiltration of local anesthesia (LA) at the biopsy site; despite the use of LA, pain relief is often incomplete especially during aspiration of bone marrow. In this study we evaluated the effectiveness of combined oral administration of analgesia and anxiolysis in reducing the pain experienced by patients undergoing BMAB. Methods and Results: 107 consecutive ambulatory adult patients seen at our hematology clinic in 2010 underwent BMAB after LA with 10 mL of 2% lidocaine injected in the left posterior superior iliac crest. All patients gave treir informed consent to participate in the study. Analgesia was obtained with 200 mcg of Fentanyl via the transmucosal route and anxiolysis was obtained with midazolam 5 mg, both given 30 min before the procedure. 52 patients received LA alone (group A) and 55 patients received an oral administration of analgesia and anxiolysis in addition to LA (group B). We assessed pain level with the Numeric Rating Scale which distinguishes 10 levels of pain, from 0 to 10 at 5 time points of procedure (baseline, start LA T1, aspiration T2a, biopsy T2b, 5 min after the end of the procedure T3). At the end, all were given a questionnaire on efficacy, satisfaction, comfort with three levels (1/low – 2/medium – 3/high). Medium values of pain were as follows: at time T1 the medium level of pain was 0.87 in group A vs 0.88 in group B; at time T2a it was 3,63 in group A vs 3,54 in group B; at time T2b it was 4,63 in group A vs 4 in group B ($p<0,05$); at time T3 it was 0,41 in group A vs 0,16 in group B ($p<0.05$). In addition 21 patients, who had already undergone the procedure without sedoanalgesia, preferred the procedure with the new medication. Conclusions: Our preliminary results underline the different subjective perception of pain in two groups of patients and, above all, they show a main level of satisfaction and comfort in patients undergoing BMBA with sedoanalgesia. The combination of fenatanyl and midazolam is safe and, given the different mechanisms of action of the two drugs, helps patients achieve both anxiolysis and analgesia, which is very important in view of a possible repeat of examination.

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BORTEZOMIB THERAPY AT HOME IN FRAIL HAEMATOLOGIC PATIENTS: HOME CARE SERVICE EXPERIENCE OF NIGUARDA

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Introduction Multiple Myeloma (MM) represent the more frequent diagnosis for patients (pts) followed by Oncohematologic Home Care of Niguarda Hospital. In the last years we treated an increasing number of elderly patients with refractory or relapsed MM and comorbidities with Bortezomib (Vel) and Dexamethasone (Dex). Aim of the study Feasibility and tolerance of Bortezomib therapy at home in frail pts, with poor performance status (PS). Patients and Methods From Jan 2008 to Apr 2011 we followed 31 pts with MM; 7 of them were treated with Vel-Dex. Vel (1.3 mg/m2 iv) was given once (on days 1 and 8) or twice (on days 1, 4, 8, 11) a week in a 21-day cycle, up to a maximum of 8 cycles, combined with Dex (10-20mg/die po on days 1-4 and 8-11). The Hospital pharmacy prepares chemotherapy(cht). Our equipe transfer cht in special containers with staff-car. A medical team with expert nursing support administer cht. The entire process is regulated by a procedure defined by the hospital Prevention Department. Results Median age was 75 years (range 69-88). All pts were in late stage disease (mainly stage III) and their PS was classified as 2-3. Pts had received various therapies before Bortezomib, with only one pt in RP. The other pts obteined 3 NR and 3 PD from prior therapies.The pts received a median number of 6 cycles (range 1–8) and Vel was given once a week in 6 pts and twice a week in 1 pt. Response assessment was available for 4 patients (one pt refused treatment after first dose and 2 pt are ongoing): 2 pts obtained a VGPR and the other 2 pts had NR. Among the 2 pts now in treatment, one is in VGPR at seventh cycle and the other is in MR at third cycle. Drug related AEs were reported in 5/6 pts during treatment, including hematologic AEs (at least grade 3), peripheral neuropathy (at least grade 2), HZV. Most AEs were predictable and manageable, without hospital admission. None serious AEs occurred. All pts received supportive therapy at home. None received transfusion support. For everyone, the median number of home physicians and nurses visits per cycle was 4 (range 3-8) and 6 (range 2-13) respectively; the median number of laboratory control per cycle was 3 (range 2-8). Conclusion In our experience administration of Bortezomib treatment at home in elderly or compromised pts with MM showed to be active and well tolerated. The toxicity profile was good: adverse events have been managed with appropriate supportive care and didn't require hospitalization.

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PREDICTORS OF EMOTIONAL DISTRESS IN HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS DURING PROTECTIVE ISOLATION

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Purpose: This longitudinal prospective study aims to assess anxiety and depression levels in a sample of hematopoietic stem cell transplant (HSCT) patients evaluated throughout hospitalization in protective isolation, and to identify which pre- isolation factors (including patient's sociodemographics and personality traits-, disease-, and information on illness and on HSCT-related variables), are the best predictors for emotional distress occurring during isolation The ultimate goal of this study was to establish whether any of the predictors identified could be considered as useful for targeting prevention strategies and intervention. Patients and Methods: Participants were 107. Anxiety and depression were assessed by State-Trait Anxiety Inventory (STAI) and Self-rating Depression Scale (SDS) at admission (t0) and weekly following HSCT up to t4. Personality traits were evaluated by Cognitive Behavioral Assessment (CBA 2.0) at t0. The level of information provided by physicians on illness and on HSCT were collected through a specifically developed schedule. Random-effects models were used to explore predictors for anxiety and depression. Results: Approximately 10% of patients experienced anxiety or depression at admission. Depression worsened significantly after two weeks of isolation. Among predictors, emotional lability was associated with higher anxiety, while introversion was associated with higher depression. Moreover, anxiety and depression showed a positive correlation throughout hospitalization. After controlling for the other predictors, and based on a patient-centered communication style, physician- provided levels of information did not significantly correlate with anxiety or depression. Conclusion: Depression represents a major component of the emotional distress experienced by HSCT patients during isolation. The most significant predictors of depression are some pre-morbid personality traits and anxiety. Thus, pre- transplant detection of specific personality traits, anxiety, and depression by self- administered questionnaires as those used in this study, could help preventing emotional distress via targeted psychotherapeutic and psychoeducational interventions.

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ASSOCIATION OF APREPITANT, PALONOSETRON AND DEXAMETHASONE IN PREVENTION OF NAUSEA AND VOMITING IN PATIENTS TREATED WITH BEAM AND AUTOLOGOUS BONE MARROW TRANSPLANTATION.

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Introduction: Complete protection from nausea/vomiting is currently achieved in a small number of patients receiving high-dose chemotherapy (HDC). Now the use of 5-HT(3)-antagonists and dexamethasone represent the standard of care. This study describes the addition of aprepitant to 5-HT3 antagonists and dexamethasone with the aim of preventing nausea and vomiting associated with BEAM in autologous stem cell transplantation (ASCT) patients (pts). Patients and Methods: 5 pts with Non Hodgkin Lymphoma and 1 pt with Hodgkin disease underwent HDC with BEAM (carmustine 300 mg/m(2) on day -7, etoposide 200 mg/m(2) and cytarabine 400 mg/m(2) on days -6, -5, -4, -3 and melphalan 140 mg/m(2) on day -2) and ASCT. The aprepitant was used at a dose of 125 mg p.o. on day -7 and 80 mg p.o. on days -6, -5; the palonosetron was used at a dose of 250 g on days -7 and -4; the dexamethasone was used at a dose of 12 mg e.v. on day -7 and 8 mg e.v. in the remaining days of HDC. Results: Complete remission was defined as no emesis and no use of rescue therapy during days of HDC (acute phase) and until 5 days after the end of HDC (delayed phase). For the overall evaluation phase the primary end point of CR was achieved in 3 (50%) patients. For the acute phase, CR was achieved in 4 patients (66,6%); acute nausea was observed in 2 pts from day -2, in conjunction with the melphalan. For the delayed phase, CR was seen in 3 patients (50%). Delayed nausea (grado 1 sec WHO) was observed in 1 patients (16,7%) and delayed vomito (grado 2 sec WHO) in 2 patients (33,3%). The tolerability profile of the triple combined regimen in our study is excellent. Conclusion: Despite the experience in a small subset of the pts, we believe that the combination of aprepitant, palonosetron and dexamethasone show a good response in the acute phase (66%) and limiting episodes of vomiting in only 2 pts in delayed phase. Pts treated with BEAM could benefit from an extension of the aprepitant regimen with 80 mg from day -6 until the end of HDC and/or administration of the palonosetron on day -2, before melphalan, rather than day -4, in order to reduce nausea and vomiting in conjunction with the administration of melphalan and in the delayed phase.

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INTRAVENOUS IRON SUPPORT VS ORAL LIPOSOMIAL IRON SUPPORT IN PATIENTS WITH REFRACTORY ANEMIA TREATED WITH EPO ALPHA. MONOCENTRIC PROSPECTIC STUDY

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Background Intravenous iron support simultaneous to erythropoietin administration improve response to erythropoietin in myelodysplastic patients. In fact intestinal absorption of common commercial oral iron compounds is considerably impaired. Moreover, in MDS patients, absorbed iron is frequently stored in tissues and is'nt bioavailable. Oral

liposomal iron, bypassing normal intestinal mechanism of absorption, shows an increased haematic absorption, better than usual commercial oral iron compounds. Aims Aim of this study is to verify if in MDS patient support with oral liposomal iron is not inferior to iv iron support. Methods Between july 2008 and december 2010, 24 patients affected by refractory anemia were studied. Median follow-up was 12 months (R10-24). Patients were randomized 1:1 to receive in group A sodium ferrigluconate 62.5 mg iv in NS 100 ml in 1 h/day in the day when patient received alpha erythropoietin 40000 IU sc/week + calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group B patient received lipofer 14 mg 2 tablets orally/day + alpha erythropoietin 40000 IU sc/week + calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group A median age was 70 years (R65-75), M/F: 4/8. In group B median age was 66 years (R60-70), M/F: 6/6. Caryotype was normal in group A and B patients. Median level of haemoglobin was 9 g/dl in group A (R8.5-11) and 8.8 g/dl (R8.5-11.5) in group B. Results Group A patients increased Hb level of 1 g/dl after a median time of 4 weeks (R4-7) and after a median time of 5 weeks (R4-8) in group B. Most frequent side effects in group A were erythema in site of injection in 4 patients (33%), hypotension in 1 patient (8%). Most frequent side effects in group B were grade 2-3 diarrhoea in 4 patients (33%) . During median follow-up time patients of A and B group gaigned near 3 g/dl of Hb. Summary/Conclusion Oral liposomial iron supporting erythropoietinic therapy seems to be safe, feasible and substantially not inferior to intravenous iron support in patients affected by refractory anemia.

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CHLORPROMAZINE PLUS METHOCLOPRAMIDE AND PREDNISONE IS COST-EFFECTIVE IN CONTROL OF LATE HEMESIS IN PATIENTS PRETREATED WITH PALONOSETRON THAN IN THOSE TREATED WITH TROPISETRON

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BAKGROUND Late hemesis is often difficult to resolve. Data regarding the most useful drug to employ in late hemesis on the base of the type of anti HT3 drug previously used in acute hemesis (palonosetron or tropisetron) are lacking. Aims Aim of this study is to define which is the best cost-effective antihemetic therapy in late hemesis when palonosetron or tropisetron are used in acute hemesis in the same type of patient. METHODS This study is a prospective monocentric study. We considered 95 patients in period June 2007- January 2011, receiving highly and moderate hemetogenic chemotherapeutic regimens for haematologic malignancies. 50 patients (5 HD, 25 NHL, 6 ABMT, 14 AML) received tropisetron 2 mg tid i.v. until 48 hours after the end of chemotherapy. Of these 15 received DHAP-like regimen, 15 received IGEV, 6 BEAM and 14 "3+7" regimen. M/F was 28/22 and median age was 65 years (R30-75). 45 patients (4HD, 26 NHL, 5 ABMT, 10 AML) received palonosetron 250 mcg i.v. day 1 of chemotherapy. Of these 20 received DHAP-like regimen, 10 received IGEV, 5 BEAM and 10 "3+7" regimen. M/F was 26/19 and median age was 67 years (R28-76). All chemotherapy regimens were of 5 or 7 days of duration. If patients presented nausea and vomiting 48 hours after the end of chemotherapy, they received dexametason 4 mg + methoclopramide 20 mg i.v. tid. If nausea and vomiting persisted, they added largactil 12.5 mg i.v. tid. Results were evaluated by Fisher exact test. A cost analysis was performed considering the median of global direct and indirect antiemetic expense for each patient. Results Out of 45 patients receiving palonosetron, 5 had late hemesis, 4 requiring chlorpromazine and all responded to treatment. Out of 50 patients receiving tropisetron, 8 had late hemesis, 6 requiring chlorpromazine and only one responded to treatment. No difference were noted in late hemesis between palonosetron and tropisetron (two tailed Fisher text p 0.56). Chlorpromazine was effective in control of late hemesis mainly in palonosetron group (two tailed Fisher text p 0.046) Only observed side effect was a slight drowsiness in all patients receiving chlorpromazine. In palonosetron group, median global antiemetic direct expense for each patient was 107.25euro (R107.25-834.53), while in tropisetron group was 410.5euro (R30-515). Median antiemetic indirect expense was 20 euros in palonosetron group, while in tropisetron group was 280 euros.

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DAPTOMICIN VS TEICOPLANIN, WITH OR WITHOUT IV CALCIUM CHLORIDE SUPPORT IN POST CHEMOTHERAPY FEBRILE NEUTROPENIA: MONOCENTRIC RETROSPECTIVE STUDY

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Backgrounds. Daptomicin is a bactericidal antibiotic highly effective in gram positive infection. In vitro experiments seem to show that calcium ione might henance bactericidal activity of Daptomicin. Moreover it's well known that empirical use of glycopeptides in febrile neutropenia is of scarce utility. Data regarding use of Daptomicin in febrile neutropenia, in terms of safety, feasibility clinical efficacy and pharmacoeconomy are actually lacking. Aims Aim of this study is to evaluate efficacy of Daptomicin respect to Teicoplanin in febrile neutropenia in terms of: - Days of fever, - Days of hospitalization, - Cost of antibiotic and supportive therapy per day of hospitalization, - Days of fever in patients receiving Daptomicin and Teicoplanin if supported or not with intravenous calcium. Methods This is a monocentric, retrospective study. Patients with post chemotherapy febrile neutropenia received empiric therapy with Piperacillin-Tazobactam 4.5g tid iv, Amikacin 15 mg/kg/day iv and Teicoplanin 6 mg/kg/day iv (arm A) or Daptomicin 6 mg/kg/day iv (Arm B). In A arm, 15 patients were supported with iv Calcium Chloride up to achieve 10 mEq/l of blood calcium, in B arm 20. Daily costs of antibiotic and supportive therapy for each patient were calculate dividing the global cost of entire period of hospitalization for the days of hospitalization. In 30 months 86 patients were considered (40 in arm A and 46 in arm B). In arm A all patients M/F was 28/12. Median age was 62 years (R30-78). 29 patients had NHL, 10 AML and 1 received autologous bone marrow transplantation. In arm B M/F was 36/10. Median age was 70 years (R30-81). 36 patients had NHL and 10 AML. Results In arm A median of days of fever was 12 days (R4-21) and median of hospitalization was 32 days (R7-40). Median cost of antibiotic and supportive therapy was 850 euros/day (R329-1550). No significative differences in days of fever were noted between patient supported with calcium (n=17) or not (11 days in calcium group, 12 in no calcium group). In arm B median of days of fever was 11.5 days (R4-30) and median of hospitalization was 30 days (R4-33). Median cost of antibiotic and supportive therapy was 783 euros/day (R278-1250). Patient supported with calcium (n=20) showed 13 days of fever (R4-30) vs 15 days (R14-33) in patients not supported. No adverse effects in patients receiving daptomicin were registered.

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INTRAVENOUS CONTINUOUS INFUSION ANTIHEMETIC THERAPY IN LATE HEMESIS IN PATIENTS RECEIVING HIGH HEMETOGENIC CHEMOTHERAPY. MONOCENTRIC PROSPECTIC STUDY

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Background Late hemesis is frequently present in patients receiving chemotherapy. Anti HT-3 drugs are ineffective in this subset of patients. In surgical patients with persistent hemesis midazolam in continuous intravenous infusion showed good efficacy. Aims Aim of this study is to verify efficacy of continuous intravenous antihemetic therapy with midazolam in late hemesis in patients receiving high hemetogenic chemotherapy. Methods 36 patients with persistent hemesis beyond 72 hours after high hemetogenic chemotherapeutic regimens were considered in a period from 2004 to 2010. They received in first 48 hours of treatment an adequate antihemetic treatment with anti HT-3 drugs (tropisetron 5 mg iv tid). Patients were randomized to receive two different antihemetic therapeutic regimens. In group A patients received chlorpromazine 12.5 mg + desametason 4 mg + methochlopramide 20 mg in NS

100 ml iv in 30 minuts tid. In group B patients received chlorpromazine 25 mg + desametason 12 mg + methochlopramide 60 mg in NS 250 ml iv iv ci over 24 hours. In group A median age was 60 years (R 50-66), M/F: 10/8. 10 patients received polychemotherapy with IGEV regimen, 5 with DHAP and 3 with BEAM. In group B median age was 58 years (R 48-66), M/F: 9/9. 7 patients received polychemotherapy with IGEV regimen, 7 with DHAP and 4 with BEAM. Statistical analysys was performed with Fisher's exact test. Results In group A 3 patients stopped hemesis, but 15 none. In group B 17 patients stopped hemesis, but 1 none, with a Fisher's exact test with a p 0.000002. In group A or B any patients showed severe toxicity or side effect, except a slightly increased drowsiness. In consideration of small number of patients, this work need confirmation on a larger cohort of subject. Summary/Conclusions Therapy with chlorpromazine 25 mg + desametason 12 mg + methochlopramide 60 mg in NS 250 ml iv ci over 24 hours seems safe, feasible and effective in patients receiving highly hemetogenic therapy with late hemesis.

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THE INDICATION AND SAFETY OF SPLENECTOMY IN HEMATOLOGICAL DISORDERS: MONOCENTRIC EXPERIENCE OF HEMATOLOGY DEPARTMENT OF CATHOLIC UNIVERSITY

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Introduction: Splenectomy in hematology is indicated for diagnostic and staging purposes, for refractory anemia or thrombocytopenia, or for splenic rupture due to infarction. The frequency and the severity of its complications influence the use of this procedure. The aim of this study was to examine our clinical experience with splenectomy in hematologic indications and complications which derive from this procedure. Methods: A retrospective analysis was performed on 28 pts who underwent splenectomy at our Institution from May 2004 to February 2011. For each patient, clinical information was accumulated in a database including age, sex, underlying disease, spleen size, Hb value, Plt and WBC count in the preoperative time and at day 30, the operative technique and the hematologic indication to the intervention (diagnosis or treatment). Postoperative follow-up evaluation was obtained through medical records and referring hematologist follow-up visit. Complications were classified as short term and long term complications and divided into major or minor according to WHO scale. Results: 10 pts underwent laparoscopic splenectomy; 1 patient required conversion to laparotomy; laparotomic procedure was attempted in the other 17 pts. Indication for splenectomy included lymphoma (n=13), chronic idiopathic thrombocytopenic purpura (ITP) (n=8), myelodysplastic syndrome (MDS) (n= 3), autoimmune hemolytic anemia (n=2), multiple myeloma (MM) (n=1) and not otherwise defined splenomegaly (n=1). Splenectomy was therapeutic in 16 pts and diagnostic in 12 pts. Early postoperative complications occurred in 8 pts (28%): infectious in 4 cases (2 pneumonia, 1 pleural effusion, and 1 patient gram+ sepsis); hemorrhagic complications in 4 cases. No patient had acute thrombotic complication. In late postoperative time only 2 pts presented complication (1 portal thrombosis and 1 wound superinfection) (7%). It was revealed no hemorrhagic late complication. On the whole only 1 patient early died for hemorrhage. In laparoscopic group was observed only 1 infectious complication, all the other events affected the laparotomic group. Stratifying the complication according to WHO grading, 4 where WHO 1, 5 WHO 2 and 1 WHO 3. Conclusion: Our data indicate that splenectomy can be performed safety in a multitude of hematologic indications, and the mild complications (infectious and hemorrhagic) that could occur usually result well controlled.

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PALONOSETRON FOR PREVENTION OF ACUTE AND DELAYED NAUSEA AND VOMITING INDUCED ABVD IN PATIENTS WITH HODGKIN LYMPHOMA: A SINGLE CENTER EXPERIENCE.

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Introduction: ABVD is still the standard treatment in most patients Hodgkin Lymphoma. Although a significant improvement has been achieved in the prevention of ABVD-induced emesis, today this treatment is still characterized by a relevant percentage of patients, experiencing uncontrolled acute and delayed emesis, with the standard antiemetic treatment (consisting in the combination anti-serotoninergic drugs plus dexamethasone). Palonosetron, a new selective inhibitor of 5-HT3 receptors, in combination with dexamethasone showed a high antiemetic activity in pivotal trials enrolling patients treated with moderately or high emetogenic chemotherapy regimens. Basing on these data we planned to evaluate the activity and safety of palonosetron plus dexamethasone in patients affected by Hodgkin Lymphoma, receiving the ABVD schedule. Patients with any stage Hodgkin Lymphoma, and condidate for ABVD therapy have been considered eligible. A single pre-treatment dose of palonosetron 0.25 mg intravenously (iv) followed by dexamethasone 8 mg iv was administered before starting chemotherapy . Complete response was defined as the absence of acute vomiting and no need of rescue therapy during the entire period of observation (5 days, starting from the day of ABVD); we also evaluated intermediate and severe nausea (grade 3-4 according WHO). Adverse events were evaluated according to the NCI-CTC criteria. Results: forty-seven patients were evaluable. The absence of vomiting on the study period (days 1–5) was observed in 43 (91.5%) patients: the complete control of emesis during the acute phase (on day 1 of chemotherapy) was observed in 45 (96.7%) patients and in 43 (91.5%) during the late phase (from day 2 to day 5 of chemotherapy). The incidence of grade 3-4 (WHO) nausea during the days 1-5 was observed in 7 (15%) patients. The main side-effects (G1 grade) were: constipation observed in 3 (6%) patients, headache in 3 (6%) patients, vertigo and insomnia in one patients. Conclusions: a single dose of palonosetron and dexamethasone is a well tolerated and very active anti-emetic treatment for the prevention of nausea and vomiting induced by ABVD-regimen in patients with Hodgkin's Lymphoma.

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PERIPHERALLY INSERTED CENTRAL VENOUS CATHETERS (PICCS) IN HEMATOLOGICAL PATIENTS: A SAFE AND EFFECTIVE ALTERNATIVE TO THE CENTRAL VENOUS ACCESS

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The use of peripherally-inserted central catheters (PICC) as an alternative to central venous catethers (CVC) is becoming very frequent in different clinical settings. To highlight the role of PICC also in hematological patients, we revised our single Institute experience from September 2009 to January 2011. During this period, 340 PICCs (BARD Groshong 4 Fr) were inserted in 331 patients (M/F: 160/171) with a median age of 56 years (range 5-89), for a total number of 32.637 PICC-days (median 75 days, range 4-458). Among the 331 patients, 99 (29.9%) had acute myeloid leukemia, 29 (8.7%) acute lymphoid leukemia, 89 (26.8%) non-Hodgkin lymphoma, 69 (20.8%) Hodgkin lymphoma, 47 (14.2%) myelodysplastic/myeloproliferative disorders and 7 (2.1%) multiple myeloma; with regard to the phase of the disease, 138/331 patients (41.7%) were at diagnosis, 39/331 (11.7%) at disease relapse, 118/331 (35.64%) in advanced phase and 45/331 (13.5%) patients prior to a stem cell transplant. A PICC was successfully inserted in all cases under ultrasound-guide (in 241 cases via the basilica vein, in 97 via the brachial vein and in 2 via the cephalic vein). At insertion, the platelet count was <20 x 109/l in 54 (16.4%) patients, 20-50 x 109/l in 30 (9%) patients and >50 x109/l in 247 (74.6%) patients. Overall, we recorded 1.5% (5/331 patients) thrombotic complications (range 7-39 days) and 12.4% (41 patients) mechanical complications. No severe bleeding was observed in patients with a high hemorrhagic risk related to severe thrombocytopenia or anticoagulant therapy. A catheter-related bloodstream infection was observed in 6.6% of cases (22/331 patients): 50% due to Gram+ bacteria, 45% to Gram- bacteria and 5% to Candida. All PICCs were removed after a median period of 73 days (range 4-458), due to completion of treatment in 115 patients, death unrelated to the PICC in 71 patients and catheter-related complications in 50 patients (22 due to infection, 3 to thrombosis, 7 to obstruction and 18 to accidental extraction). Currently, 104 PICCs are still in use for the treatment of patients.

In conclusion, PICCs represent a safe and effective alternative to conventional CVCs for hematological patients associated with a low risk of complications at insertion and during the follow-up. They can also be used successfully for cyclic chemotherapy, high dose chemotherapy and stem cell transplant.

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PROTONIC PUMP INHIBITORS USE IN HOME CARE MANAGED HEMATOLOGICAL PATIENTS

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Introduction. Protonic pump inhibitors (PPI) are broadly used drugs. Indications are prophylaxis of severe upper gastrointestinal tract complications during treatment with acetil salicilic acid (ASA) or non-steroidal anti-inflammatory drugs (NSAIDs) in high risk patients (pts) or to manage some conditions such as gastric or duodenal ulceration or esophageal reflux disease; however outside above mentioned conditions PPI are used in other situations such as steroid usage and chemotherapy-related gastric and esophageal mucositis. Side effects are rare; however PPI safety may be influenced by drug-drug interaction. PPIs represent one of the first categories for overall drug spending in Italy, as well as in the most other countries. Aim. Data about PPI use in hematology are scarce. Our study explore the PPIs use in home care managed hematological pts. Material and methods. Clinical data of 177 pts registered in our home care service from January 2010 to March 2011 were examined retrospectively. 117 pts, with adequately recorded therapy details, were considered evaluable for further analysis. Pts treated with PPIs were extracted, indication and dosage were explored. PPIs prescription, brand name and major drug interactions were examined among the whole sample. Dosage, indications, moderate or mild drug interactions and cost analysis were conducted on a 12 randomly selected pts sample. Results. Demographic data of the 117 evaluable pts were: 50 male, 67 female, median age 83 (20-98); diagnosis and disease phase are reported in table. PPIs drugs were administered to 68/117 pts (58%). 11 pts were treated with 2 different drugs. Lansoprazole, rabeprazole, omeprazole, esomeprazole and pantoprazole were used in 35 (30%), 24 (21%), 12 (10%), 5 (4%) and 3 (2%) pts, respectively. Indication was ASA/NSAIDs in 5/12 (41%), CHT in 3/12 (25%), steroids in 2/12 (17%), no clear indication in 2/12 (17%). No major PPIs-drug interaction were noted; out of 12 randomly selected pts, 8 (3 moderate, 5 mild) interactions were identified in 8 pts (67%). PPIs related daily spending (DS) / pt was 1,10 euro, representing 2.19% of total DS (50,19 euro). Conclusions. PPIs are frequently used and represent a significant amount of pharmaceutical expenditure in HC hematological pts. The virtually constant association with other drugs needs a careful evaluation of interactions. Open points remain the frequent, but off-label use in the setting of gastrointestinal mucositis.

Diagnosis	n	%
AL	16	14
MM	13	11
Lym	9	8
cMPD	17	15
MDS	37	32
Other	25	21
Disease status	**n**	**%**
Terminal	22	19
Advanced	32	27
Chronic	51	44
Active treatment	12	10

AL: acute leukemia; MM: multiple myeloma; Lym: Lymphoma; cMPD: chronic myeloproliferative disorders; MDS: myelodisplastic syndrome.

Table. Diagnosis and disease phase

P393

FEASIBILITY AND SAFETY REDUCING COLONY-STIMULATING FACTORS (G-CSF) IN DOSE DENSE R-CHOP THERAPY

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Reducing the cycle length from 3 to 2 weeks (CHOP21 vs CHOP14) in treatment of aggressive non Hodgkin lymphoma (NHL) has been demonstrated to increase OS in young patients (pts) with good prognosis, OS and EFS in elderly pts. In these studies use of G-CSF was for 10 days (from +4 to +13) as recommended by ASCO guidelines 2006. Our prospective study was to demonstrate that less number of vials of G-CSF don't significantly increase the risk of haematological toxicity and infection even if we use a dose-dense chemotherapy. Starting from 2002 we included 100 pts with a newly diagnosed NHL (92% DLBCL; 8% FL grade IIIb). The median age was 61 years (range 20-75) and 40% of pts had an high-intermediate or high IPI. Pts were treated with immunochemotherapy every 14 days (R-CHOP14) followed by G-CSF (lenograstim). In first 10 pts we used 7 vials of G-CSF (from +5 to +11) after each cycle of chemotherapy. According to well-tolerance of therapy we prospectively decided to administer 5 vials of G-CSF (from +7 to +11). Further reduction of vials, at least 3 for cycle (in 25% of pts), was accepted if pts at the moment of therapy, reached a number of leucocytes over 20.000/mmc. We used a median of 25 vials of G-CSF (range 10-35) for each pts that correspond to 5 vials for each cycle (range 1-10). Due to occurrence of severe adverse events (neutropenia, piastrinopenia, infective episodes) therapy was delayed in 18% of pts and 3 pts switched to R-CHOP21. Incidence of neutropenia grade 3-4 was 5 %, thrombocytopenia grade 3-4 was 5%, febrile episodes 8% and hospedalization in 5 % of pts. Leucocyte nadir was 3525/mmc (400 –13000), anemia nadir was 10.55 gr/dl (5,8 – 15,5) and thrombocytopenia 144.500/mmc (43000 – 328000). No myelodysplasia has been reported so far neither in young nor in elderly. We evaluated response to therapy in 98 pts who completed the R-CHOP14: complete remission rate was 86% with an ORR of 97%. OS was 82% after a median follow-up of 36 months (range 4-107). In our experience R-CHOP14 supported by G-CSF has been well tolerated also in elderly pts. We confirm that the reduction from 10 to 5 or less GCS-F vials has not determined an increase of neutropenia, febrile episodes, delays in the therapy and hospedalizations confirming an 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.

PUBLISHED ONLY

PU001

RETROSPECTIVE ANALYSIS OF 20 PATIENTS WITH HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA UNDERGOING FRONT-LINE INTENSIFICATION WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION: A ROME TRANSPLANT NETWORK STUDY

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Background Despite it is highly effective, in high risk diffuse large B-cell lymphoma (DLBCL) conventional treatment results in a global overall survival (OS) of only 30-45%. Nevertheless, autologous hematopoietic stem cell transplantation (ASCT) is so far indicated only in patients with relapsed DLBCL aged less than 65 years. Therefore, several studies have recently been performed to establish the role of front-line intensification with ASCT in this setting of patients. The aim of this study was to evaluate the safety and clinical outcome of high risk DLBCL patients undergoing front-line intensification with ASCT. Patients and Methods From January 2004 until June 2010, 56 DLBCL patients underwent ASCT in three Institutions of the Rome Transplant Network. Front-line intensification with ASCT was performed in 20 patients who were high risk (IPI 2-3) with a median age of 43 years. The remaining 36 underwent ASCT after two (n=28) or three (n=8) lines of treatment. Results Of these 20 patients, 14 were treated with R-CHOP and 6 with R-CODOX-M/R-IVAC: 15 patients (75%) obtained a complete remission (CR) whereas the remaining 5 a partial remission (PR). All 20 patients underwent one (n=16) or two (n=4) lines of mobilization chemotherapy followed by hematopoietic stem cells harvest. Conditioning regimen was BEAM in 17 patients, MITO-MEL in 2 and BU-CY in 1. Median number of CD34+ cells infused was 7,5 x 106/Kg. Median times to neutrophilis and platelets engraftment were 10 and 14 days, respectively. Three patients experienced a symptomatic reactivation of Cytomegalovirus after ASCT, requiring intravenous antiviral treatment. One patient died during aplasia because of Gram negative septic shock; global Transplant-related Mortality (TRM) was 5%. A relapse after ASCT, was experienced in 3/19 (15.8%) assessable patients; median time to relapse was 199 days from ASCT. With a median follow-up of 2 years, DFS and OS rates are 84.2% and 85%, respectively. Conclusions Our results indicate that a front-line intensification with ASCT in patients with high risk DLBCL is feasible, with a TRM of 5%. This type of approach in a limited cohort of high risk DLBCL patients is able to determinate a 2-year DFS and OS of 84.2% and 85%, respectively. Therefore, randomized cooperative studies are needed to clarify the real role of front-line intensification with early ASCT in this setting of patients.

PU002

PRIMARY HEPATIC LYMPHOMA AND CONTRAST-ENHANCED ULTRASONOGRAPHY

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Primary hepatic lymphoma (PHL) is a rare disease, but it should be considered in the differential diagnosis of focal liver lesions (FLLs), mainly in patients with chronic HCV or HBV infection. Ultrasonography is the first examination performed for screening of FLLs and contrast-enhanced ultrasonography (CEUS) can help discriminate between HCC and other lesions (such as regenerative nodules) in patients with chronic liver disease as well as between benign and malignant lesions in patients without liver disease. On ultrasonography, hepatic lymphoma usually appears as a hypoechoic lesion. Few data are available about the role of CEUS in the diagnosis of PHL. We describe 2 cases of PHL of the liver associated with hepatitis B virus (HBV) infection. A 62-year-old man was referred to our hospital for the incidental detection of hepatitis B surface antigen positivity; liver function test results and serum -fetoprotein values were within normal limits.The second case, a 58-year old man, was referred because of a slight increase of serum transaminase levels; other liver function and serum -fetoprotein parameters were normal; the serum HBV DNA levels were low in the first patient (830 IU/mL) and fairly elevated in the second (186.584 IU/mL). Both patients underwent B-mode ultrasonography for screening. After detection of an FLL, CEUS performed with a rapid bolus of a contrast agent (2.4 mL of sulfur hexafluoride; SonoVue; Bracco SpA.) in the antecubital vein followed by a 5-mL saline flush, contrast-enhanced computed tomography (CT), and biopsy were performed. In both cases,ultrasonography showed a hypoechoic liver lesion (4 and 3 cm, respectively) with irregular margins. On CEUS, these lesions were inhomogeneously hyperenhanced in the arterial phase and hypoenhanced in the portal and late phases. CT in both patients showed slight hyperenhancement in the arterial phase and hypoenhancement in the remaining phases. Needle biopsy showed marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue type in both patients. Conclusions. Contrast-enhanced ultrasonography and CT did not help us differentiate PHL from HCC, in both cases we saw the characteristic findings of primary HCC. Primary hepatic lymphoma is a rare condition, but it should always be considered in the differential diagnosis of FLLs. We stress the important role of liver biopsy when imaging indicates HCC in patients without underlying cirrhosis.

PU003

ASSESSMENT OF LOSS OF NUCLEATED BONE MARROW (BM) CELLS DURING THE HANDLING PROCEDURE FOR BONE MARROW ALLOGENEIC TRANSPLANTATION

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Introduction: Allogeneic transplantation procedure is based on manipulation and cryopreservation of an high number of bone marrow (BM) nucleated cells. This procedure is associated with a weighty loss of BM nucleated cells, as higher as more cycles are performed. Aims: First aim of this report is to identify an acceptable percentage of loss of BM nucleated cells during the procedure, either in simple deplasmation or in more elaborate manipulation, ie ABO mismatch. Secondary aim is to acquire an acceptable and standardized range in Jacie operative instructions. Materials and methods. From 2003 to 2011 we performed 23 bone marrow collection and manipulation. we evaluated both type of manipulation (deplasmation, deplasmation plus AB0 multistep handling), and cryopreservation at –192 C°). In 95.7% of cases we performed deplasmation plus criopreservating at –192 C°, while 4.3 % was only deplasmation plus AB0 multistep handling and cryopreservating at –192 C°. All procedure were performed in steril transfer bag 400 (Grifols), steril cryobag 250 (Grifols), blood administration set of 75 cm. Dimethil sulphoxide cell culture grade (DMSO) was applied in cryopreservation, while Haesteril solution was handled in AB0 mismatch, as indicated in standard procedures. Results. For all procedures the loss was estimated in 18% of manipulated total nucleated BM cells (range: 2%-42%). The major loss (26%) was observed in ABO mismatch samples. Moreover, the major loss (42%) was associated with a minimal final volume infusion (57%). Conclusions. Our data suggest that an acceptable loss of manipulated total nucleated BM cells should not be higher to 23% (range: 18%-23%). These data indicated that during explantation is maybe necessary an higher amount of nucleated BM cells, sufficient to obtain the minimum for BM engraftement. The range of acceptability is also important for Jacie procedure.

PU004

RETROSPECTIVE ANALYSIS OF 41 NON-HODGKIN LYMPHOMA (NHL) PATIENTS AGED ≥ 75 YEARS UNDERGOING CHEMO-IMMUNOTHERAPY

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Background Treatment of NHL of very elderly patients is often diffi-

cult for the frequent presence of comorbidities and solid data regarding the best appropriate approach are lacking; a Comprehensive Geriatric Assessment (CGA) could be useful in these patients to establish intensity of treatment. Patients and Methods From January 2003 to January 2011, 53 patients aged ≥ 75 years (median age 77, range 75-88) were diagnosed with a NHL (aggressive: 29; indolent: 24) in two Institutions. Chemo-immunotherapy was administered to 46/53 patients; of these, 41 are so far evaluable. A CGA was performed in all patients before treatment. The aim of the study was to describe the outcome of very elderly patients achieved outside clinical trials, to identify specific prognostic factors and to analyze the influence of CGA. Results 35 out of 41 patients (85%) presented comorbidities at diagnosis, 5 (12%) presented a low Activity of Daily Living (ADL) index and 12 (29%) were identified as frail by CGA. Overall response rate was 75% (CR 58%, PR 17%), 11 patients experienced a relapse/progressive disease (29.7%) and 15 died (36.5%). Two hundred eighteen cycles of chemotherapy were evaluated: febrile neutropenia occurred in 6/41 patients (14.6%) and in 9/218 cycles (4.1%). The main cause of death was progressive disease (53%) whereas global toxic death rate was of 7.3%. OS and PFS rates were 63.4% and 70% with a median observation time of 1.6 years. In univariate analysis, stage III-IV (p=0.034), high IPI/FLIPI/MIPI (p=0.015), presence at diagnosis of Hb level < 12 g/dL (p=0.04), a low ADL (p=0.032) and condition of frailty according to CGA (p=0.006) significantly affected survival. Significant prognostic factors (p<0.05) for PFS were an extranodal disease, presence at diagnosis of Hb level < 12 g/dL and of creatinine level above the normal, a low ADL and a condition of frailty according to CGA. Administration of anthracyclines did not influence OS, PFS and the occurrence of febrile neutropenia. Conclusions Our results show that, even outside clinical trials, very elderly NHL patients can be safely treated with curative chemo-immunotherapy programs, presenting similar prognostic factors and outcome than younger patients. In our experience, CGA is able to identify a group of frail patients with a worse clinical outcome, in which is useful to limit intensity of treatment. However, these results need to be confirmed by prospective cooperative studies.

PU005

EFFICACY AND SAFETY OF A WEEKLY CONSOLIDATION REGIMEN: BORTEZOMIB, DEXAMETHASONE, CYCLOPHOSPHAMIDE (VCD) AFTER A BRIEF INDUCTION WITH BORTEZOMIB, DEXAMETHASONE AND PEGYLATED DOXIL IN PATIENTS NOT ELIGIBLE TO ABMT INTOLERANT TO BORTEZOMIB

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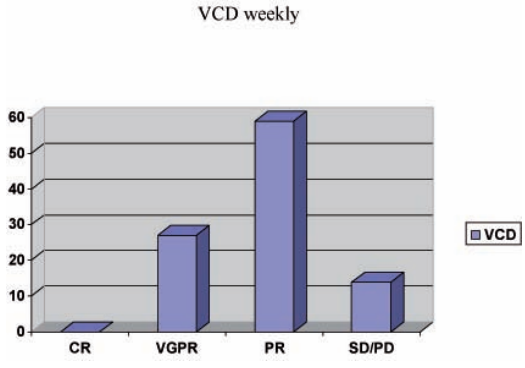
Objectives: we refer about our preliminary experience, using a weekly treatment with Bortezomib, Dexamethasone and Cyclophosfomide. Patients characterisc: pts. over 65 years old or not eligible to ABMT with neuropathic toxicity from bortezomib. Endpoint: demonstrate adequate response to VCD in six consecutive patients. Treatment: Four three-weekly cycles with Bortezomib, Dexamethasone and peghilated Doxil (only on the fourth day like Orlowsky schedule) followed by Four/eight cycles with Bortezomib, Dexamethasone plus Cyclophosphamide 500 mg total dose (d1-d8-d15-d22). Results : CR, VGPR, PR, SD/PD VCD monoweekly.



Conclusions. The VCD weekly regimen (d1-d8-d15-d22) is safe and efficacy showing more compliance and less neuropathic toxicity in this subgroup of patients.

PU006

HEMATOLOGIC IMPROVEMENT WITH DEFERASIROX AND ZOLEDRONIC ACID IN A FRAIL ELDERLY PATIENT WITH MYELODYSPLASTIC SYNDROME AND CONCOMITANT MULTIPLE MYELOMA: A CASE REPORT

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Background. Myelodysplastic syndromes (MDS) and Multiple Myeloma (MM) tipically affect the elderly, representing today a challenge for haematologist because of the frailty of these patients. Deferasirox is an oral iron chelator, aimed at preventing organ damage due to transfusional iron overload; inhibition of NF-kB seems to be another pharmacological effect of the drug. Zoledronic acid is a biphosphonate used in supportive care in patients with MM and lytic bone lesions. Recent studies suggest an antineoplastic effect by acting on bone marrow microenvironment. We describe a frail patient who developed erythroid response under deferasirox and zoledronic acid treatment. Case Report: A 79 year-old female patient was diagnosed as having refractory anemia (RA) in March 2010, normal karyotype (low IPSS and WPSS); Hb level at diagnosis was 7.2g/dl, with serum erythropoietin levels of 705 mIU/ml. Concomitant monoclonal gammopathy of undetermined significance (MGUS IgG K) was observed. Median transfusion requirement was 2 RBC units/month; in June 2010 serum ferritin (SF) was 2718 ng/dl, so she started deferasirox treatment at the dose of 10 mg/kg/day. In August 2010 a massive pulmonary thromboembolism occurred and patient was hospitalized: in that occasion evaluation of bone marrow was repeated, displaying an evolution from MGUS to MM (plasma cells 22%). Lytic bone lesions were observed, so that zoledronic acid was started (4 mg monthly). She was treated with melphalan-prednisone regimen, promptly interrupted because anemia dramatically worsened (Hb 5.2 g/dl); considering the frailty of the patient, we continued only with dexamethasone 20 mg weekly. Transfusion requirement significantly increased up to 4 RBC units/month; therefore in October 2010 deferasirox dose was increased to 20 mg/kg/day. Subsequent follow-up was unremarkable and, unexpectedly, her transfusion need decreased to 1 RBC unit/month, achieving an Hb level of 10 g/dl in April 2011. SF was 2310 ng/dl; bone marrow aspirate documented a percentage of 20% of plasma cells, with no progression on lytic lesions. According to IWG 2006 criteria, we can assume that the patient has obtained an erythroid response. It is appealing to suppose a double role of deferasirox-mediated NF-kB inhibition, both on MDS cells and on NF-kB activation in MM cells. Hematologic improvement can be partly attributed to a synergic antineoplastic effect of zoledronic acid by limiting the clonal plasma cells proliferation.

PU007

A POSSIBLE PATHOGENETIC ROLE OF LARGE RENAL CYSTS IN IDIOPATHIC ERYTHROCYTOSIS

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Background: Idiopathic erythrocytosis (IE) identifies a group of patients with absolute erythrocytosis (ES) in whom no primary or secondary cause (acquired or congenital) has been identified. 1/3 have EPO levels below the normal range. The other 2/3 have normal or elevated EPO levels, thus presenting a secondary ES of unknown cause. Different defects have been identified in some patients in these groups; however, no mechanism for the ES has been recognized in the majority of patients. In acquired ES setting, is known that tissue hypoxia leads to increased EPO production. EPO is produced by the kidney, and any process that leads to hypoxia in the kidney (renal artery stenosis, hydronephrosis or polycystic kidney disease) can result in increased EPO production and thus ES Aims and Methods: We report 5 cases of IE who curiously presented large renal cysts in association to low or normal (no high) EPO level. These patients were selected from our registry of 25 patients classified as EI. Patients were included if they did not fulfill the criteria for Polycythemia Vera (PV) and if no secondary cause of ES was evident Results: Median age (56 years), Ht and Hb levels (52% and 18,6 g/dl, respectively) at the presentation of disease in these patients were

similar to remaining IE population. To note a history of arterial hypertension was common to all 5 patients. No patient had splenomegaly, all 5 patients had diffuse hepatic steatosis and ultrasound imaging showed monolateral (3) or bilateral (2) large renal cysts (diameter between 3,5 to 10 cm) with EPO levels low (1) or normal (4). Screening for JAK2V617F and JAK2 exon 12 mutation was also negative. We did not find other causes of acquired or congenital ES. All patients received low doses aspirin as prophylaxis against thrombotic events, in association to phlebotomy to maintain a Ht target < 50%. No patient experienced arterial or venous thrombosis or evolution in PV or other chronic myeloproliferative disorders Conclusions: We retain that the presence of large renal cysts may be a potential cause of secondary ES in the forms still classified as IE. Incidence in our series of this hypothetical correlation is 20%. It would be interesting to evaluate the possible resolution of ES in these patients after surgical removal or aspiration of renal cysts in further confirmation of this pathogenetic hypothesis. Further studied could be designed to identify any molecular defects in this renal homeostatic pathway

PU008

DEVELOPMENT OF JAK2V617F POSITIVE POLYCYTHEMIA VERA AFTER CHEMOTHERAPY-INDUCED-REMISSION OF CEREBRAL NON-HODGKIN'S LYMPHOMA: A CASE REPORT

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Background: Chronic myeloproliferative disorders (MPD) are thought to derive from the transformation of a multipotent hematopoietic stem cell. Interest in MPD has been rising since the discovery of JAK2V617F mutation, but its exact role in the complex pathogenesis of these diseases is still under investigation The coexistence or the developing of MPD after non-Hodgkin's lymphoma (NHL) is an extremely rare event. We report a case of a 72 year old man who developed JAK2V617 Polycythemia Vera (PV) three years after the diagnosis of cerebral diffuse large B-cell Non Hodgkin's-lymphoma (DLBCL) for which he had received high dose intensive combined chemotherapy Case-report: A 72 year old man was diagnosed as having DLBCL with isolated cerebral localization in April 2002. Complete remission was obtained after 3 chemotherapy courses with high-dose cytarabine and high dose methotrexate. Clinical and radiological follow-up performed for DLBCL were unremarkable until February 2005, when the patient displayed pruritus, paresthesias and plethora. Peripheral blood count showed high Hb and Ht level (18,5 g/dl and 58%, respectively), with normal WBC count (5.43 x 109/l) and platelet count (441 x 109/l). The screening tests for PV showed low erythropoietin level (1,8 U/l) and no evidence of secondary causes of erythrocytosis. The Allelic specific PCR analysis of JAK2V617F mutational status resulted positive, consistent with diagnosis of PV according to WHO criteria; the research of clinical and radiological signs of relapse of DLBCL remained negative. The patient has been initially treated with phlebotomy and anti-aggregation with aspirin. From November 2007, following the progressive platelet increase (890 x 109/l), cytoreduction with hydroxyurea (500mg/day) has been initiated. The patient obtained phlebotomy independence with normalization of Ht level and platelet count. No arterial or venous thrombosis have been reported Discussion: The occurrence of a secondary MPD after high dose-chemotherapy, particularly for treatment of lymphoproliferative disorders, is an extreme rarity in literature; therefore more data are necessary to better define the possible pathogenetic role of previous chemotherapy regimens in the expansion of JAK2V617F mutant clone responsible of secondary MPD phenotype

PU009

THROMBOTIC THROMBOCYTOPENIC PURPURA IN A PATIENT WITH METASTATIC COLON CANCER IN CHEMOTHERAPY: A CASE REPORT

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Introduction.: Thrombotic thrombocytopenic purpura (TTP) is a associated with high mortality rates characterized by a reduced blood level of a protease, the disintegrin and metalloprotease with thrombospondin motifs (ADAMTS)-13, formerly known as von Willebrand factor circulating polymers. A case with TTP during chemotherapy is discussed. CASE REPORT: A 67-years-old man with metastatic colon cancer in second-line antineoplastic-antiangiogenetic treatment (capecitabina and cetuximab) was admitted to our hospital presenting fatigue, mild dispnea, tachycardia without fever;physical examination indicated pale face. Laboratory examinations revealed: haemoglobin 7.2 grams per deciliter, mean cell volume 93 fentoliters, platelets 30000 per millimeter cubic, serum indirect bilirubin level 1.12 milligrams per deciliter, gammaglutamyltransferase 120 unit per liter, serum aspartate aminotrasferase 357 unit per liter, serum alanine aminotransferase 145 unit per liter and incrased serum lactate dehydrogenase to11248 unit per liter with a negative direct Coombs test. Nor were there signs of renal and neurological dysfunction. All microbiologic tests were negative. The patient was trasfused with 5 units of red blood cells. Chest x-ray was negative, abdominal ultrasound showed multiple liver metastasis. Hemostasis screening revealed a reduced PT, prolonged aPTT and D-Dimer levels; peripheral smear showed anisopoikilocitosis, thrombocytopenia and fragmentation of erytrocytes such as microangiopathic hemolytic anemia. The patient was treated with steroids and plasma exchange without clinical results and he died in few days. The plasma level of ADAMTS-13, calculated after he died, were lower than range value such as hereditary form or acquired TTP. The plasma exchange removed the anti-ADAMTS-13 auto-antibodies and infused of active ADAMTS-13. CONCLUSION: we found a severe deficiency ADAMTS-13 in TTP and in some, but not all, patients with idiopathic thrombocytopenic purpura, acute leukemia, sepsis, pregnancy, disseminated intravascular coagulopathy, vascular disease or during radiotherapy and transplantation-immunosuppression treatment. In addition similar thrombotic microangiopathies sometimes occur weeks to months after exposure to the following multiple chemotherapic or antiangiogenetic-agents.In some studies patients were treated with PE by means of continuos-flow cell separation with the exchange of one plasma volume, with clinical improvement .

PU010

IN HEMATOLOGIC PATIENTS DEPOCYTE THERAPY IS EFFECTIVE AND PERMITS TO OVERCOME PANCITOPENIA DUE TO SYSTEMIC HD MTX AND ARAC WITHOUT SIGNIFICANT NEUROTOXICITY.

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Liposomal cytarabine (DepoCyte) is a slow release-formulation of conventional cytarabine (AraC). DepoCyte permits to decrease the frequency of lumbar punctures, without loss of efficacy, because intrathecal levels of the drug remain cytotoxic for up to 14 days. In the literature, there are data that suggest a serious potential neurotoxic side-effect in patients receiving intrathecal DepoCyte as part of a multimodal regimen including high-dose (HD) systemic methotrexate (MTX) and AraC. We investigated the efficacy and safety of therapeutic intrathecal DepoCyte in 9 pts, with Central Nervous System (CNS) localizations, admitted to our Division between March 2009 and December 2010. Six pts (67%) were female. The median age was 44 years (range 16-70). Our pts were affected by Acute Myeloid Leukemia (AML, 2), Acute Lymphoblastic Leukemia (1) and Non-Hodgkin Lymphoma (NHL, 6). Two pts (22%) presented only a CNS parenchimal mass; the other 7 pts had a leukemic or lymphomatous meningitis. The median lumbar punctures performed were 2 (range 1-6). The first pt, affected by AML, who underwent allogeneic transplant and relapsed only on CNS, was treated with intrathecal therapy (DepoCyte, 3 administrations) because of leukemic meningitis, obtaining a complete response until now after 11 months follow up. Among the remaining 8 pts, 6 were treated concomitantly with DepoCyte and HD systemic MTX and AraC. In these pts DepoCyte was used to overcome the aplastic phase (median duration 7 days, range 0-20) following HD chemotherapy. The remaining 2 pts received DepoCyte before and after the HD systemic chemotherapy respectively. None of our pts presented neurological toxicities related to DepoCyte. One of the two NHL pts with a CNS parenchimal mass obtained a complete response, without recurrence. Among the other 7 pts with meningitis, 6 pts obtained complete response, but later 3 of them relapsed. The Overall Survival of our population was 40.6% at 12

months, the Desease Free Survival 62.5% at 3 months. The median follow up was 8 months (range 1-16 months). In our experience regarding the treatment of CNS localizations of hematologic malignancies DepoCyte seemed to be effective and well tolerated, on the contrary to previous reports, also as part of a multimodal regimen including HD systemic MTX and AraC. The slow liposomal cytarabine release seems to permit to overcome pancitopenia secondary to HD chemotherapy, without reduction of intrathecal drug concentration and efficacy.

PU011

MAINTENANCE TREATMENT WITH AZACYTIDINE IN THREE ELDERLY PATIENTS WITH SECONDARY ACUTE MYELOID LEUKEMIA IN COMPLETE REMISSION AFTER INDUCTION CHEMOTHERAPY: EXPRERIENCE OF A SINGLE CENTER

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Background. The acute myeloid leukemia (AML) following MDS is associated with poor prognosis in elderly patients: the conventional chemotherapy (CHT) is burdened by high toxicity and mortality, especially with the presence of comorbidities. The consolidation CHT does not prolong the duration of remissions. It is known that in elderly patients (pts) with low marrow blast count (20% to 30%) AML, azacitidine (AZA) prolongs OS . Patients. We treated 3 pts elderly (mean age 70 years) with secondary AML and with comorbidities. The blasts in the bone marrow ranged between 40-80%. 1 of 3 pts presented skin locations and another presented the t [12; 17](q24,q21) in 12/20 metaphases. Induction CHT consisted of a regimen "3+7"(daunorubicin 45 mg/m2 i.v. day 1-3 and cytarabine 100 mg/m2 i.v. days 1–7). All 3 pts have achieved complete remission (CR); but, the achievement of CR after induction CHT was complicated by severe septic diseases. Therefore, we have started maintenance treatment with AZA (75mg/m2/d for 7 days of every 28-day cycle). Results: To date, they have been given 4, 9 and 12 cycles of AZA. In one pt, from the sixth cycle, for hematologic toxicity (grade 3 thrombocytopenia, grade 3 anemia requiring transfusion support), the dose of AZA was reduced to 60 mg/m2/d for 5 days of every 28-day from sixth cycle, resulting in a net lowering of haematological toxicity. Another pt experienced grade IV neutropenia after the third cycle of AZA. All 3 pts maintained CR of disease during the maintenance AZA. Conclusions: Considering the limit of the small number of patients and short follow-up, our clinical observations confirm that the maintenance treatment with AZA after induction CHT in secondary AML at a dose of 75 mg/m2 may be responsible for haematological toxicity grade 3-4 (sec. WHO). Is not possible to draw definitive conclusions and it is assumed that there is needed for prospective randomized studies to confirm what we have observed about the benefits and safety of AZA in maintaining post-induction CHT.

PU012

STEVEN-JOHNSON SYNDROME ASSOCIATED WITH LENALIDOMIDE TREATMENT IN A MULTIPLE MYELOMA PATIENT.

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Stevens- Johnson syndrome (SJS) is a severe and life-threatening condition and is considered medical emergency. The average reported mortality rate of SJS is 1-5%, but it can be even higher in elderly patients. Results of several studies revealed that more than 100 different causative drugs have been reported. Among hematologic drugs recently introduced into the market, drugs such as rituximab, imatinib, and bortezomib are reported. Here, we describe a patient with SJS while receiving lenalidomide in combination with prednisolone for treatment-naïve multiple myeloma. A 69-year-old woman was admitted because of back pain caused by the compression fracture of the lumbar spines. Serologic tests and bone marrow studies confirmed the pathologic diagnosis of multiple myeloma, immunoglobulin G (IgG K) type, stage III. Patient was included in a controlled trial and a lenalidomide-based combination regimen was employed, consisting of lenalidomide 10 mg daily on days 1 to 21, followed by 7 days off. Prednisolone was cycled at 25 mg on alternate days. Treatment cycles were repeated every 28 days. After the end of the first cycle she presented erithema, mucocutaneous tenderness and hemorrhagic erosions. Two days later an epidermal detachment appeared presenting as blisters and areas of neduded skin on the legs (about 5 percent of the total body surface area). Histological analysis confirmed SJS diagnosis. Subsequently, lenalidomide treatment was stopped. We started a symptomatic treatment with diphenhydramine hydrochloride, itraconazole and intravenous fluids. Steroid medication therapy was continued with 80 mg methylprednisolone. All dermal and mucosal lesions healed after 30 days. SJS has been reported rarely as an adverse reaction to Lenalidomide. Celgene Corporation has received 12 reports of SJS among approximately 57,000 patients who received lenalidomide from market launch on December 27, 2005, through June 26, 2008. However Lenalidomide should be considered in the etiology of SJS. Although several cases of SJS are described, exact mechanisms of the disease are not elucidated. However, increasing data have revealed that the genetic predisposition and immune mediators play important roles in drug hypersensitivity. The high sensitivity/specificity of some markers provides a plausible basis for developing tests to identify individuals at risk for drug hypersensitivity, and a need exists to guide Lenalidomide prescription.

PU013

223 CONSECUTIVE PATIENTS WITH HODGKIN'S LYMPHOMA; AN OUTCOME STUDY BASED ON SINGLE CENTER EXPERIENCE.

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Introduction. The treatment of Hodgkin's Lymphoma (HL)is considered one among the greatest success of cancer therapy with a cure rate of about 80%.Sparing toxicity and quality of life became the main goals;long-term side effects may cause a death-risk exceeding that from HL after 15 y of follow-up.While a large number of clinical trials based on very selected patient population are available,there are few data about the long-term outcome in unselected population.We reported a single center experience collected in the last sexteen years. Patients and Methods.Since April 1994 until December 2004, a total of 223 consecutive pts, M/F 114/109, median age 33 y(13-88) has been recorded. Stage I-II 161,III-IV 60,undetermined in 2;symptoms were present in 76,not reported in 2.Chemotherapy plus Radiotherapy (CT)in 174,Cht in 35, RT in 9,no therapy in 5.192 pts were treated with full-dose curative intent ABVD,MOPP/ABV, Stanford V,31 were approached less intensively or with palliation based on ChlVPP,VBM or steroid.Young relapsing/refractory pts were trasplanted with autologous stem cell.Results. After a median follow-up of 6y (7d-16y),191/223 (85%) obtained CR,16 (8%) relapsed,11 were refractory,7 obtained PR;14(8%)died during induction/palliation.34/223(15%)needed a second-line therapy;of them 12(35%)obtained CR,19 died for disease progression/toxicity,1 was alive at the last follow-up with active disease,1 refractory was lost at the last follow-up. We recorded a total of 44 deaths,11 in CR,33 for disease progression/toxicity.28/223 were 65y or older(13%);4/28 were treated with palliation only for comorbidity and died after a median follow-up of 2 months,24/28 were treated with full-dose ABVD (10)or with less intensive therapy (14).18/24 obtained CR (75%),3 of them relapsing,4 died while on therapy,1 was refractory,1 obtained PR.At the last follow-up,8/28(28%)are alive in CR,1 alive with active disease,19 died. Conclusions. Our results confirm brilliant worldwide reported data;OS of the whole population was 75% after 6y of follow-up.14% of the whole population was treated with less intensive therapy for comorbidity or pure palliation. Induction death rate is higher than previously reported in clinical trials. Pts >=65y account for 43% of the mortality rate; among them,only a minority (38%) were treated with full-dose ABVD, however a large quote obtained at least a transitory CR. Outcome of older pts seems to induce a limited influence on the HL whole population's one.

PU014

ANAGRELIDE THERAPY DURING PREGNANCY: A REPORT OF A SUCCESSFUL OUTCOME

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Essential Thrombocythemia (ET) presents two peaks of incidence, at 60 and 30 years, thus the diagnosis is not unusual in pregnancy women.

Anagrelide (ANA), a second line therapy for ET, crosses the placental barrier and is contraindicated during pregnancy because the effects on human fetus are largely unknown. Here we describe the successful outcome of a 32-year-old woman with ET treated with ANA before and during pregnancy. B.A. presented with recurrent headaches, a severe piastrinosis (PLT 3482 x109/L), WBC 10,8 x109/L, Hb 11,2g/dL, normal biochemistry and a JAK2 wild-type. A bone marrow biopsy exhibited normal cellularity, an increase in megacaryocytes with clustering in absence of fibrosis. Patient first underwent two platelet apheresis and started therapy with hydroxyurea (HU) that was interrupted because of a diffuse skin rashes. She turned to Interferon alpha (IFN-alpha) 3M.U.s.c. daily and Aspirin (ASA) 100mg daily. The patient had to switch the IFN-alpha to ANA (2-3mg daily) because of the onset of an iatrogenic hypothyroidism. Adequate birth control measures were suggested for the possible teratogenic effects of ANA; nevertheless, she was found to be pregnant (8th week of pregnancy) in one of the follow-up visits. The persistent high platelet count (from 566 x109/L to a maximum count of 1400 x109/L) compelled us to continue ANA therapy all along pregnancy and to begin at the third trimester a prophylactic low molecular weight heparin therapy. The development of fetus was normal and a caesarean section was performed at the 35th week of pregnancy, delivering a 2.400 grams newborn male with a normal blood count and no evidence of congenital malformations. After delivery B.A. needed to continue therapy with ANA associated with HU and ASA and she renounced the breastfeeding. The use of ANA in pregnancy is a controversial issue. Preclinical data have shown that the drug is fetotoxic in animal studies, but there are not evidences that the recommended human doses are teratogenic. Few clinical reports can be found in literature and these data are insufficient to confirm the safety of ANA in pregnancy. We suggest that women of child-bearing age in treatment with ANA should adopt adequate birth control measures. IFN-alpha remains the first line therapy in ET pregnancy women. Nevertheless our experience proposes ANA as an alternative treatment in pregnant ET patients with intolerance to IFN and a high thrombohemorrhagic risk.

PU015

PROPOSAL FOR THE MANAGEMENT OF THE OUTPATIENT WITH MGUS

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The term monoclonal gammopathy of undetermined significance (MGUS)indicates the presence of a monoclonal protein(MC)without features of multiple mieloma, Waldenström's macroglobulinemia, primary amyloidosis or other lymphoproliferative disorders (LPD). MGUS is characterized by a serum MC <30g/L, plasma cells in the bone marrow <10% and absence of end-organ damage: anemia (hemoglobin < 10g/dl.), renal failure (creatinine > 2 mg/dl), hypercalcemia (serum calcium> 12 mg/dl), bone lesions (lytic lesions or osteoporosis with compression fractures). Even if MGUS don't require any therapy, the risk of progression to a malignant lymphoproliferative disorder is 1% per year, does not diminish over time 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 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. Serum protein electrophoresis is the most common method to detect and quantify a MC. The immunofixation allows to confirm and to type the MC. Once a MC is detected the primary care physician needs: 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 Bence Jones proteinuria did not predict progression). In this phase beta-2 microglobulin, serum quantitative immunoglobulins, urine protein electrophoresis, Bence Jones proteinuria are not necessary. Once a MGUS is diagnosed the primary care physician will attend patients with MC < 15 g/L if IgG and patients with MC < 10 g/L if IgA or IgM, without end-organ damage or signs and symptoms of LPD (lymphocitosis, thrombocytopenia, lymphadenopaty, hepatosplenomegaly, constitutional symptoms, hyperviscosity, unexplained heart failure, polyneuropathy). Six-month follow-up testing is suggested, it includes full blood count, serum creatinine, serum calcium, serum protein elecrophoresis and 24 h urine total protein. An hematological evaluation instead is recommended for patients with MC IgG>15g/L, or MC IgA>10g/L, or IgM>10g/L, or any MC with end-organ damage (not attributable to any others causes) or with signs and symptoms of LPD, or MC with prompt increase (> 5 g/L per year).

PU016

LONG TERM EXTRACORPOREAL PHOTOCHEMOTHERAPY AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION TO PREVENT GRAFT VERSUS HOST DISEASE: PRELIMINARY DATA OF A PHASE II PROSPECTIVE STUDY

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Introduction: Extracorporeal photopheresis (ECP) is well recognized to be effective in the treatment of steroid refractory/dependent graft versus host disease (GVHD). So far, only few data exist about the role of ECP in preventing acute and/or chronic GVHD. Aim of our study is to prospectively evaluate whether a long term ECP schedule given prophylactically after reduced intensity conditioning (RIC) allogeneic stem cell transplantation (ASCT) can reduce (i) GVHD incidence/severity and (ii) the need of steroid treatment during the first year after transplant. Methods: According to the study protocol, 28 consecutive patients submitted to RIC allogeneic SCT will be enrolled, soon after the falling of cyclosporine blood level to levels < 100 mg/dl after dosage tapering. ECP schedule is as follows: 8 weekly procedures, followed by 4 procedures every other week, followed by 8 monthly procedures, for a total of 12 months. Results: As yet, 6 patients have been enrolled in the study (lymphoblastic lymphoma: n=1; acute myeloid leukemia: n=2; chronic myelogenous leukemia. N=1; myelofibrosis: n=2) after RIC ASCT (from a sibling donor: n=3; from matched unrelated donor: n=3). RIC SCT had been performed at a median of 165 days before enrollment (range: 104-222 days). A median of 10,5 procedures (range: 6-20) per patient have been safely administered. After a median of 421 days (range: 234-730 days) from transplant, 4 out of 6 patients are alive. Two patients have completed the treatment course: one of them is in continuous complete remission, while the other one died due to disease relapse, 730 days after transplant. One patient is on treatment and is disease and GVHD free, while the remaining three patients have been dropped out, due to relapse (1), infectious death (1) and hepatic GVHD (1). In summary, only one out of six patients developed clinically relevant GVHD until now. Conclusions: These preliminary data encourage the use of long-term ECP in the prevention of GVHD after RIC ASCT.

PU017

PRIMARY CUTANEOUS B-CELL LYMPHOMAS: A SINGLE CENTRE EXPERIENCE

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Cutaneous B-cell lymphomas (CBCLs) are rare and are usually secondary to systemic nodal lymphoma. These lymphomas account for about 20% of all cutaneous lymphomas. Only recently has the existence of B-cell lymphoma presenting clinically in the skin without evidence of extracutaneous involvement been accepted as Primary CBCL (PCBCL). The pathology and classification of this heterogeneous group of lymphoproliferative disorders is reviewed. The main entities comprise marginal zone lymphoma, lymphoplasmacytoid lymphoma (immunocytoma), follicular center cell lymphoma, mantle cell lymphoma, large cell lymphoma (immunoblastic and anaplastic), intravascular lymphomatosis, plasmacytoma, and lymphoblastic lymphoma. In our institution we observed over the last 4 years, 8 cases of PCBCLs: 4 Diffuse B Large Cell Lymphoma (DBLCL), 3 Marginal Zone Lymphoma (MZL) and 1 Follicular center cell Lymphoma (FL). The patients (M: 5, F: 3; median age: 48 years, range: 35-78) were treated as follows: those with DBLCL were treated with R-COMP (Rituximab, Cyclophosphamide, Liposomal doxorubicin, Vincristine, and Prednisone for six cycles at intervals of 21 days), those with MZL and FL underwent monotherapy with Rituximab (4 weekly doses in the first month and thereafter, a monthly

administration for another 6 months). All patients achieved complete remission after the end of treatment. One patient (MZL) relapsed one year after the end of therapy. This patient started second-line therapy with R-COMP ongoing. At present all patients are alive and 7 out of 8 are still in complete remission. From our experience seems to be an increase in the incidence of PCBCL. In addition, the histological type most frequently appears to be the DBLCL. However, the data in the literature are still few, and especially there are currently no standardized treatment protocols.

PU018

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 2010, in our Haematology Unit ASL NA-1 we treated 34 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: 21 male and 13 female; median age 69 years (range: 66-84); istology: 23 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. All patients received 6 cycles of therapy at regular intervals of 21 days. There have been no delays in administering of therapy, including because of the use, starting from the second cycle, of pegfilgrastim. One month after the last cycle all patients were subjected to a disease re-staging: 87% of patients were in CR, 10% 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 (+36 months) was 81%. 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.

PU019

BILATERAL BREAST INVOLVEMENT IN MULTIPLE MYELOMA DURING BORTEZOMIB TREATMENT: A CASE REPORT

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Breast localization in multiple myeloma is a very rare event. The published data on this manifestation include predominantly case reports and do not provide any statistical information. A young Chinese woman, admitted at our department in 2009 with diagnosis of Multiple Myeloma IgA-kappa with bone marrow plasma cell infiltration greater than 60%, started therapy with PAD protocol (Bortezomib, Adriamycin and Dexamethasone). Complete remission was achieved after four cycles of chemotherapy. After 18 months, during maintenance therapy with Bortezomib, presented initially a mass in right breast and subsequently multiple bilateral nodules. Median size was 30mm, ranging from 10 to 90mm at radiological examination. Fine Needle Aspiration (FNA) revealed numerous plasma cells, plasmablasts and giant plasma cells. Biopsy confirmed diagnosis of multiple myeloma localization. Patient started therapy with VAD protocol, but, during this treatment, breast localization increased with a skin infiltration. Surgery was performed with bilateral mastectomy. Multiple Myeloma is a slow growing plasmacells neoplasm. The introduction of new drugs has increased the number of treatment options. Bortezomib has shown a significant antitumor activity. In this report we describe a patient who developed a fulminant extramedullary localization during treatment. More data are needed to determine whether continuous treatment with bortezomib may cause these extramedullary localizations, transforming Multiple Myeloma into a solid tumor sarcoma-like.

PU020

VENOUS THROMBOEMBOLISMS PROPHYLAXIS IN MYELOMA ELDERLY PATIENTS TREATED WITH LENALIDOMIDE

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Venous thromboembolisms (VTE) may occur in patients with cancer and cause substantial morbidity and mortality. Multiple Myeloma (MM) confers a high risk of thrombosis, with a VTE rate of nearly 10%. Multiple factors are involved, such as iperviscosity, high levels of immunoglobulin, procoagulant activity of monoclonal protein, and inflammatory cytokines. Introduction of IMiDs (such as thalidomide and lenalidomide) for the treatment of Multiple Myeloma has emerged VTE as one of leading complications. IMiDs based treatments are associated with a VTE rates of 14-26%, particularly when dexamethasone is added. In our department we have treated with Lenalidomide 40 Myeloma elderly patients, for refractory/relapsed disease or maintenance/continuous therapy. Moreover, 7 out of 40 patients showed high thrombotic risk (previous thrombotic events, ischemic heart disease and mutation of MTHFR gene). Because VTE appears to be a common complication of MM, mostly during IMiDs therapy, we used, in all patients, prophylaxis with anticoagulant agents, particularly Enoxaparin (4000 iu daily s.c. continuously). In all patients we found no thrombotic event. The optimal prophylaxis for patients receiving IMiDs agents is still a matter of debate. Moreover, anticoagulant or antithrombotic therapies are problematic because of the high incidence of contemporary thrombocytopenia or bleedings. In our hands Enoxaparin seems to be effective and safe, not inducing thrombocytopenia and not influencing the trend of minor bleedings.

PU021

CLINICAL ROLE OF IL-6 AND IL-1B IN IGG AND IGA MULTIPLE MYELOMA

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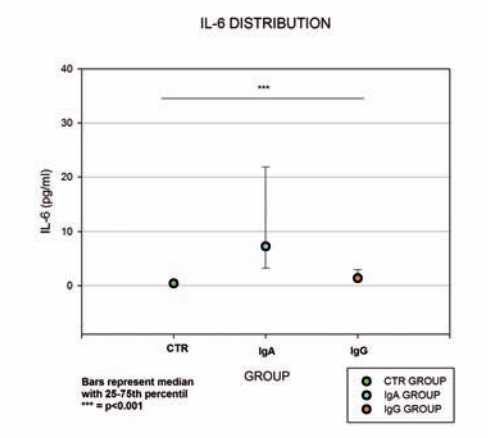
Background. Multiple Myeloma (MM) is an hematological malignancy characterized by abnormal plasma cells proliferation with typical bone-marrow involvement. Osteolytic lesions and compression fractures, chiefly seen in the axial skeleton and in proximal long bones, are caused by an increased osteoclastic activity mediated by cytokines such as interleukin-1beta (IL-1B) and interleukin-6 (IL-6), that are also implicated in survival and expansion of myeloma cells. Among different types, IgA MM is more severe prognostically than IgG MM, with an increased risk of evolution in plasma cell leukaemia. A rare hyperviscosity syndrome due to the dimeric and polymeric IgA molecular structure could lead to oral bleeding, epistaxis, blurred vision, headache, sausaging of retinal veins, paresthesias or congestive heart failure. Aims. The aim of present study was focused to evaluate the role of IL-1B and IL-6 respectively in IgG and IgA MM at presentation. Methods. IL-1B and IL-6 serum levels were detected in 5 patients with IgA MM and 6 patients with IgG MM. Ten age-matched individuals were selected as healthy controls. One Way Analysis of Variance on Ranks test and non parametric correlation were used to compare data. Results. IL-6 levels were significantly higher in IgA MM than those in IgG MM ($p < 0.001$). No differences in IL-1B serum levels were observed between the two groups, as well as no correlations were found. Conclusions. Results seems to support the role of IL-6 as unfavorable marker in the prognosis of IgA MM. IL-6 is essential for the survival and growth of malignant plasma cells and for the bone lesions in MM. Moreover in our patients the Bence-Jones positivity in IgA MM (80%: 60% k, 20% lambda) was more frequent than IgG MM (66,6%: 16,6% k, 50% lambda). The low serum lev-

els of IL-6 in IgG MM could explain the lack of such a severe symptoms respect to IgA MM patients. IL-6 could be considered a marker in the prognosis evaluation of IgA MM.



PU022

ELEVATED LEVELS OF CPK IN CML PATIENTS TREATED WITH IMATINIB

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In the past decade, the advent of imatinib, tyrosine kinase inhibitor (TKI), has completely modified the prognosis and quality of life of patients with chronic myeloid leukemia (CML). Several studies showed that this drug has an impact on some biochemical parameters such as reduction of cholesterol, phosphorus, etc. In our patients, we observed an increase of CPK. The aim of this study was to evaluate the incidence of increased level of CPK in 47 consecutive patients with CML followed in our Division and treated with imatinib. From 2003 to 2010 we followed 47 patients (25 M and 22 F) median age 45 years (range 17-61 years), 26 low, 15 intermediate and 6 high-risk. All patients started therapy with imatinib 400 mg/day. Every 3 months were monitored, in all patients, biochemical parameters and in particular the values of CPK. 44 are the evaluable patients, 25/44 patients (57%) showed increased CPK values, this increase was variable between 1.2 and 2.5 superior the normal values. In particular, 28% (7/25) of patients showed an increase ≥ 2 normal value. In all patients with abnormal CPK, cardiac function was evaluated with electrocardiogram and echocardiography and the results have been normal. In conclusion, our study showed that 57% of patients with CML treated with imatinib showed a significant increase of CK values, this increase can be correlated with a direct toxicity of the drug on myocytes as has been demonstrated with other TKI (eg sorafenib, etc..) or secondary to edema of the muscle fibers as described in the literature. Would be interesting to compare the plasma levels of imatinib with CPK

PU023

THE ALTERATION OF CPK AS A SURROGATE FOR ADHERENCE TO THERAPY IN CML PATIENTS TREATED WITH IMATINIB.

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The observation of increase of CPK in 57% of patients with CML treated with imatinib and followed in our Division, and a subsequent steady normalization of this parameter in a small percentage of patients, has raised the question whether this alteration could be valuated for poor adherence to treatment. The aim of this study is assess whether a normalization of CPK is correlates with poor adherence to therapy. From 2003 to 2010 we observed 25 patients, 21 M and 4 F with a median age of 41 years (range 17-70 years), in which there was evidence of a steady increase in CPK. 6/25 patients (24%), in follow-up, had to a costant normalization of CPK. Such standardization has been correlated with an increase in media by 0.5 log of bcr-abl transcript. These changes have led us to investigate the compliance of these patients and their adherence to treatment, 4/6 patients (67%) confirmed a lack of adherence to treatment or for poor compliance or for carelessness. These observations made us conclude that in patients with abnormal CPK and subsequent normalization of these values can be correlated with poor adherence to treatment. In conclusion, the dosage of CPK may be a surrogate for monitoring adherence to therapy in patients with CML treated with Imatinib. Would be interesting to compare the plasma levels of imatinib with CPK

PU024

THE IMATINIB TREATMENT HOW IMPACT ON BONE METABOLISM?

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Imatinib mesylate is a rationally designed tyrosine kinase inhibitor that has revolutionized the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. Although the efficacy and tolerability of imatinib are a vast improvement over conventional chemotherapies, the drug exhibits off-target effects. An unanticipated side effect of imatinib therapy is hypophosphatemia and hypocalcemia. However, emerging data suggest that imatinib also targets cells of the skeleton. The aim of this study was to evaluate in a cohort of patients with CML treated with imatinib, with Bone Mineral Densitometry (BMD), the effect of this TKI on bone metabolism. From January 2009 to January 2011 we evaluated 16 patients with CML treated with imatinib 400 mg/day, with a median follow-up of 60 months. 6 F (median age 57 years) and 10 M (median age of 41 years). All have made a BMD after a median follow-up of 36 months. In 11/15 patients, BMD was results to be normal, also in females over the age of 55 years. 4/15 BMD showed an increase in bone density and 2/4 has developed osteonecrosis of long bones of the lower limbs; in a female patient the BMD showed reduced bone density. In conclusion, therapy with imatinib appears to act on the physiological bone turnover and in particular to improve the post-menopausal osteoporotic problems and seems to show (in young male) a increased risk of osteonecrosis secondary to increased bone density. Further studies are needed to confirm these observations.

PU025

DIFFERENCES IN TERMS OF RESPONSE AND TOXICITY BETWEEN 2 INDUCTION CYCLES FOR AML PATIENTS; EXPERIENCE OF A SINGLE CENTER

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The standard induction treatment for young patients with acute myeloid leukemia (AML), in a recent review, seems to be the 3 +7 (anthracycline with aracytin c.i.). The aim of the study was to evaluate retrospectively if there are differences in terms of toxicity and response between 2 cycles of induction for AML patients(FLAI-5 and ICE) conducted in our Division. From January 2008 to January 2011 we treated 28 patients with AML. 12 male and 16 female median age of 38 years (range 14-60 years). 19 patients (8 M and 11 F with median age 37 years) were treated with the scheme ICE (idarubicin 10 mg/sqm day 1-3-5, etoposide 100 mg/sqm days 1-5 and ARA-C 100 mg/sqm c.i. days 1-7) while 9 patients (4 M and 5 F with median age of 48 years) were treated with the scheme FLAI-5 (Fludarabine 25mg/sqm days 1-5; ARA-C 2g/sqm days 1-5 and Ida 10mg/sqm day 1-3-5). With a median follow-up of 12 months we observed a major intestinal toxicity in the ICE group, 6/19 (31%) patients with toxicity grade III-IV (sec. CTC) which necessitated parenteral nutrition vs 0% in FLAI-5 group. There were no difference in TRM in induction between the 2 groups (10% in both groups). In terms of treatment response 12/19 (63%) patients achieved CR in the ICE group vs 80% (7/9 pts) in FLAI-5 group , this difference is significant even if this group is small. In conclusion in the induction treatment of AML patients, a scheme with aracytin c.i. seems to give intestinal toxicity more than a treatment with higher doses of ARA-C and not in c.i.; this affects the quality of life of patients and costs of treatment. Furthermore, although the number of patients is small, the FLAI-5 group seems to get a percentage significantly higher of CR than in ICE group. Needed a prospective randomized trial to confirm these data.

PU026

THE RITUXIMAB MAY HAVE A ROLE IN THE TREATMENT OF ALL PATIENTS?

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About 50% of acute lymphoblastic leukemia (ALL) expressed CD20 antigen. This expression is usually associated with a disease with poor prognoses. The aim of this study was to evaluate the action of rituximab in combination with chemotherapy or not in this group of patients. From 2006 to 2010 we treated 7 ALL patients with rituximab with CD20 expression >20% of abnormal cells (FAB: 5 L2 and 2 L3) 5 male and 2 female with a median age of 45 years (range 17-74 years). In 4 patients (FAB: L2: 2 and L3: 2; three at diagnosis and one in second relapse) was administered in combination with chemotherapy (Hyper C-VAD and CODOX-M) in 3 patients with a median age of 75 years (L2: 3; one patient in partial response; one patient in initial relapse and 1 patient in maintenance) was administered alone at a dose of 375 mg/sqm weekly for four weeks every 3 months. All patients achieved complete remission especially, in two elderly patients, rituximab has been used as single agent (1 in partial remission and 1 in initial relapse) and has been achieved a complete remission, 3 patients had transplanted 2 AUTO 1 ALLO; with a median follow-up of 22 months 6/7 (85%) are alive and in CR, one patient is dead but in CR. In conclusion, rituximab appears to be active in the treatment of ALL,the use would be recommended for high-risk ALL (elderly patients, FAB L3 etc.). Would be interesting to evaluate the action of rituximab in maintenance cycles, particularly in patients not FIT for aggressive treatments.

PU027

EFFECTIVENESS OF LENALIDOMIDE PLUS RITUXIMAB IN A PATIENT WITH RELAPSED FOLLICULAR NHL: CASE REPORT

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Follicular lymphomas are characterized by good sensitivity to chemotherapy but also by frequent relapse. Lenalidomide is a potent immunomodulator agent with anti-angiogenic effect, approved for the treatment of patients with relapsed or refractory Multiple Myeloma and 5q- Syndrome. Moreover, has been shown that lenalidomide is also effective as monotherapy in indolent and aggressive lymphomas, in Chronic Lymphocytic Leukemia and Cutaneous T cell lymphomas. Recent studies have shown the efficacy and safety of lenalidomide in combination with rituximab in patients with refractory or resistant indolent NHLs. We report the case of a 63-year-old male, affected by follicular NHL diagnosed in 2005, and treated with several lines of chemotherapy over the years (R-CHOP, R-IEV, 90Y-ibritumomab tiuxetan, auto-SCT, R-FM), achieving always transient and partial remissions. For disease progression, in 2009 we started treatment with lenalidomide and rituximab. Lenalidomide was administered at a dose of 25 mg/day orally for 21 days every 28 days. Rituximab was administered at 375 mg/sqm on day +15 of each cycle for a total of 6 cycles. A month after the sixth cycle of therapy, the patient underwent clinical restaging (PET/CT, bone marrow biopsy), which showed a complete remission. The following restaging after six months and one year after the treatment, confirmed the complete remission. The patient is still alive and in complete remission. Lenalidomide in combination with rituximab seems to be a very effective therapy in the treatment of indolent refractory and/or relapsed NHL, and without serious side effects.

PU028

COMPLETE REMISSION AFTER 9 YEARS FOLLOW-UP IN PATIENT WITH INDOLENT FOLLICULAR LYMPHOMA TREATED WITH RITUXIMAB ALONE: A CASE REPORT

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Follicular lymphoma (FL) is characterized by slow growth and initially good response to therapy, but later the patients relapsed or become refractory and are treated again. We report a case of one patient with indolent FL in progressive disease after III line chemotherapy, treated with Rituximab alone and in complete remission after 9 years follow-up. In March 1997 a 52year old male was referred to our Division of Hematology with an istologic diagnosis of indolent FL. Successive staging showed enlarged lymph nodes over and under diaphragmatic, splenomegaly, bone marrow infiltration (Stage IVA). He was treated with 8 cycles CEOP (epirubicin used for cardiologic disease), achieving complete response. Maintenace therapy with Interferon (3 MU x 3/w) was performed for 12 months. In February 2000 for relapse of disease, the patient was treated with II line chemotherapy (6 R-CEOP), achieving complete response until to August 2001. Between August 2001 and December 2001 was performed a new treatment for relapse, III line chemotherapy with ProMACE/Cyta BOM, achieving no response and progressive disease for massive mediastinic lymphoadenopathies. The critic cardiologic condition of patient (recent myocardial acute infarction), was no permissive to aggressive chemotherapy. In February 2002 we started a therapy schedule with Rituximab alone at a dose of 375 mg/m2 every month for 12 months. With this program we achieved after 6 months a complete response and was performed for others 6 months. At the moment the patient is in complete remission after 9 years follow-up. The use of Rituximab alone in the treatment of FL was not indicated in the time when we observed this patient and our terapeutic decision was determined by the presence of a progressive disease in cardiologic critic patient previously treated with III lines therapy. No treatment of progressive and massive mediastinic lymphoadenopathies was a negative prognostic factor and the life of patient would been compromised. Whereas a new traditional chemotherapy was very risky in a patient with acute cardiologic disease. Further this case report is indicative that complete remission in FL caused by Rituximab alone can be stable and very long, also in cases when other chemotherapeutic lines failed.

PU029

EXJADE IN PATIENTS WITH TRANSFUSION HEMOSIDEROSIS

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Background. EXJADE (deferasirox) is indicated for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in patients suffering with Beta Thalassemia Major. Exjade is used in the Myelodysplastic Syndromes (MDS) and other rare congenital or acquired anemias, that require repeated transfusions, in which the treatment with Desferal turns out inadequate or with serious side effects. Exjade is the only oral chelator capable to produce a continuous chelation of the iron in excess, in the 24 hours with a single daily dose. The clinical studies of phase III completed for patients with Beta Talassemia, Sickle Cell Anemia, and MDS, have demonstrated that Exjade remarkably reduces the iron concentration in liver (LIC), the most accurate indicator of the iron content in the body. Methods. Exjade has been used in three patients followed by the Hematology Ambulatory of the Vimercate Hospital, requiring trasfusional regimen for Myelodysplasia (patients 1; 2), and Myelofibrosis (patient 3). All three patients suffered from post-transfusional syderosis of moderate degree. Patient 2 suffered from steatohepatitis with low degree of fibrosis. The medium daily dose of Exjade has been of 20 mg/Kg, except in the case of the hepatopatic patient for whom it has been evaluated to not exceed 10 mg/Kg. Results. The effectiveness of the iron-chelating treatment has been evaluated after a year of therapy with Exjade. Serum ferritin levels have been practically halved in two patients, while in the hepatopatic patient the levels remained stable. The drug has been well tolerated except for persistent nausea in the hepatopatic patient. Conclusion. The iron-chelating treatment has demonstrated to be effective in two patients who have assumed the standard dose of EXJADE. The patient with MDS that has assumed a reduced dose of the drug, because of his concomitant hepatopathy, does not seem to have received substantial benefits, in terms of reduction of ferritin levels. This could be imputable to: 1) lower doses of Exjade compared to the ones assumed by the other two patients, 2) to concomitant hepatopathy, 3) to the low transfusional regimen 4) or as a consequence of inadequate compliance in the patient. It is therefore necessary to evaluate the minimum effective dose of the iron-chelating treatment in those patients who, because of concomitant pathology or poor compliance, do not tolerate the treatment with recommended standard doses of the drug.

PU030

A CASE OF INFECTION BY ASPERGILLUS FUMIGATUS DIAGNOSED BY TRANSBRONCHIAL LUNG BIOPSY IN A PATIENT WITH NON-HODGKIN LYMPHOMA/CLL

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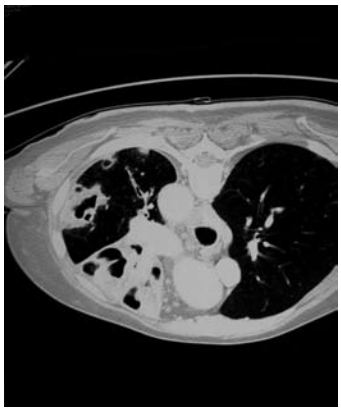
Invasive Aspergillus infection is still a major complication in immunocompromised patients. Case Report. We describe the case of an 81-year-old female patient with Stomach cancer; she was admitted with high fever, cough and leucocytosis. The patient had been in remission for gastric cancer. Methods. Lymphocyte immunophenotyping diagnosed non-Hodgkin lymphoma. Pulmonary aspergillosis was documented radiologically with chest CT. Examination of sputum culture was not performed because of lack of patient cooperation; transbronchial lung biopsy was performed. The histology of the specimens collected showed colonies of *Mucor hyphae*. Results. LNH/CLL was treated according to the chemotherapy protocol chlorambucil alone. Lobectomy was not performed because of the age and condition of the patient .The patient was treated with Itraconazol and Amphothericin B. Conclusions. We believe diagnostic tests should be performed aggressively, especially when pulmonary aspergillosis is suspected.



PU031

LABORATORY INDEX IN EVALUATION OF MYELOPEROXIDASE DEFICIENCY IN HEMATOLOGY PATIENTS

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Introduction: Congenital deficiency of MPO is the most common biochemical defect of neutrophils, it is as an autosomal-recessive defects, and her prevalence is estimated to be 1/2000-4000 individuals in Italy and the USA. The myeloperoxidase (MPO) is a heterodimeric glycoprotein, synthesized in the early stages of differentiation in bone marrow. The MPO is a powerful microbicidal enzyme,three different isozymes are known and at least nine different mutations in the MPO gene that could cause people with partial or total deficiency of the enzyme.The deficit can be completely asymptomatic or be associated with an abnormal susceptibility to infections caused by bacteria or mycetes. In several malignant hematological disorders this deficiency may occur in adults in the acquired form, and this seems related to the degree of impaired myelopoiesis, also many drugs used in chemotherapy treatment can be cause of MPO deficiency. Aims: To acquire acceptable range of laboratory MPXI index and to evaluate myeloperossidase flags in MPO deficiency patients at last to verify presence of MPO enzyme with specific cytochemical staining. Materials and methods. From 2010 to 2011 in Hematology Department we studied MPO enzyme activity in 9 patients (5 men and 4 women) through the ADVIA 2120 Hematology System, all cases were with congenital or acquired MPO-deficiency, were studied subsets of white blood cells (WBC) and MPXI Index. For comparison of WBC subclasses, we carried out a study on another hematology Analyzer COULTER without myeloperossidase citochemistry.MPO enzyme deficit verification were carried out by a specific cytochemical staining, (like that of Hattori-modified method). Results. On 9 cases, 33.3%(3) were with total absence of MPO enzyme, with cytochemical staining, the rest 66.3% (6) show variable quantity of enzyme (15%-60%) on neutrophils cells. MPXI index range was -15 (-25; -5); only in 44.4% (4) of cases we have saw MPO flags from ADVIA 2120 Hematology System, while in all other cases the percent of poli morphonucleated cells (PMN) and percent of mononucleated cells (MN) corresponds with hematology Analyzer COULTER evaluation.ADVIA 2120 under estimate (absolute and percentual) quantity of neutrophils and lymphocytes and can overestimate same quantity of blasts. Therapeutic approach in patients with malignancies diseases can be changed or can even suggest in healthy subjects presence of hematologic diseases. Conclusions. ADVIA 2120 Hematology System is based of the MPO enzyme citochemistry, total WBC were evaluated in the same for both haematological sistems, but the ADVIA 2120 technology was extremely inaccurate, if compared with the Coulter analyzer in patients with MPO deficiency. Our study underline that the evaluations with Hematology Systems based of the MPO enzyme is a method with limitations when the laboratory has to analyze patients with this particular deficiency. Acquired forms are often undergoing chemotherapy like the habitual users of a hematology laboratory. But, both MPXI index and MPO(myeloperoxidase flags) in ADVIA 2120 Hematology System are important and irreplaceable parameter for myeloperossidase deficency evaluation, while the basophils channel shows a split among the mononuclear cells and polymorphonuclear cells. Also absolute/percent quantity of cells subset of peripheral blood smear evaluation with an other Hematology System evaluation plus wit.

PU032

TREATMENT OF REFRACTORY/HIGHER-RISK MYELODISPLASTIC SYNDROMES AND ACUTE LEUKEMIA WITH 5-AZACITIDINE: A MONOISTITUTIONAL EXPERIENCE

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Myelodysplastic syndromes (MDSs) are a clonal bone marrow disorders characterized by ineffective hemopoiesis, leading to cytopenias, with a high risk of progression to acute myeloid leukaemia (AML). The recent expansion in therapeutic choices and biologic understanding has led to an increasing attention to MDS and to highlight the need for more consistent clinical trial. We reported our experience in patients with refractory-higher-risk MDS/AML treated with azacitidine (AZA). Between July 2009 and March 2011, we observed 7 patients with MDS/AML (6 male, 1 female). Median age was 71 years (range 70-75 yrs). WHO diagnosis included 2 cases of RA resistent to EPO treatment, 2 cases of RAEB-1, 1 case of RAEB-2, 1 patient had secondary AML occurring after myeloproliferative neoplasm (MPN), 1 patient with RAEB-t/AML. Cytogenetic risks were good in 3 pts, intermediate in 2 and poor in 2 patients. IPSS was intermediate-2 in 3 pts, high in 4 pts. Median duration of MDS before the onset of AZA was 3 months. Azacitidine was administered subcutaneously at the approved FDA/EMEA schedule (75 mg/m^2 during 7 days every 28 days) but with a 5-0-2 day administration (week-ends skipped). The median number of cycles of AZA received was 7 (1-20). Response was evaluated after 4-6 cycles by blood count and marrow aspirate according to the IWG criteria. The best response was complete response (CR) in one patient (alive in CR after 20 months), a stable disease with haematological improvement in five patients. One patient, with neutrofil count before starting treatment less than 500/mm3, had a failure after one cycle because of severe sepsis. Many factors must take in account before treating myelodysplasia: host factors in these mainly older patients, disease heterogeneity, feasibility of performing clinical trial. We think the importance of including haematological improvement as meaningful endpoint in future clinical trials of hypomethylating agents in higher risk MDS. This improvement, which means a decrease in transfusion requirements and reduction of cytopenia, suggests also an improvement in quality of life and this is a requisite to declare unequivocally a beneficial treatment effect in this particularly cohort of patients with advanced age. Noteworthy the patient who achieved a CR was the one having processed to MDS/AML from

a MPN, suggesting that AZA can induce a substantial response with a low toxicity in a cohort of patients for whom prognosis remains very poor.

PU033

5-AZACITIDINE TREATMENT RESTORES THE RESPONSE TO EPOETIN IN HIGH RISK MYELODYSPLASTIC PATIENTS

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5-Azacitidine(5-Aza) is an extremely valid therapeutic option in int-2/high risk MDS patients who can not undergo a bone marrow transplant procedure and is able to induce hematological responses and reduce the progression to acute myeloid leukemia in most of patients. Epoetin(Epo)therapy, differently by low risk MDS patients who obtain a high rate of erythroid responses, has no role in the setting of higher risk patients.5-Aza response is not rapid but it generally takes at list three cycles to become evident with a progressive hematological and QoL improvement.For these reasons we have thought that the administration of Epo in int-2/high risk MDS patients who are on 5-Aza treatment and who begin to show some sort of erythroid response(that is expression of the MDS clone proliferative advantage reduction and of the normal hematopoiesis expansion)could help to accelerate the hemoglobin rise, with a more rapid improvement in patients QoL. Three int-2,three high-risk MDS patients and one AML patient have been treated with 5-Aza at our institution; some of them had a history of Epo therapy with no response. All patients were transfusion-dependent when they started 5-Aza.We decided to begin Epo therapy as soon as the patients showed some sort of initial erythroid response(Hb ≥ 8 g/dl for three consecutive determinations and an initial reduction in transfusional support need). All but one patient received -Epo at a dose of 40.000 IU/wk. All patients have shown a response to Epo (only one patient had to increase the dose to a twice weekly schedule)with a rapid improvement in QoL; in two of them the response has been extremely rapid and so impressive that we had to stop the drug. What is surprising is that these two patients had a very poor prognosis: - A 72 years old man with a high-risk MDS(complex cariotype, severe cytopenia,high transfusional need:6 red blood cell unit/month) -A 60 years old female with a high-risk MDS secondary to high dose chemotherapy and bone marrow transplantation for NHL(-7 cariotype, severe cytopenia and transfusional need:3 red blood cell unit/month) Cause of the low number of patients and the fact that it has been just a clinical study, it is not possible to draw any reasonable conclusion. But as a regard to the results that we obtained it is our department policy to begin Epo therapy in the setting of int-2/high-risk MDS patients who are on 5-Aza and begin to show some sort of erythroid response

PU034

DEFERASIROX EFFICACY IS INDIPENDENT BY IMPROVEMENT OF IRON OVERLOAD BIOCHEMICAL MARKERS IN TRANSFUSION DEPENDENT MYELODYSPLASTIC PATIENTS

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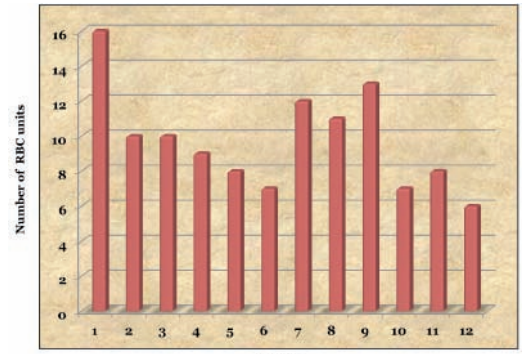
Iron chelating therapy(ICT) has become an important therapeutical opportunity in chronic transfusion dependent anemia as the one most myelodysplastic (MDS) patients suffer from, since the availability of oral chelating drugs as Deferasirox. ICT allows these patients to be prevented by iron overload organ damage (heart, liver) which has been demonstrated to negatively affect their life expectancy. There's evidence that some transfusion dependent chronic anemic patients who are on ICT show an improvement in hematological parameters and that a few of them experience a reduction in transfusional need (probably related to the reduction of: oxidative stress in erythroid bone marrow precursors, ROS generation, lipid peroxidation and free iron levels. It is also thought that iron chelating agents can let iron become available for erythropoiesis). That's what we have seen in eleven transfusion dependent MDS patients(5 RA, 2 RARS-T, 1 CMMoL, 1 RARS, 1 RCMD, 1 RAEB-1)who started oral ICT (Deferasirox). We have seen an improvement in hemoglobin level and a reduction in transfusional need. This effect has appeared to be evident in most of the patients within a few months; none of them went on to other therapies that could be able to modify erythropoiesis. What seems interesting is that these positive effects appeared to be independent by significative modification of iron overload biochemical markers as the most widely used ferritin concentration. Another result we have noted has been a progressive reduction in liver enzymes concentration in patients who showed biochemical evidence of liver damage before beginning ICT. Also this effect appeared to be independent by a significative modification of ferritin levels. All these results have been noted also in those patients who had to be treated with ICT doses lower than conventional ones because of sever comorbidities they were affected by (as renal function impairment). The possible explanation to our results is that Deferasirox is able to induce(especially at lower doses as demonstrated in "in vitro" models)intracellular oxidative stress processes reduction (independent by the effects on ferritin concentration, marker of iron overload). According to this we think that ICT may have a role in the setting of transfusion dependent anemic MDS patients even if used at low doses especially if we keep in mind that most of these patients are old and have comorbidities that can prevent them by using conventional doses.



Total number of Red Blood cells units transfused per month

PU035

POSSIBLE IMPLICATIONS OF AN "ACUTE" IRON OVERLOAD IN BONE MARROW TRANSPLANTATION(BMT): EXPERIENCE OF A SINGLE CENTER

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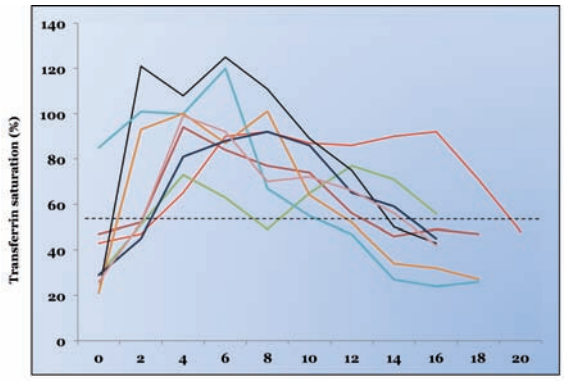
BMT complications are thought to be related to high-dose chemotherapy(HDC) and radiotherapy toxicity causing severe and prolonged host immune system disorders. It has been shown that there are iron homeostasis alterations in the peri- and post-BMT period causing an iron overload able to induce free non transferrin bound iron(NTBI)increase and ROS generation associated with complications as mucositis, infections, idiopathic interstitial pneumonia and hepatic disease. This "acute"iron overload is the consequence of cellular iron release, increased intestinal iron absorption, reduced transferrin synthesis, erythropoiesis suppression, transfusional support which are all related to HDC. On these basis we designed a study to evaluate iron homeostasis alterations in the setting of autologous and allogenic BMT. Eight adult patients have been enrolled in this study (four Multiple Myeloma, three non-Hodgkin's Lymphoma and one Hodgkin's Lymphoma); all underwent HDC and peripheral blood stem cells infusion. These patients have all been studied for serum iron, serum transferrin, transferrin saturation , serum ferritin, C reactive protein. Serum samples have been collected before HDC, the day of the beginning of HDC and every other day up to patients discharge. In all patients we observed a steep rise in transferrin saturation just a few days after HDC with the highest levels during days 3 to 6; these levels persisted high for at least two weeks, then showing a progressive decline. All patients have also shown a progressive serum ferritin increase(not always associated with an increase in serum inflammation markers). We also noted that complications as diarrhea, oral mucositis and fever occurred in the period of elevated transferrin saturation(which has been shown to be always correlated with high levels of free NTBI for a cut-off above 80%)and not always associated with

HDC induced severe neutropenia. Although the number of patients enrolled in our study is low(we are carrying on to include other patients)and we can not draw any conclusions our datas agree with those reported in other studies: there's a condition of "acute" iron overload in patients who undergo a BMT procedure. According to other authors we think that studies concerning the effect of binding NTBI with chelators as Deferasirox (likely with doses lower than those used in conditions of chronic iron overload)over the incidence and outcome of BMT-related complications would be highly indicated.



Transferrin saturation increase during bone marrow transplantation

PU036

DESCRIPTION OF TWO CASES OF HYPEREOSINOPHILIC SYNDROME: A MONOISTITUTIONAL EXPERIENCE

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Hypereosinophilic syndrome (HES) is a rare condition with different features at the presentation. We report two cases of HES, myeloproliferative-type, with a different history and symptoms. CASE 1: A 37 yrs old male came to our hospital because a chest pain and paresthesias of superior arms. At first presentation and during the course of 32 days in hospital, patient had been complaining very hard pain in the back and left arm requiring opioid treatment that was tapered only after discharge. TC scan and MR shoved two masses, one close to cervical column and another in the left paravertebral region at D12-L1 level, with hypo-density central zone. The peripheral blood cell examination showed an increased eosinophil cell count (14.090/mm3) and his bone marrow biopsy showed an increased cellularity with eosinophilic iperplasia. A search for FIP1L1/PDGFR was positive. CASE 2: A 42 yrs old southamerican male, in Italy since 8 yrs before, came to our hospital because a 4 months complaining of asthenia, dyspnea and progressive weight loss. At first presentation a peripheral blood cell examination showed an increase eosinophil cell count (18443/mm3) and no spleen enlargement. Search for FIP1L1/PDGFR resulted negative. A steroid treatment was started unsuccessfully followed by hidroxiurea. No response was obtained and progressive weight less and dyspnea continued till right retinal vein thrombosis appeared. A new molecular study was performed using FISH method which demonstrated FLIP1L1/PDGFR mutation in 20% of the peripheral eosinophilic cells. Both cases were treated with imatimib 100 mg/day which resulted in a complete resolution of peripheral hypereosinophilia after a week. Both cases showed that HES is a rare condition with different features at presentation, with extra medullar mass in the first and with hematological hypereosinophilia without expansive lesions in the second. Treatment with imatimib can resolve rapidly symptoms and hematological picture.

PU037

ROLE OF 18F-FDG PET–CT IMAGING FOR THE DETECTION OF PRIMARY OR SECONDARY INVOLVEMENT OF EXTRANODAL SITES IN HODGKIN AND NON HODGKIN LYMPHOMA

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Lymphoma may originate in extranodal sites. The most common extranodal sites of involvement being the stomach, spleen, Waldeyer ring, lung, bone, and cutaneous extranodal lymphoma may also be secondary to nodal disease. Fluorine-18 fluorodeoxyglucose (18F-FDG) imaging has an essential role in the staging of lymphoma, in monitoring the response to therapy, and may be specifically beneficial for the detection of primary or secondary extranodal sites of disease or exclusion of disease in the presence of PET/TC studies nonspecific extranodal CT findings. Methods: The study population consisted of 34 consecutive patients with biopsy-proven of extranodal lymphoma disease (9 gastric, 7 lung, 6 cutaneous, 6 orbital, 2 tonsillar, 2 oral mucosa, 1 bone, 1 spleen, 1 small intestine lymphoma). Each patient underwent a PET scan, carried out according to a standard procedure (6 h of fasting, i.v. injection of 370 MBq of 18F-FDG and image acquisition with a dedicated PET-CT scanner for 4 min per bed position, FDG avidity, patterns (focal/diffuse), and intensity (visually vs. the liver and SUVmax were assessed). Same patient underwent a CT study, same others underwent a MR study (orbital disease). Results: PET-CT studies proven nodal involvement in 5 cases (2 gastric, 2 lung, 1 cutaneus, lymphoma) uncertain on CT studies; denied the presence of residual disease in 1 orbital case, found on RM study. Five cases of suspect nodal involvement on TC studies were found as negative. On 3 years of follow up evaluation post-therapy 2 case of nodal and extranodal localization (1 lung, 1 cutaneous) are died; the other patients are in remission. Conclusion: primary extranodal lymphoma can still be categorized as stage I or II, whenever there is secondary involvement of extranodal distant from primary nodal disease, the disease is considered to be stage III or IV, since the correct staging of disease may change treatment strategy and overall prognosis. FDG- Whole body PET-CT is a potent primary staging tool. It also has application as an instrument for evaluation of follow-up and response to therapy, showing greater sensitivity and specificity of CT and MR to define the extension of disease.

PU038

NON-HODGKIN'S LYMPHOMA OF THE ORAL TONGUE: COMPLETE REMISSION AFTER R-CHOP CHEMOTHERAPY.

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Introduction: Primary non-Hodgkin's lymphomas (LNH) of the oral region are extremely rare and only few cases have been reported in the literature. Case Report: We describe a case of 57 years old woman presented with a history of a slowly growing painless swelling on right side of the tongue for a lot of months without other symptoms. Local examination revealed hard, nodular lesion involving the lateral margin of left half of the oral tongue. The mobility of the oral tongue was unaffected. On examination, she was pale, with upper cervical non-tender lymphadenopathy. Examination of oral cavity revealed proliferative growth on the left and posterior part of the tongue. Margins were indistinct. The lesion's biopsy demonstrated atypical large lymphoid cells infiltrating the skeletal muscles of the oral tongue. A diagnosis of diffuse LNH was given. Bone marrow aspiration, CSF examination, abdominal CT scan and ultrasonography were performed. No other sites of body were involved by the disease. Thus, a final diagnosis of the primary LNH, diffuse large cell type, B cell of the oral tongue was established and her disease was staged as I-E. The patient was treated with 8 cycles of CHOP and she is actually in complete response with resolution of pain and with good motility of oral tongue. Discussion: 20 to 30% of LNH arise from extranodal sites. Involvement of various parts of the oral cavity is very uncommon. To date, only 12 cases of the LNH of the oral

tongue has been mentioned in the literature . It generally affects the elderly over the 6th decade of life. There aren't characteristic clinical features of oral LNH. The most common presenting symptoms are local swelling, pain or discomfort and ulcer. The oral LNH may mimic more commonly benign oral and dental pathologic conditions . Thus, these lesions may be easily misdiagnosed. Most of the head and neck LNH including oral lesions are of B-cell origin and diffuse large cell type being the most common. Accurate diagnosis requires excisional biopsy of the extranodal mass. Conclusion: LNH of oral region is infrequent but it should always be considered in differential diagnosis of a variety of benign and malignant lesions in this region. A correct clinical evaluation, histopathologic as well as immunohistochemical evaluation of biopsy specimen may aid in the diagnosis and thus, help in proper management.

PU039

SIMULTANEOUS OCCURRENCE OF NON HODGKIN'S LYMPHOMA AND KAPOSI'S SARCOMA IN A PATIENT WITHOUT HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

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Introduction: The simultaneous non Hodgkin's lymphoma (LNH) and Kaposi's sarcoma (KS) within the same patient is a rare and interesting occurrence and has been described since 1920; the presence of the two pathologic entities is frequent complications also of renal transplantation (KS occurs in 0.2-5% of renal transplant recipients) that usually occur as separate entities: the immunosuppression consisted of cyclosporine and prednisone has a important role. Only sporadic cases of lymphoma of Hodgkin have been described in patients without the acquired immune deficiency syndrome (AIDS); this suggests an association of AIDS with both KS and malignant lymphomas and raises the question of a common pathogenetic mechanism. The altered immune status, human herpesvirus-8 infection and Epstein-Barr virus (EBV) might have played a role in the pathogenesis of this LNH in KS in AIDS and non-AIDS patients. Case report: A 70 years old man , has undergone skin examination to diffuse erythematous lesions of the lower limbs, trunk and arms, then performed a skin biopsy that showed a Kaposi's sarcoma in "early patch" phase. The patient performed the investigations necessary to detect the presence of a systemic involvement of disease . The total body PET with 3D technique with 18 FDG has been highlighted: 1) same lymph nodes with an SUV max 22.8 in axillary bilaterally, in the mediastinum, in the lumbar aorta, external iliac level, 2) an intense accumulation in skeletal with SUV 22.8 in costal, dorsal and lumbar spine, pelvis, vertebrae L1, 3) build the right psoas muscle, 4) hyperaccumulator in the spleen. The lymph node biopsy showed the presence of Non-Hodgkin's large cell-rich T-lymphocytes Lymphoma (CD20 +, CD 30 + / -, CD 15 negative, ALK neg; elements lymphocyte T CD3 +, CD5 +, CD20 + / -). The bone marrow biopsy showed a diffuse infiltration of lymphoma cells. The patient haven't the systemic symptoms. Lymph node biopsy hasn't evidenced KS: evidently we are faced with a double cancer and KS is confined to the skin without systemic involvement. Staging: LNH IV A. We have starting chemotherapy with R-COMP protocol that could be beneficial for both forms for the presence of liposomal doxorubicin (drug of choice for Kaposi's sarcoma). Conclusion: Synchronous development of KS and malignant lymphoma is a rare phenomenon and has a special interest; the coexistence of the KS and NHL is probably the result of immunosuppression.

PU040

ARE LOW DOSES OF LENALIDOMIDE EFFECTIVE IN 5Q- SYNDROME? A CASE REPORT

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Background: The "5q- syndrome" is characterized by isolated del 5q and no excess of marrow blasts. It usually presents with severe anemia, thrombocytosis, typical dysmegakaryopoiesis. The outcome is favorable, with prolonged survival and rare progression to AML. However, the anemia often requires multiple RBC transfusions with hemochromatosis risk. CASE REPORT: We report a 77-year-old woman with severe macrocytic anemia. Personal history and physical exam only revealed anemia-related symptoms and signs. Complete blood cell count showed Hb 6.1 g/dL, MCV 102.3 fL, WBC 2800/ L, PLT 519000/ L. All biochemistry values, including Vitamin B12 and folate, were normal. Bone marrow biopsy and aspirate revealed increased mononuclear micromegakariocytes with no blast excess (2.6%). Cytogenetic analysis detected only a clone with interstitial del 5q. Serum erythropoietin was 150 mU/mL. She received RBC transfusions. Epoetin alfa (EPO) 40000 UI twice a week was started. Due to no response, EPO administration was stopped 6 months later. During the first year, her transfusion requirement increased from 4 to 9 RBC concentrates bimonthly. Subsequently, lenalidomide became available for the 5q- syndrome and the patient started with 10 mg daily (days 1-21 every 28 days). After 5 months of therapy, she achieved the complete transfusion independence. Eight months after the beginning, the patient had to discontinue lenalidomide due to G4 neutropenia. After several cycles of restart-discontinuation and dose reduction, she definitively discontinued lenalidomide. Simultaneously, her transfusion requirement increased till 9 RBC concentrates bimonthly. Cytogenetics showed the following karyotype: 46xx, der (5)(q14q33)[21]/46,idem,i(8)(q10), der (18) t(8;18) (q?;q12)[8]/46,idem, i(8)(q10), -18[3]. Morphological, molecular and immunophenotypic findings allowed the diagnosis of RAEB-2 (blasts: 17%). A therapy with azacitidine was started, but no response was obtained. Patient's clinical status worsened and she died 2 months later. Discussion AND Conclusions: Lenalidomide improved our patient's quality of life, determining transfusion independence. Unfortunately, the dose reduction was followed by a rapid increase of transfusion requirement and disease progression.In 5q- syndrome patients, lenalidomide has a positive effect on transfusion requirement, but less is known about its impact on the natural course of the disease. It is currently being assessed in various clinical trials.

PU041

PRIMARY BONE MARROW LYMPHOMAS (PBML): CLINICAL PRESENTATION, HISTOPATHOLOGICAL FEATURES AND OUR EXPERIENCE.

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Introduction: Primary bone marrow lymphomas (PBML) are rare and only few cases are reported in the literature. In order to assess whether or not primary bone marrow lymphoma (PBML) could be considered as a real entity among extranodal lymphomas, a retrospective analysis is ongoing. The definition of PBML comprised the following specific clinico-pathological features: 1) isolated involvement of bone marrow (with or without peripheral blood) 2) no evidence of extra-marrow involvement on imaging studies 3) no evidence of splenic infiltration 4) absence of localized bone tumors 5) exclusion of lymphoma subtypes primarily affecting bone marrow, such as SLL/CLL, lymphoplasmacytic, splenic marginal zone, Burkitt and lymphoblastic lymphoma, ALL Case report: We describe 3 cases of PBML. The median age was—— years; two patients were men and one case was female. B-symptoms weren't present in all patients; no leukopenia , no anemia and thrombocytopenia were observed; high serum LDH was observed in 100% cases. The Platelet count < 100x109/L and high serum LDH predicted poor OS. Histologically, two cases were large B-cell lymphoma and 1 case was follicular lymphoma. Immunohistochemically, all cases were positive for B-cell markers. All patients are in observation without therapy from 1 year. Discussion: Secondary involvement of malignant lymphomas in bone marrow is relatively common. However, primary occurrence of lymphomas in bone marrow is quite rare, except in chronic lymphocytic leukemia or small cell lymphoma. Large cell lymphoma with primary bone marrow involvement is exceptional, although secondary involvement is sometimes observed. PBML initially manifesting in the BM to be a unique entity because the neoplastic cells proliferate mainly in BM, with infrequent involvement of the sinusoids and occasional leukemic infiltration in various organs. Conclusion: PBML is a very uncommon extra-nodal lymphoma often associated with cytopenias, aggressive disease, poor outcome and heterogeneous histological features, some of them revealing a putative germinal centre origin. we consider DLBCL initially manifesting in the BM to be a unique entity because the neoplastic cells proliferate mainly in BM, with infrequent involvement of the sinusoids and occasional leukemic infiltration in various organs.

PU042

FOLLICULAR LYMPHOMA "IN SITU " : CASE REPORT AND REVIEW OF LITERATURE

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Introduction: The designation " in situ " localization of follicular lymphoma (FL) is for lymph node biopsies with focal germinal centers containing centrocytes staining strongly for bcl-2 protein, a finding that supports their neoplastic nature, whereas most of the remaining lymph node showed bcl-2–negative follicular hyperplasia. No clear guidelines for diagnosis and management of these patients have been defined so far. Case report: We describe an apparently healthy 50 years old man, who underwent biopsy of left inguinal lymph node swelling. Histological examination showed non Hodgkin follicular lymphoma "in situ". CT total body not documented elsewhere and the bone biopsy result was negative. The staging, therefore, led to the conclusion that a limited I A stage of follicular lymphoma "in situ". After discussion with the patient about the particular feature of the lymphoma we decided to conduct regional inguinal radiotherapy 30 Gy with a curative intention. Actually the patient is in complete remission. Discussion: The "in situ" lesions are often incidental findings in an otherwise reactive-appearing lymph node. Is not yet fully known for these focal lesions the risk of progression to clinically appreciable lymphoma. The staging of lymphoma exclude other site of nodal or extranodal involvement and it is highly recommended for the possible coexistence of an overt lymphoma. Biopsy of all sites of suspicious involvement should be mandatory. There isn't support for starting therapy also in the presence of multifocal "in situ" lymphoma exists and a "wait and see policy" is strongly suggested. A follow-up strategy reserving imaging evaluation only in the presence of disease-related symptoms or organ involvement. The recognition of in situ FL is of utmost importance because it is associated with localized early stage disease (stage I), which according to standard regimens is amenable to local radiation therapy with a good chance for inducing remission. Initial work-up has included a computed tomography and a bone marrow biopsy. An additional positron emission tomography scan was not proposed because it is not recommended according to the updated consensus. The staging is given according to the Ann Arbor system with mentioning of bulky disease. For prognostic purposes, a Follicular Lymphoma-specific International Prognostic Index should be determined.

PU043

DRUG - INDUCED PULMONARY TOXICITY IN HEMATOLOGICAL PATIENTS. REVIEW OF LITERATURE AND EXPERIENCE OF A SINGLE CENTRE

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Malignancies hematological patients may develop numerous pulmonary manifestations, as a complication of either the disease or the diverse agents used to treat the disease. Many antineoplastic drugs may injure the respiratory system with varied clinical pattern of involvement; the drugs involve mainly the parenchyma, and less frequently the airways, pleura or the pulmonary circulation. Clinical, radiographic, and physiological features of drug-induced and radiation-induced pulmonary damage are often difficult to distinguish from other pulmonary infiltrates. Bronchoscopy with bronchoalveolar lavage is essential to exclude infectious etiologies. In some cases, surgical lung biopsies are required to establish a specific etiological diagnosis. The antineoplastic drugs that might cause lung injury are: Amphotericin B, Antithymocyte Globulin, All-Transretinoic Acid, Arsenium Trioxide, Bleomycin, Busulfan, G-CSF, Corticosteroids, Cyclophosphamide, Cytosine-Arabinoside, Deferoxamine, Etoposide, Fludarabine, Gemcitabine, Hydroxyurea, Interferons, Imatinib, Methotrexate, Nitrosourea, Oxaliplatin, Procarbazine, Rituximab, Thalidomide, Vinca Alkaloids. The most common symptom is the dyspnea, the treatments determine a resolution of lung's damage. The patterns of drug-induced respiratory involvement are various: infiltrative lung diseases, nonspecific interstitial pneumonia, pulmonary infiltrates and eosinophilia, organizing pneumonia, diffuse alveolar damage, pulmonary fibrosis, granulomatosis and sarcoidosis, pulmonary edema and capillary leak syndrome, diffuse alveolar hemorrhage, acute chest syndrome, bronchospasm, pleural involvement, pneumothorax. Last year in our Hematology Unit we had five cases of severe pulmonary toxicity in patients who receiving chemotherapy for hematological malignancies. The cytotoxic drugs responsible were bleomycin, fludarabine, gemcitabine, oxaliplatin and rituximab. In all five cases the clinic was characterized by dyspnea and hypoxemia. CT scan of the lung showed different pictures. The patients underwent steroid therapy which determined an improvement of symptoms and of radiological picture. Bronchoscopy was performed in all five cases. Conclusion: The respiratory system can be involved by drugs used in the treatment of malignant and nonmalignant hematological diseases in many ways. Clinicians should be aware of the potential of most novel antineoplastic agents to cause lung toxicity.

PU044

5-AZACYTIDINE INDUCED LONG-TERM TRANSFUSION INDEPENDENCY (51 MONTHS FOLLOW-UP) IN AN OLD PATIENT AFFECTED BY MYELODISPLASTIC SYNDROME (CASE REPORT)

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Introduction.. The treatment of elderly patients affected by myelodisplastic syndrome (MDS) with azacytidine has been described to reverse the need of transfusions and prolong overall survival. The schedule (dose and timing) according to indication can induce toxicity, implying dose reductions with the risk of loss of response. We report the case of a patient treated with a 'dose and time-reduced' schedule of azacytidine, in persistent transfusion independency (51 months). CASE REPORT. S.S., male 78 yy old, received diagnosis of MDS, RCMD type, karyotype 44X -Y -15,46XY, IPSS 1, in Sept 2006. After one month erythropoietin and transfusion of erythrocytes and platelets were started because of anemia and thrombocytopenia. The transfusion need was 5.6 erythrocytes unit and 15 platelets unit per month . In the same period the patient was admitted to hospital four times for cardiac complications due to severe anemia and FUO. On Jan 2007 azacytidine therapy at the standard dose of 75 mg/m2 for 7 days monthly was started. After the first cycle a 50% dose reduction was applied for haematological and non-haematological (hepatotoxicity gr. IV WHO) toxicity. The third cycle was carried out at the planned full dose, with haematolocigal toxicity gr. IV. From the fourth cycle the dose reduction to 60 mg/die was restored, without any toxicity, but with persisting, although reduced, need of erythrocytes and platelets transfusion (average 1,5 and 4.5 per month). After 8 cycles the patient became transfusion independent. At cycle +15 the schedule was furtherly reduced, in that the complete hematological response observed prompted us to carry out treatment for five days only, instead of the planned 7. Moreover, starting from cycle +35 the therapeutic interval was prolonged to 6 weeks, because of the marked elevation of hb rate (15 g/dL). At the present time 44 cycles have been administered in 51 months of therapy. The last bone marrow examination showed persistent RCMD with normal karyotype. Discussion. Our patient, given the severe toxicities observed with the scheduled dose of azacytidine, necessarily received dose-reduced treatment, obtaining, notwithstanding, transfusion independency. At the same time the applied dose has reduced further toxicity and increased cost-effective therapy. This experience prompts everyone to focus on 'tailored' therapies in elderly patients, in order to preserve the safety profile and reach, at the same time, the planned clinical benefit.

PU045

DISSEMINATED AND FATAL EXTRAMEDULLARY RELAPSE OF IGA KAPPA MULTIPLE MYELOMA AFTER BORTEZOMIB COMBINATION THERAPY

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Extramedullary (EM) spread of Multiple Myeloma (MM) may occur either at diagnosis or during the course of the disease and it's rare. The

contemporary extensive EM involvement is described only in cases report. In more recent years has been observed a higher incidence of EM involvement. The risk seems not be influence by prior exposure to high-dose therapy, but the role of expanding use of novel biological agents will must be investigated. We report a case of devastating, disseminated, multifocal and fatal EM relapse of IgA/kappa MM after bortezomib combination therapy. In literature, to our knowledge, there is only isolated cases of rapid and fatal EM relapse of MM despite recent exposure to bortezomib therapy.A 77 year old men was diagnosed IgA/kappa MM stage III A in October 2007.The patient was treated with bortezomib, melphalan and prednisone (VMP) and achieved a complete response (CR). In January 2010 the patient presented with dyspnea, asthenia and abdominal pain. CT scan demonstrated a soft mass in polmonary right region with pleural effusion, diffuse peritoneal and subcutaneous abdominal nodular masses, enlarged para-aortic and retrocaval lymph nodes. PET exhibited middle FDG uptake. Fine-needle aspiration-biopsy (FNAB) from pulmonary and abdominal soft mass revealed atypical plasma cell CD 45+/CD38+/CD138+, MIB1>50%. There was anemia, since evidence of plasma cell in periferal blood , elevation of LDH, Beta2microglobulin and monoclonal (M) protein. Chemotherapy with bortezomib, dexamethasone and pegylated liposomal doxorubicin (PAD) was started but between the first and second cycle the patient presented with obstructive jaundice. Abdominal ultrasonography revealed two large masses of the head of the pancreas. The patient began quickly PAD therapy with reduction of jaundice and completed six cycles.After CT scan demonstrated extension of enlarged lymph nodes at axillary and esophagus and progression of the nodular masses at pancreas, mesentery, pelvis, rectum and breast. PET-FDG revealed higher uptake.Was started salvage therapy, but the patient died in November.This was a rare case of fulminant and disseminated EM spread of IgA MM occuring after CR with bortezomib based therapy. The disease relapse was responded to bortezomib yet, but was progressive and fatal. We proposed that the role of novel agents for EM disease during follow-up must be investigated becouse it is associated with unusual aggressive course and very short survival.

PU046

ASSOTIATION OF CYCLOPHOSPHAMIDE 100 MG/DIE ORALLY TO LENALIDOMIDE AS SALVAGE THERAPY IN PATIENTS IN PROGRESSION DURING LENALIDOMIDE THERAPY

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Lenalidomide is efficacy as salvage therapy in patients with multiple myeloma.It is a continouos therapy until progression of the disease occurs, but progression is a difficult challenge. Here we present 3 cases with disease progression during salvage therapy with lenalidomide,in which the addiction of cyclophosphamide (Cy) orally 100 mg/day days 1 to 21 induced a new response. Case 1:Male,aged 45 years,diagnosis of micromolecular myeloma in February 2006.After 3 lines of therapy (Thal/dex, MPV and ID-EDX)and two failed attempts of collection of peripheral blood stem cells,in april 2010 patient began treatment with lenalidomide 25 mg/die.Response was present till November 2010 when progression of disease was documented (monoclonal urine componet 1820 mg/dl, bone marrow plasmacells 65%).Cy was added 100 mg/day from day 1 to 21.After three cycles reduction of more than 75% of the urine component and reduction of bone marrow plasmacells from 65% to 20%. Case 2:Female,aged 76,diagnosis in 2008.MPT for 8 cycles then thalidomide 50 mg/day till progression that occurred in may 2010.Patient was not eligible to other treatments because an invalidating vertebral fracture and living so far from hospital. Monoclonal component was 4,75 gr/dl.Lenalidomide 25 mg/day was began with partial response till November 2010 when progression occured with anemia,transfusion requirement,new vertebral fractures and increase of monoclonal component to 4.80 gr/dl. Cy 100 mg/day 1 to 21 was added to lenalidomide, and after 3 cycles no more transfusion requirement and decrease of the monoclonal component from 4.75 to 2.5 gr/dl was the response. Case 3:Male,aged 72,diagnosis in 2008,MPT as first line therapy and then lenalidomide 25 mg/dat for progression.The addiction of Cy 100 mg/day day 1 to 21 resulted in decrease of monoclonal component from 4.4 gr/dl to 1.5 gr/dl.Response is stable and patient has already completed 9 cycles. All three patients experienced progression of the disease during treatment with lenalidomide.There were objective difficulties in planning other treatments because all patients live so far from hospital.The responses observed has been very encouraging in continouing this approach.Patients are still in treatment without any adverse event recorded.We are now planning for the younger patient a new attempt of collection of peripheral blood stem cells. Cyclophosphamide and lenalidomide could be sinergic,but the schedule of the assotiation is stil to be found.

PU047

HOME CARE MANAGEMENT OF TRANSFUSIONS IN PATIENTS WITH MYELODISPLASTYC SYNDROMES

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Background. Anemia is the most common problem afflicting the majority of patients with myelodysplastic syndromes (MDS). Erythropoletin-stimulating agents (ESAs) are widely used for treating MDS_related anaemia with the objective of avoiding or, at least, of reducing the need for red blood cell (RBC) transfusions and improving the quality of life (QoL). However, for ESAs-failed patients or in those unsuitable for this option, RBC transfusions remain the only available measure. Although RBC transfusion is a relatively safe procedure from a clinical point of view, some logistic and practical problems may concern these patients, most often being older and frail individuals, and their families. Therefore, in this setting home care (HC) may represent a valuable option allowing to preserve the patient's QoL and to avoid useless hospital admissions. Purpose. Evaluating the safety, the feasibility and reliability of a two-year home care program focusing on RBC transfusion management. Material and methods. There were 62 MDS (23 male) with a median age of 86 (69 – 98) years. Patients were followed at home for a mean of 8, 7 (1 – 24) months. ESAs were given to 40 (65%) patients. Results. Overall, 49 (79%) patients required transfusions, for a total of 628 RBC units; the median number of RBC transfusions for patient was 9 (1-62). Older age, a lower baseline Hb concentration, the duration of MDS and the time from the primary diagnosis to its transformation into acute myeloid leukemia strongly correlated with the number of transfused RBC units. All transfusions were safely administered at home without any untoward effect. Conclusion. QoL is a particularly important issue for older MDS patients. With this regard, management of chronic patients requiring multiple and repeated hospital /day hospital admissions to receive RBC transfusions may be a concern for the affected individual and for their families. Our experience demonstrated that the administration of RBC transfusion at home is feasible, reliable and effective in our older MDS patients, avoiding social and economic costs due to an inappropriately frequent removal from their domestic environment; moreover, our findings may provide the basis of future and more comprehensively studies to perform in this setting also in order to explore other correlated issues, such as the influence of a HC transfusion program on patient's and relative's QoL.

PU048

ASSOTIATION BETWEEN RITUXIMAB AND BENDAMUSTINE IS HIGLY EFFICACY IN PATIENTS WITH NON HODGLIN FOLLICULAR LYMPHOMA HEAVELY PRE-TREATED: A SINGLE CENTER EXPERIENCE

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In the last 30 months 7 patients con relapsed follicular lymphoma have been treated with a regimen of Rituximab 375 mg/m2 day 0 and Bendamustine 90 mg/m2 day 1-2 for a total of 4 cycles. Their median age was 65 years (range 41-76) and previous lines of therapy were 3.7 (range 3-5), including R-Flucy (all of them), Zevalin (1), Autotranplant (1), R-CHOP (5). All patients were treated in some of the therapy lines with maintenance with rituximab. The main characteristics are as follows: Sex Age Previous Response Follow up months Treatments 1. m 71 5 PD 28

2. m 74 4 RC 13 + 3. m 70 3 RC 23 4. m 71 3 RC 9 5. m 41 4 RC 10* 6. f 55 3 RC 17 7. m 76 3 RC 20 6/7 are alive, and 5 of them in complete remission. One patient (+) died for complications due to surgery for basalioma that infiltrate la cranic bones. This patient was in RC from 13 months. The younger patient (*) after the complete response received allotransplant. Follow up is from 9 to 28 months. Treatment has been well tolerated, without any serious adverse event. Only grade 2 neutropenia, anaemia and thrombocytopenia was noted. In only one case and in one cycle neutropenia was grade 4. Other toxicities include fever in 2 patients. No other toxicities were recorded. Remarkly, the younger patient, affected by chronic hepatitis from HBV resistant to lamivudine did not show any hepatic toxicity. Even considering this small numbers rituximab-bendamustine resulted, in our experience, very efficacy in heavily pre-treated patients, and with an excellent toxicity profile.

PU049

LONG LASTING REMISSION IN MULTIPLE MYELOMA AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION COMPLICATED BY ITP. THE POTENTIAL IMMUNOMODULATION ACTIVITY OF AUTOIMMUNE DISEASE

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A 49-year male patient was diagnosed as having Ig A k stage IIIA multiple myeloma (MM) in october 2000 and treated with 3 cycles of VAD. After achieving a partial remission (PR) according to IMWG criteria he was considered suitable for peripheral blood stem cell (PBSC) procedure. Two PBSC procedures (in april and july 2001,respectively) were carried out using a conditioning regimen based on melphalan (200 mg/m2).Since the patient failed to reach complete remission (CR) , a third PBSC procedure was performed without improvident in the deepness of response. Furthermore, maintenance treatment with thalidomide was early stopped due to grade III peripheral neuropathy. While on follow-up, in june 2002, patient developed symptomatic bone progression with increase in either number or size of lumbar lytic lesions as demonstrated by MRI imaging. A second-line treatment consisting of 6 cycles of cyclophosphamide and high-dose dexamethasone made it possible to achieve a very good partial remission. In July 2003 patient was admitted to our hematology department because of cutaneous purpura without major bleedings. Platelet count was 9.000/µL and clinico-hematological evaluation including bone marrow biopsy confirmed that MM was still in VGPR. A diagnosis of immune thrombocytopenic purpura (ITP) was performed and patient was treated with standard doses of prednisone. Initially refractory to standard doses of prednisone, he achieved a complete response (Platelet count 119.000/µL) with intravenous methylprednisolone (1.0 g/d for 1-3 consecutive days) combined with IV Ig. At the time of ITP relapse in may 2004 serum and urine immunophixation was negative therefore suggesting an improvement of deep response that shifted from VGPR to CR. Treatment with Rituximab 375 mg/m2/weeky x 4 doses did not succeed. After preoperative pneumococcal and meningococcal vaccination patient underwent elective splenectomy in February 2005. Interestingly, such a procedure provided a sustained remission maintained at the time of present report. A clinico-pathologic restaging of MM carried out in January 2011 confirmed the CR status. We may speculate that the autoimmune reaction, most probably triggered by autologous bone marrow transplantation and presenting clinically as ITP, has resulted in the elimination of an incurable malignant condition in our MM patient that has lasted for over 7 years so far. Interestingly, the cascade of events related to ITP improved the deep of MM response supporting the immune-mediated control of malignancies as an attractive approach for future research in oncology.

PU050

THE SECONDARY PROPHYLAXIS IS PROPOSABLE IN ADULT/ELDER HEMOPHILIACS HIV- WITH NEOPLASMAS AFTER SURGERY WHEN CHEMOTHERAPY AND/OR RADIOTHERAPY PROGRAMMES CANNOT BE DEFERRED

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Background/Aims. The life expectancy of adult/elder hemophiliacs is clearly approaching that of the general population as results of purer plasma concentrates or recombinant products employed more frequently to achieve good quality of life. Nevertheless,from years the secondary prophylaxis in those patients is proposed in well defined clinical setting. In this scenario emerging evidences demonstrate that in aging hemophilia a large spectrum of tumoral co-morbidities often related to HCV+ and/or HIV+ co-infection as well as other neoplasmas can occur in time (http://www.ingentaconnect.om/content/bsc/haem/2010/00000016/00000003/art0004). In this regard, the magnitudo of the incidence of these latter remains controversial as well as the approach to management by secondary prophylaxis or 'on demand' regimens. Material and Methods.We here report the notable clinical history of a severe haemophiliac A,aged 45yrs, treated in the past with human plasma and after with commercial concentrates until 1993,HBV+ and HCV+, HIV1-. The patient undergone to bilateral elbow synoviectomy in 1981 and in 1983 to orthopaedic surgery at right femur owing post-trauma fracture. From 193 he was treated 'on demand' with recombinant FVIII BDD. Iny November 2004 he was successfully operated for severe right hip arthropaty with total arthroplasty with r-FVIII BDD infusions and in April 2005 he undergone to left total arthropasty so gaining his complete mobility. Since then, a regular prophylaxis with r-FVIII BDD (3000 I.U. twice a week) was enthusiastically started and no haemorrhages were documented. In March 2010 the patient referred agonizing scrotal pain and we observed in the right testis a large mass extended to inferior side which the patient had from months judged as hematoma. A prompt abdomen sonography followed by CT scan showed a tumor of right testicle and several lumbosdacral/pelvic lymph nodes (2.5-1.5 cm). Results.Laboratory findings were:reduced platelets 95.000/ul, AST 131 IU and ALT 259 IU both increased, -fetoprotein 5.4 IUnormal, -HCG 5.4/ml increased, FSH 24.6 mU/ml ,LH 33.6 mU/ml increased,prolactin 6.3 ng/ml normal, total testosterone 402 ng/ml and free testosterone 1.7 pg/ml. Therefore, funiculus-orchidectomy was successfully performed by r-FVIII BDD bolus infusion (5000 IUx2 daily), and 5000 IU/daily for 5 days. The histology accounted for testicular seminoma and funiculus both with haemorrhagic necrosis, pathologic stadium T1, clinical stadium IIB-pT1 N2 MO dx. In April 2010, PET total body by 18-FDG demonstrated minimal lymphonodal uptake in the abdomen as well as in shoulders and back owing to aspecific muscle uptake. By multiprofessional team decision a transcutaneous X photonic 6 MV 3D conformational radiotherapy was performed for 2 months. In our case the chemotherapy was excluded because of active persistent HCV+/HBV+ infection and thrombocytopenia (71.000/mmc). From May 2010 a prophylaxis regimen by r-FVIII BDD (3000 IU twice a week)is going on. No haemorrhages were seen and patient's follow-up is very satisfactory Conclusion.To date, germ cell tumors have been only reported among men with HIV/AIDS particularly testicular seminoma and the increased risk remains unexplained in this non-hemophilic population, even if an impairement of tumor immunosurveillance by HIV infection has been considered as underlying mechanism. Furthermore, in 1998 an advanced testicular seminoma has been mentioned in a Japanese severe haemophiliac HCV+ a

PU051

THE EXPERIENCE OF CASTELFRANCO VENETO-TREVISO NETWORK FOR AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION

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High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) has required in the treatment of different haematologic malignancies. Castelfranco Veneto General Hospital is settled in the peripherical area of the Treviso province and does not have all facilities for stem cell collection and transplantation. These procedures are not available in all hospitals and these patients have to be often referred to haematological departments equipped and located in cities far from

where they live. This condition produces discomfort and logistics difficulties for patients and their relatives. The Haematology of Castelfranco Veneto has implemented a protocol with the Haematology department and the Blood Bank of Treviso Hospital. Mobilization therapy and peripheral blood (PBSC) stem cells monitoring are hold in our department in Castelfranco Veneto while collection and storage of PBSC with subsequent ASCT are performed in the Haematology department in the Hospital of Treviso. From January 2008 to dicember 2010, 30 patients coming from the area of Castelfernaco have been managed with this protocol. 17 patients were males and 13 females. 14 patients were affected by NHL (mean age 49 years), 12 patients were affected by Multiple Myeloma (mean age 53 years) and 4 patients were Hodgkin Lymphomas (mean age 40 years). Mobilization cycles by using EDX 4 g/m2 were for MM while DHAP, IEV and IGEV were for lymphomas. Following the aplasia the monitoring was made in Castelfranco Veneto until the day of collection (cut off was 20 cell/ l). In total 37 leukapheresis were performed at the Blood Bank of Treviso Hospital. All procedures were well tolerated without any adverse effects. After ASCT at Treviso Haematology Department the patients from Castelfranco Veneto still remain in our Dep for their follow up. Conclusions: Our experience shows that treatments for mobilization and subsequent PBSC monitoring is feasible even in Hospitals which does not have facilities for storage and reinfusion of PBSC. This protocol seems feasible with a close collaboration between different departments also when located in a wide area when common protocols are shared. This approach minimizes the inconvenience of travelling and discomforts for patients and their families and is effective for patients.

PU052

DATA OF ADVIA 120® RBC MATRIX ARE USEFUL TO DISCRIMINATE B12/FOLATE DEFICIENCY, CHEMOINDUCED AND BETA THALASSEMIA MINOR ANAEMIA

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Background In automated peripheral blood cell analysis, ADVIA® technology (Siemens) provides a lot of information regarding also red blood cells (RBC). Among these information, those regarding red blood cells (RBC) contained in RBC matrix might be useful to define different aetiology of anaemia. Aims Aim of this study is to define if information contained in RBC matrix is useful to define different aetiology of anaemia. METHODS We evaluated 148 patients with moderate/severe anaemia. M/F was 57/91. Median age was 65 years (R23-87). 14 patients presented B12 and folates documented anaemia (9%), 68 with chemoinduced anaemia (45%), 9 with beta thalassemia minor (6%) , 57 with sideropaenia (38%). Data were analyzed using RBC matrix of ADVIA 120® automated blood cell analyzer. In this matrix volume of RBC is plotted with haemoglobin concentration of RBC. In this matrix ther'are 9 box (RBC macrocytic/hypocromic, macro/normo, macro/hyper, normo/hypo, normo/normo, normo/hyper, micro/hypo, micro/normo, micro/hyper). In each box ADVIA 120® provide absolute and percentual number of RBC with above mentioned charachteristics. Results Among 14 patients with B12 and/or folate deficiency, 11 (78.6%) presented RBC simultaneously macrocytic/normochromic >2% and normocytic/hyperchromic>2%. In remaining 134 patients with anaemia without B12 and/or folate deficiency only 10 (7.5%) showed the same charachteristics, with a Chi Square Yates corrected of 46.962 ($p<0.0001$), with an Exact Fisher text with $p<0.0001$, with a sensibility of 78%, a specificity of 92% and a predictive negative value of 97.6%. Among 68 patients with chemorelated anaemia, 10 (14.7%) presented RBC normocytic/hyperchromic >2% . In remaining 80 patients only 3 showed the same charachteristics, with a Chi Square Yates corrected of 4.2 ($p<0.04$), with an Exact Fisher text with $p<0.038$, with a sensibility of 15%, a specificity of 96% and a predictive positive value of 77%. Among 9 patients with beta thalassemia minor, 8 (89%) presented RBC simultaneously microcytic/hypochromic >2% and microcytic/normochromic>2%. In remaining 139 patients no patient showed the same charachteristics, with a Chi Square Yates corrected of 113.8 ($p<0.0001$), with an Exact Fisher text with $p<0.0001$, with a sensibility of 89%, a specificity of 100% and a predictive positive value of 100% and a predictive negative value of 99%.

PU053

EMPIRIC THERAPY WITH PIPERACILLIN-TAZOBACTAM PLUS AMIKACIN WITH OR WITHOUT TIGECYCLIN IN POST CHEMOTHERAPY FEBRILE NEUTROPENIA: MONOCENTRIC RETROSPECTIVE STUDY AND COST ANALYSIS

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Background Tigecyclin is a bactericidal antibiotic highly effective in gram positive, gram negative and anaerobes infection. Data regarding use of Daptomicin in febrile neutropenia, in terms of safety, feasibility clinical efficacy and pharmacoeconomy are actually lacking. Aims. Aim of this study is to evaluate efficacy of tigecyclin addition to piperacillin-tazobactam plus amikacin in febrile neutropenia in terms of: - Days of fever, - Days of hospitalization, - Cost of antibiotic and supportive therapy per day of hospitalization, Methods This is a monocentric, retrospective, nonrandomized study. Patients with post chemotherapy febrile neutropenia were randomized to receive empiric therapy with Piperacillin-Tazobactam 4.5g tid iv, Amikacin 15 mg/kg/day iv with (group A) or without (group B) Tigecyclin 100 mg/day iv. Daily costs of antibiotic and supportive therapy for each patient were calculate dividing the global cost of entire period of hospitalization for the days of hospitalization. Patients In two years 16 patients were enrolled. All were evaluable. In group A M/F was 5/3. Median age was 68 years (R32-72). 4 patients had NHL, 1 HD, 2 AML and 1 received autologous bone marrow transplantation. In group B M/F was 4/4. Median age was 60 years (R45-75). 6 patients had NHL and 2 AML. Results In group A median of days of fever was 10.5 days (R6-12) and median of hospitalization was 35.5 days (R15-42). Median cost of antibiotic and supportive therapy was 550 euros/day (R350-1470). In arm B median of days of fever was 13 days (R4-18) and median of hospitalization was 20 days (R15-27). Median cost of antibiotic and supportive therapy was 472 euros/day (R182-842). No adverse effects in patients receiving tigecyclin were registered. Summary/conclusion Tigecyclin seems to be safe and feasible in patients with post chemotherapy febrile neutropenia. Data regarding days of fever, cost of antibiotic and supportive therapy per day of hospitalization seem to be better in Tigecyclin group, on the contrary days of hospitalization are lower in group without tigecyclin. Nevertheless these data are difficult to evaluate because of little dimension and partial heterogeneity in two treatment groups. These data need confirmation in a larger cohort of patients.

PU054

CEREBRAL NON HODGKIN LYMPHOMA: CASE REPORT

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A 65 years old man, suffering from 10 years of IgM kappa MGUS in monitoring every six months, was admitted to our Istitution. The latest blood test showed an increase in the monoclonal component (1,9 g/dl vs 0,5 g/dl). Bone marrow biopsy was performed with a diagnosis of lymphoma-derived B cells: indolent, lymphocytic lymphoma with IgM kappa component accompaniment. The total body CT and the FDG-PET was negative for lymphoadenopathy. After about 1 month, for the onset of jargon aphasia, daytime sleepiness and mental confusion, brain CT scan was done and showed a periventricolar lesion extended to the left basal ganglion and radiate crown of the fronto-basal fronto-polar, insular and temporo-opercular region. The same lesion was also confirmed by brain MRI. A stereotactic biopsy of cerebral lesion was performed. Histopathology revealed a low grade lymphoplasmacytic B-cell lymphoma: immunohistochemical characterization, infact, showed a population of mature B lymphocytes CD19+ CD20+ CD22+ CD79b+ CD23+- CD38+- for the chain kappa monotypic surface and negative for Epstein-Barr virus encoded RNA. . The CSF cytology was negative for pathological lymphoid cells. An ultrasound of the testicle was made and it was negative as well as the examination of the fundus oculi. Laboratory workup did not show any systemic involvement. It was an indolent lymphoma affecting the brain that is an exceedingly rare finding. A general policy is that these patients should be considered to have disseminated disease and should be treated with systemic chemotherapy. In

younger patients with unfavorable features the high-dose chemotherapy with or no autologous stem cell transplantation should be used. Nevertheless, the course is rapidly progressive and ultimately fatal. The patient was treated with a first round of COP and COPADEM At the end of this course of therapy was carried out a brain MRI that showing a mass reduction and response to chemotherapy. The bone marrow biopsy showed only the persistence of monotypic kappa plasmacell component. The therapeutic program of the patient will be represented by another cycle COPADEM, 2 runs CYM and radiotherapy as the last step. This case is a rare example of indolent lymphoma with involvement of CNS. Only the follow up at the end of therapeutic procedure will allow us to fully evaluate the response.

PU055

B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH THE FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B CELL LYMPHOMA AND CLASSICAL HODGKIN 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. The PET SCAN after 2 cycles ABVD shows persistence of disease and the patient is treated with 2 cycles of salvage chemotherapy R-DHAP every 28 days to obtain a complete remission. The patient undergoes chemotherapy to mobilize the PBSC and subsequent high-dose therapy and autologous stem cell transplantation. At one year follow-up remains a complete remission.

PU056

T CELL HISTIOCYTE- RICH LARGE B CELL LYMPHOMA- CASE REPORT

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A 37-year old man was admitted in our Institute after a six months history of irregular fever, sweating, left cervical and supraclavicular lymph nodes enlargement, weigth loss, splenomegaly, rise in the indexes of cholestasis and increase trasaminasi. Cervical echography confirmed the presence of adenopaties, which was biopsied. Microscopic analysis showed a lymphoid tissue with normal structure subverted by an infiltrator who grew into large nodules and cytologically consisted of abundant histiocytes and small lymphocytes with admixture of large cells, predominantly mononuclear cells with irregular profile and often twisted, sometimes multinucleated. Immunohistochemical characterization showed that cell population expressed CD20, CD79A, EMA, Bcl6, Bcl2 (weak), LCA; whereas CD30, CD15, CD23 antigenes were absent. Small lymphocytes were CD23+ with predominance of CD4 on CD8 and few aspects of rosettes around the large cells. Lymphoid population were morphologically suggestive of Reed-Sternberg's (R-S) cells and popcorn cells with few centroblastic elements; a moderate proportion B, EMA+ , was maintained inside some nodules. T Component seems to be strongly CD4+ with rosettes PU1+; lymphoid population was PU1-; LSP1-/+; CD23 +/-; CD10- with population growth/MIB1 that highlights the nodularity and the presence of popcorn- like cells. These findings were strongly in favour of the diagnosis of lymphoma derived from peripheral B lymphocytes with morphologically characteristics of nodular lymphocyte predominant Hodgkin Lymphoma T cell rich –like. Bone marrow biopsy did not show lymphoid infiltrates. PET scan revealed lymphoproliferative disease in the over and under diaphragmatic with spleen and bone involvement. Total Body CT scan showed lymphadenopathy: lower laterocervical, submental and left subclavicular, sovrasternal ilary on the right, subcarinal, right paratracheal and axillaries. Splenomegaly (15,8x5,7 cm) with hypodence nodular lesions. We decided to treat the patient with two courses of ABVD. A short term PET scan relieved no response, so we decided to switch to R-CHOP for aggressive NHL. At the present patient is in complete remission but in a strict follow up.

PU057

IRON DEFICIENCY OR THALASSEMIA-TRAIT? A NEW DIAGNOSTIC APPROACH

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Thalassemias are a group of hereditary disorders caused by defective and imbalanced production of globin chains. The globin chains linked to heme, form the hemoglobin (Hb), the oxygen transport molecule of the red cells. Mutations in the Alpha and Beta-globin gene cause Alpha and Beta-thalassemia respectively. The commonest problem that is encountered in the diagnosis of thalassemia is their distinction from iron deficiency anemia (IDA) since their similar clinical features. Physiological IDA occurs when the requirements of the body exceed the absorptive capacity of the gastrointestinal, and is the predominant cause of IDA. Using laboratory tests, distinguishing between thalassemia and IDA is difficult, as the commonly used laboratory parameters do not discern between these pathologies in a certain way. The aim of our study is to identify a method that we allow to obtain a differential diagnosis between thalassemia and IDA. The conventional laboratory tests of iron status and in particular ferritin level are widely used in clinical practice, although they are considerably influenced by acute phase response, which complicates the clinical interpretation of the test results. High sensitive, as well as specificity, is of special importance for a test of iron status. The Soluble Trasferrin Receptor (sTfR) measurement is commonly regarded to be the more sensitive test for the measure of functional iron stores. In 1997 Punnonen described a study which for identify a new parameter for detect patients with depleted iron stores: the sTfR-index (the ratio sTfR/log ferritin). This study shows that, while serum sTfR measurements are useful in the diagnosis of IDA and in the differential diagnosis of various types or anemia, the combination of sTfR and ferritin measurements provides the highest sensitivity and specificity. We studied patients (male and female; age range 18-65 years) admitted to 'Centro Studi per le Microcitemie di Roma' divided, according to their pathology, in two groups: thalassemia-trait and IDA. We compared routine biochemical and hematological parameters and they resulted not correlated. Consequently we tested our group of patient in according to this index and obtained a statistically significant difference between the two population. sTfR-index could be a good parameter for the differential diagnosis between thalassemia and IDA but further studies are required to determine the appropriate cut-off for distinguishing thalassemia-trait and IDA.

PU058

TARDIVE DIAGNOSIS FOR ALPHA THALASSEMIA (THAL) IN A COUPLE AT RISK

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OBJECTIVE: We present a case of a couple at risk for Alpha thal, originating from Philippines, who underwent three pregnancy's interruptions (7°/8° months) with intrauterin fetal death, without any suspect of a relation with the hemoglobin (Hb) patology. MATERIALS AND METHODS: The Alpha° thal diagnosis (—PHIL) has been concluded after hematological and Hb exams routinely performed in our institute, surely helped by the knowledge of the origin of the couple. The study has been completed by molecular analysis (RDB, gap-PCR, direct DNA sequencing). Results: Both the subjects showed a slight anemia with microcytosis, polyglobulia and a normal Hb picture. The erythrocyte morphology and the osmotic globular resistances were altered. The globinosynthesis Alpha/non-Alpha ratio gave < 0.80 values. The molecular study showed an Alpha° thal heterozygosity in both the patients, due to a —PHIL deletion. Discussion: Alpha thals present an hematological picture characterized by a decreasing of MCV, MCH and Hb and an increasing of red cell's number while the Hb picture is normal. The increasing of HbA2, typical of Beta thals, is absent. Moreover Alpha thals could present milder characters and could be defined silent or subsilent. In the area of Mediterranean Sea, a typical thal-intermedia is common (HbHdisease), due to a compound heterozygosity (Alpha°/Alpha+ thal). Instead, in South-East Asia, large deletions of the Alpha clusters are found very often: they cause an Alpha° thal, in homozygosis, not compatible with life (fetal hydrops ascites). The fetal hydrops ascites is the most severe Alpha thal, caused by an homozigosity of Alpha° thal, due to the 4 Alpha genes deletion. The production of the embrional Hbs (Hb GowerI, Hb Portland) drastically reduces after the eighth week of pregnancy and, as the fetus cannot synthesize any Alpha globin chains, it cannot produce either HbF (Alpha2/Gamma2) nor HbA (Alpha2/Beta2) as well. In these conditions it is impossible to complete the natural development. A tardive abortion constitutes a serious risks for mother. Thus the genetic difference between Asian and Italian patients is really important and so there is the need that oriental women and their relatives undergo specific analysis to find out the thals. It is worth to perform an accurate Hb study in immigrant couples, as possible before a pregnancy, considering the frequent problems involving the Alpha globin genes, with mutations often not present in endemic population, and consequent specific pathological hematological and Hb pictures.

PU059

USEFULNESS OF UNICEL DXH 800 CELL POPULATION DATA IN THE DETECTION OF PLASMACELL LEUKEMIA: CASE REPORT

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Introduction and methods. Lymphoproliferative disorders (LD) are characterized and described by lymphocyte population with heterogeneous morphological features both in optical microscopy revision and in flow cytometry. Several literature report the clinical usefulness of Cell Population Data (CPD) provided by Beckman Coulter hematology analyzers. Abnormal values of CPD correlate with morphological abnormalities of leukocytes. In this work we present a case report of a plasmacell leukemia analyzed with UniCel DxH800 device. DxH800 performs leukocytes differential with the Flow Cytometric Digital Morphology (FCDM) technology, based on the measurements of Volume (V), Conductivity (C) and 5-angle Scatter light laser (MALS, UMALS, LMALS, LALS, AL2) on cells in their native state. Mean and standard deviation of FCDM measurements are collected in 56 CPD. Normal CPD values were computed from a 42 normal samples. Results. A 47-years old woman, referring continuous asthenia, was addressed to our lab with clinical suspect of LD with leukocytosis (WBC=17190/µl, LY#=3800). DxH800 analysis confirmed WBC count adding some important comments. WBC histogram showed a big peak in lymphocyte population. Differential values reported neutrophilia and lymphocytosis while scatterplot showed a lymphocyte cluster very close to the neutrophil one. CPD suggested a heterogeneous neutrophil population with low volume and low scatters (MALS, UMALS, LMALS, LALS, AL2 in arbitrary units) respectively of 106, 90, 112, 62, 75 vs normal values of 144, 137, 143, 158, 159. Examination of blood smear showed a lot of lymphocyte with nuclear immaturity and plasmoblast features. Immunophenotype revealed that 63% of the WBC were CD138+/CD38+ CD56+ CD200-, CD27- CD20-. Bone marrow biopsy confirmed the Plasmacell leukemia diagnosis. She obtained a complete remission after therapy with C-PAD (cyclophosphamide,bortezomib, dexametasone, antracycline). Initial decrease of WBC came together with high LY# and two different LY cluster in the scatterplot. CPD values confirmed this heterogeneity. After chemoterapic treatment DxH 800 reported WBC=0.359 and LI=0.1. LY CPD were close to normal values as the blood smear examination confirmed. Conclusion. We presented a case report of a plasmacell leukemia together with the useful information of the CPD provided by DxH 800. Morphological features discovered during the diagnosis and follow-up were properly correlated with CPD values.

PU060

HEMATOLOGIC RESPONSE TO LENALIDOMIDE IN JAK2 V617F-POSITIVE ESSENTIAL THROMBOCYTEMIA (TE) ASSOCIATED TO DEL(5Q) MYELODYSPLASTIC SYNDROME: A CASE REPORT

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The JAK-2 V617F somatic mutation is the molecular marker most frequently detected in the BCR/ABL negative myeloproliferative neoplasms (MPN). Deletion of long arm of chromosome 5 (del5q) is one of the most common cytogenetic abnormalities in Myelodysplastic Syndromes (MDS). Here we describe simultaneous occurrence of JAK-2 V617F mutation and del(5q) in a case of MDS successfully treated with Lenalidomide. A 62-year-old woman had been treated with hydroxyurea for 5 years because of a diagnosis of Essential Thrombocytemia (ET) before admission to our hospital for normocytic anemia (Hb 9,5 g/dL) thrombocytosis (PLT 853x109/L) and mild leucopenia (WBC 2,9x109/L). Bone marrow aspirate showed increased number of megakaryocytes with dysplastic features, dyserythropoiesis and dysgranulopoiesis, no ringed sideroblast was observed. Cytogenetic analysis performed by Fluorescence in Situ Hybridation (FISH) revealed an isolated interstitial deletion of the long arm of the chromosome 5. Polymerase Chain Reaction (PCR) was positive for JAK-2 V617F mutation. Treatment with Lenalidomide (10mg/day on days 1 through 21 of repeated 28 days cycles) was started after red blood cell transfusions. After seven cycles a complete recovery of hemoglobin and platelet concentration was obtained (Hb 13 g/dL, PLT 180x109/L), while worsened leucopenia needed supplementation with growth factor. Several authors have already reported the presence of del(5q) in MPN as well as MDS with JAK 2 V617F somatic mutation, furthermore some cases of ET with concurrent presence of del(5q) and JAK 2 mutation have been referred and finally Ingram and coll. have described six cases of MDS arboring del(5q) and JAK2 alteration. There is not yet an univocal evaluation of the prognostic significance concerning the association of JAK 2 mutation and del(5q) in MDS or in MPN, in future probably new entities will be identified with the more extensive application of molecular investigation. Here we underline the very good response of this patient to Lenalidomide therapy with normalization of thrombocytosis, previous resistant to the Hydroxyurea administration, and recovery of hemoglobin concentration with no further need of transfusion therapy.

PU061

NON MIELOABLATIVE (NMA) HAPLOIDENTICAL (HAPLO) BONE MARROW (BM) TRANPLANTATION WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE (CY) FOR RELAPSED/REFRACTORY HODGKIN'S LYMPHOMA (HL): A SINGLE INSTITUTION EXPERIENCE.

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Background. A recent GITMO study confirms that HL patients refractory to salvage therapy or auto SCT have a survival advantage if they undergo RIC alloSCT. Few patients find an available matched related or unrelated donor. Recently has been suggested that NMA-Haplo BM transplantation with Cy post-tranplant is a well tolerated and effective procedure in HL . Aims.. To evaluate toxicity and efficacy of NMA-haplo BM tranplantation with post-transplant Cy for relapsed/refractory HL. Patients and Methods. From April 2009 to March 2011, 10 NMA-haplo BM Tranplantation with post-transplant Cy for relapsed/refractory HL were performed. Conditioning regimen consisted of fludarabine

(30 mg/m2 from day -6 to -2), Cy (14.5 mg/kg/day (d) from d -6 to -5), and TBI (2 Gy d -1). Unmanipulated bone marrow cells were infused on d 0. GVHD prophylaxis consisted of Cy 50 mg/mKg/d on d +3 and +4, and tacrolimus 1 mg /d (from d +5 to +180) and MMF 15 mg/kg x 3 /d (from d +5 to +35). Results. Median age was 36 (range 25-61) 6 male and 4 male. Three patients receveid aplo because HL relapse after high dose chemotherapy. Seven patients were included in a tandem auto/allo program because refractory after 2 chemotherapy lines. Disease status before aplo transplantation was Complete Remission in 6 patients and partial remission in 4 patients. Sorror score was 0 for 9 patients and 2 for 1 patient. Median follow up was 241 days (33-739). Median number of infused MNC cells was 3.7 x 108/kg (1-4.7). One graft failure occurred. Median time to reach an absolute neutrophil count more than 0.5 x 109/L and platelet more than 20x 106/L were 22 (16-31) and 27 days (16-43). Median time to discharge was 31 days (range 23-48). Two patients (20%) experienced acute GVHD (grade II and IV) and 1(10%) severe chronic GVHD. Five patients (50%) had EBV reactivation and four (40%) CMV reactivation, 3 (30%) patients had Polioma virus cystitis and one H1N1 pneumonia, Two E. Coli bacteremia and one candida non albicans enterocolitis were observed. Eight (80%) patients are alive and in CR. Two patients died: 1 due to progressive disease and 1 (10%) due to H1N1 pneumonia. Conclusions. NMA-haplo BM transplantation with post-transplant Cy for relapsed/refractory HL is a well tolerated procedure with low NRM rate and aGVHD incidence. Only one patient had graft failure. More patients and a longer follow up are needed to analyze OS and PFS. Prospective immunological reconstitution analysis is ongoing.

PU062

VISCERAL LEISHMANIASIS: A CASE OF EX-JUVANTIBUS DIAGNOSIS

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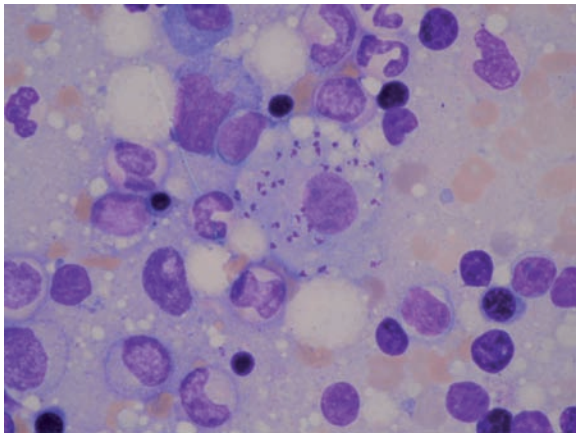
A 44 year-old man clinically suspected for haematological malignancy was referred to our Institution because of persistent fever (up to 39°-40° C), dyspnea, abdominal pain, diarrhea, vomiting, marked spleen enlargement, thrombocytopenia, leukopenia, moderate anemia and oligoclonal gammopathy. Blood test results at admission showed several abnormalities: WBC 3.010 x 10^3/mmc (neutrophils 50%), hemoglobin 10.4 g/dl, platelets 71 x 10^3/mmc, prothrombin time 50%, Dimer 8.587 ng/ml, AST 183 U/L, ALT 88 U/L, LDH 1.837 U/L, C-reactive protein 250,1 mg/l, proteins 6.5 g/dl, albumin 40.2%, alpha-1 7.7 %, alpha-2 15.8 %, globulin 26.8%. A chest radiograph was clear. Total body computed tomography showed hepato-splenomegaly without lymphadenopathy. A blood smear was negative for parasites. Bone marrow microscopy revealed slight lymphocytosis and plasmocytosis, myeloid and megakaryocytic hyperplasia.



The patient received for a week steroids, broad-spectrum antibiotics (piperacillin, amikacin, teicoplanin, imipenem, levoxacin), antifungal (fluconazole) and antiviral (acyclovir) drugs, without any improvement. Blood, urine and stool cultures obtained were negative for bacteria and fungi. An extensive diagnostic work-up ruled out hematologic causes and eliminated infectious etiologies as legionella, tuberculosis, mycoplasma pneumoniae, thypus, cytomegalovirus, mononucleosis, viral hepatitis, HIV. Because of fever persistence, the patient was transferred to the Infectious Diseases Division and ex-juvantibus liposomial amphotericin B (L-AmB) was added to the therapy. A quickly fever defervescence and organomegaly resolution was observed. Serology for leishmaniasis resulted positive confirming the diagnosis. Reviewing bone marrow aspirate we found multiple leishmania amastigotes inside mononuclear phagocytic cells (fig.1). Visceral leishmaniasis may be elusive if the diagnosis goes unsuspected and patients may be left untreated. In complex situations, L-AmB may be helpful both in immunocompetent and immunosuppressed patients and provided the key diagnostic clue in this case.

PU063

ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING IN PATIENTS WITH RELAPSE-REFRACTORY LYMPHOMAS. A SINGLE CENTRE EXPERIENCE.

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Allogeneic stem cell transplantation (allo-SCT), mainly with reduced-intensity conditioning (RIC) has a potential curative role in patients with Malignant Lymphoma , due to the potential benefit of an immune-madiated graft-versus-tumor effect. From 2005 to 2010 we transplanted 25 patients with relapsed-refractory lymphoma (7 follicular, 6 aggressive, 6 Hodgkin and 6 T lymphomas). The median age was 46 years, 21 patients had an advanced stage at diagnosis and an high risk score, the median number of prior therapies was 3, 10 patients had undergone prior autologous stem cell transplantation, the median time from diagnosis to allo-SCT was 16 month. Chemoresponsive patients at the time of transplantation were 16: 5 pts transplanted in complete remission (CR), 11 patients in parzial remission (RP) 1 patient in stable disease (SD) and 6 patients in progressive disease (PD). The graft source was peripheral blood in 24 patients, 24 had a related donor (two of them were mismatched). All patients were conditioned using a non mieloablative regimen. Thiotepa (10 mg/kg), Fludarabine (60 mg/mq) and Cyclophosphamide (60 mg/mq) was utilized in 23 cases. Methotrexate and cyclosporine was the most used prophylaxis regimen for graft versus host disease (GVHD). Six patients had a transplant related mortality (26%). The incidence of acute GVHD was 20% (5 patients, but only in one case the grade was 4) and the incidence of cronic GVHD was 36% (9 patients, 6 of them had an extensive cronic GVHD). With a median follow up of 2 years 8 patients are still in continuos CR, 1 patient has a SD, 2 patients have a progressive disease and 14 patients are dead (5 of them for lymphoma). Three-year Overall Survival is 46% (OS) and three-year Progression Free Survival is 35% (PFS). We analized the differences in terms of OS and PFS in patients with cGVHD or not, Previous Autologous stem cell-transplantation or not, chemioresponsive disease at time of transplantation or not. Only DFS in patients with cGVHD compared with patients without cGVHD demonstrated a difference statistically significant (p 0.03).

PU064

CASE REPORT: DEVELOPMENT OF TWO BREAST CANCERS AFTER TREATMENT FOR HODGKIN LYMPHOMA

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Hodgkin Lymphoma (HL) survivors face substantially elevated risks of breast cancer and cardiovascular disease. They and their physicians are often unaware of these risks and surveillance recommendations. We describe the case of a forty-two year old women treated with splenectomy and radiotherapy at age of twenty-two for a HL obtaining complete remission. The patient did not report a family history of breast cancer. Ten years after the diagnosis of HL she developed a right breast carcinoma in situ, underwent upper external quadrantectomy and then treated with aromatase inhibitors for five years. Twenty years after the diagnosis of HL the patient developed a second left breast ductular lobular carcinoma pT2 N1 (1/11) M1 (bone), G2, estrogen receptor positive,

progesterone receptor negative and HER 2 negative. The patient was subjected to upper external quadrantectomy and ipsilateral lymphadenectomy. Then she was treated with chemotherapy with taxanes and monoclonal antibody that recognizes and blocks vascular endothelial growth factor with bone progression and meningeal localizations.

PU065

MYELODYSPLASIA AS PRIMARY MANIFESTATION OF HIV INFECTION

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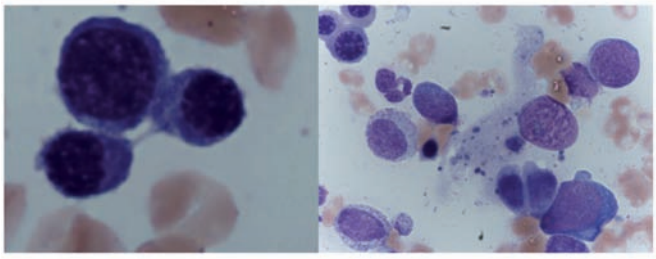
Introduction. Hematologic abnormalities are found in most patients with human immunodeficiency virus (HIV) infection, particularly in later phases of the disease. Patient. In March 2010, a man of 40-years old was interned in our Center for macrocytic anemia, leucopenia with neutropenia and slight splenomegaly. He referred a former use of cocaine and alcohol abuse after a careful anamnesis, while he denied the use of drugs and recent infections. The peripheral blood smear exam underlined a light dysplasia hypogranular granulocytes. The levels of endogenous erythropoietin was totaled 272 mU/mL. The bone marrow aspirate and the osteomedullary biopsy showed the erytroid hyperplasia with dyserythropoiesis, marked hemophagocytosis and dysmegakaryopoiesis. The karyotype was normal (46,XY). The patient tested positive for HIV antibody. After excluding other infectious pathogens (e.g. parvovirus B19, Leishmania, etc), it was supposed the diagnosis of mielodysplasia secondary to HIV infection. It was started the antiretroviral therapy (tenofovir-emtricitabina 200/245 mg/d, darunavir 800 mg/d boosted with ritonavir 100 mg/d) and with the purpose of reducing the blood-transfusion support, epoetin alpha was somministrated to the dose 40000 UI twice at week for 3 months without benefits. A significant decrease was observed in the number of viral copies three months after starting treatment, without improvements in hemoglobin and a further worsening of leukopenia. The bone marrow aspirate was repeated: the cytomorphology was similar to the previous examination and the PCR was negative for opportunist germs. The cytogenetic re-evaluation showed a trisomy of chromosome 18 in 4/20 metaphases. Therefore, the patient underwent 3 cycles of treatment with azacitidine (75 mg/m(2)/d for 7 days every 28 days), without benefits to the persistent severe pancytopenia and the need of transfusion support. The same patient underwent iron chelation therapy with deferasirox (20 mg/kg/d), suspended for a slight increase in the indices of hemolysis. Conclusion. We report a rare case of myelodysplasia as a first manifestation of HIV infection, which subsequently assumed the characteristics of the clonal disease with cytogenetic abnormalities. We have not observed any answer to the therapy with azacitidina, that we have also employed for the suppository ability to inhibit HIV replication.

Image 1: cytoplasmic bridge between erythroblasts Image 2: hemophagocytosis



PU066

ERITHROCYTOSIS AS PROBABLE ADVERSE EFFECT OF SORAFENIB THERAPY IN A PATIENT WITH AML

Metafuni E, Bellesi S, Sorà F, Giammarco S, Za T, Laurenti L, Leone G, Sica S, Chiusolo P

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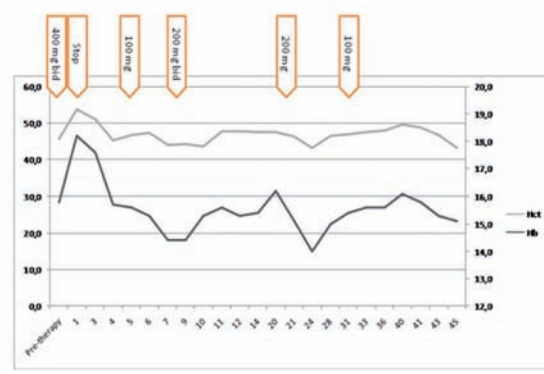
The mutations of FMS-like tyrosine kinase-3 (FLT3) occur in about 1/3 of adult patients (pts) with acute myelogenous leukemia (AML) and determine the poor prognosis of these pts. Recently, some studies tested sorafenib, a multikinase inhibitor, in pts with FLT3+ AML. In leukemic pts leucopenia and thrombocytopenia were the mainly adverse events reported during sorafenib administration, while erithrocytosis was reported during treatment of solid tumors. We reported the case of a 21-years old man with FLT3+ AML, treated with high dose of cytarabine and daunorubicin and submitted to allogeneic stem cell transplant (SCT). Graft versus host disease (GVHD) prophylaxis consisted of cyclosporine A (CSA) and short course of methotrexate. Patient (pt) obtained a complete remission (CR) with a full donor chimerism, but 5 months after SCT, he was re-admitted for leukemia relapse. Pt received a conditioning regimen with cytosine arabinoside, fludarabine and idarubicin and a second SCT. No GVHD prophylaxis was adopted in order to elicit graft versus leukemia (GVL) and a dose of donor lymphocytes was administered at day +30. CR was achieved and pt presented acute GVHD III grade of liver and skin appropriately treated. Unfortunately a further BM and extramedullary (skin) relapse occurred 8 months after new SCT. Cytosine arabinoside and gemtuzumab ozogamycin were administered and radiotherapy (RT) was then applied for refractory skin lesions. Four months later, pt developed a muscular localization of leukemia in the jaw and RT was administered. Shortly after, he relapsed newly in BM and progressed at extramedullary sites. Compassionate use of sorafenib (400mg bid) was offered after informed consent and IRB approval. Pt achieved complete molecular and hematological remission with extramedullary lesions resolution. A full donor chimerism was re-established and the pt is maintaining CR for 15 months on sorafenib. During treatment, our pt presented a recurrence of chronic skin GVHD; skin rash and liver toxicity; and erythrocytosis (Hct 53.8% and Hb 18.2 g/dl) with normal erythropoietin level and absence of JAK2 mutation. This is the first report on erythrocytosis occurring in AML patient during sorafenib administration. We thought that the vascular endothelial growth factor blockage by sorafenib could induce an hypoxia-induced stimulus on donor erythropoiesis, but the real mechanism of erythrocytosis induced by sorafenib was already unknown.

Figure 1. Characteristics and timing of erithrocytosis



PU067

LEUKEMIC EVOLUTION OF LOW RISK MYELODYSPLASTIC SYNDROME IN A PATIENT AFFECTED BY MELANOMA INFILTRATING BONE MARROW

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We report the case of a 70 year old man affected by Myelodysplastic Syndrome (MDS) since 2006. Bone marrow (BM) evaluation at diagnosis showed, according to WHO 2008 classification, Refractory Cytopenia with Multilinear Dysplasia with normal karyotype. Patient was therefore classified as intermediate-1 by means of IPSS scoring system. He received supportive care including successful high dose Erythropoietin, which was interrupted after 14 months because of loss of haematological response. Since 2007 he became blood transfusion dependent and he received daily iron chelation with oral deferasirox. In January

2010 nodular melanoma was diagnosed on his left shoulder stage IIb (2.6 mm thick with ulceration, Clark IV, mitosis rate 6/mm2). Total body Computed Tomography (CT) and Positron Emission Tomography (PET) scan did not show evidence of metastasis. He underwent complete surgical excision with histologically negative margins and negative sentinel node. Total body PET after 6 months and CT scan after 12 months follow up were negative. After 13 months he presented with cough, back pain and mild fever not responsive to antibiotic therapy. Chest X ray showed 2 discrete nodular lesions and other millimetric lesions. Chest CT scan performed seven days later revealed diffuse multiple pulmonary nodulations suggesting spread of melanoma. At the same time myeloid blast cells were shown in peripheral blood (12%) and, for this reason, BM evaluation was performed and revealed relevant infiltration (50%) of malignant melanoma cells with reduction of hematopoietic tissue and increased CD34 positive cells (25%) with patchy distribution. Cytogenetic analysis revealed a complex karyotype. We completed staging of disease (abdomen and brain CT scan) with evidence of multiple distant metastasis in spleen, vertebral and rib bones . Clinical course was rapidly worsening with the onset of dyplopia and severe dyspnea requiring oxygen support. Chest CT scan performed after additional seven days showed an important increase of number and dimension of metastatic lesions, pleural effusion and new bone metastasis. After a comprehensive clinical evaluation the patient was addressed to palliative care. This case suggests that the bone marrow metastatic infiltration of melanoma may modify the microenvironment favouring leukemic progression of MDS. Recent studies have in fact provided insights into the role of aberrant microenvironment signalling leading to disease pathology.

PU068

CASE REPORT: IMATINIB TREATMENT IS RELATED TO DECREASED ESTIMATED GLOMERULAR FILTRATION RATE IN A CHRONIC MYELOID LEUKEMIA PATIENT

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Imatinib Mesylate (IM) is the treatment of choice in patients with newly diagnosed Chronic Myeloid Leukemia (CML), irrespectively of their age. Nevertheless, information regarding tolerability and responses in advanced-age patients, a subgroup in which co-morbidities and other factors may influence outcome, is scarce, since they were excluded from most clinical trials. In literature there is evidence that introduction of IM therapy in non-clinical trial CML patients is associated with potentially irreversible acute renal injury, and the long-term treatment may cause a clinically relevant decrease in the estimated glomerular filtration rate. We describe the case of a eighty-five year old man with a CML diagnosed in chronic phase. In medical history he reported: ischemic heart disease and acute myocardial infarction at age of forty-three, aortic stenosis of moderate degree, systemic arterial hypertension in drug treatment, prostatic hypertrophy in drug treatment and chronic renal insufficiency III stage NKF. Two weeks after the beginning of treatment with IM 300 mg/day the patient developed acute renal failure which required initiation of dialysis. The introduction of IM has also induced a low volume heart failure (with edema of the lower limbs, bilateral pleural effusion and swelling of the jugular veins), refractory to medical therapy. The echocardiogram showed moderate left ventricular systolic dysfunction (ejection fraction 43%), mild to moderate insufficiency of mitral and tricuspid valve and moderate aortic stenosis. These echocardiographic data were similar to previous ones. An ultrasound of the kidneys and urinary tract was negative for urinary outflow obstruction. Despite initial hematologic response, medical therapy and dialysis the patient's condition gradually became worse. The death occurred after one month and a half since the start of IM therapy.

PU069

CASE REPORT: DEVELOPMENT OF METHICILLIN-RESISTANT STAPHYLOCOCCUS HAEMOLYTICUS OSTEOMYELITIS OF THE JAW AFTER BIPHOSOPHONATE TREATMENT IN MULTIPLE MYELOMA

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Bisphosphonates have been widely, efficiently, and safely used for the treatment of osteoporosis, malignant hypercalcemia, bone metastasis of solid cancers, and Multiple Myeloma bone diseases. However, an undesirable effect associated with bisphosphonates is osteonecrosis of the jaw. We describe the case of a eithy-eight year female patient affected by Multiple Myeloma IgG/kappa stage III (multiple osteolisis, vertebral collapse, monoclonal component, anemia) A, evolved from a plasmocitoma of the second cervical vertebra diagnosed six years before. In medical history the patient reported: systemic arterial hypertension in drug treatment, chronic atrial fibrillation and chronic renal failure stage III sec. NKF. Previously the patient was subjected to laminectomy, chemio and radiotreated and treated for years with bisphosphonates. She was recovered in our Hospital for a submental abscess, incised and drained with positive bacterial culture examination for Methicillin-Resistant Staphylococcus Haemolyticus. CT scan and magnetic resonance imaging showed a suffusion of subcutaneous submental fat, a suffusion of subcutaneous fat localized lateral to the anterior belly of the left digastric muscle and the presence of signs of erosion at left hemimandible related to osteomyelitis. Prolonged intravenous antibiotics resulted insignificant improvement in symptoms and imaging after eight weeks of treatment.

PU070

CASE REPORT: DEVELOPMENT OF A PRIMARY MALIGNANCY OF THE BRAIN AFTER TREATMENT FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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Understanding risk patterns for developing a second primary malignancy after treatment for lymphoproliferative diseases has implications for both research and clinical practice, including, in some cases, cancer screening. We describe the case of a seventy-two year old man affected by chronic lymphocytic leukemia advanced in four stage sec. RAI six years before with associated immune thrombocytopenia. Previously he was repeatedly treated: three cycles of chlorambucil and steroids, alentuzumab 90 mg/week for twelve weeks, four cycles of fludarabine plus ciclophosfamide plus rituximab, splenic radiotherapy and with periodic cycles of steroids for immune thrombocytopenia. In medical history he reported: heterozygosity for factor V Leiden, chronic gastropathy and asthmatiform bronchitis. The patient was hospitalized for the onset of cognitive impairment and disorientation space/time. The radiological CT and MRI showed the presence of a left thalamic mass of 2.2 cm diameter, characterized by intense enhancement of mdc and compression of surrounding structures, indicative of primitive cancer of the brain. Instead, there were no clinical, laboratoristic and radiological evidence of progression of chronic lymphocytic leukemia. The patient was treated with dexamethasone and evaluated by the neurosurgeon that, given the comorbidity and the four grade WHO thrombocytopenia, advised against surgery. Pending the visit with the radiation oncologist patient developed right facial-brachial-crural hemiparesis with a rapid worsening of clinical conditions. Death occurred fifteen days after the onset of neurological symptoms.

PU071

MYELOABLATIVE CONDITIONING REGIMEN WITH BUSULFAN AND FLUDARABINE IN ALLOGENEIC STEM CELL TRANSPLANTATION: INCIDENCE OF COMPLICATIONS AND FOLLOW-UP. A SINGLE CENTRE EXPERIENCE

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Association of endovenous (i.v.) Busulfan and Fludarabine (Bu-Flu) is widely employed in conditioning regimen for Allogeneic Hematopoietic Stem Cell Transplantation (HSCT). Nevertheless dosage and administration times are very variable in the different schemes and safety and efficacy of these regimens are not actually well defined. Aim of the study: Here we present our experience with the following Bu-Flu myeloablative conditioning regimen: Busulfan i.v. 0,8 mg/Kg x 4/daily from day -5 to day -2 associated to Fludarabin 30 mg/m2 once a day from day -5 to day -2. Anti Thymocite Globulin (ATG) 3,75 mg/Kg at day -3 and -2 has been added in Matched Unrelated Donor (MUD) transplants. We performed 28 HSCT (14 MUD and 14 Sibling donor) from August 2008 to April 2011 using Bu-Flu as conditioning regimen. Diseases were: Acute Myeloid Leukemia: 12 patients (pts), Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma: 7 pts; Myelodisplasia: 6 pts; Myelophibrosis: 2 pts; Peripheral T-cell Lymphoma:1 pts.17 pts were in complete remission, 5 pts in partial remission and 6 pts with stable or

active disease. Results: 23/28 (82%) pts are still alive, 22/28 (76%) pts are in complete remission. 5/28 (18%) pts died: 3 for transplant related mortality (TRM) and 2 for relapse. We observe severe mucositis (WHO grade 3 or 4) in 10/28 (36%) pts and low-moderate mucositis (WHO grade 1-2) in 14/28 (50%) pts. Incidence of grade 2-4 acute Graft versus Host Disease (GvHD) was 4/28 (14%) pts (1 died) and incidence of chronic GvHD was 6/28 (21%) (2 extended and 4 limited). 22/28 (79%) pts experienced fever in aplasia phase, but only 4 of them had inflammatory localisation, (pneumonia) with 2 related deaths. After median time of observation of 13 months overall survival (OS) is 79% , and Disease Free Survival (DFS) 76%. Median of OS and DFS is not still reached. Conclusion: in our experience Bu-Flu regimen is safe. The major side effects are mucositis of different entity and fever. We observed a low incidence of TRM, severe infective complications and acute GvHD. Also DFS and chronic GvHD have a low incidence and OS is good, but the median time of follow-up is still too limitated for these last conclusions.

PU072

EARLY EXTRAMEDULLARY RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PH+ ACUTE MYELOID LEUKEMIA EVOLVED FROM A CHRONIC MYELOID LEUKEMIA.

Parma M, Bozzani S, Gambacorti-Passerini C, Pioltelli P, Isimbaldi G, Terruzzi E, Colnaghi F, Colombo A, Mottadelli F,Levati L, Elli E, Pogliani EM

Divisione di Ematologia e Centro trapianti e Centro di Ricerca Tettamanti, Ospedale San Gerardo, Monza

We report the case of a 53 y old man, affected by an Acute Myeloid Leukemia evolved from a latent Chronic Myeloid Leukemia (LMC) who suffered for an early extramedullary LMC relapse after allogeneic Hematopoietic Stem Cell Transplantation (HSCT). At onset he presented leg bone pain, hepato-splenomegaly and peripheral leukocytosys (16.82 x 109/L) with immature red cells an dacryocytes. Nuclear Magnetic Resonance (NMR) displayed bone abnormalities in femurs and pelvis. The marrow biopsy revealed a grade 3 fibrosis with a massive infiltrate of blasts, expressing alternatively MPO or Factor VIII antigens. The cytogenetic study showed a complex caryotype associated to t(9;22) and 7 monosomy. He received an induction chemotherapy according to the ICE regimen associated to Imatinib. He achieved a cytogenetic remission and a 3-log reduction of bcr-abl hybrid transcript, whereas the femur and pelvis lesions persisted on NRM re-evaluation. Maintenance with Imatinib and diphosphonates was planned till HSCT in November 2010. The patient received an HSCT from an HLA identical unrelated donor after a conditioning with Busulfan and Fludarabin, and a GVHD prophylaxis with CyA, MTX and ATG. At day +60 he showed a 95% donor mixed chimerism and the blood group shifted toward donor (from 0 positive to A negative). Skin acute GVHD grade 2 occurred on day +79, requiring a steroid treatment. On day +95, a NMR was performed for the occurrence of pain on the right leg with difficulties in walking associated to a thigh swelling. It demonstrated an increase of the lesions and an irregular thickening of the bone at the proximal third of the right femur with the presence of solid tissue around the diaphysis, infiltrating the muscle. Biopsy of the lesion showed the presence of extramedullary CML tissue, with evidence of t(9;22). The aspiration and biopsy of marrow demonstrated a morphologically remission with the same low level of abl/bcr transcript present before HSCT and the persistence of full donor chimerism. The patient has been treated with local radiotherapy (50 Gy in 5 daily fractions), he resumed 400 mg/day of Imatinib and he tapered the immunosuppression. On day +137 the physical examination revealed the regression of the thigh swelling and remission of the bone pain without other complications. We retain this case of interest because it reveals the difficulties in eradicating extramedullary CML localisation with HSCT and the efficacy of Imatinib in their treatment.

PU073

A RARE CASE OF PRIMARY PLASMABLASTIC PLASMACYTOMA OF LYMPH NODES QUICKLY FATAL

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Primary plasmacytoma of the lymph nodes is very rare hematologic neoplasm (0.5 % of lymph node malignant neoplasms) .Usually manifests as an enlargement of the lymph nodes above the diaphragm with no evidence of metastatic multiple myloma. Have a better prognosis than other extramedullary plasmacytomas with rare recurrence and no progression after treatment. The plasmablastic morphologic form is even rarer, associated with disseminate and relapse disease, unresponsive to myeloma-chemotherapy and quickly fatal. In July 2010 we observed a 73-year-old men with fever and enlarged inguinal lymph node. Was detected double serum monoclonal protein IgG and IgA Kappa , elevation of Beta2microglobulin and LDH. The patient was without lytic lesion and bone marrow examination was without myeloma cell. Total body computed tomografy (CT) scan demonstrated multiple enlargement lymph nodes on both sides of diaphragm with maximum size of 6 cm in iliac region. All lymph nodes was PET positive with high SUV. He had surgical excision in inguinal site. Histological examination demonstrated lymph node architecture effaced by a diffuse proliferation of plasmablasts. Immunohistochemical characterization revealed Kappa light-chain restriction, KWS, S100, LCA, CD 56 and CD 20 was negative; CD 79and HTPD52 was positive with KI 67= 90%.Epstein-Barr viral (EBV) RNA was not detected by polymerase chain reaction. Was made diagnosis of primary plasmacytoma high grade. Chemotherapy with bortezomib, dexamethasone and pegylated liposomal doxorubicin (PAD) was started but the patient showed thrombosis of iliac vein for compression by extrinsic of massive enlarged lymph nodes abdominal. Salvage chemotherapy began , but was unresponsive and patient died in October 2010 after only three months by diagnosis. The primary plasmablastic plasmacytoma of lymph nodes is a rare and very aggressive neoplasia that is not responsive to myeloma-type therapy. The clinical evolution is fast and fatal for high cellular proliferation rate. We proposed that the initial therapeutic program should to be very strong and effective including high-dose therapy also.

PU074

TRISOMY 14 IN A PATIENT WITH MYELODISPLASTIC/MYELOPROLIFERATIVE NEOPLASM

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The occurrence of trisomy of a chromosome is reported in 6-7% of human malignancies; the most commonly reported in myeloid neoplasm are trisomy of chromosome 8, 9, 13, 15 and 21. Trisomy 14 (+14) has been reported as rare, non random recurrent chromosomal abnormalitie in this setting too. In particular, the trisomy 14 is reported in Myelodisplastic Syndrome (MDS), followed by Myelodisplastic/Myeloproliferative Neoplasm (MDS/MPN) and Acute Myeloid Leukemia (AML). Here, we report a case of trisomy 14 in a 64-yo man that was admitted to our hospital in November 2010 with a history of fatigue, anemia and leucocytosis. Physical ex-amination revealed the presence of splenomegaly, while the peripheral blood count showed: Hb 8,6g/dL, WBC 124,7x103/µl with neutrophilic count of 83,6x103/µl, and platelets 89,0x103/µl. Bone marrow aspirate was hypercellular, with 5% blast cells and trilineage dysplasia. The case was classified as MDS/MPN based on the WHO 2008 classification. The cytogenetic analisys was performed on bone marrow cells by a direct technique and after a 24-hour coulture. Giemsa-banded methaphases were analyzed and the karyotype on 20 methaphases was 47 XY,+14, according to standard nomenclature (ISCN 2009). The Fluorescence In Situ Hybridization (FISH) analysis, performed using the IGH probe for chromosome 14 (VYSIS), confermed the trisomy of chromosome 14. The patient died one month after diagnosis during therapy for cardiac complications. Trisomy 14 as an non- random karyotipic abnormality has been identified in association with myeloid malignancy in other published cases. Certainly the trisomy 14 play an essential role in the initiation of neoplastic processes; the dosage effect of extra copy of genes is potentially associated with cell proliferation and the following acquisition of additional genetic mutations may contribute to disease progression or leukemic transformation. The clinicopathologic features and prognostic significance of trisomy 14 must be further defined and described.

PU075

A CASE OF MYELODISPLASTIC SYNDROME WITH TRISOMY 15 AS THE SOLE CYTOGENETIC ABNORMALITY

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Introduction. Trisomy 15 as the sole cytogenetic abnormality is uncommon in hematopoietic disorder but could be preferenzially associated with myelodisplasia (MDS) in older subjects, often with sex chromosome loss. The clinicopathologic features and the prognostic significance of isolated trisomy 15, however, have not well described. Case report. Here, we report the case of a 81 yo man that was admitted to our hospital in April 2010 with anemia, low WBC count and normal platelets counts. A bone marrow aspirate showed a normal cellularity with <1% myeloid blast cells, with signs of trilineage dysplasia features. In particular, they were observed erythroblasts with nuclear aberrations, cytoplasmic vacuolization; granuloblasts with hypogranularity; and megakaryocytes in large mononuclear form, with fragmented nuclei or micromegakaryocytes. A diagnosis of Refractory Cytopenia with Multilineage Dysplasia (RCMD), WHO 2008 classification, was made and treatment started with erythropoietin alone. At the moment, the patient is still alive in good condition, with stable disease. At the time of diagnosis cytogenetic analysis was performed on bone marrow cells by a direct technique and after a 24-hour unstimulated culture. The methaphases were banded by the GTG method. The cytogenetic analisys revealed all abnormal metaphases with karyotype 47,XY,+15 on all 20 metaphases observed. The trisomy was confermed by FISH (Fluorescent In Situ Hybridization) analysis, performed using the whole chromosome painting probe for chromosome 15 (Human IDetech Chr. 15 Paint Probe Green, Cambio) on fixed cells from the cytogenetic culture, according to the manufacturer's instructions. Conclusion. Few cases of MDS and Acute Myeloid Leukemia (AML) were described with clonal trisomy 15 as a sole cytogenetic abnormality or in combination with loss of X or Y chromosome. The trisomy 15, with the dosage effect of extra copy genes, is potenzially associated with the cell proliferation and the initiation of the neoplastic process; but, at the time, this cytogenetic abnormalitie is associated with any definitive morphologic or clinical features of MDS or any hematologic specific disease.

PU076

ACUTE GRAFT-VERSUS-HOST DISEASE AFTER REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION OF T-CELL REPLETED HLA IDENTICAL PERIPHERAL STEM CELLS: PRE-TRANSPLANT HYPOGAMMAGLOBULINEMIA MAY BE A PREDICTIVE FACTOR

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Reduced intensity conditioning (RIC) has decreased the transplant-related mortality (TRM) in patients affected by relapsed haematological malignancies undergoing an allogeneic stem cell transplantation (alloSCT). Nevertheless, after RIC alloSCT from a sibling donor TRM still remains above 12-15%. A further decrease of the TRM rate is hampered by the development of acute graft-versus-host disease (GVHD). In order to investigate the predictive factors of acute GVHD in the setting of RIC alloSCT we performed a retrospective analysis in 89 patients with the following characteristics: i) having an HLA identical sibling donor; ii) receiving T-cell repleted allogeneic peripheral stem cells; iii) taking the immune suppressive therapy without an earlier withdrawal due to progressive disease or other causes. The median age was 50 (range, 18-68). Diagnoses were the following: multiple myeloma n=24, acute myeloid leukaemia n=14, non–Hodgkin's lymphoma n=36, Hodgkin's lymphoma n=14, sarcoma n=1. The median number of previous chemotherapy was 2 (range, 0-7). The conditioning regimens and GVHD prophylaxes were: fludarabine/cyclophosphamide+/-thiotepa with short course methotrexate/cyclosporine, n=52 (42/10); low-dose total body irradiation (TBI) +/- fludarabine with mycophenolate mofetil/cyclosporine, n=32 (19/13); fludarabine/busulfan with short course methotrexate/cyclosporine, n=5. All the patients were evaluated until day +100 and acute GVHD was assessed using the international standard criteria. The rate of acute GVHD on day +100 was 39.3%. In univariate analysis we included both clinical and laboratory parameters: female donor/male recipient (p=0.89), > 55 years old patients (p=0.50) and donors (p=0.69), female donor previous pregnancy (p=0.15), >3 previous therapy lines (p=0.17), CMV-positive patient/CMV-negative donor (p=0.38), diagnosis (lymphoma vs myeloma vs others, p=0.31) the conditioning regimen (TBI+/-Fludarabine versus others) (p=0.19), IgG value < 600 mg/dl before transplant (p<0.001), ABO mismatch (p=0.54), infused CD34+ cells/kg > 8 x10^6 (p=0.89), infused CD3+ T-cell/kg > 5 x 10^8 (p=0.20), serum level of cyclosporine < 200 ng/ml on day 0 (p=0.17), and fever > 38°C lasting > 2 days before the engraftment (p=0.39). The multivariate analysis confirmed as predictive factor of acute GVHD the IgG value < 600 mg/dl (p<0.001). In conclusion, hypogammaglobulinemia should be investigated in a larger sample as a risk factor of acute GVHD in the setting of HLA-identical T-cell repleted RIC alloSCT.

PU077

INTERMEDIATE DOSES OF CYCLOPHOSPHAMIDE AS SALVAGE THERAPY IN PATIENTS WITH MULTIPLE MYELOMA AND PLASMACELLULAR LEUKEMIA PREVIOUSLY TREATED WITH BORTEZOMIB AND/OR LENALIDOMIDE

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The advance of new drugs in the treatment of multiple myeloma has gave to haematologist good prospectives. Despite the efficacy shown by new drugs refractory and/or relapse still represent a challenge. In our institution in the last two years 4 patients affected by multiple myeloma relapsed or were refractory to bortezomib and/or lenalidomide containing regimens and were treated with intermediate doses of cyclophosphamide 1 mg/m2 days 1 and 3 assotiated with dexametasone 40 mg days 1 to 4 (ID-EDX) for 2 cycles. Patients were 3 women and a male, median age was 56 (range 45-60). One patient was affected by plasmacellular leukaemia at diagnosis, and another one, female and aged 60 too relapsed as plasmacellular leukaemia after treatments with lenalidomide and bortezomib. The previous lines were 3 for 2 patients and 2 for the other 2 patients and included bortezomib in all patients, bortezomib and lenalidomide in 3 patients and in 2 of them also thalidomide. One patient relapsed after autotranplant while the other 3 before it could be performed. 2 patients obtained complete response of the disease, and one a partial remission according to the EBMT criteria response. The fourth patient, the only one who relapsed after autotransplant, have completed the first cycle of ID-EDX and had reverted transfusion requirement and the reduce of the monoclonal component of 50%. Second cycle will be performed within two weeks. The 2 patients who obtained complete response, of interest the one plasmacellular leukaemia and the relapsed as plasmacellular leukaemia, underwent to autotransplant. After 4 months to autotransplant one is still in complete remission, while the patients who relapsed as plasmacellular leukaemia after 5 months from second autotransplant relapse and died for meningeal localization of myeloma. The other patient, male and aged 45 failed two times to collect peripheral blood stem cells, and is now in treatment with lenalidomide, with a very good partial response. This results seem to show that this schedule of cyclophosphamide is able to induce complete response in patients pre-treated and with very aggressive disease, and could represent a good choice in the strategy of the treatment of multiple myeloma.

PU078

AN UNUSUAL CASE OF SPLANCNIC THROMBOSIS

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A 64 year old woman was admitted to Hospital for abdominal pain and rectal bleeding. An emergency abdominal CT was performed which resulted in portal cavernoma, splenic, portal and superior mesenteric veins thrombosis with peripancreatic, perigastric, perisplenic hypertrofic collateral flows. The patient was then transferred to the Surgery Depart-

ment where she underwent ileal resection for venous ischemia. Blood biochemistry showed normal parameters: HBG 11.4 g/dL, CMV 73 fl, RBC 5020 x 10*6/mm3, WBC 5440 x 10*3/mm3, platelets 237 x 10*9/mm3. Gastric endoscopy proved the presence of esophageal varices F1-2 and colonscopy reported sigma diverticulosis. Subsequently the patient was addressed to our Haematology Service for the assessment of thrombophilic parameters. Patient's medical history was investigated , giving evidence of a previous hospital admission (3 years earlier) for acute diverticulitis; on such occasion an abdominal CT evidenced a suspected portal vein thrombosis, but the patient was not evaluated any further nor administered anticoagulant therapy. These data were suggestive of Budd-Chiari Syndrome, so the JAK2 mutation was searched; it positivity lead us to perform bone marrow biopsy, which resulted comparable with polycythemia vera. Finally, based on age, previous thrombosis and the presence of the V617F JAK2 mutation the patient was treated with cytoriductive and anticoagulation therapy with good response.

PU079

CASE REPORT: ASSOCIATION BETWEEN MIXED CONNECTIVE TISSUE DISEASE AND DEVELOPMENT OF AN HODGKIN LYMPHOMA

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A 54-year-old male patient complained hight fever and night sweats. Anamnestic he reported a mixed connective tissue disease with interstitial pneumonia, diagnosed 7 years before and successfully treated with corticosteroid, arterial hypertension in drug treatment and a previous deep vein thrombosis of the right leg, from that time in oral anticoagulant treatment. Objectively he presented one hard and not sore neck's lymph nodes (1.5cm), the lower liver and spleen margins were palpable. The chest X-rays showed a normal mediastinal size and no parenchymal lesions. The blood exams showed a mild normocromic normocitic anemia with the features of ACD, a II WHO grade thrombocytopenia, an increase of hepatic transaminase (AST 133U/L, ALT 130U/L), total (1.43mg/dl) and direct bilirubin (0.71mg/dL), alkaline phosphatise (363U/L), VES (71mm/h) and PCR (16.14mg/dl). There was evidence of iron overload (iron 10ug/dL, transferrin 144mg/dL sat 5%, ferritin 11804ng/mL). The assay of CEA, CA 19-9, PSA, AFP, renal function, LDH sieric immunoglobulins and B2 sieric microglobulin was normal. The nucleus autoantibodies were positive (1:640). The viral serology for Leptospira, HIV, HBV, HCV, HZV were negative, instead the for Leptospira, EBV, Cytomegalovirus, Toxoplasma Gondii and HSV were compatible with remote infection. All cultural samples were negative. The Tc scan documented neck (1-1.5cm), mediastinal (1-1.5cm), abdominal (1-1.5cm) lymph nodes, an hepatomegaly and a marked splenomegaly (longitudinal diameter 20cm) with multiple hypodense lesions inside in a radiologically suspicion for lymphoproliferative disease. It was performed an osteomedullar biopsy which didn't showed a lymphoid infiltrate. Despite treatment with antibiotics and antifungal therapy, there wasn't defervescence, but the patient four days after the admission developed a grade IV WHO leucopenia and thrombocytopenia, a rapid increase of LDH (1010U/L) and an hepatorenal syndrome (creatinine 3.5mg/dL, total bilirubin 13.9mg/dL and direct 8.35mg/dL). There was no change in clinical patterns even with the steroid treatment at the dosage of 1mg/kg/day. In few days there was a rapid evolution towards a multi-organ failure; dialysis treatment and ventilatory support became necessary. The neck lymph node biopsy and the hepatic biopsy were diagnostic for an Hodgkin Lymphoma, classic type. Chemotherapy treatment was start while the patient was in intensive care. Death occurred 20 days after admission.

PU080

FONDAPARINUX FOR HEPARIN INDUCED THROMBOCYTOPENIA (HIT) ASSOCIATED WITH ACUTE RENAL FAILURE: A CASE REPORT

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Heparin-induced thrombocytopenia (HIT) is a serious complication that occurs in ~1–5% of patients treated with heparin and may be associated with severe thrombotic events. HIT is mediated by antibodies directed mostly to epitope(s) formed by complexes between heparin or other anionic mucopolysaccharides and platelet factor 4 (PF4). In patients affected by HIT the high risk of thrombosis requires an anticoagulant treatment, but Vitamin K antagonists are contraindicated and the other anticoagulant drugs are difficult to manage. Fondaparinux (FDX) has been used in small groups of patients with HIT, but there is still not general agreement about its use in this disease. We report here the case of a 74 years old patient, treated for abdominal aortic aneurysm, receiving calciparine as anticoagulant prophylaxis. After few days is received a second surgery for intestinal infarction, associated with acute renal failure, requiring hemodialysis; therefore he was exposed to heparin, which was required also for the extracorporeal blood flow in the blood circuit. We observed a rapid drop of the platelet count (from 232,000 to 7,000), associated with the presence of Anti-PF4/heparin IgG antibodies Basing on this finding, we used FDX at 2.5 mg/day. FDX was also used at 1.25 mg for the preparation of blood circuits. 8 days after the start of FDX the platelet count raised 140,000 and after 20 days af treatment the platelet count remained stable >200,000. We did not observe further thrombotic episodes. The treatment with FDX was continued up to day 19 when the patient experienced a hemorrhagic erosive gastritis. The following course was good and now the patient maintains a normal platelet count and do not need hemodialysis (the actual creatinine level is 3,2 mg/ml). This case report suggests that FDX can be used in patients with HIT, requiring dialisys, also for the extracorporeal blood flow, but this drug should be administered carefully, due to the risk of a late bleeding.

PU081

EARLY ASSOCIATION OF IRON CHELATORS AND R-HUEPO IMPROVES ANEMIA AND REDUCES TRANSFUSIONAL REQUIREMENT IN MYELODYSPLASTIC SYNDROMES: REPORT OF TWO CASES

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Background: Patients suffering from myelodysplastic syndrome (MDS) have transfusion-dependent anemia and consequently an increased risk of developing iron overload. R-HeEPO, the gold standard of therapy for patients with IPSS low- intermediate-1 risk, inhibits the apoptosis of the erythroid precursors, but its activity requires an adequate amount of functional iron available. Strategies to manage iron overload include the use of iron chelator therapy that seems to improve ineffective erythropoiesis by decreasing the ROS and NF-kB activity. On this basis, it seems convincing that a combined administration of r-HuEPO and oral iron chelators may potentiate the effect of the former throughout the mobilization of iron from deposits. Aims./Methods:we evaluated 2 trasfusion- dependent patients (M/F:1/1) affected by RCMD (WHO classification) and IPSS Int-1 who received a combination of r-HuEPO and Iron Chelation Therapy (ICT). At baseline, both patients had Hb<9 gr/dl, serum EPO<200 mU/ml, transferrin saturation>20% and ferritin>850 ng/ml. Treatment consisted of r-HuEPO 40000 UI twice a week. ICT was initiated as soon as ferritin level were higher than 1500 ng/ml (Deferasirox 10 mg/Kg/die, increased to 20 mg/Kg/die in absence of adverse reactions). Results: After 2 months of therapy r-HuEPO and Deferasirox both patients became transfusion independent and Hb level increased up to 10 g/dl. Since ferritin levels were steadily below 500 ng/ml, only r-HeEPO 40000 UI was continued using a flexible administration schedule (weekly or every other week). Conclusions: Although the concomitant use of r-HuEPO and defarosirox makes results difficult to be interpretered, from a clinical standpoint appears intriguing to extend the experience to a more consistent patient series. Another relevant point raised by present report that might affect the success of r-HuEPO therapy concerns the early use of Deferasirox in patients with MDS.

PU082

PERICARDIAL EFFUSION COINCIDENT WITH SIROLIMUS THERAPY IN PATIENT AFFECTED BY ACUTE MYELOID LEUKEMIA AND SUBMITTED TO ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION

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Sirolimus (SRL) is an immunosuppresant drug, approved for prophylaxis of acute rejection in renal transplant patients (pts) but it is also used

in liver, heart and hemopoietic stem cell transplant (SCT) as an effective alternative to calcineurin inhibitors (CNI) in pts with drug related nephrotoxicity or neurotoxicity. It suppresses cell growth in B and T-lymphocytes, vascular endothelium and fibroblasts by mTOR inhibition. SRL side effects are leukopenia, thrombocytopenia, dyslipidemia, mouth ulceration, edema, joint pain and wound dehiscence. A 42 years-old caucasic male, affected by acute myeloid leukaemia, diagnosed at our centre on September 2008, was submitted to ablative SCT on March 2009 from related male donor. The regimen of GvHD prophylaxis was methotrexate and cyclosporine (CSA). Because of aGvHD with cutaneous and hepatic involvement on day 97 he started steroid therapy and was submitted monthly to ECP. At 24 months after SCT, CSA was interrupted for GvHD worsening and he started SRL. After two weeks pt was admitted to emergency room for dyspnea and asthenia. He was afebrile and tachycardic with jugular venous distension, distant hearth sounds and peripheral edema. Laboratory data showed a decreased platelet count. An electrocardiogram showed sinus tachycardia and decreased voltages; a chest radiograph revealed a massive cardiomegaly and an echocardiogram confirmed the large pericardial effusion with hemodynamic compromise. He underwent urgent pericardiocentesis and a catheter was left in place for 15 days. Pericardial fluid was serosanguinous. Blood, urine, stool, throat and pericardial fluid culture were negative for bacteria, BK, virus and mould. Bone marrow aspirate showed a blast percentage of 1% and a complete donor chimerism. SRL was immediately discontinued: platelet count slowly increased and pericardial effusion did not develop again. He was discharged after 1 month and continued CSA and steroid. The mechanism of this complication remains unclear: SRL antiproliferative action on lymphocytes, fibroblasts and endothelial cells, may favour delayed wound healing and suppression of hemangionesis and neovascularisation, by inhibition of VEGF. As consequence inhibition of lymphoangiogenesis may predispose to development of pericardial effusion. SRL should be considered for GVHD prophylaxis and treatment, but it is not devoid of side effects so it is important the early recognition and prevention of drug related complications.

PU083

HODGKIN LYMPHOMA AND HYPOTHERMIA: A CASE REPORT

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We describe a case of advanced Hodgkin lymphoma (HL) who developed hypothermia during chemotherapy. A 56 year-old man, treated 15 months before with 4 courses of fludarabine and cyclophosphamide (FC) for Chronic Lymphocytic Leukemia (CLL), was diagnosed with stage IV (involving bones, bone marrow and liver) HL, nodular sclerosing type. Before treatment the patient had fever (38°C). Bacteriological investigations gave negative responses. Therefore, we started chemotherapy with doxorubicin, dacarbazine, vinblastine, bleomycin (ABVD). During the infusion of chemotherapy, the patient had fever (39 °C), treated with hydrocortisone. About twelve hours later, his body temperature decreased to 33°C and his systolic blood pressure fell to 90 mmHg; he had malaise, profuse transpiration and deep apathy. On the first day he received high dose of steroid and hot air flows. His body temperature returned to normal within six days. Laboratory and radiological investigations excluded hypothyroidism, hypoglycaemia, hypopytuitarism, hypoadrenalism. Further chemotherapy was not associated with hypothermia. The development of hypothermia in HL is rare. These are other 12 cases reported in the literature; it's generally associated with the administration of chemotherapeutic agents and advanced disease. The mechanisms of this disorder has not been clarified. Hypothermia is not a recognised side effects of any of drugs administrated. The absence of metabolic disease, hypothalamic or central nervous system dysfunction suggests that the hypothermia was related to HL. The mechanism could be central, originating from the hypothalamus, or peripheral, with acute autonomic neuropathy; it could be triggered by high release of cytokines after first course of chemotherapy in advanced disease.

PU084

IMPACT OF ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY OF CHEMOTHERAPY FOR PATIENTS WITH AGGRESSIVE LYMPHOMA PREVIOUSLY TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION. THE SINGLE INSTITUTION EXPERIENCE

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Introduction. The autologous stem cell transplantation (AUSCT) is a viable option for the treatment of early relapsed lymphoma patients (pts) or at the beginning as first-line treatment in poor prognosis of lymphoma. The high relapse rate after AUSCT needs a more aggressive strategy to achieve significant and sustained clinical responses. Aim of the study. To assess the outcome of 26 pts with aggressive Hodgkin or non Hodgkin lymphoma undergoing to allogeneic transplant (AlloSCT) after AUSCT. Patients and Methods. The median age was 35,2 y (r. 23-67); 14 patients were male. The conditioning regimens were with reduced intensity and mainly based on Fludarabine, Thiotepa and Cyclophosphamide. The GvHD prophylaxis was based mainly on Cyclosporine and Methotrexate. At the time of AUSCT disease status was: 10 in complete remission (CR), 12 in partial remission (PR) and 4 in progression disease (PD). The median interval between AUSCT-AlloSCT was 6,8 months (r.2,5-115). Disease status at AlloSCT was: 23 in CR, 2 in PR and 1 in PD. Twenty-four pts were transplanted from HLA identical sibling donors, 2 from a matched unrelated donor. The median number of CD34+cells infused was 5,4 (r.3,3-7,2). Results. The main toxicities observed were: mucositis (16 pts WHO grade 1-2); fever (15 pts WHO 2). The median time to reach PMN>500/microl and PLT>30.000/mmc was 12,5d (r.7-21) and 13d (r.10-39), respectively. The TRM was 0.3%. Seven pts developed grade 1-2 of acute GvHD while 4 pts experienced chronic GVHD. After a median follow up of 48.4 months (r. 2-107) from AlloSCT, 18 pts are still alive. Of these, 12 are in CR and 6 relapsed. Five patients died for late complications (2 for bleeding disorders, 1 for cerebral vasculitis, 1 for ARDS, 1 for interstitial pneumonia). Three pts died for disease recurrence at 10, 17 and 36 months post alloSCT, respectively. Comments. This retrospective study shows that patients with aggressive lymphomas and heavily pretreated have a low toxicity after alloSCT with reduced intensity chemotherapy. Despite the poor prognosis, the 69.3% of these patients were long-survivors and with a good quality of life. To consolidate these results could be interesting to extend the study to other centers.

PU085

LOCALIZED CONJUNCTIVAL AMYLOIDOSIS IN A 25 YEAR-OLD MAN: A CASE REPORT

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Background Amyloidosis is a disorder characterized by deposition in tissues of amyloid, an extracellular protein in an abnormal fibrillary form with a -sheet structure. The clinical presentation can vary from a focal lesion to extensive systemic disease involving almost any organ of the body. We report a case of a young man affected by localized conjunctival amyloidosis. METHODS A 25 year-old man had noted conjunctival hyperemia and a growth on conjunctiva in his left eye; as the lesion persisted for three months and a conjunctival neoplasm was suspected, an excisional biopsy was performed. The patient consulted our Institution because the biopsy revealed an amyloid deposition. Clinical examination was performed by photograph slit lamp. The patient underwent further studies to exclude a systemic impairment. The work up included a complete blood count, serum protein electrophoresis, serum cardiac enzymes, urinalysis with urinary protein immunoelectrophoresis that were all within normal limits. Serum free light chain assay was also into normal range. Furthermore, minor salivary glands biopsy and echocardiogram were performed. Results No clinical, analytical or instrumental data supported a diagnosis of systemic amyloidosis, so the patient was treated with topical dexamethasone only. The management of localized conjunctival lesion via excisional biopsy had been crucial to prevent an enlargement of lesion and involvement of other tissues. Moreover a

topical consolidation therapy with dexamethasone had to be applied in order to prevent any recurrences. We treated him with 3 courses of topical dexamethasone (1 mg/mL eye) 4drops/4 times daily for four days every twenty-one days. The patient underwent monthly ophtalmological examination and throughout 1 year of follow up he has not shown any recurrence and he is currently asymtomatic. Conclusions This case is very interesting for the patient's young age and for rarity of this disease. The exclusive conjunctival localization allowed taking a topical therapy without the need to refer the patient to systemic steroid therapy. Dexamethasone is a corticosteroid actually used in the treatment of plasma cell dyscrasias, often associated with systemic side effects. Topical therapy avoids these effects, though it is necessary to perform periodic inspections of the intraocular pressure in order to exclude the occurrence of iatrogenic glaucoma.

PU086

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)IMPROVE OUTCOME OF SEVERE REFRACTORY SYSTEMIC SCLEROSIS: A CASE REPORT

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Systemic Sclerosis (SSc) is an autoimmune disease with limited (lcSSc) or diffuse (dcSSc) skin involvement and different pattern of organ involvement. In patient with dcSSC, the 5-year mortality is estimated to be 40-50% and no conventional treatment has been shown to influence the skin-score (surrogate marker of mortality). ASCT has been employed as a new immunosuppressive therapeutic strategy in patient with a poor prognosis desease. In Phase I/II trials has been shown a positive influence of ASCT on skin score, despite a higher Transplant Related Mortality (TRM). We report on a 41-years old woman with dcSSc, complicated by pulmonary fibrosis and polymiositis, who was given. Despite non continuous therapy with Iloprost, corticosteroids and cyclophosphamide therapy for two years the clinical condition of patient rapidly worsened (skin score 30 to 50 in 6 months and progressive disphagia, asthenia and dyspnea). In March 2010 the patient underwent peripheral stem cell mobilizing therapy with Cyclophosphamide 4 gr/mq + G-CSF 5 µg/Kg daily. Despite an acute respiratory insufficiency needing tracheostomy, two apheretic procedures were performed and 3.4x10E6/Kg CD34+ cells collected and cryopreserved in 4 bags without positive selction. In May 2010 the patient underwent ASCT conditioned with EDX 3,5gr daily given for 3 consecutive days (-5,-4,-3). Rabbit Antithymocyte Serum 100 mg day (-3 -2) was also given and the PBSC infused at day 0. Pegfilgrastim 6 mg was subcutaneously given at day +2. Febbrile neutropenia appeared for 3 days (+5 +6 +7) and II-III WHO oral mucosytis was also observed without diarrohea, epatic and renal toxicity. Neutrophil (> 500/µL) and platelet (> 20.000/ µL) engraftment was observed at +9 and +10 days, respectively. Only one packed red cell unit and 2 platelet apheretic concentrates were given. The patient was finally discarded from the Hospital at +14 day from PBSC infusion. At + 6 months the patient was given Rituximab 1000 mg intravenously because of joint pain and small swellings at the lower limbs. At a follow-up of 12 months, the patient is with a good skin score (< 11), capillaroscopy, respiratory function (and normal CPK values (from 2000 U/L to < 100 U/L). This case report shows that this therapeutic procedure is feasible and efficacy in the setting of severe systemic sclerosis. Despite the high TRM reported, ASCT may be a valid alternative approach for patient with a very poor prognosis, but in controlled clinical trials

PU087

DASATINIB: SINGLE DOSE FOR ALL CML PATIENTS? A CASE REPORT

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Dasatinib is approved for the treatment of chronic myeloid leukemia(CML) in patients with resistance or intolerance to imatinib.Dasatinib has a reduced half-life and no active metabolites. In a randomized, open-label, phase III trial, dasatinib 100 mg once daily demonstrated similar efficacy and a better tolerability profile than 70 mg twice daily. This unexpected result has been confirmed in recent studies, in which a dose of dasatinib 100 mg once daily was sufficient to trigger apoptosis in leukemic cells. Furthermore, cytogenetic responses correlate with BCR/ABL inhibition. Data suggest dasatinib 100 mg once daily achieves oncogenic shock and chronic inhibition of BCR/ABL activity. But this action is the only justifiable and is the same for all patients? 54 year old woman received a diagnosis of CP CML in December 2003. For that reason it has started therapy with imatinib 400 mg/d. The patient, during follow-up was not adherent to therapy and this has resulted, in May 2006, to increase the dose of imatinib (600 mg/d) and then in November 2008, for hematological progression, and the appearance of cytogenetic mutation Y293N, has shifted to the second generation TKIs (dasatinib 100 mg/d). At Month +1 after dasatinib, the patient was in hematological and cytogenetical remission and in suboptimal molecular response, no evident mutation . At+3 months the patient showed cytogenetic progression (metaphases Ph + 30%) and increment molecular of CML, no mutation is evident. The patient had two siblings HLA identical but also 60 years, it was decided not to amend or change the dose of dasatinib but only change the administration of the drug (50 mg/twice daily). At month+6 by dasatinib start, the patient was in complete cytogenetic and major molecular response. Currently the patient is at+30 months by dasatinib start in major molecular response.This case report shows that the patient does not respond to dasatinib single dose, but at the same dose twice daily. This evidence suggests that the activity of the drug on the disease is not only direct but probably (to be administered as a single dose) triggers a series of actions or immunological or inhibiting other molecules that act indirectly on the CML clone. In patients who do not respond to single dose treatment, probably for the absence of indirect action of dasatinib, more than change to other treatments or increase the therapeutic dose would be useful to carry out the treatment by dividing the dose twice daily.

PU088

BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A SINGLE-CENTER EXPERIENCE

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In recent years, new first line therapeutic strategies induce a high percentage of remissions in multiple myeloma (MM) patients, but, eventually, a great amount of patients relapses and requires further treatment. The aim of this study is to retrospectively evaluate response to bortezomib and related toxicity, in patients with refractory or relapsed Multiple Myeloma; patients were not enrolled in clinical trials and were treated in a single center. From September 2006 until February 2011, 33 MM have been included: 14 males, 19 females; median age 69 years (range, 53-88); 16 IgG, 6 IgA, 9 light chain, 2 non secretory. According to Durie -Salmon staging system 15% were stage I, 21% were stage II, 64% were stage III. Seven patients (21%) had renal failure. Median time from diagnosis was 46 months (range 5-92 months) and 12 patients had received autologous bone marrow transplantation (ABMT) as first line approach. In 15 patients we used bortezomib as second line (45%), in 15 patients as third line (45%), in 1 patients as sixth line (3%), while 2 patients were refractory (6%). Several combination were used: 26 VD, 3 VTD, 2 PAD, 1 MVTD, 1 MPV. Median number of cylces was 4,8 (range, 1-12). According to IMWG criteria, 23patients (70%) achieved a response [6 (18%) CR, 7 (22%) VGPR, 10 (30%) (PR)], 8 patients (24%) had a stable/progressive disease (SD/PR), while 2 patients (6%) die during the first cycle. Median PFS at 15 months was 25% and OS from the onset of bortezomib was 47% at 24 moths. With a median follow up of 27 months, 21 patients had progressed (63%) and 14 of them (42%) have deceased, . Median response was 8,5 month (range, 1-23). No differences in obtaining response or in length of PFS due to number of previous line of therapy (1 vs ≥2) or age (< vs > 65) were found. While median duration of response is significantly better in CR/VGPR patients (11,7 m) vs ≤ PR patients (4,4 m)(p<0,05), no differences has been shown in OS of the two groups . Grade 3/4 adverse event included 8 patients with thrmbocytopenia (24%), 2 patients with peripheral neuropathy (6%), 1 patient with leucopenia, cardiac failure, pneumonia (3%). Seven patients (21%) delayed or discontinued therapy due to toxicity. This retrospective analysis shows effectiveness and safety of bortezomib when used

in actual clinical practice similar to those reported in clinical trials. In addiction it confirms the importance of obtaining a good quality of response in order to improve patients' PFS.

PU089

5-AZACITIDINE FOR THE TREATMENT OF MYELODISPLASTIC SYNDROME. A SINGLE – CENTER EXPERIENCE.

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Introduction.: Myelodysplastic syndrome (MDS) is a clonal disorder of hematopoiesis that results in peripheral blood cytopenias and a marked propensity to progress to acute myelogenous leukemia. Over the last decade, treatment approaches for patients with MDS have improved significantly. Azacitidine (AZA) is the first drug FDA-approved for the treatment of MDS that has demonstrated improvements in overall survival and delaying time to progression to acute myelogenous leukemia for patients with International Prognostic Scoring System score of intermediate-2 or high-risk MDS. It appears to be well tolerated, with the most common adverse effects being myelosuppression. METHODS: Between March 2009 and February 2011, AZA was admistered as single agent in eight patients affected by intermediate-2 risk or high-risk myelodysplastic syndromes, according to the International Prognostic Scoring System. Azacitidine was administered subcutaneously at 100 mg daily for 7 days every 28 days. The characteristic of the patients were the following: median age = 68 years (66-79), M/F = 3/5; interval from diagnosis was 4 weeks (3-12). International Prognostic Scoring System categories were intermediate 2 (n = 2), and high (n = 6). Results: A median of eight cycles of azacitidine was administered (range 6–10 cycles). No dose-limiting toxicities occurred. The administration-related events such as nausea and vomiting occurred typically in the first week of drug delivery, resolved with antiemetics. The most relevant observed toxicity (grade 3-4) were myelosuppression included febrile neutropenia (n = 3). The median time to transformation to AML in 6 patients was 15 months (9-18); and of 6 patients who were red blood cell (RBC) transfusion-dependent at baseline, 5 of those became RBC transfusion-independent during the treatment period. CONCLUSION: 5-AZA is an attractive option for patients with intermediate-2 risk or high-risk myelodysplastic syndromes.

PU090

EFFICACY OF A SEQUENTIAL TREATMENT WITH PENTOSTATIN AND RITUXIMAB IN PATIENTS WITH HAIRY CELL LEUKEMIA

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Rituximab addicts a vantage in all indolent lymphomas and especially in follicular lymphomas, but little is known in a specific subset as hairy cell leukemia. Here we report seven cases of hairy cell leukemia who obtained response after pentostatin and subsequently were treated with rituximab every one or two months respectively for one or two years. At diagnosis the characteristics of the patients were: median age 50 (range 41-66); male/female ratio 5/2; diagnosis was confirmed by bone marrow aspiration or biopsy in all patients; splenomegaly was present in all cases but one, mean longitudinal spleen diameter was 167 mm (range 139-200); peripheral pancytopenia was 3/3 and 1/3 respectively in two cases, 2/3 in five cases. Induction therapy was pentostatin for 6 cycles and subsequently rituximab for 12 doses every one or two months. Rituximab was administered each month in 5 patients, while every two months in the remaining two patients. Actually we will not consider these last two patients, because rituximab therapy is ongoing. After pentostatin all patients achieved complete haematological responses with pancytopenia disappearance, although a minimum rate of hairy cells was still present in the bone marrow (until 5%). In 4 out of 6 patients splenomegaly continued, although it was improved. After rituximab therapy, the bone marrow involvement and the splenomegaly disappeared in all patients. All the patients have been maintaining these responses for 9,10,11,13 and 83 months from the rituximab stop respectively. In none patient adverse effects realized. Here we have shown: after pentostatin therapy all patients showed residual bone marrow disease and persisting splenomegaly, after rituximab treatment all patients resolved these pathological aspects. Although the follow up time is very short but in one patient, we suggest that the disappearance of the residual disease can avoid the recurrence of this disease. Nevertheless the number of patients is too small and these data need to be confirmed in a prospective trial with a longer follow up.

PU091

THERAPEUTIC LEVELS OF POSACONAZOLE IN ALLOTRANSPLANT PATIENTS TREATED WITH PROTON PUMP INHIBITORS: A SINGLE CENTRE EXPERIENCE

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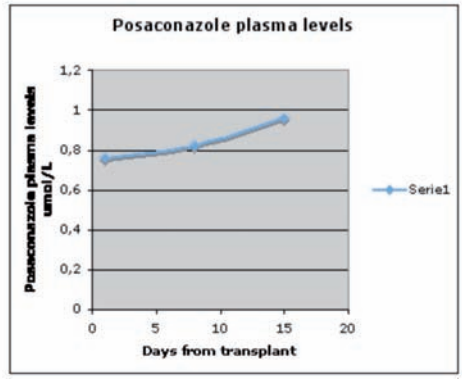
Introduction. Posaconazole (PSZ) is a triazole antifungal agent which blocks the synthesis of ergosterol, through the inhibition of the enzyme lanosterol 14a-demethylase and accumulation of methylated sterol precursors. It is specifically indicated for the treatment and prophylaxis of invasive fungal infections in immunocompromised subjects. Because of the variability in absorption, therapeutic drug monitoring (TDM) is a useful tool to optimise outcomes. Concomitant use of proton pump inhibitors may decrease PSZ plasma concentrations. Methods. From April 2010 to February 2011, 27 consecutive patients (20 male, 7 female, age range: 8-66 years) underwent allogeneic BMT. The donor was a sibling in 10 and unrelated in 17 cases. The conditioning regimen was mostly myeloablative (20/27). PSZ was administered at the dose of 200 mg of oral solution, 3 times a day to all patients, starting on day -7 from transplant. According to our policy, all patients were on pantoprazole 40 mg iv. In case of severe mucositis, nausea or vomiting PSZ was substituted with fluconazole iv. Levels of PSZ were detected using a high-performance liquid chromatography (HPLC) assay (Chromsystems, range 0,4-7 micromol/L) on day +1, +8 and +15 and then every week until the discharge from the clinic. Galactomannan assay was obtained twice a week. High definition CT scan was performed in case of suspected invasive fungal infection. Results. The administration of PSZ was possible in 25/27 patients (92%). In 2 cases it was stopped because of severe mucositis. In 21/25 cases (84%) the drug level reached the therapeutic range (median values: on day +1, + 8, +15, 0,7, O,8 and 0,9 micromol/L, respectively). In 3 patients with nausea and vomiting and in 1 with diarrhoea the drug level was below the threshold level. No significantly side effects were registered. GVHD was diagnosed in about 30% of patients (8/27). Twenty patients are still alive, 7 have died because of relapse (5) or GvHD (2). No invasive fungal infections (proven or probable) were observed. Conclusion. Our data confirm the efficacy of PSZ in preventing invasive fungal infections in allotransplant patients; many factors (nausea/vomiting, diarrhoea, drug interactions) may influence the drug availability, so that it's important to detect plasma levels of the drug. Proton pump inhibitors assumption doesn't seem to impact significantly the plasma levels of the drug. However, if concomitant administration is required, close monitoring of plasma levels and breakthrough fungal infections is recommended.



PU092

ALLOGENEIC TRANSPLANTATION FOR PH POSITIVE ALL

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Introduction. Allogeneic hemopoietic stem cell transplantation (HSCT) is a therapeutic option for patients with Philadelphia positive acute lymphoblastic leukemia (Ph+ALL). We report 48 consecutive patients who underwent an allogeneic HSCT. Patients. We analyzed 48 patients who received an allogeneic HSCT between 1989 and 2010. The median age was 41 years (16-64). 25 patients were in CR1 and 23 patients were in more advanced disease. The median year of transplant was 2001. The conditioning regimen was cyclophosphamide (CY) and total body irradiation (TBI) in the majority of patients (n=41) . The median follow up for surviving patients is 3.8 years (range 6 months- 20 years) Results. Acute GvHD developed in 44% (0-I), 45% (II) and 10% (III-IV) . In patients in CR1 there was no survival difference between acute GvHD grade 0-I and II (52% and 54%). Chronic GvHD was diagnosed as minimal (34%), moderate (45%) or severe (5%). Moderate and severe cGvHD had a detrimental effect on survival . (p=0.07) Sixteen patients are alive (33%) and 32 died of relapse related complications (RRD) (29%) or transplant related complications (TRM) (38%). The proportion of patients who died of RRD in CR1 was 24% , vs 35% for patients with more advanced disease. TRM was 24% and 35% respectively . The actuarial 10 year survival is 37% for CR1 patients and 0% for patients with more advanced disease. There was no major difference in patients grafted from matched related (n=26) (survival 34%) vs alternative donors (n=22, survival 32%) Favourable predictors of outcome in multivariate COX analysis were male donors and patients in CR1. There was no effect of transplant year. Conclusions. This study confirms a proportion of patients with Ph' positive ALL can be cured with an allogeneic transplant, especially if grafted in CR1. There does not seem to have been a major effect of the introduction of thyrosine kinase inhibitors on outcome.

PU093

EFFICACY OF LAMIVUDINE PROPHILAXIS TO PREVENT HEPATITIS B VIRUS REACTIVATION DUE RITUXIMAB REGIMENS IN HEPATITIS B CARRIERS WITH B LYMPHOPROLIFERATIVE MALIGNANCIES.

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Hepatitis B virus (HBV) reactivation is a recognized potentially fatal complication in patients undergoing chemotherapy for hematologic malignancies. Rituximab-based regimens induce long-lasting effects on B lymphocytes increasing the probability of HBV reactivation. Lamivudine prophylaxis reduces the risk and severity of chemotherapy-related HBV reactivation in patients with B lymphoproliferative diseases. We report our experience as single Institution to asses the efficacy of preemptive lamivudine to prevent HBV reactivation due to rituximab therapy in lymphoproliferative malignancies. Nine patients, two female and seven male, HBV positive at diagnosis, consecutively admitted to our Institution for B lymphoproliferative malignancies, received prophilaxis with lamivudine 100 mg once daily at least seven days before starting chemotherapy until six months after completation of chemotherapy. Six patients were affected by diffuse large B cell lymphoma (DLBCL), three by B-cell chronic leukaemia (B-CLL).A patient received a liver transplantation for hepatic carcinoma secondary to HBV infection before DLBCL onset. Five patients were treated by R-CHOP, one by R-COMP, one by R-FluCy and two by R- Bendamustine. Hepatitis B virus carriers were HBsAg and/or HBcAb positive at diagnosis, liver function and HBV DNA levels were controlled before every cycle of chemotherapy and monthly after completation of chemotherapy. No hepatitis B reactivation was observed, lamivudine was well tolerated and chemotherapy was administered as planned. Conclusions: based on our experience, lamivudine prophilaxis is efficacious in preventing HBV reactivation in patients treated by different chemotherapy regimens including rituximab for B lymphoproliferative diseases, close monitoring needs to be performed during and after anti-CD 20 antibody treatment.

PU094

A PROSPECTIVE RANDOMIZED COMPARISON BETWEEN ATG-GENZYME – ATG-FRESENIUS IN ALLOGENEIC MATCH UNRELATED DONOR (MUD) TRANSPLANTS ; IMPACT ON GVHD, INFECTIONS, RELAPSE AND SURVIVAL

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In vivo T cell depletion with rabbit anti-thymocye globulin (ATG) is a common strategy for graft versus host disease prevention in allogeneic setting. Because its use seems to correlate with an increase risk of opportunistic infections and relapse, we evaluated efficacy of 2 preparations of ATG (ATG-Genzyme Thymoglobulin "ATG-G" and ATG-Fresenius "ATG-F") in two cohorts of transplanted patients. The aim of this study was to evaluate acute and chronic GvHD, infections, relapse incidence and overall survival. Since July 2005 to March 2011, 43 consecutive patients undergoing allogeneic MUD transplants with myeloablative or reduced intensity conditionings entered the study. Underlying diseases were advanced and/or high risk hematologic malignancies. Twenty-two (22) patients received ATG-G at a total dose of 6 mg/kg. Twenty-one (21) patients received ATG-F at a total dose of 30 mg/kg. Both ATGs were administered on days -3,-2,and -1; additional GVHD prophylaxis was uniformly performed with Cyclosporin and sMTX. Groups were comparable as regards age, underlying diseases, conditionings, HLA compatibility and stem cell sources. Infusion related side effects occurred more frequently in the ATG-G group (80% vs 65%). Time of engraftment was similar. Acute GVHD grade 3-4 occurred in 22% of ATG-G patients versus 14% of ATG-F patients, while Cr GVHD all grades was 16% in the first group and 55% in the second (p=0.03, Fisher exact test). One patient rejected in the ATG-G group, none in the ATG-F group. Early and late post transplant bacterial infections and Cytomegalovirus infections were similar in both groups. At 5 years post-transplant overall survival was 54% for patients in the ATG-G cohort vs 57% for patients in the ATG-F cohort. Probability of relapse was 27% in the ATG-G group vs 24% in the ATG-F group. In this preliminary analysis no statistical difference in the incidence of infections and acute GVHD was observed between the two ATG preparations. Even if Cr GVHD was higher in the ATG-F cohort overall survival and probability of relapse were similar after 5 years of follow up. Further study is warranted in order to confirm difference in cr GvHD incidence.

PU095

THE TOLERABILITY OF THE TREATMENT WITH HYPERCVAD IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS.

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The HyperCVAD alternating with high doses of MTX and Ara-C+/-rituximab is a dose-intensive chemotherapy regimen used effectively in the treatment of ALL and aggressive NHL,but associated with considerable toxicity.We evaluated the tolerability of this regimen in patients with newly diagnosed aggressive lymphoproliferative disease or relapse.Patients received an average of 5 cycles (range 1-8),all as inpatients; they were subjected to profilaxis with antiemetics, antibiotics, antifungal, antiviral, and G-CSF in cycle A. In cycle B was provided a rescue with calcium folinate and a control of the methotrexatemia to 48 hours.The carriers of HBcAb were treated with lamuvidine. Prophylaxis with cotrimoxazole was provided for those treated with Rituximab.The cycles were accompanied by appropriate intravenous hydration, alkalinization and use of allopurinol/rasburicase to prevent tumor lysis syndrome. From 2007 to 2011 we treated 13 patients (7 males and 6 females) aged between 30 and 63 years (median 46 years) of which 6 (46%) MCL patients with stage III-IV, 1 (8%) patient with lymphoblastic lymphoma, 2 (15%) patients with follicular non-Hodgkin's peripheral T lymphocytes, 1 (8%) patient with stage IV Burkitt's lymphoma, all in the 1st line, while 3 (23%) patients had acute lymphoblastic leukemia in relapsed or refractory. 78% of patients achieved a complete remission (6 MCL 1st line, 1 lymphoblastic lymphoma and 3 ALL relapsed).Of the remaining 22%, 1 patient has not yet assessed and 2 patients died of PD.During the treatment, all patients had hematologic toxicity grade IV (min 2 days - max 13 days).In patients treated several times, toxicity had lasted longer(11 days grade IV neutropenia, 13 days grade IV thrombocytopenia). Only 1 patient had febrile neutropenia with sepsis and 1

patient,treated several times,developed CMV infection. 2 patients had grade II mucositis during cycle B.We didn't observe treatment-related mortality.Other extraematological toxicity (elevated transaminase, conjunctivitis, nausea, vomiting) didn't exceed WHO grade II.There was no need dose adjustments.The results showed a hematologic toxicity comparable to that reported in literature, but with incidence of infectious and/or fatal complications significantly lower in our cases.The "preemptive" management of side effects and toxicity of the treatment and the adoption of effective measures and prompt management of complications allow a dose-intensive treatment with acceptable toxicity.

PU096

A PARTICULAR MUTATIONAL STATUS IN A CHRONIC MIELOID LEUKEMIA PATIENT TREATED WITH DIFFERENT TIROSIN-KINASE INHIBITORS

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The most common mechanism of resistance to imatinib in chronic myeloid leukemia is the presence of BCR-ABL kinase domain mutations. Some of them could be pre-exsting, while some other could be a new mutations. The mutation have different sensitivity to different TKIs in vitro. Here we describe a case of CML patient, who never achieved cytogenetic remission and developed two mutations, resistant to different TKIs. A 68-year-old female was referred to our department in January 2006 with high WBC (179.900/mmc) and platelets count (619.000/mmc) and severe spleen enlargement. The cytogenetic analysis showed 100 % Ph+ cells and molecular evaluation revealed BCR-ABL fusion transcript (b3a2). The patient was diagnosed to suffer from CML in chronic phase and was treated with imatinib mesylate 400 mg until August 2006 when the dose was reduced to 200 mg daily due to leucopenia. In November 2006 imatinib was sequentially escalated to 400 mg then 600 mg daily because of the lost of CHR which had been achieved 3 months after the initial diagnosis. 21 months after initial diagnosis any cytogenetic or molecular response was seen; so the patient switched to Dasatinib at 100 mg daily until a month later when it had to be stopped because of pulmonary edema. After resolution of this latter event, the therapy was restarted at lower dose of 50 mg/day. In April 2008 F317L mutation was detected and Nilotinib 800 mg daily was initiated according to the in vitro bcr-abl mutants sensitivity to tirosin-kinase inhibitor (TKI). 3 months later, the F317L mutation disappeared and a new one was detected: E255K. The patient was switched to Dasatinib 50 mg daily. Interestingly the patient developed the previous mutation F317L exactly 3 months later and continued to alternate these two mutations until now. So we changed therapy when the blood count started getting worse, taking into account the regular periodicity of the two mutations. Unfortunately the patient had never achieved cytogenetic or molecular response although the use of the 3 TKI avaible. This case is an example of the complex mechanism of resistance to TKI and shows how the TKI can influence the clones selection: sensitive clones are suppressed while other resistant clones emerge. This case demonstrates the potential use of a concomitant combination of ABL kinase inhibitors to prevent the so specific alternance. Moreover the introduction of new TKIs with any known resitance will be help in this case.

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MAIN PROGRAM

THE ANEMIA OF MIGRANT PEOPLE

Cianciulli P

Centro di Riferimento regionale per le Talassemie Regione Lazio, Ospedale S. Eugenio, Roma

Worldwide it is approximately estimated that 7% of the population is carrier of hemoglobinopathies including thalassemias, hemoglobin E (HbE) and hemoglobin S (HbS). More, near 300.000–500.000 children are born annually with a severe hemoglobin disorder and the majority of them comes from developing countries[1]. Hemoglobin disorders represent 3.4% of deaths in children under their fifth year. Globally, around 7% of pregnant women carry + or 0 thalassaemia or HbS, C, D Punjab or HbE and over 1% of couples is a risk[2]. Worldwide most of hemoglobin disorders is carried out because of mutations of beta globin chains while in Southeast Asia because of alfa globin chains mutations. In heterozygosis conditions they mostly appear with no clinical relevance (HbD, HbO, HbC). Some can lead to morphological changes of red cells (HbS, HbC) and become pathological conditions in homozygosis (HbS/HbS) or double heterozygosis (HbS+HbC). The spread of this problem on a global scale is due to simultaneous existence of the same hemoglobin disorders risen de novo in different countries while the clinical variability is linked to different genes interactions (thal, gamma genes, genes responsible for neutral mutations i.e. HbD, HbO) or to combination of hemoglobin variants with thalassemia or sickle cell trait. Globalization facilitates hemoglobin disorders development because of decreased child mortality in developing countries as a result of growing use of antibiotics, greater refractory of Plasmodium malariae to quinine and marriages between different ethnic groups. More consanguinity mostly focused in malarious areas is an important factor for the expansion of a recessive disorder[3]. The spread of these diseases in the West is manly due to immigration of ethnic groups at high risk of hemoglobin disorders in reproductive age: since the year 2000 the percentage for foreigners in the European Union has presented an exponential increase[4]. In fact, immigrants usually do not implement preventive strategies for cultural, religious or economic reasons and address social and health services only in case of emergency or established disease. Therefore this behaviour complicates diagnosis, treatment and prognosis and management costs. In Western countries, despite the reduction of new indigenous cases due the prevention, the influx of immigrants has led to an increased number of these diseases. A national census sponsored by Italian Society of Thalassemia and Hemoglobinopathies (SITE) (Cianciulli P., unpublished data), has shown that immigrant children with hemoglobin disorders represent 4,6% of the overall patients in Italy (78% of registered cases is mainly concentrated in the North while the most often "imported" disease is sickle cell anemia, particularly from Africa and Albania). In Western countries financial resources are aimed at medical therapy with improvement in life expectancy and quality of life and at sophisticated therapy procedures such as bone marrow transplant or gene therapy and early diagnosis of iron overload by RMT2*[5]. On the contrary in developing countries, "safe"transfusion and expensive iron chelation therapy are not always guaranteed[1]: the above mentioned conditions lead to a pediatric population with severe clinical problems which cause high mortality rates. Considering the high cost of hemoglobin disorders, prenatal diagnosis and bone marrow transplantation centers should be promoted in immigrants native countries[6]. At the same time, industrialized countries must not lower the attention level to hemoglobin disorders. Dedicated screening for newborns, pregnant women and people in reproductive age coming from risk areas, seems to be the most effective approach to stem the increase of these diseases.

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SICKLE CELL DISEASE, AN EMERGING PROBLEM: HOW TO TREAT SICKLE CELL RELATED CRISIS

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Sickle cell disease (SCD) is an autosomal recessive genetic red cell disorder, distributed worldwide. A mutation in the gene for globin, a subunit of adult haemoglobin A (HbA), is the cause of SCD. In the United States approximately 75,000 people suffer from SCD. In Europe, immigration from developing countries has increased the prevalence of SCD through the second half of the twentieth century and now almost 20,000–25,000 SCD patients have been registered[1,2]. Sickle haemoglobin (HbS) shows peculiar biochemical properties, polymerizing when deoxygenated.The cyclic HbS polymerization-depolymerization is responsible for the severe red cell membrane damage involving both membrane proteins and lipid bilayer, which is associated with abnormal activation of membrane ion pathways generating dense, dehydrated red cells[3,4]. This results in the two main clinical manifestation of SCD: the acute vaso-occlusive crisis, characterized by ischemic-reperfusion tissue damage and the chronic haemolytic anemia, release of free Hb that binds nitric oxide (NO) reducing NO bioavailability[3,4]. The acute sickle cell related vaso-occlusive crisis (VOCs) are the major cause of morbidity for the large part of sickle cell patients[5,6] and make SCD patients high utilizers of emergency department (ED) compared to other severe hemoglobinopathies[7,9]. The most common acute events responsible for bringing SCD to ED are shown in Table 1. Sickle cell related acute events might be underserved and frequently SCD pain is undertreated. Studies on SCD patients rehospitalization within 14-30 days, used as quality indicators for SCD care, have shown that the rate of rehospitalization of SCD is high and the more frequent causes are reported in Table 1. The SCD population more exposed to the risk of rehospitalization is represented by the young-adult SCD patients related to the worsening of the disease and the passage from pediatric to adult clinical care[10,11].

Acute painful crisis

The sickle cell related pain crisis are the first cause of admission to ED of SCD patients. Pain is a devastating experience for SCD patients and the memories of acute events markedly affect patients behaviour and

quality of life sometimes more than the complications related to SCD[10,11]. The PiSCES study (Pain in Sickle Cell Epidemiology, 2002-2004) has shown that in SCD patients the mean pain is not affected by gender or genotype but the patient age (older than 45 years) and the presence of depression as comorbidity markedly affects the patient perception of pain and the number of pain sites during the acute painful crisis[12]. The most common pain sites are lower/back, knee/shin and hip[12]. Needleman et al have recently shown that SCD patients with thoracic pain have increased respiratory rate associated with smaller levels of alveolar ventilation, which is significantly ameliorated by analgesia[13]. Thus, in management of sickle cell related acute pain crisis, the effective pain relief is one of the primary objectives to achieve in order to reduce the possible development of more severe sickle cell related complications such as the acute chest syndrome (ACS). Fig.1 shows the flowchart of managment of acute sickle cell related pain crisis in young-adult patients. First step: clinical examination, immediate brief history of the pain crisis and identification of the pain sites with pain scoring by a visual analog scale (VAS) from 0 (no pain) to 10 (worst pain) and blood tests[14]. Since the VOCs are generally associated with reduced fluid intake and increased water losses, the patient's hydration is crucial, but overhydration should be always avoided. Based on the pain VAS score the analgesia should be started using either first level analgesic therapy (pain VAS<7) or second level analgesic therapy (pain VAS score >7). Since SCD pain is generated by somatic, neuropathic and vascular mechanisms[14], the effective analgesia is based on the use of molecules with different mechanism(s) of action and different pharmacological targets as the non-steroidal anti-inflammatory agents (NSAIDs), which affect pain signal transduction, and opioids which influence transmission and modulation of nociception and pain perception. In the ED the differential diagnosis should also consider other possible cause(s) of pain involving bone-muscle district(s) in SCD patients such as bone infarction, aseptic necrosis of the bone (epiphyseal segments of the humeri and expecially the femoral) or osteomyelitis. When the pain is in a joint and is associated with fever and local sign of arthritis, a septic arthirtis must be excluded. In the more severe form of acute sickle cell related pain crisis transfusion strategy might be required as partial manual red cell exchange, erythrocytoapheresis or simple red cell transfusion. The management of transfusion therapy in SCD patients during acute events requires monitoring both total hemoglobin levels and HbS percentage. The goal is to restore the patients hemoglobin levels (not more than 10-11 g/dL) before the acute events in order to prevent possible complications related to blood hyperviscosity, while the percentage of HbS reflects the success of transfusion therapy, with HbS levels being maintained below 40% to avoid sickle cells complication. The exchange strategy should always be considered in patients with hemoglobin levels higher than 9 g/dL. A growing experince on managment of uncomplicated SCD acute painful crisis in day-hospital is well accepted by patients and represents an interesting alternative cost-effective care delivery system.

Acute chest syndrome

One of the major clinical complications of SCD is the ACS, which is associated with high mortality rate in SCD patients defined by the appearance of new pulmonary infiltrate and acute respiratory symptoms. It is of note that radiographic evidences might lag the respiratory clinical manifestations. However, 61% of SCD patients with fever and chest pain admitted to the ED have a pulmonary infiltrate, indicating that the presence of fever is per se an indication for a chest X-ray. Table 2 summarized the pathogensis of sickle cell related ACS. Lungs are particularly vulnerable to the vaso-occlusive events because of their anatomic features. In the pulmonary microcirculation, dehydrated and sickled red blood cells are trapped, before reoxygenation and unsickling can occur, and this phenomenon results in frequent and diffuse microinfarction that results in severe acute and chronic lung disease. Subsequently, ischemic lung areas interested by vaso-occlusion of large vessels are more prone to infection, which are recognized in the pathogenesis of ACS. The ACS with superimposed infection by Chlamydia pneumoniae or Mycoplasma pneumoniae are generally associated with a more severe clinical course. Since hypoxiemia is present in almost 70% of ACS, incentive spirometry alone or in combination with intermittent positive endrespiratory pressure (PEEP) is important to ensure a rapid resolution of ACS[8,15].

Figure 1. Flow-chart for evaluation and managment of acute painful crisis in SCD patients admitted to the emergency department (ED); HbS: sickle hemoglobin; DH: day hospital. Inset: visual analog scale for pain (VAS) from 0 (no pain) to 10 (worst pain).

Similarly to acute painful crisis, the hydration by intravenous infusion should always be started. While the transfusion approach in ACS should be consider as either simple or exchange transfusion whenever there is respiratory compromise or general clinical deterioration. Vichinsky et al. have shown that the patient outcome following ACS is mostly related to age: when the patients were older than 20 years of age, more complications appeared than in the younger SCD population[9]. Similarly to

what indicated for acute painful crisis, the antalgic therapy with coumpounds from second level analgesia (i.e.: ketorolac, morphine, tramadol) is also required in treatment of ACS. This might be obatined by balance analgesia, which is based on the co-administration of drugs with different pharmacological mechanism of action such as NSAID(s) and opioid(s) controlling pain of different origin and reducing drug side effects such as respiratory depression. The use of corticosteroid in more severe form of ACS is still matter of discussion within the scientific comunity.

Acute abdominal pain

Abdominal pain is frequently experienced by sickle cell patients and might be related to (i) hepatobiliar disease; (ii) spleen (infarction, sequestration, absescess); (iii) mesenteric ischemia/infarction; (iv) renal origin (obstructive uropathy, stone, clot, papillary necrosis, cystitis); (v) bone infarction; (vi) gynecological origin; (vii) osteoporosis and vertebral collapse. Studies on SCD population have shown that more than half of the acute abdominal pain are related to vaso-occlusive abdominal episodes, 40% of the episodes are due to biliary tract dysfunction (60% at the age of 30 and more than 40% at the age of 20) or appendicitis, 10-15 % are related to renal events, the others are related to gynecologic disease(s) or pneumonia. An easy tool to be use in ED for SCD patient admitted for acute abdominal pain is the routinely use of urinary stick which is rapid instrument for differential diagnosis. Abdominal ultrasonography is required in the differential diagnosis of acute abdominal pain episode(s) in SCD patients, in complicated clinical picture biliary tract MRI should be considered to exclude the presence of gallstone in the common duct.

Conclusions

The high biocomplexity of SCD requires reference centers with dedicated multidisciplinary medical teams devoted to treatment of SCD related acute clinical manifestions and chronic SCD complications. One of the mission of these teams is also to keep up to date the physicians of the ED in order to ensure the rapid identification of acute sickle cell related events and the intensive treatment of pain and sickle cell related complications.

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THE ANEMIA OF ELDERLY IN THE TWENTY-FIRST CENTURY

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The number of elderly individuals is expected to increase significantly in the XXI century all over the world. The Italian population is getting older with a rate among the highest in Europe. In Italy the hope of living is minimum 75 years for men and 81.3 years for women (in 1950 these figures were 57 and 62). In 2025 there will be 6.9 millions of young people (under 20) and 17.7 millions of elderly people over 65; fifthy years back it was the opposite (Source: ISTAT 2010).Generally speaking by 2050 the number of people in EU aged 65 and above is expected to grow by 70% and the number of people aged over 80 by 170%. This raises important challenges for the 21st century: meet the higher demand for healthcare; adapt health system to the needs of an ageing population while keeping them substainable in societies with smaller workforce. One of the key challenge for EU is to promote healthy and active ageing for European citizens (EIPAHA: European Innovation Partnership on Active and Healthy Ageing, July 2011) Anemia of any degree is recognized as a significant independent contributor to morbidity, mortality and frailty in elderly patients[1]. The incidence of anemia in men and women older than 65 years was reported 11% and 10% respectively from the Third National Health and Nutrition Examination Study (NHANES III).The study highlighted that even when "mild" anemia is present, it either causes and/or is associated with both significant functional impairment and, perhaps, increased patient mortality[2]. Although the prevalence of anemia (using the World Health Organization (WHO) definition of anemia) is greater in women than men before the age of 75 yrs, by age 75 the prevalence is similar in both sexes even higher in men. Furthermore the NHANES III showed a significant difference in the prevalence of anemia among ethnic groups, with elderly non-Hispanic black having a prevalence of anemia of 27.8% versus 9% in elderly non –Hispanic white[2]. These observations prompted Beutler and West[3] in 2005 to re-evaluate the stated normal range for hemoglobin, hematocrit and mean corpuscular volume in African-American individuals. Moreover the prevalence of anemia varies quite significantly according to the settings where elderly persons live and are cared. (Table 1). Several studies have shown decreased physical performance and strength in elderly anemic patients. To remain in Italy, the in CHIANTI study conducted in the Chianti areas, found a significant reduced knee extensor and hand grip strength among anemic residents compared with their non-anemic peers aged 65 to 102 [4]

Anemia in the elderly: Causes

Although anemia has often been considered a normal consequence of aging due to impaired erythropoietin (EPO) responsiveness of the hemopoietic stem cell, three major causes have been recognized for anemia in elderly:1) blood loss/nutritional deficiences, 2) chronic illness/inflammation or chronic renal failure and 3) unexplained anemias. The 3 causes are nearly equal in frequency.

1. Among the nutritional deficiences, Vit B12 deficency is common in elderly but only rarely causes megaloblastic anemia. Low levels of B12 occur in 10% to 15% of the elderly but it is estimated that only 1%-2% of the elderly are anemic due to vitamin B12 deficiency. Folate deficiency varies in different populations and settings: elderly residents in nursing homes often receive nutritional supplements, thus megaloblastic anemias due to folate deficiency appear to be rare: when they occur are often related to alcohol abuse. Iron deficiency anemia of different origin mainly nutritional is frequent in petients over 75.

2. The high frequency of chronic comorbidities in elderly patients (Cardiovascular diseases, diabetes, renal insufficiency, cancer ecc) responsible for a chronic proinflammatory state, contributes to the pathophysiology of one-third of anemias in subjects over 65. The inflammatory response in the elderly is often aberrant, prolonged, even after the initial inflammatory stimuli have resolved. There is strong evidence that many markers of inflammation, including tumor necrosis factor-alpha (TNF-alpha) and interleukin (IL)-6 are increased in the elderly population, regardless the health status[5]. Recently, the pathophysiology of anemia of inflammation most

commonly seen in chronic diseases has been elucidated by the discovery of hepcidin, a key regulator of iron metabolism. Hepcidin inhibits intestinal iron absorption and blocks the release of iron from macrophages. Hepcidin is an acute phase reactant potentially induced by IL-6 and it is partially responsible for the iron-limited erythropoiesis in patients with acute and chronic inflammatory status.[6] A recent analysis of a subgroup of participants in the inCHIANTI study, surprisingly showed no association between hepcidin levels and anemia in elderly although IL-6 and C-reactive protein were elevated. This observation do supports a recent hypothesis that anemia especially in elderly may be mediated through hepcidin-indipendent proinflammatory pathways such as TNFalpha[7].

3. Approximately 34% of anemia in elderly patients is "unexplained" and its pathophisyology is still poorly understood. The diagnosis is mainly by exclusion. Vanasse[7] postulate that overexpression of proinflammatory cytokines is an important determinant of unexplained anemia in elderly patients, and that they induce anemia by suppression of erythroid colony formation on one hand and impairment of iron utilization on the other. It has been shown that EPO production increases with age in healthy, non-anemic elderly as compensatory mechanism to maintain normal erythrocyte production. The sensing mechanism hypoxia/erythropoietin may become deficient with age requiring higher levels of EPO in elderly. Patients with anemia had a lower slope of rise, suggesting that anemia reflected a failure of a normal compensatory rise in EPO levels with age. Stem cell physiology also changes with age. The bone marrow cellularity declines with age and whether this is due predominantly to an absolute decrease in stem cells and/or altered stem cell functional characteristics remains to be determined[8]. Furthermore it is well known that myelodysplasia (MDS) increases with age, thus occult MDS may be an important cause of "unexplained" anemias in the elderly. Other additional factors deserve to be taken into account when considering unexplained anemia in advanced age such as changes in estrogen or testosterone levels, propensity for polypharmaceutical usage, alcohol abuse, careful enumeration of all significant medical conditions that may contribute to development of anemia.

Anemia in the elderly: population based study

Tettamanti et al. recently conducted a population-based observational study of all elderly resident in the municipality of Biella a town with a population of about 46,000 inhabitants. The study was specifically aimed at investigating the epidemiology of mild anemia in an unselected population of elderly. Findings from this large survey indicate that more than one out of ten elderly persons are anemic and that most of the cases are of mild grade. The prevalence of mild anemia steadly increase with increasing age, affecting more than two out of ten people over 80 years old.9 The prevalence of anemia was 13.2% overall, 12,6% in women and 14.1% in men, similar to that reported in other population-based study. In subjects over 85 years the prevalence reached 31.3%. According to recent estimates, the worldwide prevalence of anemia is 23.9%. Few data are available on mild anemia. In the Tettamanti study, mild grade anemia accounted for approximately 84% of the cases: the majority of the subjects were unaware of being anemic. This confirms that mild anemia in the elderly is often undiagnosed or disregarded leading progressively to moderate-severe anemias that could compromise the subject health status.

Anemia among hospitalized elderly

In a prospective evaluation of 276 patients admitted in a period of 6 months to an Internal Medicine Unit, it was shown that 78% were over 65, and among those 48% were anemic although anemia was not the cause of admission (personal data).Patients admitted with onco- hemalogical conditions were excluded. The anemia was mild (10.1 + 1.4 g/dl) and in 36% of the cases was due to nutritional deficiency (iron deficiency without loss, or Vit B12). In 10% of the patients a combined iron, Vit.B12 and folic acid deficiency was observed. 27% of the anemic patients had anemia associated to chronic diseases and/or inflammation and for the remaining 27% the anemia remained "unexplained". The presence of anemia impacted significantly on the length of hospitalization. (Fig 1). These data, although in a selected population reflects the prevalence reported in several studies.

Conclusions

Anemia is a significant problem in elderly patients, it affects some 164 million elderly people worldwide, representing a public health problem. Although many anemic elderly can be diagnosed with nutritional deficiency, the etiology of anemia in a significant fraction remains obscure. A better understanding of the causes of haemoglobin decline with age and ,in particular of unexplained anemia, should provide critical pathophysiological entry points, and would represent an important progress for effective strategies of anemia control that will improve survival and quality of life in the aging population.

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MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

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The 2008 WHO classification of tumors of hematopoietic and lymphoid tissues[1] includes within myeloid neoplasms myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN) and acute myeloid leukemia (AML).

Myelodysplastic/myeloproliferative neoplasms are clonal myeloid neoplasms that have some clinical, laboratory or morphologic findings that support a diagnosis of myelodysplastic syndrome, and other findings more consistent with myeloproliferative neoplasm.[2,3]

These disorders include chronic myelomonocytic leukemia,[4] atypical chronic myeloid leukemia (BCR-ABL1 negative),[5] juvenile myelomonocytic leukemia,[6] and myelodysplastic/myeloproliferative neoplasms, unclassifiable.[7] The best characterized of these latter unclassifiable conditions is the provisional entity defined as refractory anemia with ring sideroblasts associated with marked thrombocytosis.[8]

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PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: TALKING POINTS ON DIAGNOSIS AND THERAPY

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Until few years ago, diagnosis of Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF), by far the most frequent Ph-negative Myeloproliferative Neoplasms (MPNs), was performed by excluding secondary forms of polyglobulia, thrombocytosis or bone marrow fibrosis. It was evident that these diseases arose from an unregulated myeloproliferation, consequent to the hypersensitivity to erythropoietin or to other growth factors[1] of the neoplastic clone. Nevertheless, except the so called "endogeneous erythroid colonies", until 2005 no other marker was available for the diagnosis of classical Ph-negative MPN. In 2002, Kralovics et al. demonstrated that 33% of patients with PV had a recurrent genetic lesion, consisting of the loss of heterozygosis of chromosome 9p:[2] it was the is the most frequent chromosomal lesion described in PV. These findings seemed to suggest that MPN were sustained by a deregulation of one of the signaling transduction pathways, through an alteration possibly involving the chromosome 9p. Spurred by the convincement that the molecule involved should be a kinase, in analogy with other chronic myeloproliferation as myeloid leukemia or chronic eosinophilic leukemia, in 2005 five laboratories from USA and Europe independently found the $JAK2^{V617F}$ mutation.[3] Not only this genetic marker allowed physicians for the first time to diagnose MPN through a positive criterion, but, in addition, the quantitative assessment of mutated alleles set the foundation for identifying different prognostic groups of patients or for evaluating the efficacy of various therapeutic approaches. Paralleling the genetic studies, also the diagnostic pathology of MPN has deeply revisited over the last decade,[4] and it has been definitely highlighted that each MPN subtype associates with specific bone marrow findings and that their correct recognition may impact survival, disease complication rates and prognosis, by distinguishing between ET and early/prefibrotic PMF.[5] Indeed, the discovery of genetic abnormalities that could be used as diagnostic markers and the definition of histologic features that aid in the identification of MPN subtypes, led in 2008 to the revision of the WHO diagnostic criteria for Ph negative MPN.[6] Soon after the discovery of the JAK2V617F mutation, other mutations involving the exon 2 of the JAK2 gene, or the exon[10] of MPL gene or the exon 2 of LNK gene, were reported. This body of knowledge spurred the development of drugs inhibiting the pathways which were activated in consequence of all these mutations. However, the reports of deregulated genes in MPN, either by mutations or promoter hypermethylation, progressively increased (SOCS, TET2, IDH1 and HID2, CBL, ASXL1, EZH2, DNMT3A).[7] These findings definitely evidenced that, none of the aforementioned gene abnormalities, is the primary MPN-initiating mutation, and indicated that other pathways, in addition or instead of the JAK/STAT, have to be targeted to abrogate the clonal proliferation. As predictable, recent clinical trials on MPN demonstrated that all JAK/STAT inhibiting drugs fail in inducing the molecular remission of the disease. Although the JAK2 mutation testing is routinely used for diagnosis, to date the presence of the mutation itself or the quantification of mutated alleles have not been validated for the risk stratification of MPN patients; furthermore their impact on the clinical management and on the assessment of the therapy efficacy are still to be validated. However, the availability of these specific genetic assays resulted worthwhile in particular setting of clinical situations. For example, testing JAK2 or MPL acquired mutations in children with previous diagnosis of ET or PV, allowed to conclude that in childhood PV is more frequently JAK2 wild type than among adults and that children with JAK2V617F positive PV have a very low burden of mutated alleles in comparison with adults.[8] These findings agree well with the rarity of symptoms, of thrombosis and of leukemic transformation and with the reduced cytoreductive therapy needing typical of these patients (manuscript in preparation). Moreover, genetic characterization of Italian children with familial forms of ET demonstrated that they frequently have a hereditary thrombocytosis due to the germ-line mutation of MPL gene, particularly frequent in central Italy where this mutation spread after a founder effect.[9] Another particular setting in which testing $JAK2^{V617F}$ mutation demonstrated a great utility by unraveling an underlying "occult" or "latent" form of MPN is represented by idiopathic splanchnic venous thrombosis. Thrombosis of either portal or hepatic veins might be frequently accompanied by pancytopenia due to spleen enlargement and plasma volume expansion with hemodilution. For these reasons, easily the underlying myeloproliferation may progress unrecognized. The search for $JAK2^{V617F}$ mutation in these cases plays a fundamental role, allowing patients to undertake a MPN tailored treatment beyond anticoagulant therapy.

The discovery of $JAK2^{V617F}$ mutation was the first step towards the comprehension of molecular pathogenesis of MPN. Nowadays, we cope with several molecular alterations in MPN, but it is impossible to tie them to one specific subgroup. Similarly, it appears very hard to incorporate one or more mutations in a prognostic system able to predict vascular complications, fibrotic evolution or acute leukemia transformation. In addition, about 20% of MPN patients exhibit familial recurrence of phenotipically different MPN, whereas they carry genetic abnormalities indistinguishable from the sporadic MPNs. Epidemiologic studies highlighting that first relatives of MPN patients exhibit a risk for MPN several times higher than the general population, clearly generate the suspects that this risk is linked to the genetic familial background. Nevertheless, one of the lessons we learned by studying JAK2 - or maybe MPL- mutated patients, is that they share the predisposition to acquire these somatic mutations through the presence of the JAK2 46/1 haplotype, which confers wide genomic instability. Although the JAK2 46/1 haplotype is not more represented in familial than in sporadic cases of MPN, these families show also an increased clustering of other neoplasms (i.e. lymphomas). These

findings denote that these individuals have a predisposition to somatic mutagenesis that is not restricted to myeloid hematopoietic cells. Since the only treatment so far available for the cure of patients with primary or secondary myelofibrosis still remains the allogenic stem cell transplantation, the genetic predisposition to mutagenesis of familial stem cell donors should be addressed by specific counseling, and, overall, should be considered during the donor follow up.

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NEW DRUGS FOR THE TREATMENT OF PRIMARY OR SECONDARY MYELOFIBROSIS

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The Philadelphia chromosome (Ph)-negative classic myeloproliferative neoplasms (MPNs) are clonal disorders that originate at the level of a multipotent hematopoietic stem/ progenitor cell, and are characterized by exaggerated proliferation of terminally differentiated myeloid cells.[1] The diagnostic criteria have been revised in 2008 by the World Health Organization, (that also changed the name from "disorders" to "neoplasms") prompted by discovery of first recurrent mutation in JAK2.[2] The classic MPNs include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Both PV and ET can evolve, usually late during the course OF disease, to post-PV/post-ET myelofibrosis (PPV-/PET-MF); at that stage, clinical presentation and therapeutic/management issues are substantially the same as for PMF, and the two entities are usually considered together. However, it should be stressed that most rigorous studies, particularly for prognosis assessment, have been performed specifically in patients with PMF only.

PMF is characterized by significantly reduced overall survival. In a multi-institutional series of 1,001 patients with PMF collected by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) median survival was 69 months,[3] ranging from a minimum of 23 to 181 months. The International Prognostic Scoring System (IPSS) was designed in order to allow identification of patients with projected shorter survival who could be candidate to more aggressive therapies, including stem cell transplantation, a potentially curative approach that is still burdened by significant toxicity and mortality. This system employs five variables, estimated at diagnosis or during the disease course (named Dynamic IPSS, DIPSS)[4] for accurate prediction of survival: age >65 years, hemoglobin level <100 g/L, leukocyte count >25x10^9/L, ≥1% blasts in the peripheral blood and presence of constitutional symptoms (night sweats, loss of >10% body weight in the last six months, not-infectious fever) (Table 1). Survival ranged from 135 months (low-risk category; no risk factors) to 95 months (the intermediate-1; one risk factor), 48 months (intermediate-2; two risk factors) and 27 months (high-risk group; three or more risk factors). The DIPSS includes the same five variable as the IPSS with the only difference that acquisition of anemia has a value of 2. Finally, the recent "DIPSS-plus" score[5] incorporates three additional variables for improved prognostic categorization: these are red cell transfusion need, thrombocytopenia and unfavorable karyotype (Table 1).

The number of clinical problems a patient with MF has to face with is remarkably long and complex (Table 2). These include anemia, either moderate or transfusion dependent, splenomegaly and/or hepatomegaly, foci of nonhepatosplenic hematopoiesis, marked leukocytosis or thrombocytosis, an increased risk of thrombohemorrhagic complications and debilitating constitutional symptoms. Sadly, none of available treatments available to date is able to approach satisfactorily the entire spectrum of these disease-related manifestations; hence, the ELN stated that "if prolongation of survival or cure [in MF] is not possible [which is currently only achieved by SCT] symptom-orientated palliation and quality of life are the main goals" of treatment.[6]

The discovery of Janus kinase 2 (JAK2V617F) mutation in greater than 95% of patients with PV and approximately 60% of those with ET or PMF, and the appreciation of the fundamental role that activated JAK/STAT pathway plays in the pathogenesis of MPNs has promoted a number of clinical trials with low molecular mass, ATP-competitive, drugs under the prospective of a targeted therapy. These studies enrolled patients with MF, both PMF and PPV-/PET-MF, with the characteristics of intermediate-2 or high risk disease. Most of currently available JAK2 inhibitors are actually active against both JAK2 and JAK1, with a greater selectivity towards JAK3; furthermore, some have overlapping activity against other kinases, particularly Flt3, and none is specific for mutated JAK2 but targets also the normal protein. These shared characteristics of the JAK inhibitors are at the basis of their most common toxicity (ie, myelosuppression owing the role of JAK2 plays in normal hematopoiesis), help to reasonably explain the most disappoint results of their use, ie the lack of an Imatinib-like effect on the molecular disease-driven abnormality, and at the same time represent an added value for their clinical efficacy due to the tampering of the JAK1-mediated abnormal release of inflammatory cytokines.

The largest experience to date has been produced for Ruxolitinb (INCB018424), with two already completed phase III trials. A phase I/II trial established 15 mg twice daily as the most effective and safest dosing regimen in 153 patients with MF; dose limiting toxicity was thrombocytopenia.[7] Approximately half of the patients had a rapid (within one month) ≥50% reduction of splenomegaly and rapid improvement of individual symptom score, based on the Myelofibrosis Symptom Assess-

ment Form (MFSAF). Response rate was independent of the presence of JAK2V617F mutation and occurred regardless of diagnosis of primary versus PPV-/PET-MF. There was no significant effect on the JAK2V617F allele burden, suggesting that clinical efficacy was minimally mediated by a selective cytotoxic effect on mutated cells and rather it was ascribable to functional inhibition of JAK/STAT pathway in neoplastic as well as normal cells. In particular, a marked inhibition of a large panel of inflammatory cytokines was demonstrated after treatment, that could likely account for the reported clinical benefits. Non-hematological toxicity was minimal, typically grade 1 or 2 in less than 10%; grade 3 or grade 4 anemia and thrombocytopenia occurred in less than 10% of patients, and were dose responsive.[7] Results for the COMFORT-I and -II phase III trials (Table 3) have been preliminary reported. The two trials produced very similar results: nearly all patients on Ruxolitinib experienced reduction of spleen volume of some degree and 41.9% vs 0.7% (placebo group) in COMFORT-I and 28.5% vs 0% (BAT group) in COMFORT-II ($P<0.0001$ for both) achieved a ≥35 volume reduction. A significant improvement in total symptom score (MFSAF) and global health status/quality of life (EORTC QLQ-C30 score) was demonstrated in the two trials, respectively. Thrombocytopenia and anemia were the most common adverse events, that very rarely led to discontinuation; noteworthy, there was no difference in mean unit transfused per month versus BAT in COMFORT-II.

Complete results of a phase I/II study have been reported also for TG101348 (Targegen/Sanofi), an ATP-competitive inhibitor with high selectivity for JAK2V617F mutated cells *in vitro*. In the phase I study portion, the dose-limiting toxicity was reversible asymptomatic grade 3 or 4 amylasemia/lipasemia and the maximum tolerated dose was 680 mg. In the phase II extension, 65% of the 43 subjects who continued treatment beyond 6 cycles achieved a spleen response and the large majority an improvement in constitutional symptoms. Interestingly, a median 62% decrease in JAK2V617F burden was observed among JAK2V617F mutated subjects, although conversely in 20% of patients a 18% to 58% increase of V617F allele burden occurred. There was no consistent change in inflammatory cytokine levels. Non-hematologic adverse events were mainly gastrointestinal, while grade 3/4 hematological adverse events included treatment-emergent anemia (35%), thrombocytopenia (24%) and neutropenia (10%). Finally, CYT387 (Cytopia) has been used in a phase I/II study, still ongoing and reported only partially. Treatment was effective in reducing splenomegaly of some degree in 97% of the subjects, with 37% achieving the target level for a clinical improvement (IWGMRT criteria). Symptoms improved in most. However, the most intriguing effect of the drug was a total 63% response on anemia: of the 22 subjects evaluable for anemia, 9 (41%) achieved a clinical improvement, and an additional 5 patients experienced a >50% reduction in transfusion requirement. The drug was overall well tolerated. Thus, CYT387 seems to differ from other JAK inhibitors for its unique activity against anemia.

Other JAK inhibitors that have been/are being used in clinical trials are CEP-701, or lestaurtinib, a JAK2 and Flt3 inhibitor; XL019 (Exelixis), that has been withdrawn from clinical trials because of neurotoxicity of variable severity; SB1518 (S*Bio) that is currently in Phase I/II trial in 33 MF patients. At least two other molecules are now in phase I/II trials (AZD1480, Ly2784544). In a phase II clinical study with CEP-701, 27% of 22 patients with JAK2V617F mutated MF had clinical improvement with remarkable side effects of myelosuppression (grade 3 or 4 anemia, 14%; thrombocytopenia, 23%) and gastrointestinal disturbances Thus, the drug resulted overall modestly effective but somewhat toxic. At the safest and effective daily dose of 400 mg of SB1518, 97% of the subjects presented a spleen volume reduction by MRI, with 57% and 23% showing a reduction of ≥25% and a complete resolution of splenomegaly, respectively. Improvement in symptoms occurred in 40-65% of the patients treated for 6 months. The drug was well tolerated also in thrombocytopenic subjects; most adverse events were grade 1 or 2 gastrointestinal toxicity, likely related to Flt3 inhibition.

The appreciation that enhanced activation of the mTOR/AKT signaling pathway occurs in cells harboring the JAK2V617F mutation and that RAD001, an orally available macrolide derivative of rapamycin prevented proliferation of cells from JAK2V617F mutated cells lines and primary MPN cells,[8] provided the rationale for studying the clinical efficacy of RAD001 in a phase I/II study in 39 patients with intermediate/high risk myelofibrosis. A rapid and sustained splenomegaly reduction of ≥50% and ≥30% occurred in 20% and 44% of subjects, respectively. Sixty-nine per cent of the patients experienced complete resolution of systemic symptoms while pruritus disappeared in eighty per cent Response in leukocytosis, anemia and thrombocytosis occurred in 15% to 25%. Clinical responses were not associated with reduced JAK2V617F burden, circulating CD34+ cells or cytokine levels, while CCDN1 mRNA and phospho-p70S6K level, known targets of mTOR, and WT1 mRNA were identified as possible biomarkers associated with response. Overall, response rate was 60% using EUMNET criteria and 23% using IWG-MRT criteria. RAD001 was generally well tolerated; commonest toxicities were grade 2 mouth ulcers and grade 1/2 hypertrigliceridemia. Hematological toxicities were represented mainly by worsening of anemia in about 20% and infrequent grade 2 neutropenia or thrombocytopenia; however, improvement of anemia was seen in some patients. Thus, RAD001 has an overlapping spectrum of activity compared to JAK inhibitors but lower hematological toxicity, and could represent a valuable alternative in selected categories of patients with MF.

Novel immunomodulators with pleiotropic action mechanisms are being evaluated for the treatment of anemia in MF patients. In previous studies, low dose thalidomide associated with low-dose prednisone produced some responses in anemia (20% to 40%), but with very poor tolerance. Therefore, new immunomodulators with improved activity and expected lower toxicity are seen with particular interest. Lenalidomide has been successfully used in anemic patients with MF and a del(5q31) abnormality; occasional evidence of molecular remission has also been reported (9). These data were not reproduced in a multicenter ECOG phase 2 trial in PMF patients lacking the del(5q), and the treatment was very poorly tolerated. Thus, use of lenalidomide is restricted to the very rare MF patients with the 5q deletion. Pomalidomide has been used in a phase 2 randomized, double-blind trial in 84 MF anemic patients. About 25% of them showed sustained (>1 yr) responses for anemia; myelosuppression and neuropathy were infrequent and mild. A preferential response in JAK2V617F-positive disease, unlike in JAK2 wild-type

subjects, has also been reported. An ongoing phase 3, multicenter, double-blind, placebo-controlled study in transfusion-dependent MF patients plans to randomize 210 subjects in a 2:1 ratio to the active arm.

A novel avenue in the treatment of MF is represented by drugs targeting deregulated epigenetic gene expression regulation. The discovery of mutations affecting proteins normally involved in epigenetic gene regulation has further strengthened the interest in epigenetically active drugs.[10] The hypomethylating agents azacitidine and decitabine have both been used in small clinical trials in patients with myelofibrosis. In brief, overall results with this class of agents have been quite disappointing and it is unclear whether they still deserve to be tested in this clinical settings unless eventually as combination modalities. The histone deacethylase inhibitors Givinostat and Panobinostat have been shown to down-regulate JAK/STAT signaling through still largely unknown mechanisms; a synergism of panobinostat with the JAK2 inhibitor TG101209 has also been reported. In a phase II trial with Givinostat that included 16 patients with MF 3 major responses were obtained in MF patients. Reduction of splenomegaly was observed in 38% of MF patients. Studies with the HDAC inhibitor LBH589 (panobinostat) in myelofibrosis are ongoing, and preliminary results are of interest.

In summary, these are exciting times for the therapy of MF, since we are now in the position to test several compounds belonging to different pharmaceutical categories that might represent, for the first time, targeted drugs. It is indeed remarkable that in a very short time after the original description of the involvement of the JAK/STAT pathway, mainly following the discovery of JAK2V617 mutation, consistent data have been produced on the novel class of JAK2 and JAK1 inhibitors. The latter drugs have demonstrated significant improvement in the management of patients with symptomatic myelofibrosis, with acceptable toxicity, compared to conventional therapy. However, they are not able to produce molecular remission, and future work should be done in this direction. It is also anticipated that combination of drugs acting on different targets needs to be evaluated for combined/synergistic activity and improved safety.

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GERIATRIC EVALUATION OF HEMATOLOGIC PATIENTS WITH ACUTE LEUKEMIA

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Acute leukemia in the elderly: a "different entity"

"I am older, not elderly" said the patient with acute myeloid leukemia". This editorial of Dr. Schiffer, published last year in the Journal of Clinical Oncology, clearly underlines one of the most frequent clinical problems in the daily practice of hematologists: how to treat older (elderly?) patients with acute leukemia (AL), providing benefits in terms of survival and quality of life and avoiding undue toxicities in a condition where there is a strict correlation between age and probability of survival[1]. The standard treatment of adult AL mainly consists of two phases: induction and consolidation. Induction aims to achieve complete remission (CR), which is considered an absolute prerequisite for long-term survival and cure; consolidation aims to maintain CR and may also include transplantation of autologous or allogeneic stem cells. Accordingly, the possibility of adopting tailored and individual therapeutic approaches in young adult patients is not generally considered. AL, however, is a typical disease of the elderly and older AL patients achieve CR less likely than younger patients and have higher probability of primary resistance, early relapse and early death. Tumor biology and patient's specific factors, in fact, negatively influence the prognosis, inducing less effectiveness and higher toxicity of chemotherapy. On the other hand, intensive therapies can still result in a significant survival advantage even in subsets of very elderly patients; thus, establishing prognostic models for CR achievement, induction mortality, and survival rates, based on simple, readily available baseline characteristics, useful in the daily practice to avoid unnecessary toxicity not balanced by actual survival advantage, represents a specific issue in elderly patients with AL.[2-4]

To treat or not to treat: how to provide benefits avoiding toxicity

Importantly, not only survival but also quality of life of these patients must be considered. Patients in CR have a much better quality of life than patients during palliative treatment, with less hospital visits. However, induction therapy with some consolidation requires at least some months of hospitalization. Most patients with less than 6 months of expected survival with treatment should therefore not be considered unless highly motivated. This is likely to exclude most of the older patients with advanced performance status (PS) from intensive treatments. Others should be offered one course of intensive treatment, and if this fails, an experimental low-toxicity treatment could be an option. However, if a CR or a near CR is achieved, a consolidation adjusted to the individual response to initial treatment, regarding toxicity, recovery, and remission status, would be recommended. Second-line treatments and intensive relapse therapies are rarely useful in older patients with high risk features.

To achieve the best therapeutic results, avoiding unnecessary toxic effects, is therefore a primary objective. To this aim, it is particularly important to establish which elderly patients will do better with specific, intensive or less intensive, treatments. In particular, it may be useful to recognize the so-called "unfit" patients, in other words those not amenable with standard intensive treatments and needing modified or attenuated therapies without altering the natural history of the disease. On the other hand, there are "frail" patients in whom true best supportive care and no toxic drugs can be exclusively administered. Generally speaking, the latter category includes the vast majority of patients aged over 80 years, independently from PS and co-morbidities, the former those patients aged 70-80, in whom there is a greater border of uncertainty and physician's experience plays a major role. On a clinical ground, this is particularly relevant in acute myeloid leukemia (AML), whose median age is around 70 years, with about one fourth of patients who are older than 80 years. Contrarily to AML, acute lymphoblastic leukemia (ALL) in adults is an uncommon disease and specific studies in older patients are really rare.[4] The median age at diagnosis is, however, > 60 years. Of interest, the proportion of patients with Philadelphia positive ALL rises with age: these are probably the only elderly leukemic patients who, in the recent years, have significantly improved their clinical outcome receiving inhibitors of tyrosine-kinases (TKI).

Several prognostic indexes, specifically designed or adapted for AML, have been recently proposed in order to obtain a sufficiently reliable prediction of CR rate and survival in intensively treated elderly patients (Table 1).

Etienne et al. identified unfavorable karyotype, leukocytosis

>30x10^9/L, CD34 expression on leukemic cells, and Charlson Comorbidity index (CCI) >1[5] as risk parameters (Table 2). These authors generated a score allowing the stratification of 133 elderly AML (median age 73 years) into low-, intermediate-, and high-risk groups with CR rates of 87%, 63%, and 37%, respectively. The risk of early mortality and the probability of survival also were different in the 3 risk groups.

Malfuson et al. developed a decisional index derived from the analysis of 416 elderly AML patients treated in the ALFA-9803 trial that included high-risk cytogenetics and/or the presence of at least two of the following criteria: age > or =75 years, PS > or =2, and white cell count > or = 50x10^9/L. This simple two-class decisional index, which was validated in an independent patient set, enabled to discriminate about a quarter of patients who had an estimated overall survival of only 19% at 12 months.

Multivariate analyses performed on 909 patients with AML (AML96 study) with a median age of 67 years (range, 61-87 years) revealed that karyotype, age, NPM1 mutation status, white blood cell count, LDH and CD34 expression were of independent prognostic significance for survival. In particular, four prognostic groups were distinguished: favorable risk, good intermediate, adverse intermediate risk, and high risk. The corresponding 3-year OS rates were 39.5%, 30%, 10.6%, and 3.3%, respectively.

Weatly *et al.* developed a risk index based on the analysis of 2483 patients with AML aged > 60 years entered into two UK trials. MRC AML11 trial (n = 1071) was used to develop the index; this was validated using data from the LRF AML14 trial on 1137 intensively (AML14I) and 275 non-intensively (AML14NI) treated patients. In AML11, cytogenetic group, age, white blood count, PS and type of AML (de novo or secondary) were all highly significantly related to prognosis in multivariate analysis. The regression coefficients were used to define good, standard and poor risk groups, with 1-year survival of 53%, 43% and 16% respectively.

At MDACC 446 AML patients ≥ 70 years of age treated with cytarabine-based intensive chemotherapy were analyzed. A multivariate analysis for 8-week mortality identified the following adverse prognostic factors: age ≥ 80 years, complex karyotypes, (≥ 3 abnormalities), poor PS (2-4 ECOG), and creatinine > 1.3 mg/dL. Patients with none (28%), 1 (40%), 2 (23%), or ≥ 3 factors (9%) had estimated 8-week mortality rates of 16%, 31%, 55%, and 71% respectively. The model also predicted for differences in CR and survival rates. In this study the prognosis of most patients (72%) ≥ 70 years of age with AML treated with intensive chemotherapy was particularly poor (8-week mortality ≥ 30%; median survival < 6 months).

The same group used the hematopoietic cell transplantation comorbidity index (HCT-CI), which predicts non relapse mortality and survival post-stem cell transplantation[6] (Table 2), in 177 AML patients over 60 years of age receiving intensive induction therapy. HCT-CI score was 0 in 22% of patients, 1-2 in 30%, and > or =3 in 48%. In patients with scores of 0, 1-2, or > or =3, early death rates were 3%, 11% and 29%, while median OS was 45, 31 and 19 weeks, respectively.

More recently, a web-based risk score, able to predict the chance to achieve CR and the risk of early death in medically "fit" older patients (aged ≥60 years) with AML treated with intensive chemotherapy, has been proposed. In this study multivariate analysis identified body temperature, age, secondary disease, hemoglobin, platelet count, fibrinogen, and LDH serum concentration as factors significantly associated with CR or early death. The probability of CR with knowledge of cytogenetic and molecular risk ranged from 12% to 91%, (from 21% to 80%. without such a knowledge). The predicted risk of early death was comprised between 6% to 69% and between 7% to 63%, respectively. The predictive power of the risk scores was confirmed in an independent patient cohort. This score could be of particular usefulness for identification of patients with a very low predicted CR rate (i.e. <25%).

Interestingly, this year the Pavia group has proposed a time-dependent, modified HCT-CI score (Myelodysplastic Syndrome-Specific Comorbidity Index, MDS-CI) to evaluate the prognostic impact of comorbidity on the natural history of myelodysplastic syndromes[7]. The study population included two cohorts of 1344 patients, about 25% of whom had higher risk disease. Co-morbidity was present in 54% of patients, cardiac disease being the most frequent and the main cause of non-leukemic death. In multivariable analysis, comorbidity had a significant impact on both non-leukemic death and overall survival. Cardiac, liver, renal, pulmonary disease and solid tumors were found to independently affect the risk of non-leukemic death. A score model attributing the values of 2 to cardiac disease and 1 to each of other identified parameters, stratified the patients in three risk groups for non-leukemic death: low (0-1), intermediate (2) and high (> 2). The application of this simplified, HCT-CI-derived model to AL could represent an interesting tool to investigate in the near future.

Finally, it should be remembered that, among the possible factors affecting the therapeutic decision in older patients with AL, patient's or relative's compliance and logistic problems (i.e. distance from the Hospital) may influence the practical feasibility of a given treatment in daily practice; attitude and scientific interest of the physician may also play a relevant role in this setting.

Is geriatric assessment useful and feasible in AL patients?

The simple evaluation of physical function provides information that can be independent of comorbidity in older AL patients, given that not rarely the disease by itself represents the major cause of poor PS; in daily practice, physical function is typically assessed by using the Eastern Cooperative Oncology Group (ECOG) or Karnofsky scales. The clinical relevance in AL of PS at presentation is clearly demonstrated by the close relationship between it and mortality within 30 days of initiation of induction. In a large study from unselected patients conducted in Sweden, it was showed that the mortality rate in the AML population over 75 years with favorable PS can be lower than younger population with worse PS [8]. In addition, in this study it was also suggested that standard intensive therapy decreases rather than increases early death rate and is a prerequisite for long-term survival in most patients up to the age of 80 years.

A potential misinterpretation in AML derives from the possibility that poor PS may specifically depend on the disease by itself. In other words, PS classification in a number of cases fails to differentiate between functional impairment resulting from leukemia, which is reversible with intensive supportive treatment, compared with irreversible comorbidity unrelated to leukemia; therefore, comorbidity scoring may have a greater potential for providing basis for treatment decisions than PS, which would be in turn reassessed after correction of anemia and control of sepsis and other complications specifically induced by AML. Indeed, in a minority of cases, patients can improve with the simple reduction of leukemic burden and other supportive measures and it is hard to assess whether poor clinical conditions were dependent on disease itself, concomitant diseases or both. On the other hand, a PS > 2 not reversible after supportive treatment should represent a contraindication to any therapy aiming at CR achievement, either aggressive or attenuated.

Aging determines a reduction of the expected life and of functional reserves of different organ and systems, inducing a reduced tolerance to any type of stress, including neoplastic disorders and their treatments. Aging, however, is an individualized process that allows to people of the same age to have a life expectancy and stress tolerance very different. Thus, it is important to determine the "physiological" age of AL patients to reach a correct evaluation of the risk/benefit balance [9].

To this purpose, one well validated strategy commonly used in geriatric medicine is the Comprehensive Geriatric Assessment (CGA), which refers to a multidisciplinary evaluation of geriatric domains, able to determine the medical, psyco-social and functional capabilities of frail elder-

ly persons in order to develop a coordinated and integrated plan for treatment and long term follow up (9). While integrating standard medical diagnostic evaluation, CGA score emphasizes quality of life, functional status, prognosis and outcome that require a work-up of more depth and breadth. A through assessment of cancer patients can help to determine those who are candidates for standard treatments to prolong life, as well as those in whom the potential benefits of such treatment are out-weighted by its risk. Indeed, CGA can identify unsuspected vulnerable patients that may still benefit from adequately modified regimens; it can also identify non- medical factors that can be managed to make the treatment safer and more convenient. Frailty is therefore a guide for simple pharmacologic palliation of symptoms or low dose chemotherapy for tumor control. The NCCN guidelines recommend that all patients older than 70 years be screened with some form of geriatric assessment (10). In order to simplify CGA, a consensus panel established that such an evaluation may address at a minimum of areas, as reported in Table 3.

The clinical relevance of each of these parameters variably correlates with risk of death, chemotherapy tolerance and complications, need of a care-giver, need of adjunctive treatments, iatrogenic comorbidity, compliance with the treatment and management of chemotherapy complications. CGA is therefore useful to plan antineoplastic treatments in cancer patients, as it allows an estimation of the life-expectancy and tolerance to treatment in single patients. In addition, it allows to detect previously unknown conditions favoring not appropriate management of the patient or unsuccessful/too toxic treatments, facilitates a common definition of the elderly patient, and is useful for retrospective or prospective studies, permitting evaluation of long term results.[9]

Thus, the use of a CGA, initially developed and validated in the general geriatric population, may allow more accurate assessment of the likelihood of chemotherapy-induced complications and proactive risk minimization. The situation, however, is more complex in AL, given that CGA is time consuming, costly, and requires a multidisciplinary approach not available at many institutions for a decision that must often be taken in few days. In order to at least partially obviate to these drawbacks, some shortened forms of assessment have been developed.[9]

Take home messages

Elderly patients with AL have really a "different entity", with higher prevalence of both disease and patient-related adverse prognostic factors with respect to younger patients. While chronologic age alone is inadequate in predicting early toxic death rate and response to therapy, any effort should be made to define well-established and universally accepted criteria for the definition of unfit/frail AL patients.

The integration of PS with standardized evaluation of comorbidity, integrated in a CGA, would help in identifying patients who are less likely to benefit from standard therapies and who must receive reduced treatments or only supportive care. On the other hand, a careful evaluation allows to avoid a therapeutic nihilism that could exclude from effective therapies those patients who are instead eligible for intensive (and possibly curative) approaches, even at high age. Molecular and, above all, cytogenetic parameters also may play a role in this setting. The best way to manage these patients, however, still remains to perform any attempt to enroll them in clinical trials in which new drugs and new experimental therapeutic strategies are planned.

Perhaps the most complete definition holds aging as "loss of entropy" and "loss of fractality".[9] The former implies a progressive decline in functional reserve of multiple organs and systems and, consequently, reduced tolerance of stress, while loss of fractality correlates with a progressive decline in the ability to coordinate different activities and to negotiate the environment. In absence of precise measurements of entropy and fractality, aging is best assessed by its consequence, including progressive loss of function and emerging comorbidities.

Chronology, therefore, reflects very poorly the physiologic age of each patient, which can only be estimated on the basis of individual assessment. Thus, potentially curative therapies should not be withheld from older patients because of their age alone. The routine use of simplified CGA may provide valuable information about patient condition,

functional status and overall health risk.

Based on the CGA, it is possible to recognize patients who are functional and independent, patients who are partially dependent in one or more functional aspects with associated comorbidities, and patients who are really very frail. When facing frail patients, symptoms palliation and preservation of quality of life are the most important tasks for the health-care team.

Considering that the incidence and prevalence of changes associated with aging increases dramatically after the age of 70, it is reasonable practice for all patients aged 70 or older to have a complete CGA, but it could also indicated in persons younger than 70 years with decline in physical and cognitive performance. Pace and severity of aging is a completely individualized process that is influenced by the environment and genetic background of each individual.

Since frailty is poorly correlated with chronological age, functional ability is a better marker for initiating the multidisciplinary evaluation of older patients afflicted with a AL diagnosis and an important step to make rational treatment decisions. Decisions that, however, never should forget the patient's opinion.

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ACUTE MYELOID LEUKEMIA

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The clinical outcome of acute myeloid leukemia (AML) is extremely variable, ranging from survival of a few days to cure, and a number of clinical and biological features at presentation have been reported as useful for the prediction of final outcome. However, age represents the most relevant prognostic factor in AML, in that the prognosis of the disease steadily declines with increasing age. This is clinically relevant, given that more than half of AML diagnoses are currently made in patients aged over of 65 years. Comorbidities strictly related to advanced age as well as unfavourable biologic characteristics at diagnosis, including adverse cytogenetic findings, presence of an antecedent hematologic disorder and high rates of MDR1 expression in tumor cells account for the dismal prognosis of older patients with AML (Table 1). Of note, in advanced age unsatisfactory therapeutic results have been reported within any cytogenetic prognostic subgroup, including core-binding-factor AML and NPM1 positive/FLT3 negative AML, which in young adults are considered as the most favorable subgroup (1).

Patients' selection in AML of the elderly

Currently, in older patients conventional induction therapy results in complete remission (CR) rate in the range of 45–55%, and fewer than 10% of intensively treated patients survive for a minimum of 5 years . Most available results derive from multicenter trials based on aggressive treatment aimed at CR achievement and do not take into account a consistent proportion of elderly AML patients who are only given best supportive care (BSC) with periodic treatment with hydroxyurea (HU) to control the peripheral white blood count. Such population accounts for at least 40 % of older AML patients and, apart from anecdotal cases, median survival does not exceed few months. Accordingly, patients selected for randomized studies are not representative of the entire patient population, given that very elderly patients (> 75 years) and those with severe comorbidity often are not included in studies. Other reasons for excluding patients from clinical trial participation may be logistical aspects such as geographical distance from specialized institutions and lack of a caregiver, patient refusal, or an arbitrary decision of the treating physician. In daily practice, a combination of these factors determines relevant selection and jeopardizes the general applicability of study results, which are often diffused in the web or the media, finally inducing overoptimistic expectations in the patients and their caregivers. As an example, in a survey undertaken in the USA, 74% of patients estimated their chance of cure to be 50% or greater, but for 89% of patients the physician estimates of cure were 10% or less (2).

The search for prognostic factors in AML has been generally addressed toward biologic parameters able to predict relapse when CR has been achieved. As a consequence, in young adult patients the possibility of stratifying induction chemotherapy is not taken into account. A unique issue in elderly patients consists of establishing prognostic models for CR achievement, induction mortality, and survival rates that would be used in the clinical practice in order to avoid unnecessary toxicity not balanced by actual survival advantage. On the other hand, because aggressive chemotherapy can also result in a significant survival advantage in a minority of very elderly patients, models based on standard, readily available baseline characteristics have been developed for with the aim of predicting CR, induction mortality, and survival rates. Different studies identified consistent, independent poor prognostic factors for CR, early mortality, and survival, including age > 70-75 years, unfavorable karyotypes, poor PS, longer duration of antecedent hematologic disorder, and abnormal organ function. Recently, Krug and co-workers presented a web-based risk score application, which is able to predict the chance to achieve CR and the risk of early death in medically healthy older patients (aged ≥60 years) with AML, who were selected for treatment with intensive chemotherapy (3). In a multivariate analysis, body temperature, age, de-novo versus secondary disease, hemoglobin, platelet count, fibrinogen, and lactate dehydrogenase serum concentration were significantly associated with CR or early death. Overall, the risk scores accurately predicted the likelihood of CR between 12% and 91% with knowledge of cytogenetic and molecular risk profile, and between 21% and 80% without this knowledge. Similarly, the risk of early death was accurately predicted. While this score could be very helpful for patients with a very low predicted CR rate (i.e. <25%), for the remaining patient population the final decision will remain with the individual patient's hematologist and will be still affected by several factors including the psychological attitude of patients and their relatives, the practical feasibility of a given treatment in daily practice and, last but not least, the physician's attitude and scientific interests

Treatment

Induction and consolidation therapy. At least three different approaches are currently pursued in AML of the elderly: palliative treatment, attenuated chemotherapy and standard intensive chemotherapy. Notwithstanding, the most appropriate remains a highly controversial issue and the therapeutic choice for an individual patient is not rarely based on the physician's attitude rather than on objective criteria. If we consider the limit according to which aggressive induction and post-induction therapy is specifically designed by age, a threshold of 60-65 years seems reasonable. However, if this is true, we would suppose that large and well-designed randomized studies have succeeded in establishing an ideal induction and post-induction therapy. Unfortunately, this is not the case, and the 3-plus-7 regimen, introduced 20 years ago and based on the combination of 3 days of daunorubicin with 7 days of continuous-infusion cytarabine, remains the induction treatment of choice in AML independent of age. In a recent study AML patients aged over 60 years were randomly assigned to receive cytarabine, at a dose of 200 mg/m2 by continuous infusion for 7 days, plus daunorubicin for 3 days, either at the conventional dose of 45 mg/m2 (411 patients) or at an escalated dose of 90 mg/m2 (402 patients compared). This treatment was followed by a second cycle of cytarabine at a dose of 1000 mg per square

meter for 6 days. A clinical advantage was found in the age range 60-65 and in patients with CBF-AML, who accounted for 4 % of the entire patient population.[4]

Once CR has been achieved, there is substantial evidence that further intensive therapy is needed to prevent leukemia relapse. However, in most cases elderly patients cannot withstand the side effects of repeated courses of intensive consolidation chemotherapy as usually adopted in young or adult subjects; in addition, even though age of AML patients at stem cell transplantation (SCT) is continuously increasing, SCT is feasible in a small minority of highly selected elderly patients (see later). Finally, in older patients, the preferential leukemic involvement of stem cells, their intrinsic resistance to most cytotoxic agents and the age associated derangement of immune system, will ultimately result in a reduced efficiency of current consolidation strategies in eradicating residual disease. Accordingly, a formal study addressing the actual benefit from different options of intensive post-remission therapy has never been conducted up to now. Probably, intermediate dose cytarabine (1 gr/sqm) for 5-6 days represents a reasonable approach.

Stem cell transplantation. Reduced-intensity conditioning (RIC) regimens allow older and debilitated patients to undergo allogeneic stem cell transplantation (allo-SCT). Several studies have shown outcomes and complication rates comparable with myeloablative SCT in younger patients. However, as with myeloablative transplantations, questions have arisen as to the relevance of RIC-SCT to AML in CR1. In particular, to the extent that various selection biases lead to selection of only the "best " older patients for RIC-HSCT, the results of the procedure may be irreproducible in the vast majority of older patients in CR1. To address this issue prospectively, two studies explored the feasibility of allo-SCT in large cohorts of consecutive older patients with AML. In both studies, allo-SCT was actually given to 5 % of patients. Reasons for not allografting patients included failure to achieve CR, toxicity after induction/consolidation chemotherapy and early relapse before allo-SCT (4-5). Autologous SCT (ASCT) represents a further possibility of post-remission treatment in AML of the elderly, given that overall toxicity of the procedure has been consistently lowered since the introduction of peripheral blood SC (PBSC). Either registry data or single institution studies suggested that ASCT is feasible in older individuals with acceptable morbidity and mortality and exciting results. Notwithstanding, the proportion of patients who actually receive ASCT is not higher than 20 %; apart from poor response to induction therapy, early relapse and toxicity, failure to mobilize PBSC represents and additional obstacle to perform ASCT (6).

In conclusion, SCT can result in an improvement of therapeutic results in AML of the elderly, but it is feasible in a minority of patients, who are highly selected for best response to induction, consolidation and mobilization as well as for minor non-hematologic toxicity (Figure 1).

Attenuated therapies. Low-dose cytarabine (LD-ARA-C) has been for many years the prototype of attenuated chemotherapy however aimed at CR achievement. Previous data demonstrated that LD-ARAC is able to induce CR in about 20 % of older AML patients; hematologic toxicity of this approach is substantial and many patients experience prolonged cytopenia. The only randomized study present in the literature specifically designed for AML patients considered as unsuitable for intensive chemotherapy was conducted by Burnett and coworkers (7). 217 patients were randomized to receive LD ARA-C or HU, with respect to efficacy, toxicity, and supportive care require¬ments. No specific criteria for defining such patients were used, except that patients aged < 70 years should have a documented comorbidity that precluded chemotherapy. Overall, LD ARA-C treatment was superior to BSC in that 13 of 71 patients achieved CR, for an overall CR rate of 18%, whereas only one on the HU arm achieved CR; in addition, survival was significantly longer in the LD ARA-C arm. Of note, the benefit in survival was not seen for patients with adverse karyotype, in whom CR was never achieved. In addition, there was some evidence that patients with a poor PS did not benefit from the treatment. Therefore, LD ARA-C appears inadequate in terms of risk/benefit ratio for the really frail patient population as well as in patients with unfavorable cytogenetics. More recently, a potential benefit for older patients with low bone marrow blast count (20-30%) AML has been reported with the use of hypomethylating agents (HMA), such as azacytidine (AZA) and deoxyazacitidine (DAC). These agents are viewed as mechanistically similar DNA hypomethylating agents; however, while the two drugs share mechanisms of action on DNA-mediated markers of activity , different effects on cell viability, protein synthesis, cell cycle, and gene expression have been demonstrated. On a clinical ground, the novelty of HMA in AML relies in the possibility of disease control, without necessarily achieving CR. In addition, the risk/benefit profile of either AZA or DAC allows to offer a treatment potentially able to alter the natural history of the disease to a patient population otherwise candidate to receive BSC only. Overall, response rate (namely CR rate) seems to be superior with DAC as opposed to AZA in AML of the older patients; notwithstanding, data on OS are poorer in all DAC studies as compared to Fenaux's trial based on AZA (8). The selection of patients with hypoproliferating disease, age less than 70 years in a considerable number of patients, few high risk karyotype and, the low number of patients, do clearly account for the above difference. A head-to-head comparison of AZA vs. DAC is therefore warranted. Data demonstrating survival advantage in absence of CR are exciting and suggest an alternative mechanism of disease control, but still need a careful and definitive confirm in larger and well-conducted new trials. Furthermore, a true superiority with respect to intensive CHT or other new agents

such as clofarabine, cloretazine and voreloxin, reported as potentially useful for poor risk older AML patients remains to be definitively demonstrated.

New drugs for AML of the elderly

In the attempt to overcome the poor prognostic relevance of adverse cytogenetics in AML of the elderly, new drugs have been developed (9) with exciting results (Table 2).

Clofarabine is a next-generation nucleoside analog, active in acute leukemia at doses not associated with severe extramedullary side effects, particularly neurotoxicity, in contrast to other deoxyadenosine analogues fludarabine an cladribine. Two phase 2 multicenter studies explored the potential utility of single agent Clofarabine as front line treatment for older patients with AML unlikely to benefit from conventional induction chemotherapy. In the CLASSIC 2 study, conducted in US, eligible patients included adults >60 years with >1 adverse prognostic factor: >70 years, AHD, PS 2, and/or intermediate/unfavorable risk karyotype. Enrollment completed with 112 patients with a median age of 71 years (range 60-88). The overall response rate (ORR) was 46%, 38 % CR, 8% CRp (complete response with incomplete platelet recovery). Of note, the presence of adverse karyotype was associated with a remarkably 42% CR. The 30-day all-cause mortality was 9.8%. The most common non-laboratory drug-related toxicities (20% patients) were nausea, febrile neutropenia, vomiting, diarrhea, rash, and fatigue.

In the study conducted in Europe, a total of 106 patients were treated in two monotherapy studies. The median age was 71 years (range, 60 to 84 years) and 36% had a WHO performance score 2 or higher. All patients were considered unfit for standard intensive chemotherapy based primarily on age (> 65 years) and/or performance status. None of the patients had a favorable cytogenetic profile, with all having either intermediate (70%) or adverse (30%) cytogenetics. Forty-eight percent had a complete response (32% complete remission, 16% complete remission with incomplete peripheral blood count recovery), and 18% died within 30 days. Of note, response and overall survival were not inferior in the adverse cytogenetic risk group. The conclusion was that Clofarabine appears superior to current standard treatment (low-dose Ara-C) in both unfavourable cytogenetic AML (>65 years) and AML patients > 70 years (all cytogenetic risk groups). Once again, most patients (75 %) were in PS 0-2 according to ECOG scale. While the two above studies do clearly demonstrate the efficacy of Clofarabine in biologically unfit patients, it is clear that in both trials selected patients in favorable PS were accrued; differences into selection do probably account for the difference early death rate reported (9.8% in the US study opposed to 18 % in the European study). As a consequence, single agent Clofarabine does not seem to be appropriate to the really frail older AML population given that it results in substantial myelotoxicity with prolonged and severe bone marrow aplasia as well as in life threatening liver injury. Probably, clinically fit older patients (PS ≤ 2, age > 70 years) with unfavorable cytogenetics could represent the ideal AML population candidate to receive this drug.

Laromustine (Cloretazine) is a novel sulfonylhydrazine alkylating agent which preferentially targets the O6 position of guanine resulting in DNA cross-links. Results of an international phase II study conducted in 85 elderly patients with previously untreated poor-risk AML were reported. Patients were aged 70 years or older or older than 60 years with at least one additional risk factor such as unfavorable AML karyotype, PS of 2, and/or cardiac, pulmonary, or hepatic comorbidities. Of note, 96% of patients had at least two risk factors, and 39% had four risk factors. The overall response rate (ORR) was 32%, with 20 patients (23%) achieving CR and 7 (8%) achieving CRp. ORR was 20% in patients with adverse cytogenetics, suggesting that single agent Laromustine has the ability to induce remissions in elderly AML patients with unfavorable cytogenetics. Early death rate was 14%, and main adverse events were predominantly myelosuppressive and respiratory. Unfortunately, development of Laromustine was halted based on issues with study design as well as clinical outcomes.

Voreloxin is a first-in-class anticancer quinolone derivative, or AQD, a class of compounds that has not been used previously for the treatment of cancer. Voreloxin both intercalates DNA and inhibits topoisomerase II, resulting in replication-dependent, site-selective DNA damage, G2 arrest and apoptosis. Voreloxin is currently being evaluated in a Phase 2 clinical trial (known as the REVEAL-1 trial) in previously untreated elderly AML patients. In the REVEAL 1 study the initial dose regimen, voreloxin 72 mg/m^2 qw x 3, was established in a phase 1 dose-escalation study in relapsed/refractory leukemia pts. Overall remission rate (CR+CRp) was high (41%), but this regimen was less well tolerated in the frontline elderly population . The protocol explored 2 alternative voreloxin schedules, based on safety data from the 72 mg/m^2 cohort and from an ongoing Ph 1b/2 study of 90 mg/m^2 voreloxin d 1,4 in combination with 1 g/m^2/d cytarabine x 5d. Eligibility criteria required: newly diagnosed AML (*de novo* or secondary AML), pts age ≥ 60 with ≥ 1 additional adverse risk factor (age ≥ 70, secondary AML, intermediate or unfavorable cytogenetics, or PS 2). Best results in terms of risk/benefit ratio were achieved with the days 1,4 schedule (CR + CRp:38%, toxic death rate: 7%), 24 % CR in unfavorable cytogenetics. The conclusion was that voreloxin demonstrates clinical activity with 3 dosing schedules in previously untreated elderly (age ≥ 60) AML pts who are unlikely to benefit from standard chemotherapy. Responses were seen in each risk factor category and with multiple risk factors.

Conclusions

While current therapeutic results in AML of elderly patients remain overall unsatisfactory, research is in fast progress and future strategies would include the development of well-designed random¬ized phase II trials based on multiple outcomes and including novel target-based agents. The unfit category of older AML, either clinically or biologically defined, would represent an ideal field of clinical experimentation, given that ethical issues might allow first-line experimental clinical trials fully justified by the dismal prognosis of these patients upon conventional treatments. In the design of such studies, however, the great clinical heterogeneity, the typical multiple comorbidity of elderly patients, the psychologic attitudes of patients and their relatives, and the practical feasibility of a given treatment in daily practice need to be seriously considered to properly evaluate the global clinical effect of novel strategies. In this way, rather than merely recording response rates and survival times of new drugs or drug combinations, one might hopefully identify novel approaches actu¬ally able to modify the current practice for patients.[10]

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ACUTE LYMPHOBLASTIC LEUKEMIA IN THE ELDERLY

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The challenge of elderly ALL: long-term survival still close to 10%

Acute lymphoblastic leukemia (ALL) is a rare disease with a peak incidence in the pediatric population and a reported average incidence of 0.9/100.000 per year in adults. In the elderly this figure seems higher but nevertheless the disease is rare and clinical trials in the elderly are difficult to perform because of the increasing morbidity and mortality from other causes. The definition of elderly ALL is commonly applied above 55 years in Europe, although from this threshold to 65 years the term "older adult" would be more suitable and "elderly" more appropriate from 65 years onwards. However this difference is of little practical significance, because outcome of patients aged >55 years is uniformly poor, although general management problems become extremely serious above age 75 and in so called "frail" patients (see later). To document this point, the long-term results of a cumulative database including more than 700 patients of the Northern Italy leukemia Group (NILG) since 1979 (Figure 1) demonstrates how an increasing age impacts on 10 year survival rates, these figures being consistently below 20% for all age groups >45 years and close to 10% for those aged >55 years. These data are duplicated by others. Survival in elderly ALL changed little compared to younger patients in the past 20-25 years. It has been estimated that in patients aged above 60 overall survival at 5 years increased from 8.4% in 1980-4 to only 12.7% in 2000-4 (+4.7%), that is less than 0.25% per year, compared to an increase of 15-20% for younger age groups.[1] The large Medical Research Council-Eastern Cooperative Oncology Group (MRC-ECOG) trial indicated a survival rate of 15% at 5 years for 108 patients aged 50 years and greater. Excellent review papers on elderly ALL were published, reporting similar figures and reviewing in detail the results obtained with different treatment protocols.[2,3]

Why age matters (I): two basic limitations

There are several reasons why treatment results are so poor in the elderly. Two general concepts help understand how difficult it is treating an elderly patient. First, age acts as a continuous independent and adverse prognostic variable. Thus in pediatric ALL outcome is worse in patients aged >10 years, and in adults in those aged >35 years when this is used as cut-off value.[4] Therefore in the elderly, not only outcome is anticipated to be worse but, although differences may be minor, it is expected to worsen progressively in older age groups. The other consideration pertains to treatment intensity, that has been the mainstay of therapeutic progress in children and younger adults over the past decades. No therapeutic improvement was ever achieved without pursuing this paramount concept, in any age group. Therefore the design and interpretation of clinical trials in the elderly must take into account that, with "reduced intensity"chemotherapy as frequently used in the elderly, it may not be possible to demonstrate any improvement. Whether low toxicity, targeted therapies will change this dogma is now the major challenge in the treatment of these patients.

Endpoints and design of clinical studies for elderly ALL

Because substantial treatment intensification is basically not applicable to the elderly, several studies addressed the role of de-intensified schedules variously denominated as "age-adapted", "moderate-dose", "flexible intensity" and "outpatient therapy" programs, sometimes in small series and mostly focusing on complete remission (CR) rates and/or encouraging early survival results at 1-2 years. In general, CR rates were >60-70% and 1-year survival rates between 30-50%. All these results can be regarded with interest but also require caution in the context of a disease whose risk of recurrence persists for 2-5 years (T- vs B-cell precursor phenotype), and where any reliable therapeutic information is normally obtained after 3-5 years of follow-up in series of hundreds. The problem with randomized trials is even worse. Owing to the disease rarity phase III trials were seldom attempted. This decision requires a careful study design with adequate statistical power. A recent trial from the Group for Research on Adult ALL (GRAALL) compared continuous infusion doxorubicin to pegylated liposomal doxorubicin in induction-consolidation therapy (Hunault-Berger et al, Haematologica 2011; 96:245). Patients in each arm were only 31 and 29, respectively, and despite these small numbers the study primary endpoint was a rather complex, composite one consisting of a joint evaluation of both efficacy and toxicity at 140 days. Unfortunately, despite the relevance of the clinical question posed, no conclusion was drawn due to statistical and numerical weakness of the trial. Although randomized clinical trials are desirable in elderly ALL, they will remain very difficult to perform without the necessary patient number and the appropriate statistical design. To this purpose only international collaborative efforts can warrant a chance of success. The European Working Group on Adult ALL (EWALL, formed within the EuropeanLeukemia Net) aims to facilitate these initiatives.[5] An alternative is the adaptive design of clinical trials, applicable to both phase II and III. This allow rapid testing of several new agents, with subsequent study expansion when an active drug is identified. This method is attractive when the patient number is limited like in elderly ALL, as already demonstrated in acute myeloid leukemia (reviewed by Estey in Haematologica 2009; 94:1435).

Why age matters (II): disease subsets, treatment tolerance and comorbidities

The negative effect of higher age on outcome is immediately perceived in the clinical practice and is mediated by three independent mechanisms. First, ALL-related adverse prognostic factors predominate in the elderly compared to younger patients, whatever the definition of high-risk ALL. This is traditionally related to a significant increase in Philadelphia (Ph) positive ALL, a highly unfavorable subset before the advent of tyrosin-kinase inhibitors (TKI), and seemingly by the concurrent decrease in T-ALL incidence, the disease subset benefitting most from recent chemotherapy advancements. This point is illustrated by the risk subset distribution in a NILG trial enrolling 404 adult patients up to an age of 65 years (Figure 2A and B). The graphs show the increased incidence of HR subsets (Ph+, and Ph- HR-B and HR-T) with age, with a net prevalence >55 years. Similar data are provided by others and are rather well known. The second mechanism is a reduced tolerance to intensive treatments. Most ALL active drugs are toxic to the elderly because of the reduced drug metabolism that normally occurs with age and/or the several comorbidities that can aggravate the natural toxicity of these compounds. Apart from the inevitable case of myelotoxicity (counterbalanced by the postchemotherapy application of myeloid growth factors), anti-ALL drugs can be extremely toxic to major organs. Among induction drugs vincristine is neurotoxic (peripheral neuropathy, constipation), anthracyclines are cardiotoxic (heart failure, dysrythmias), corticosteroids are diabetogenic and L-asparaginase is hepatotoxic (liver failure and dyscoagulation including ATIII deficiency with an increased risk of thrombosis). Among consolidation drugs, high-dose methotrexate can lead to severe mucositis and kidney failure (and is not employed at >1 g/m^2 as 24 hour infusion) while high-dose cytarabine exerts central nervous system toxicity and is therefore used in the elderly at 0.5-1 g/m^2 instead of 2-3 g/m^2 like in the adult setting. Third, the chapter on comorbid diseases can be a dominant one. Because any concurrent illness of some gravity is a contraindication to inclusion into trial, the majority of elderly patients have not access to the best available treatment and receive palliative-intent regimens. This was demonstrated by population-based registry analyses, and implies that current trial results, already so dismal, are obtained in positively selected patient groups.[3] The ECOG performance scale (0-4) is a useful general tool to identify patients with a poor outlook (ECOG >2 before treatment), unless this is caused by ALL itself and is rapidly modifiable by the institution of therapy. Charlson *et al.* introduced a specific score system for the elderly, taking into account separate morbidities of all major organs as well as discrete age groups (J Chron Dis 1987; 40:373). This system is widely used in studies involving aged cancer patients and allows comparisons of results among different studies and risk groups.

Which induction therapy? Gentle is safer, as with the EWALL/GMALL schedule

An age-adapted de-intensified regimen is better tolerated and results in higher CR and early survival rates compared to adult-type unmodified schedules.[3] The latter can be associated with induction or remission

death rates of 30% and greater, mainly caused by infections, as reported by a French study (Thomas et al, Am J Hematol 2001;67:73), a study from PETHEMA (Program for the Study and Treatment of Malignant Hemopathies, Spanish Society of Hematology) (Sancho et al, Eur J Hematol 2006;78:102), and by the M.D. Anderson Cancer Center with the HYPER-CVAD regimen (O'Brien et al, Cancer 2008;113:2097). It has therefore become customary to apply reduced intensity schedules (compared to standard adult ALL regimens), aiming first to obtain high CR rates with as little toxicity as possible, to allow subsequent consolidation in uncompromised patients. One such program shared by EWALL members is derived from the German Multicenter Group for Adult ALL (GMALL) elderly study (Figure 3). This type of treatment, incorporating subsequent modifications from GMALL (see later), can yield a remarkable 75-85% CR rate, and although 3-5 year results are still missing, 2-year survival is as promising as 50% like in the most recent GMALL version. This common European protocol can allow stepwise improvements by the addition of new drugs. GMALL eventually reported a low 4% early death rate in induction. In the investigators' opinion,[3] this followed the substitution of intrathecal methotrexate (x5 weekly), which is reabsorbed and contributes to neutropenia and sepsis, with liposome-encapsulate cytarabine that has no untoward systemic effect. They also found that patients aged 75 years and less and/or with ECOG score <3 or Charlson score <4 fared better, this being once more related to lower induction mortality.

Which postinduction therapy? Putting SCT in perspective with MRD

Once in CR the expectation for the average elderly patient with Ph- ALL is to relapse within a year, even receiving EWALL consolidation blocks with methotrexate/asparaginase and intermediate/high-dose cytarabine. Owing to drug intensity limitations, with this treatment and others like HYPER-CVAD only 20% or less total patients are expected to remain in CR long-term. The use of stem cell transplantation (SCT), either allogeneic or autologous, the latter followed by maintenance like in protocol LAL 1104 from the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA), was advocated as a means to overcome these therapeutic constraints at least in fit patients aged less than 65 years, but as yet no evidence in favor of this approach emerged (as in general for autologous SCT in ALL) from any prospective trial and the indication remains both subjective and experimental. The toxicity problem is particularly cogent with allogeneic SCT, whose effect against gut, lungs, skin and liver in the form of acute and chronic graft-vs-host disease is particularly feared of in the elderly, in association with the increased infection risk. Although with some selection bias due to age ranges (median 50-56 years and range 45-68 for myeloablative conditioning and 46-73 for reduced intensity conditioning [RIC]), an interesting report reviewed the European experience with allogeneic SCT in these patients [6]. Leukemia-free survival in first CR was 40% with myeloablative conditioning and 35% for the RIC group, indicating that even with a (less toxic) RIC SCT cure may be possible for at least one third of the patients. In those aged >60 years, transplantation-related mortality was 20% with RIC, and leukemia-free survival 25% including patients in and beyond CR1.

Long-term analysis of NILG database on >700 patients with ALL (1979-2000), by age groups. Overall survival rates at 10 years are indicated. Comparative data at 5 years from MRC-ECOG study on >1500 patients were as follows: age <20 (n 234) 45%, 20-29 (n 301) 44%, 30-39 (n 217) 34%, 40-49 (n 163) 23%, >50 (n 108) 15% (Rowe et al, Blood 2005;106:3760).

A) Prevalence of patients with age >55 years in high risk (HR) group in NILG trial 09/00 (Bassan et al, Blood 2009;113:4153; Bassan et al, J Clin Oncol 2010;28:3644). B) Distribution of HR subsets according to age groups in NILG trial 09/00. HR subsets: 1) Ph+, i.e. t(9,22) or BCR-ABL1 rearrangement; 2) HR-B, i.e. WBC (white blood cells) >30, pro-B phenotype, adverse cytogenetics/genetics, late CR (any criterion); HR-T, i.e. WBC >100, pro/pre/mature-T phenotype (CD1a-), adverse cytogenetics/genetics, late CR (any criterion). Adverse cytogenetics/genetics in HR-B/T: t(4;11) or MLL-rearranged, +8, -7, del6q, t(8;14), low hypodiploidy/near triploidy, complex (>5). SR patients with none of above features. C) Treatment protocol for patients aged >60 years (NILG 09/00). D) Outcome according to MRD response in patients aged >55 years (NILG 09/00).

Therefore RIC can be considered for elderly ALL patients with an indication for allogeneic SCT. Who are these patients? To answer this question it is essential to turn to the topic of minimal residual disease (MRD). The study of MRD allows, at the individual patient level, to refine the clinical risk classification and assess the risk –if not the exact timing- of recurrence. The MRD-based information was used therapeutically in children and adult protocols, with great success. The information available for elderly patients with ALL is scanty but will be reviewed because potentially useful when critical treatment decisions are to be taken, like allotransplantation with attending morbidities (or autotransplantation). The NILG conducted a prospective MRD-guided treatment study in both SR and HR patients, including older adults aged 55-65 years, whose final treatment allocation depended solely on MRD study results (standard chemotherapy if MRD negative, allografting or autografting if MRD positive) (Figure 2C). The trial confirmed the usefulness of the MRD analysis even in older patients (Figure 2D). The study of MRD can assist the clinician facing the decision to prescribe SCT or not. Patients with complete, durable MRD response are best treated with standard chemotherapy and may thus experience a prolonged survival without the risk of SCT-related mortality. On the contrary those showing persistence or reappearance of MRD are at greatest risk of relapse and could be considered for experimental, age-adapted allogeneic SCT programs. The role of autologous SCT is undefined in ALL, however it may be used when allogeneic transplantation is not possible. Posttransplantation chemotherapy may be attempted but is usually difficult to administer to an elderly patient.

The unique case of Ph+ ALL

The data so far reviewed concern Ph- ALL. Ph+ ALL has the highest incidence in the elderly population and deserves a unique therapeutic approach. The introduction of TKI imatinib, dasatinib and nilotinib changed substantially the prognosis of this highly malignant disease, turning it into an intermediate-risk condition with 5-year survival probabilities around 40% [4]. The contribution of TKIs was oustanding, demonstrating for the first time the value of targeted therapy in ALL. Because chemotherapy-TKI associations may be toxic particularly in the elderly, GIMEMA piloted a simplified imatinib-prednisone induction with excellent results (100% CR rate), setting a reference regimen for patients definitely at high risk of chemotherapy-related complications [7]. This first study was followed by a similar one with dasatinib (Foà et al, Blood 2008;112:305a) and lastly by a rotational schema alternating imatinib and nilotinib for 6 weeks rounds (LAL 1408, GIMEMA 2011 Report, WP Leucemie Acute e Sindromi Mielodisplastiche). Despite excellent early results, TKI treatment alone does not yield high PCR negativity rates like TKI-chemotherapy combinations (>30%), hence debate per-

sists as to the best therapeutic approach (median CR duration was 8.4 months in the imatinib alone study), because PCR negative patients on TKI-chemotherapy combinations experience much longer remissions, sometimes even without SCT. For this reason EWALL piloted a modified elderly chemotherapy protocol plus dasatinib (8), obtaining an 89% CR rate (63/71), a high rate of molecular response (BCR-ABL/ABL ratio <0.1%: 56% postinduction and 71% postconsolidation; PCR negativity: 23% and 28%), and a remarkable survival rate of 54% at 2 years (70% in patients with a molecular response <0.1%). Relapses were almost always associated with point mutations (19 relapses: 15 T315I, 1 F317L, 1 V299L), which in turn were more frequent in cases with additional chromosome abnormalities. Future collaborative trials will clarify which is the best treatment option for Ph+ ALL in the elderly. Drugs, strategies and ALL subsets: balancing the good and the news

A) Schematic representation of induction and consolidation phases of European protocol (EWALL) for elderly ALL (Goekbuget et al, Blood 2008;112:304a). B) Results of the EWALL/GMALL protocol for elderly ALL.

The nihilistic mantra of incurability of elderly ALL is slowly giving up to a more optimistic view in which, provided "curable" patients are identified first (age <75 and acceptable performance profile), CR is achieved in about 80% of them and survival is 50% at 1-2 years. Good strategies were and are as important as direct therapeutic innovations. The modified GMALL induction (EWALL regimen) keeps toxicity within acceptable ranges while providing an effective early antileukemic treatment. The MRD study allows to separate chemotherapy-sensitive patients from those in whom SCT or other experimental treatment should be considered, preferably within a clinical trial, in view of the poor response to standard treatment.This is the next level of the strategy, otherwise all these patients will relapse in weeks or months. Ph+ ALL can be easily brought into CR by TKI therapy alone but then requires a more vigorous, chemotherapy-integrated approach to improve the molecular response rate which is the key to obtain a prolonged survival. These themes are subject to change due to the introduction of effective new drugs. Pegylated asparaginase, liposome-encapsulated drug preparations (vincristine, anthrayclines, cytarabine), nelarabine and forodesine (for T-ALL) are of great interest because highly active and/or less toxic than parent compounds. The shift to intrathecal liposomal cytarabine (DepoCyte) may reduce the number of prophylactic lumbar punctures and improve treatment compliance [5]. Targeted therapy with monoclonal antibodies is the next promising area. Monoclonal antibodies against CD20 (rituximab) and CD52 (alemtuzumab) antigens were added to chemotherapy in some trials (GRAALL, GMALL, HYPER-CVAD: rituximab; Cancer and Leukemia Group B: alemtuzumab), so far with uncertain or conflicting results in the elderly [4]. Others are planned (MRC: rituximab and epratuzumab). Rituximab in combination with short intensive chemotherapy (GMALL NHL 2002 protocol adopted by NILG and other EWALL participants) is highly active in Burkitt lymphoma and B-ALL. In patients aged >55 years and/or with an ECOG score >2,treatment-related toxicity was severe, and overall and disease-free survival were 37% and 54%, respectively [9]. The new bispecific monoclonal antibody blinatumomab (activating CD3+ T-cells against CD19+ ALL cells) may represent the next therapeutic breakthrough, to be successfully employed in patients with MRD+ ALL for the reinduction of a durable molecular remission [10]. New blinatumomab-based first line programs are eagerly awaited. Intergroup collaborations like those promoted by EWALL will continue to provide the best treatment opportunities to patients of all European countries, and warrant the necessary accrual rate to innovative trials specifically designed for different ALL subsets and clinical conditions.

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PATHOBIOLOGY OF PERIPHERAL T-CELL LYMPHOMAS

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Peripheral T-cell lymphomas (PTCLs) correspond approximately to 12% of lymphoid neoplasms, being definitely rarer than B-cell tumours but slighter commoner than Hodgkin lymphoma in Western Countries [1]. They represent a heterogeneous group of diseases that can be roughly sub-

divided into specified and not otherwise specified (NOS) forms. However, three subtypes represent about 70% of all cases: PTCL/NOS, angioimmunoblastic T-cell lymphoma (AITL) and anaplastic large cell lymphoma (ALCL) with or without expression of the ALK protein (ALK+ or ALK- respectively) as consequence of the t(2;5) translocation and variants.

PTCLs usually occur in the fifth-sixth decade of life, without sex predilection, more often in advanced clinical stage, with both nodal and extra-nodal involvement [1]. A haemophagocytic syndrome is at times encountered. On clinical grounds, they are among the most aggressive non-Hodgkin lymphomas (NHLs), their response to conventional chemotherapy being frustrating with 5-year-relapse free and overall survival rates not exceeding 26% and 20%, respectively. In particular, conventional chemotherapeutic regimens do not appear as effective as in B-NHLs and the addition of anthracyclins does not provide significant benefits [2]. Noteworthy, resistance to anthracyclins as well as to other conventional agents has been demonstrated in experimental models by our group [3].

In the light of the above, the better knowledge of the pathobiology of PTCLs is warranted if one aims to positively modify the outcome of these neoplasms. To this hand, our Group has been intensively working during the last few years. In 2006, we published an extensive report based on the construction of tissue micro-arrays from 193 PTCLs (148 NOS and 45 AITL) that were tested by immunohistochemistry and EBER in situ hybridization [4]. Both tumors demonstrated frequent loss of CD5 and CD7, with CD3 being the conventional marker most commonly expressed in NOS, and CD2 in AITL. CD4 was detected in 46% of cases and CD8 in 15% of cases. Interestingly, a huge number of PTCLs/NOS and AITLs (55%) turned out to be either CD4/CD8 double-negative or, more rarely, double-positive. Such profiles, which are usually observed during intra-thymic T cell development, had previously been reported in isolated PTCL cases and a proportion of cutaneous T-cell tumors. This study demonstrated that in the setting of PTCLs there is no marker that – like the Ig light chain restriction of B-cell lymphomas – can surrogate a clonality assay. Else, it is the aberrancy of the global profile that supports the neoplastic nature of a given population. Interestingly, in this study high Ki-67 expression, EBV positivity and CD15 staining were associated with the worst outcome among PTCLs/NOS. No other phenotypic marker alone or in combination was associated with a poor prognosis, although patients with tumors expressing a CD57 or CD4+/CD8– profile showed a tendency towards a more favorable course. Based on such observations and previous publications in the literature, a new score for PTCL/NOS was developed integrating patient- and tumor-specific characteristics (age ≥60 years, high performance status, elevated LDH values, and Ki-67 marking >75%): this score identified three clear-cut groups of patients with different prognosis and seems to be more effective than previous indices, including the IPI and PIT [4].

In 2007, we published the first comprehensive study illustrating the gene expression profile (GEP) of PTCL/NOS [5]. It was based on 28 lymph node biopsies containing an amount of neoplastic cells exceeding the 70% value of the whole examined population. The messenger RNA extracted from these cases was hybridized on the HG U133 2.0 Plus gene chip. The results obtained were compared with those of six AITLs, six ALCLs (two ALK+ and four ALK-) and 20 samples of normal T-lymphocytes, which had been purified from the peripheral blood and tonsil and corresponded to the main T-cell subsets (CD4+, CD8+, resting and activated). Such study significantly differed from the previous ones of Martinez-Delgado et al. [5] and Ballester et al. [6] who had evaluated very heterogeneous cases, often containing a prominent reactive component that had influenced the global signature. Notably, for the first time, our study provided the rationale for possible targeted therapies in PTCL/NOS by offering clear evidence of their ex vivo effectiveness. In particular, GEP indicated that PTCL/NOS are distinct from normal T- and B-lymphocytes and are more closely related to activated rather than resting T-cells. As in normal mature T-lymphocytes, it was possible to identify two main subgroups of PTCL/NOS, with GEPs related to either CD4 or CD8 elements. Notably, this characteristic did not reflect the expression of CD4 and CD8 molecules. More importantly, two small subsets were identified provided with cytotoxic and follicular T-helper (FTH) profile. The former was shown to herald a very poor prognosis by Iqbal et al. two years later [7]. The latter will be further discussed in the following.

In addition to histogenetic information, our analysis provided several insights into the functional alterations of PTCL/NOS. A careful comparison of PTCLs/NOS with the closest normal counterparts revealed the systematic deregulation of 155 genes controlling functions that are typically damaged in malignant cells, such as matrix remodeling, cell adhesion, transcription, proliferation and apoptosis. In particular, our findings might explain the dissemination pattern of PTCL/NOS, with frequent extranodal and bone-marrow involvement and spread to peripheral blood, by showing the up-regulation of genes that promote local invasion and metastasis in different types of human cancer. In addition, it revealed the deregulation of genes involved in apoptosis (e.g., MOAP1, ING3, GADD45A and GADD45B) and chemo-resistance (such as CYR61 and NNMT). Immunohistochemistry provided in situ validation of the genomic data by showing correspondence between messenger RNA and protein expression, as seen, for example, with PDGFRalpha and BCL10. In addition, by comparison with normal tissues, immunohistochemistry allowed the identification of staining patterns corresponding to the synthesis of ectopic or para-physiological products by neoplastic cells. Finally, the phenotypic test highlighted the possibility that some of the results obtained by GEP may depend on non-neoplastic components present in the analyzed sample, as seen for Caldesmon. In the course of the same study, we found that all ALCLs tended to cluster together – irrespective of their ALK positivity or negativity – showing a signature distinct from those of PTCL/NOS and AITL. Some of these findings have been the object of further research activities and will be detailed in the following.

In the same year and almost at the same time, our own and De Leval's GEP analyses revealed that AITL has a gene signature that is indeed close to that of FTH cells, i.e. of T-lymphocyte taking part in the regulation of the germinal center B-cell life [8,9]. This explains why AITL expresses CD10, Bcl-6, CXCL13, PD1, ICOS, SAP, and CCR5 in variable combinations. In fact, such molecules are physiologically carried by FTH lymphocytes. This was regarded as a tool for the straightforward differentiation of AITL from PTCL/NOS and the staining for one of the above mentioned markers as the diagnostic proof of AITL. Unfortunately, both concepts turned out wrong. In fact, further studies carried out by our Group have demonstrated on a large series of cases that the FTH phenotype can also be found in tumours that lack the hallmarks of AITL, i.e. hyperplasia of follicular dendritic cells and high endothelium venules and are thus classified as PTCLs/NOS [10]. This suggests that a new histogenetically homogeneous category of PTCLs can be envisaged that includes morphologically different pictures. In addition, on immunohistochemistry the expression of one single FTH marker is not enough to sustain the derivation of the process from FTH-related cells: in fact, at least three of these markers must be simultaneously detected since they can be singly expressed as the result of cell plasticity [11]. In the course of the GEP study on AITL, in agreement with De Leval et al. [9], we observed up-regulation of the VEGF gene. However, by immunohistochemistry on tissue microarrays, we showed that neoplastic cells strongly express both VEGF and its receptor KDR. This fact suggests the possible existence of an autocrine loop and sensitivity to anti-angiogenetic drugs.

Three additional findings merit attention. Firstly, we found that PTCL/NOS presents global down-regulation of NF- B genes in comparison with normal T- lymphocytes [3]. This observation was corroborated by a subsequent SNP array study carried out in cooperation with Martin Hansmann's Group [12], as well as by the consistent cytoplasmic localization of NF- B molecules, the latter moving to the nucleus in the case of NF- B pathway activation. Our data differ to some extent from those reported by other Groups that displayed up- or down-regulation of NF- B molecules, with possible prognostic implications, not confirmed in our series [5,6]. However, such discrepancies might reflect the fact that other studies included a limited number of PTCLs/NOS or anyway cases with prominent non-neoplastic components. Secondly, since silencing of certain genes (such as GADD45A and GADD45B) can be regulated by epigenetic mechanisms including acetylation, we tested a histone deacetylase inhibitor against PTCL/NOS primary cells. Notably, the compound induced dramatic G0–G1 cell cycle arrest and apoptosis at therapeutic concentrations, suggesting a possible role for this class of drugs in PTCL/NOS therapy. This issue has been developed by Owen O'Connor and co-workers: their results that will be the object of a presentation at the next ASH meeting, support the efficacy of histone deacetylase inhibitors in the treatment of PTCLs (personal communication). Thirdly, the regular detection of PDGFRalpha over-expression at the messenger RNA and protein levels, as well as its consistent phosphorylation prompted us to design ex vivo experiments aimed testing the sensitivity of PTCL/NOS cells to Imatinib, a well-known PDGFRalpha inhibitor. The results obtained were of interest, with about 50% cytotoxic effect seen at 48 h with a 1 mol concentration. Such an effect

became even higher (75%) with a 10 mol dose. Notably, Imatinib exerted a limited effect on the viability of normal lymphocytes.

PDGFRalpha has been one of the main objects of our most recent research activity. In fact, the same alteration has been found in T-cell tumors other than the NOS and AITL ones, such as extranodal NK/T-cell lymphoma nasal type, mycosis fungoides and ALCL, thus suggesting that it can represent an important pathogenetic mechanism. For this reason, we sequenced the whole gene and promoter and looked for rearrangements or amplifications by FISH and ad hoc constructed probes: neither mutations nor chromosomal abnormalities at the PDGFRalpha locus were found. This observation, further supported by the above mentioned SNP array study, prompted us to consider the possibility of an autocrine loop, as previously shown in breast cancer. With this in mind, we focused on the expression of PDGF ligands. In particular, the gene encoding for PDGFa was found to be over-expressed in neoplastic cells by comparison with normal T-lymphocytes as its plasmatic levels were significantly higher in PTCL patients than in healthy individuals. Once again, the molecular data were supported by the immunohistochemical determination of PDGF ligands in TMAs from 200 PTCLs. At this point, we adopted the PTCL-derived FE-PD cell line as a model. The ELISA assay demonstrated a significantly higher concentration of PDGFa in the supernatant of the cell line. The usage of anti-PDGF ligands neutralizing antibodies produced PDGFRapha de-phosphorylation in a dose dependent manner as demonstrated by flow cytometry. Notably, no effect was observed in the EOL1 cell line where PDGFRalpha activation is due to the fusion gene FIP1L1/ PDGFRalpha. The hypothesis of an autocrine loop was further supported by the restoration of phosphorylation by the addiction of PDGFa as well as by transfection experiments. Notably, the neutralizing antibodies produced an important effect downstream by causing de-phosphorylation of STAT5. This is in keeping with the observed up-regulation of the corresponding gene and – to a lesser extent – of the one encoding for STAT1. Both these signal transducers and activators of transcription are downstream PDGFRalpha in contrast to STAT3 which is affected by PDGFRbeta. Interestingly, the gene encoding for the latter is down-regulated in PTCL/NOS cells by comparison with normal T-lymphocytes. A further hint to an autocrine mechanism was provided by the dramatic reduction of proliferation in the FE-PD cell line following PDGFRalpha and STAT5 de-phosphorylation. Last but not least, since activation of STAT5 promotes proliferation and rescue from apoptosis, in PTCLs the PDGFRalpha pathway might vicariate the NF- B one that is instead of pivotal importance in B-cell lymphomas.

All these pieces of information further sustain the original ex vivo observation concerning the potential therapeutic efficacy of TKIs. On this respect, in co-operation with Lukas Kenner's and Giorgio Inghirami's Groups, it was found that ALCL (both ALK+ and ALK-) is also highly sensitive to Imatinib [13]. This was shown in the mouse model and a limited number of patients refractory to all therapies with either ALCL or PTCL/NOS. Imatinib produced regression of the transplanted tumor in mice and disease stabilization or even complete remission in humans.

Combined GEP and TMA studies have provided additional relevant contributions as to the in vivo administration of the humanized monoclonal antibody Campath-1H (Alemtuzumab) targeted to CD52, repeatedly proposed for the treatment of patients with PTCL. Although other factors can affect its response in vivo, the lack of CD52 expression may play a major role in causing refractoriness to the compound [14]. We studied the expression of CD52 on tissue microarrays containing 97 PTCLs/NOS [14]. In addition, in 28 cases for which frozen material was available, GEPs were generated and compared with those of 20 samples of normal T-lymphocytes. We found that 60% of PTCLs/NOS showed CD52 gene expression level at least two log lower than the lowest one recorded in normal T-cells. In addition, the gene product was detected by immunohistochemistry in 41% PTCLs. Based on these findings, we think that the estimation of CD52 expression may provide a rationale for the selection of patients with higher probability of responding to Alemtuzumab, by avoiding the risk of unwanted toxicity.

In 2010, the same combined GEP and TMA approach was applied to a large series of ALK+ and ALK- ALCLs with frozen material available [15]. This study aimed to definitely clarify whether or not ALK- ALCL should be lumped with PTCL/NOS, as suggested at the time of the last WHO Classification drafting in spite of the fact that the International PTCL Trial had reveled that ALK- ALCL – although more aggressive than the ALK+ form – has 5-year failure-free and overall survival rates that are significantly better than PTCL/NOS. The profiles of ALK- ALCL were compared to other PTCLs, and 14 genes were discovered capable to distinguish ALK- ALCL from PTCL-NOS and AILT samples. Unexpectedly, all 14 ALK predictors were similarly expressed by ALK+ ALCL, suggesting the existence of a common ALCL signature. This hypothesis was subsequently confirmed comparing all ALCL samples to PTCLs. A class prediction analysis led to the identification of an overlapping list of genes which included 34 probes. The new classifier clearly separated ALCLs from PTCLs/NOS, AITLs and normal T-cells. The identified fingerprint was confirmed by Q-RT-PCR in independent cases using 4 targets (TNFRSF8, SNFT, NFATC2, and PERP), which resulted differentially regulated in ALCL patients. As predicted, also the immunostaining revealed weak/rare expression of NFATC2 in ALCL, while it was consistently expressed in the neoplastic compartments of PTCL/NOS samples. Notably, by massive parallel sequencing Feldman et al. have recently observed the occurrence of the $t^{(6;7)}$(p25.3;q32.3) translocation in about one third of ALK- ALCLs causing downstream the same effects as t(2;5) in ALK+ ALCLs [16].

The most recent developments of our research activity deal with gene expression and microRNA profiling and deep sequencing of PTCLs.

GEP studies have for a decade found a major limitation in the need for cryopreserved material. This has obviously affected the clinico-molecular correlations with special reference to rare tumors as PTCLs actually are. In fact, it is indeed difficult to collect series large enough to achieve high statistical power. We have recently applied the novel DASL technology from Illumina to 144 PTCLs. Such technique allows profiling of formalin-fixed, paraffin-embedded tissue samples by using a pool of probes spanning about 50 bases that make possible to analyze partially degraded RNA. Interestingly, all the observations published in the Journal of Clinical Investigation in 2007 [3] by frozen samples, were indeed confirmed. This – for instance – regarded the malfunctioning of the NF- B pathway. In addition, by supervised analysis signatures could be constructed clearly differentiating PTCLs/NOS, AITLs and ALK+ and ALK- ALCLs, which over all showed different regulation of relevant cellular programs. Interestingly, by comparing the signature of the different subsets of normal T-lymphocytes with that of the neoplastic cells, PTCL/NOS clusters could be identified corresponding to activated central memory T-cells, cytotoxic T-lymphocytes and FTH elements. In the same series of cases, the microRNA profile was studied by using the Applied Biosystem card A.

While the unsupervised analysis did only distinguish neoplastic cells from normal T-lymphocytes, the supervised one revealed up-regulation of miR-146 and miR-222 in ALCL and differential expression of 7 miRNAs between AITL and PTCL/NOS and 26 miRNAs between the latter and ALK- ALCL. Notably, by gene set enrichment analysis the microRNA profile turned out to significantly impact the GEP. These preliminary results demonstrate the feasibility of high-tech studies by using archival material and the usefulness of the combined evaluation of gene expression and microRNA profiles.

As to the next generation sequencing approach, at the moment we are enrolling patients who agree to provide both their normal and pathological DNA according to the guidelines of our Ethical Committee. The former is obtained from saliva, peripheral blood or skin shave biopsy, depending on the clinical manifestations and disease spread in each single patient.

By the Illumina HiScan SQ platform different types of analysis are ongoing: whole genome, whole exome and transcriptome sequencing. The aim is to identify driving mutations which can better explain the pathobiology of PTCLs and allow the development of novel therapeutic options as already happened for hairy cell leukemia.

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TREATMENT AND NEW DRUGS

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Peripheral T-cell lymphoma (PTCL) represent 8-12% of all non-Hodgkin lymphomas.[1,2] Although low, this figure is not very different from that of e.g. Hodgkin or mantle cell lymphoma. However, what uniquely characterizes PTCL as compared to other rare types of lymphoma is the marked heterogeneity between the single entities belonging to this group of lymphoid neoplasms. This probably explains the discrepancy in outcome observed between early PTCL studies, which attempted to evaluate the prognostic significance of T-cell phenotype as compared to their B-cell counterparts.[3,4]

Standard CHOP and CHOP-like regimens

With the exception of alk-pos ALCL, outcomes with CHOP in PTCL have been modest, with encouraging ORR of 60-70%, but subsequent OS rates at 5 years in the range of 25-35% and even lower progression-free survival (PFS) values. Nevertheless, CHOP or CHOP-like chemotherapy is still largely considered to be the standard treatment for PTCL outside clinical trials. Despite the modest results of CHOP, no studies have directly compared it to other regimens in the initial treatment of PTCL. In the International T-cell Lymphoma Project, the outcome of PTCL-not otherwise specified (PTCL-NOS) patients, who received anthracycline-based combination chemotherapy was compared to that of those, who received non-anthracycline containing regimens. No difference in OS could be detected,[5] suggesting that anthracycline-containing chemotherapy may not be the optimal combination in PTCL. However, this data should be interpreted very cautiously, since it was collected retrospectively from the hospital files of a large number of centers throughout the world covering a period of time of approximately two decades.

Intensified CHOP or CHOP-like regimens without stem-cell transplantation

The intensification of standard CHOP chemotherapy by either shortening the chemotherapy intervals, increasing its dose and/or adding additional drugs has been attempted. Due to their more frequent occurrence, the conclusions drawn from the results of these studies are most applicable to the subtypes PTCL-NOS, angioimmunoblastic T-cell lymphoma (AITL), and ALCL.

A retrospective subset analysis of the PTCL cases included in different randomized high-grade NHL trials performed by the German Study Group on Aggressive Non-Hodgkin's Lymphoma[6] showed that young good-risk patients (18) had an improved 3-year event-free survival (EFS) (71% vs 50%, P = .01) when etoposide was added to CHOP-14 or CHOP-21.[6,7] A fraction of these patients had ALCL, but their alk-protein status was not known. Another retrospective analysis described the experience at M.D. Anderson Cancer Center with the management of treatment-naïve PTCL within the period 1996-2002. Outcome data for the CHOP regimen were compared with more intensive regimens such as Hyper-CVAD (cyclophosphamide, mesna, doxorubicin, vincristine, prednisone, methotrexate, cytarabine), Hyper-CHOP, and a schedule alternating 3 regimens: ASHAP (doxorubicin, methylprednisolone, high-dose cytarabine, cisplatin), M-BACOS (methotreaxate, bleomycin, doxorubicin, cyclophosphamide, vincristine, methylprednisolone), and MINE (mesna, ifosfamide, mitoxantrone, etoposide).[8] There was no significant difference in 3-year overall survival (OS) between patients treated with CHOP and the intensive regimens: (i) 62% versus 56%, respectively, for all PTCL including ALCL, and (ii) 43% versus 49%, respectively, after the exclusion of ALCL. The same authors reported the results obtained with a modification of the Hyper-CVAD regimen using HCVID DOXIL (doxorubicin HCl Liposome Injection) in a PTCL cohort lacking alk-positive ALCL cases.[9] Preliminary results in 38 patients showed a high ORR of 87%, but the CR rate was similar (59%) to that obtained in their previous study. PFS and OS values of HCVID DOXIL as compared to the historical CHOP-treated cohort awaits longer follow-up.

Based on piloting observations of high single agent activity in PTCL, gemcitabine has been investigated as single agent or as part of combination regimens in both previously untreated and recurrent disease.[10,11] In the front-line setting, the intensive CHOP-EG (cyclophosphamide, doxorubicin, vincristine, prednisone, etoposide, gemcitabine) regimen was feasible in 26 patients with PTCL.[12] At a median follow-up of 1 year, 77% of the patients were alive; however, the median event-free survival was only 7 months, suggesting that remissions were not durable.

With regard to subtype-specific treatment strategies, relevant therapeutic findings have been reported particularly for the NK/T-cell lymphoma, nasal type. This entity shows a characteristic geographic distribution with endemicity in Asia and South America, whereas it is very rare in other populations. Most nasal NK/T cell lymphomas present with localized stage I/II disease and front-line radiotherapy is a mainstay for successful treatment outcome.[12-17] Chemotherapy is indicated for advanced stage disease, but due to expression of p-glycoprotein, resulting in multi-drug resistance, conventional anthracycline-based chemotherapy is often unsuccessful.[12] Several reports have described an efficacy of L-asparaginase in this rare lymphoma entity.[18-20] Other potentially effective drugs include alkylating agents, methotrexate, and epipodophyllotoxins. Based on these considerations, the Asian NK-cell Tumor Study Group has launched the new combination chemotherapy regimen SMILE (steroid, i.e. dexamethasone, methotrexate, ifosfamide, L-asparaginase and etoposide). It yielded an ORR of 67% in a recent phase I/II trial.[21]

Chemo-immunotherapy

Analogue to rituximab in B-cell lymphomas, monoclonal antibodies have also been applied in PTCL. One of the largest clinical experiences in the 1st line setting has been gathered with the anti-CD52 monoclonal antibody alemtuzumab (ALZ), either as monotherapy or in combination with chemotherapy. ALZ has shown activity in PTCL[22,23] as monotherapy in PTCL, not inferior to that of recently tested novel drugs. However, ALZ is more immunosuppressive than rituximab and requires careful monitoring for infectious complications.

In a multicenter phase II trial, Gallamini et al combined low-dose alemtuzumab (10 mg) with four-weekly CHOP for newly diagnosed PTCL.[24] Twenty-four consecutive patients were enrolled. Histology was confirmed in only 19 patients: PTCL-NOS (n=8), AITL (n=7), alk-negative ALCL (n=3), and enteropathy-type (n=1). After preliminary safety was demonstrated in the first 4 patients, the next 20 patients received 8 cycles of CHOP-28 with alemtuzumab at 10 mg intravenously on day -1 of each course. CRs were seen in 17 of 24 (71%) patients. At a median follow-up of 16 months, 13 of the 24 patients (54%) were disease-free with an estimated 2-year OS and failure-free survival of 53% and 48%,

respectively. Reported infectious episodes included 1 J-C virus reactivation, 1 aspergillosis, 1 staphylococcal sepsis + pneumonia as well as cytomegalovirus reactivation. ALZ was also given in combination with dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) in an ongoing trial at the National Cancer Institute for PTCL.[25]

New drugs

While current salvage regimens show some promise, what is more exciting is the expanding number of new drugs being studied specifically in PTCL. In particular, available drugs such as denilleukin diftitox, alemtuzumab, and gemcitabine[10,11,22,23,26,27] are being included in combination regimens for PTCL. While these new uses of approved drugs are adding to an elongating list of useful or promising therapies for PTCL, there are currently only two drugs specifically studied and FDA-approved for the treatment of relapsed/refractory PTCl: pralatrexate and romidepsin.[28,29] Pralatrexate is a novel antifolate designed for higher affinity for RFC-1 (reduced folate carrier) and increased polyglutamation, resulting in increased internalization and retention of the drug in tumors. A multicenter registration phase II study of pralatrexate in 111 relapsed or refractory PTCL has confirmed an overall response rate (ORR) of 29% including 11% of complete response.[28] Romodepsin, a histone deacetylase inhibitor (HDACi), inhibits enzymes that regulate acetylation of core necleosomal histones as well as other proteins. An international prospective multicenter phase II of romidepsin in 130 relapsed/refractory PTCL has recently completed reporting an ORR of 30% and a CR rate of 16%.[29] Other monoclonal antibodies being studied for PTCL include several anti-CD30 antibodies. CD30 is uniformly expressed in anaplastic large cell lymphoma (ALCL) and in approximately 30% of cases of PTCL not otherwise specified. Recently, an antibody-drug conjugated brentuximab vedotin (SGN-35) delivers the highly potent antimicrotubule agent monomethyl auristatin E (MMAE) to CD-30-positive malignant cells by binding specifically to CD30 on the cell surface and releasing MMAE inside the cell via lysosomal degradation. In particular, a phase II international multicenter study in 58 relapsed/refractory ALCL patients showed an ORR of 87% with a CR rate of 57%.[30]

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TRANSPLANTATION FOR T-CELL LYMPHOMA

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Patients affected by mature T-cell lymphomas, other than anaplastic large cell lymphomas (ALCL) with anaplastic lymphoma kinase (ALK) expression, have a 5-year overall survival of approximately 25% with conventional chemotherapies. In fact, most of them do not benefit from anthracycline-based therapy as a part of their induction treatment or from abbreviated chemotherapy intervals [(1)]. To improve the poor results obtained with conventional chemotherapy, autologous stem cell transplantation (auto-SCT) has been offered to these patients as consolidation of first remission or in relapsed disease. Different studies have shown that only patients in first complete remission appear to achieve long-term remission with auto-SCT [(2)]. However, autoSCT gives none or minimal

survival benefit in patients with refractory or relapsed disease [3].

In the last ten years, allogeneic stem cell transplantation (allo-SCT) has been more frequently investigated in patients with relapsed disease and a graft-versus-T-lymphoma effect has been postulated. In 2004, we firstly reported encouraging results in a small pilot study: 12 of 17 PTCL patients were alive and in CR after alloSCT performed for relapsed disease [4]. In addition, Le Gouill et al. recently analysed the long-term outcome of 77 patients with several subtypes of PTCL and observed an overall survival of 57% at 5 years [5].

We now expanded our original observation and thus we have retrospectively evaluated the long-term outcome of 52 patients receiving allo-SCT for relapsed disease. Histologies were PTCL-not-otherwise specified (n=23), anaplastic large-cell lymphoma (n=11), angioimmunoblastic T-cell lymphomas (n=9) and rare subtypes (n=9). Patients were allografted from matched related siblings (n=33, 64%) or alternative donors (n=19, 36%) following a reduced-intensity conditioning (RIC) regimens including thiotepa, fludarabine and cyclophosphamide. Most of the patients had chemosensitive disease (n=39, 75%) and 27 (52%) failed a previous auto-SCT. At a median follow-up of 67 months, 27 of 52 patients are alive (52%) and 25 (48%) died [n=19 disease progression, n=6 non-relapse mortality (NRM)]. The cumulative incidence (CI) of NRM was 12% at 5-years. Extensive chronic graft-versus-host disease (GVHD) increased the risk of NRM (33% versus 8%, p=0.04). The CI of relapse was 49% at 5-years, influenced by disease-status (p=0.0009) and treatment lines (p=0.007). Five years OS and PFS were 50% (95%CI, 36% to 63%) and 40% (95%CI, 27% to 53%), respectively. Eight of 12 patients (66%) who received donor-lymphocytes infusions for early disease progression had a clinical response. At multivariable analysis, refractory disease and age over 45 years were independent adverse prognostic factors. The long-term follow-up indicates that RIC allo-SCT is an effective and feasible salvage treatment with a better outcome for younger patients with chemosensitive disease. Future studies should be focused on incorporating new drugs and allo-SCT in the salvage setting of PTCL.

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CLINICAL AND BIOLOGICAL RISK FACTORS FOR VENOUS THROMBOEMBOLISM IN CANCER PATIENTS

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Patients with cancer have an increased risk of thrombosis of both the venous and arterial side. Venous thromboembolism (VTE), which includes pulmonary embolism (PE) and deep-vein thrombosis (DVT), has been extensively studied and is a recognized major cause of morbidity and mortality in cancer patients[1] The incidence of VTE in cancer populations has been estimated at approximately 1 in 200, 5-fold higher than that of the general population,[2] and active malignant disease has been shown to be an independent risk factor for VTE [3]. Overall, 18-29% of all VTE events in the general population are associated with cancer.[4,5]

Epidemiological and population-based studies provide convincing data on the incidence and risk factors of VTE. The incidence of VTE among hospitalized cancer patients is increasing. Studies based on hospital discharge have reported overall VTE incidences of 0.6–7.8% in patients with cancer, more than double the incidence of VTE in patients without cancer.[1,6,7] A large retrospective analysis reported a significant 28-36% increase in VTE events in this population over the period 1995-2003 (p<.0001 for the trend).[8] The increased risk of VTE associated with cancer is also reflected in an increased likelihood of treatment failure.[9] However, the incidence of VTE is probably underestimated by retrospective, population-based studies that report only symptomatic or objectively confirmed VTE events. Interestingly, among cancer patients, those incurring in a VTE event present with a worse survival outcome compared to thrombosis-free subjects.[1,6,10,11] There is a reciprocal pathophysiological link between malignancy and thrombosis, by which the malignant disease predisposes to thrombosis, and vice versa the thrombotic process favors the tumor progression.[12] The underlying mechanisms are not entirely understood and are under active investigation. Among other factors, a prominent role is played by tumor cell-specific clot promoting properties, which may contribute to thrombosis as well as to the process of tumor growth and dissemination.[13] The principal mechanisms include the expression of issue factor (TF) by tumor cells, the production of microparticles (MP) and inflammatory cytokines by tumor and/or host cells, and the direct adhesion of tumor-cells to platelets, leukocytes, and endothelial cells. In addition to their primary role in hemostasis and blood coagulation, proteins and cellular components of the hemostatic system may play several roles in tumor neoangiogenesis and metastasis formation.[13,14] Recently, molecular studies of experimental models of human cancer (i.e.: hepatoma, brain tumors and colon cancer) demonstrate that oncogene and repressor gene-mediated neoplastic transformation (e.g. activation of MET, loss of PTEN, induction of K-ras and loss of p53) activate clotting as an integral feature of neoplastic transformation[13] As a result of this procoagulant background ambient, a subclinical activation of blood coagulation is commonly present in patients with cancer, even without thrombosis. This condition, namely the 'hypercoagulable state', is characterized by alterations of the levels of circulating biological markers of clotting activation, as demonstrated by numerous studies.[15] The contribution of each of these biomarkers to the clinical risk of thrombosis in these patients is yet undefined, however a number of these parameters are starting to be incorporated in predictive models to identify patients at high VTE risk. The identification of such patients is essential for selecting the subjects, who primarily deserve prophylactic measures to prevent thrombosis, particularly before starting anti-cancer therapies or invasive procedures.

Clinical risk factors

The risk of VTE in patients with cancer steadily accumulates with each additional patient-related thrombotic risk factor. A series of clinical risk factors have been identified by epidemiological studies and include: 1. General factors; and 2. Cancer-related factors. The demographic and general risk factors contributing to the VTE rate are listed in Table 1. General factors are very common among cancer patients, for example, advanced age, prolonged immobility, a prior history of VTE, and comorbid conditions.[6,9,16] The contribution of these factors to cancer-associated thrombosis is not different from that to non-cancer-associated thrombosis.

In addition to general factors, there are risk factors which are distinctive of malignancy. Table 1 lists a number of factors strictly related to the disease, which independently increase the cancer-associated VTE risk, such as the type of cancer, the disease stage, the treatments with chemotherapy or antiangiogenic drugs, the use of erythropoietc growth factors.[1] The type of cancer appears to be relevant. In particular, the rate of VTE is consistently higher in patients with cancer of the pancreas, stomach, brain, kidney, uterus, lung or ovary, and hematological malignancies (i.e. lymphoma and myeloma disease.[17,18] Registry surveys and large epidemiological studies based on hospital discharge data have confirmed earlier autopsy series that the cancer diagnoses most strongly associated with the development of VTE are tumors of the brain, pancreas, ovary, lung, and uterus, and leukemia.[6,19] Also, the extent of malignant disease is an important risk factor for VTE. Different studies have shown an increased risk of VTE in patients with advanced-stage cancer.[17] An analysis of data from the California Cancer Registry demonstrated that metastatic disease is the strongest predictor of VTE complications.[10] Similar results have been reported by Blom et al, where cancer patients with distant metastases had almost a 2-fold increased risk of VTE compared with those without metastases.[19]

Many standard anticancer treatment strategies, including both surgical procedures and non-surgical treatments, have been shown to increase the risk of VTE complications.[20]

Surgery is a well-known risk factor for VTE in patients without cancer. Cancer patients undergoing surgery are at increased risk (i.e. 3-5 fold) for postoperative thrombosis compared to surgical non-cancer patients,

and this risk can persist for up to 7 weeks after the procedure. Data from the observational surgical @RISTOS registry identified VTE as the most common cause of death 30 days after surgery in cancer patients (46%), followed by cancer progression (12%).[21]

Non-surgical anticancer treatment strategies, including chemotherapy, adjuvant chemotherapy, hormonal therapy, antiangiogenic agents, and combination regimens, are also associated with a high incidence of VTE. Chemotherapy, either as primary or adjuvant therapy, significantly increases the risk of VTE complications in patients with cancer.[22] Hormone therapy, in combination with chemotherapy, significantly increases the incidence of VTE in women with breast cancer. Several studies have indicated that women who received tamoxifen had a 1.5- to 7.1-fold increase in the risk of developing symptomatic VTE, compared with placebo or no treatment.[23]

Differently, anastrozole, a third-generation aromatase inhibitor, may be associated with a lower risk of VTE complications than tamoxifen. It represents an alternative agent for adjuvant treatment in women with early breast cancer and a low risk of recurrent tumors.[24]

New antiangiogenic agents, such as bevacizumab, the monoclonal antibody to vascular endothelial growth factor, have shown efficacy in improving survival rates among patients with advanced disease. However, the addition of bevacizumab to chemotherapy regimens is associated with a high incidence of thrombotic events.[25] Similarly, new cancer treatment regimens involving thalidomide or lenalidomide are associated with an increased risk of VTE when used concomitantly with chemotherapy or corticosteroids in patients with multiple myeloma.[18, 26]

Long-term central venous catheters (CVC) have considerably improved the management of cancer patients. A number of retrospective studies have indicated a high risk of CVC-related thrombosis in malignancy.[27] However, recent randomized controlled trials in cancer patients have reported lower rates of CVC-related thrombosis[28]

The use of recombinant human erythropoietin (EPO) and other hematopoietic growth factors, such as granulocyte-macrophage colony-stimulating factor and granulocyte-colony stimulating factor, as supportive therapy in cancer patients appear to increase the risk of VTE [29]. The use of EPO and white cell growth factors has been shown to be independently and significantly associated with VTE risk among patients on chemotherapy.[30] Transfusions were also found to be associated with an increased risk of in-hospital mortality.[31]

Biological risk factors

Abnormal levels of a number of molecules derived from the activation of the coagulation cascade exist in patients with cancer. Furthermore, alterations of the vascular cells, including endothelial cells, leukocytes, and platelets, showing an 'activated' status with exposure of the cellular prothrombotic properties have been demonstrated in this condition.

About half of all patients with cancer, and as many as 90% of those with metastases, exhibit abnormalities in one or more routine coagulation parameters [15]. The development of novel laboratory tests with increased sensitivity has enabled the detection of subtle alterations in the hemostatic system. Levels of plasma markers of clotting activation, such as the thrombin–antithrombin (TAT) complex, prothrombin fragment F1+2 (F1+2), fibrinopeptide A (FPA), plasminogen activator inhibitor 1 (PAI-1), and D-dimer, tend to be universally elevated in patients with cancer [32], indicating the occurrence of in vivo ongoing thrombin generation and fibrin formation. These abnormalities can further worsening with the start of antitumor therapies.[33]

Until recently, only few prospective studies have evaluated the utility of serial measurements of hemostatic markers for predicting the occurrence of VTE (as confirmed by objective test) in cancer patients.[34]

Current research is focusing on a number of biomarkers (Table 2) that may be helpful in identifying cancer patients who are at higher risk of developing thrombosis who might benefit from primary thromboprophylaxis. At the moment some of these markers have been incorporated in predictive models involving clinical and biological markers, which are to be validated in prospective clinical trials[30,35,36]

Recently, plasma MP are being investigated in connection with cancer progression and thrombosis development. Variations in MP quantity and/or phenotype are relevant pathogenic markers of thrombosis and vascular damage, and are associated with a higher risk for VTE in cancer patients. MP levels are currently developed by ongoing clinical trials as a criterion to enroll high risk patients.[37-39]

In the last 2 decades, several hereditary risk factors (i.e. factor V Leiden or G202110A prothrombin gene mutation) for venous thrombosis have been identified. The influence of inherited thrombophilia in patients with cancer may be more difficult to demonstrate than in the general population, the risk of thrombosis due to cancer per se possibly outweighing the contribution of thrombophilic factors. Several studies evaluating the role of factor V Leiden or G202110A prothrombin gene mutation on the risk of thrombosis in patient with cancer have been published.[40] Overall, although conflicting results were obtained, it appears that patients with cancer and either of these mutations tend to exhibit a higher risk of thrombosis than patients with cancer without these mutations. In the largest study by Blom et al the risk of VTE in patients with both cancer and factor V Leiden or prothrombin 20210A mutation was increased 12 and 17-fold, respectively, compared with patients without cancer and factor V Leiden.[41]

Risk stratification

As previously described, several demographic, cancer-associated, and treatment-related factors are known to increase the risk of thrombosis in cancer patients. Risk prediction models for chemotherapy-associated VTE have become available and include many of the risk factors listed above, but also begin to incorporate biological markers.[42,43] The Khorana's model uses a simple scoring system. It is based on baseline clinical and laboratory data, and has been shown to accurately predict the short-term risk of symptomatic VTE in patients undergoing chemotherapy-based treatments.

The inclusion of D-Dimer and soluble P-selectin in a validated risk assessment model increased the capacity to predict for VTE.[30,35,36] This permitted a better stratification of the thrombotic risk. In particular, the hazard ratio of patients with the highest compared with those with the lowest score was 25.9.[44]

The availability of new and validated predictive models will allow the possibility of improving outcomes for chemotherapy patients by identifying those who would benefit most from thromboprophylaxis.

Conclusions

A reciprocal cancer-thrombosis connection exists, by which cancer cells support clot formation, and clotting proteins support cancer growth and dissemination. Cancer is associated with a fourfold increased rate of VTE. The risk of VTE varies according to the type of malignancy (i.e.: pancreatic cancer, brain cancer, lymphoma) and its disease stage and individual factors (i.e.: sex, race, age, previous VTE history, immobilization, obesity). Preventing cancer-associated VTE is important because it represents a significant cause of morbidity and mortality in these patients. The current development of VTE predictive models, using the increasing knowledge of the clinical and biological markers of thrombosis in cancer, is important for identifying high-risk patients and reducing associat-

ed morbidity and mortality. Areas that warrant further research include the benefit of prophylaxis in the ambulatory setting, the risk/benefit ratio of prophylaxis for hospitalized patients with cancer, an understanding of incidental VTE, and the impact of anticoagulation on survival.

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PROPHYLAXIS OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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It is widely accepted that patients with solid cancer have an increased thromboembolic (TE) risk. Recently, several guidelines have been produced recommending preventive measures, especially during surgical procedures or periods of immobility/hospitalization.[1] In contrast, there is less awareness of an increased thromboembolic risk in patients with hematologic malignancies (HM). However, rates of TE in patients with HM are comparable with those observed in many solid tumors.[3]

In particular, the incidence of venous thromboembolism (VTE) in acute leukemia ranges from 2.1% to 12.1%.[4,5] In a prospective study with 379 leukemia patients[6] the rate of thrombosis was 6.3% (80% venous and 20% arterial): all events occurred within 6 months from diagnosis. Acute lymphoblastic leukemia (ALL) showed a higher 6-month rate of thrombosis compared to acute myeloid leukemia (AML) excluding acute promyelocytic leukemia (APL) (10.6% vs. 1.7%). As in almost all series, APL had a higher incidence of thrombosis, both at diagnosis and during follow-up (9.6% and 8.4% respectively). The type of treatment has a significant impact on the TE rate: the concomitant use of steroids and asparaginase increases the TE risk in ALL, while the treatment with all-trans-retinoic acid has been associated with an increased

TE risk in APL. In all studies, upper extremities deep vein thromboses were highly associated with the presence of a central venous catheter (CVC). In patients with lymphoma, TE incidence is 5-10%, with the highest TE rate observed in patients with primary central nervous system lymphoma.[7] In a large single-center retrospective analysis of 1038 lymphoma,[8] 7.7% patients experienced at least one thrombotic event: most episodes (72%) occurred during treatment, thus confirming the well-known thrombogenic effect of chemotherapy. Histology was very important for the thrombotic risk, with the highest rate of thromboembolism in high-grade non Hodgkin lymphomas (10.6%), followed by Hodgkin lymphomas (7.25%) and the lowest risk in low-grade non Hodgkin lymphomas (5.8%). A registry-based analysis performed in California confirmed these data, showing a 2-year cumulative incidence of VTE of 2.2%, 4.7% and 4.5% in low-, intermediate- and high-grade lymphomas respectively.[9]

The presence of a benign monoclonal gammopathy of undetermined significance (MGUS) was also found to be associated with an increased risk of thrombosis (6.1-7.5% in two different studies).[10,11] In a large population-based study performed from data of more than 4 million veterans in the USA, the crude incidence of deep venous thrombosis was 3.1/1000 person-year for MGUS and 8.7 for multiple myeloma (MM).[12] The use of immunomodulatory drugs (IMiDs) like thalidomide and lenalidomide has further enhanced the TE risk in these patients. In particular, the TE risk increases when IMiDs are used in combination with high doses of dexamethasone or with chemotherapy: incidence of VTE was 26% in newly diagnosed myeloma patients treated with thalidomide and high-dose dexamethasone[13] and 28% in those treated upfront with thalidomide and doxorubicin-containing chemotherapy.[14] Similarly, in two multicenter phase III trials patients with relapsed MM treated in the lenalidomide plus dexamethasone arm showed a higher VTE incidence 11.4-14.7% as compared with 3.4-4.6%.[15,16]

Currently, no consensus exists on the optimal strategy for preventing TE in patients with HM, with the possible exception of multiple myeloma. The rapid clinical evolution of most HM requiring immediate chemotherapy with the inherent risk of ensuing severe thrombocythopenia demands a patient-adapted approach. So far, no prospective studies have been carried out in leukemia and lymphoma patients. In absence of bleeding, a platelet count above 30-50.000/mcl is generally felt as sufficiently safe to administer prophylactic doses of low molecular weight heparin (LMWH) or anti-platelet treatment. Oral anticoagulation is best avoided for these complex patients. In our center, antithrombotic prophylaxis is usually stopped when platelet count falls below 50.000/mcl and will be restarted when platelets recover to above 30.000/mcl after chemotherapy.

Most data available on prevention of thrombosis in HM come from studies in myeloma patients. In fact, the increased rate of thrombosis observed after the introduction of antiangiogenic agents in myeloma therapy warranted the introduction of antithrombotic prophylaxis in new trials with these drugs. Different approaches to prevent TE in patients treated with IMiDs plus dexamethasone or chemotherapy include LMWH, low fixed-dose warfarin, adjusted doses of warfarin (INR 2-3) and aspirin (80-100 mg/day).

In the experience of the Arkansas' group with a protocol including intensive chemotherapy with doxorubicin associated with thalidomide, fixed low-dose of warfarin (1 mg/day) did not change significantly the incidence of VTE, while enoxaparin was able to abrogate its prothrombotic effect (VTE risk reduced from 34% to 15%).[17] Similarly, nadroparin was found to be an efficacy prophylaxis in patients treated with chemotherapy and thalidomide in other centers.[18] Even if LMWH seems a very effective and safe option for thromboprophylaxis, monitoring of heparin-induced thrombocytopenia could be problematic in patients on chemotherapy with fluctuating platelet count. Aspirin has been widely used in protocols with lenalidomide/dexamethasone: reports from different centers range from 3% to 19%.[19,20] Recently, two prospective randomized trials of the GIMEMA group have compared types of thromboprophylaxis in myeloma patients. One prospective randomized trial compared enoxaparin 40 mg/d, aspirin 100 mg/d or fixed-dose warfarin (1.25 mg/d) in newly diagnosed MM patients treated with thalidomide plus dexamethasone or with bortezomib-melphalan-prednisone-thalidomide [21]: venous or arterial thromboembolic events did not differ significantly with the three type of prophylaxis: 5% in the LMWH arm, 6.4% in the aspirin arm and 8.2% in the warfarin arm. Bleeding complications were also similar in the three arms. However, in the group of elderly patients, warfarin was less effective than LMWH. The second trial compared enoxaparin 40 mg/d or low-dose aspirin (100 mg) in 342 newly diagnosed MM patients treated with lenalidomide and low-doses of steroids followed by consolidation with melphalan-prednisone-lenalidomide: VTE incidence was 2.27% in the aspirin arm and 1.2% in the LMWH arm (not statistically different).

As recommended by an international panel,[22] other personal or therapy-related risk factors should be considered in the choice of the best antithrombotic prophylaxis in the single patient: obesity, previous VTE, presence of CVC, diabetes mellitus, chronic renal or cardiac disease, immobilization, acute infection, surgery, use of erythropoietin.

Use of erythropoietin is considered an additional risk factor for VTE, even though there is discrepancy in different studies reported in literature: after a preliminary observation of thrombosis in 3 out of 7 patients with myelodysplasia treated with darbepoetin-alpha and thalidomide [23], an increased risk of VTE was not observed in some studies with MM patients treated with thalidomide or lenalidomide,[16,24] whereas others found an increase of VTE risk from 5% to 23% in patients treated with lenalidomide and erythropoietin.[25]

At the moment, prophylaxis with low molecular weight heparin (LMWH) or adjusted-dose warfarin (INR 2-3) has been suggested only in ambulatory myeloma patients treated with thalidomide (ESMO and ASCO guidelines) or lenalidomide (ASCO guidelines) in combination with dexamethasone or chemotherapy.[26,27]

We think that more awareness on the thrombotic risk in HM is required and hematology departments should implement a policy for TE prophylaxis in patients with HM with written institutional guidelines. Particular situations like surgery (also minor surgery); restricted mobility; treatment with chemotherapy, asparaginase or IMiDs; presence of disseminated intravascular coagulation and placement of a central venous catheter should be taken into consideration as contributing risk factors and should guide clinical decisions on thromboprophylaxis.

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TREATMENT OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH HAEMATOLOGIC MALIGNACIES WHO ARE AT HIGH HAEMORRHAGIC RISK

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The standard treatment for an initial acute episode of venous thromboembolism (VTE) consists of either low molecular weight heparin (LMWH) at a dose adjusted for body weight (100 U/kg bid) or unfractionated heparin (UFH) administered i.v. in a continuous infusion. UFH is first administered as a bolus of 5000 U, which is followed by a continuous infusion that is adjusted to achieve and maintain an activated partial thromboplastin time (aPTT) 1.5–2.5 times greater than the basal value. A recent analysis that was conducted on 23 randomised controlled trials involving 9,587 patients with VTE found a greater efficacy for LMWH versus UHF (odds ratio, OR, 0.70, 95% confidence interval, CI, 0.57-0.85). Major haemorrhages occurred in 1.1% of participants treated with LMWH compared with 1.9% of those treated with UFH (OR 0.58; 95% CI 0.40 to 0.83).[1]

Due to these results and together with the lower rate of heparin-induced thrombocytopaenia and the possibility of treating patients on an out-patient basis, LMWH has generally replaced UFH for the initial treatment of the vast majority of patients with acute VTE. After 5 to 10 days of initial treatment, long-term treatment for at least 3 months with vitamin K-antagonists (VKA) is warranted; duration can be prolonged in select patients.[2] The rate of major haemorrhagic complications during VKA treatment is estimated to be 1.1% patient-years.[2] A meta-analysis found that a three-month course of LMWH was as effective and safe as a corresponding period of VKA treatment and may therefore be considered a valuable alternative option for patients where VKA treatment is contraindicated or problematic[3] In cancer patients treatment with LMWH for 3 to 6 months after the acute VTE episode is preferred over that with VKA.[2,4-7] Similar to that estimated for heparin or VKA treatment, major haemorrhagic complications have been reported at a rate of 1 to 2% during either initial or long-term treatment of VTE with novel anticoagulant agents such as the heparin pentasaccharide fondaparinux, the oral direct thrombin inhibitor dabigatran etexilate, and the oral direct factor Xa inhibitors rivaroxaban and apixaban.[8]

The occurrence of VTE in patients who may be at greater haemorrhagic risk challenges the aforementioned strategies and increases the expected rate of major bleeding. Haemorragic risk can be circumstantial (e.g., immediate post-operative period) or can be due to constitutional factors, namely thrombocytopaenia or a deficiency of plasma coagulation factors. The latter can be distinguished between acquired (e.g., chemotherapy-related thrombocytopaenia, haematologic malignancies, immune thrombocytopaenic purpura) or congenital (e.g., inherited bleeding disorders) conditions. The present review has focused on the treatment of VTE in patients with haematologic malignancies who are at greater haemorrhagic risk due to their primary disease and/or chemotherapies.

Incidence of VTE in patients with haematologic malignancies

Cancer-associated thrombosis is a common complication for patients with malignancies. In the general population, the presence of malignant neoplasms is associated with a significant increase in the risk of VTE either with (OR 6.53, 95% CI 2.11-20.23) or without chemotherapy (OR 4.05, 95% CI 1.93-8.52). Additional independent risk factors in this setting include hospitalisation (OR 7.98, 95% CI 4.49-14.18), recent surgery (OR 21.72, 95% CI 9.44-49.93), or the installation of a central venous catheter (CVC) (OR 5.55, 95% CI 1.57-19.58).[9] A prospective cohort study reported that solid tumours, such as cancers of the kidney, stomach, pancreas, brain, and ovary, as well as lymphomas, were associated with the highest incidence of venous thrombosis (10). Nevertheless, from a recent population-based study, the overall risk for venous thrombosis was increased 6.7-fold in patients with a malignancy (95% CI 5.2-8.6) over persons without malignancy, and patients with haematologic malignancies had the highest risk of developing VTE (OR 28.0; 95% CI, 4.0-199.7), followed by lung cancer and gastrointestinal cancer. However, the majority of patients with haematologic malignancies had lymphoma or multiple myeloma, and only a small fraction of the patient population had leukaemia.[11] In another population-based study, the relative risk for VTE in comparison to the general population was greatest among patients with pancreatic (16.3, 95% CI 8.1–32.6) or brain cancer (19.8, 95% CI 7.1–55.2) or multiple myeloma (46.1, 95% CI 13.1–162.0). This last group was found to have a VTE incidence of 2.26 % patient-years and an overall prevalence of 4%. In this study the incidence of VTE among patients with leukaemia was 2.11 % patient-years, there was an overall prevalence of 2.7%, and the relative risk was 9.1-fold increased (95% CI 5.3-15.8).[12]

The incidence of VTE among patients with haematologic malignancies has been recently reviewed.[13] For patients with lymphoma, the incidence of VTE ranged from 1.5 to 14.6% and was reported to occur in 59% of the patients with central nervous system lymphoma. The incidence of VTE in patients with acute leukaemia varies with time; the incidence is between 1.4 and 9.6% at the time of diagnosis and ranged between 1.7 and 12% during induction therapy. Notably, the highest rates of VTE were reported in patients with acute promyelocytic leukaemia, and the incidences were found to be between 6 and 16% in the largest series. For patients with multiple myeloma who did not not receive antithrombotic prophylaxis, the rate of VTE increased to 26% of cases given treatment regimens including immumodulatory drugs such as thalidomide and lenalidomide.[13] Moreover, for patients who have undergone autologous or allogeneic haematopoietic stem cell transplan-

tation (HSCT), the rate of VTE has been reported between 2.9 to 9.9%, and was CVC-associated in the majority of cases.[14-16] Notably, VTE has been shown to occur even during periods of severe thrombocytopaenia; one-third of the events from a patient series occurred when the platelet count was <50 x10^9/L.[15]

Treatment of VTE in patients with haematologic malignancies

There is a general consensus based on the results of several randomised cInical trials that the administration of LMWH for three to six months is more effective and safe than treatment with VKA for preventing recurrent VTE in cancer patients with established VTE. After six months, indefinite anticoagulant therapy should be considered for select patients with active cancer, such as those with metastatic disease or those receiving chemotherapy.[2,4-7] Patients with acute leukaemia have a high risk of haemorrhage, which is mostly related to thrombocytopaenia as a result of haematologic disease and/or chemotherapy, such that the administration of anticoagulant drugs in this setting is problematic. No randomised controlled trials have addressed the issue of VTE treatment in patients with acute leukaemia, and the management of these cases is currently based only on small groups of patient series or experts' opinion.

Five reports have described in detail cases of paediatric or adult patients with malignancies and thrombocytopaenia who have undergone heparin treatment as a result of a VTE.[17-21] Out of a total of 54 cases, 32 had haematologic malignancies and a platelet nadir <50x10^9/L (Tables 1 and 2). Of the other cases, two with lymphoma had sustained platelet count >100x10^9/L, one with acute lymphoblastic leukaemia (ALL) had cerebral artery thrombosis, and the remaining cases had non-haematologic malignancies. The majority of patients (22 of 32) had CVC-related thrombosis. A full dose of LMWH bid was administered to the majority of the patients with CVC-related thrombosis (Table 1) and to the totality of patients for whom the thrombosis was not CVC-related (Table 2). All patients received platelet transfusions if the platelet count fell below <20-40x10^9/L, and the dose of LMWH was halved when the platelet count was <20x10^9/L in one report (Table 2). None of the patients had major bleeding. Rethrombosis occurred in three of 32 (9.3%) patients; two of these cases (with factor V Leiden) were associated with CVC and occurred at a site distinct from that of the first symptomatic deep venous thrombosis,[19] and the rethrombosis in the remaining case occurred in the arm after the removal of CVC and the withdrawal of heparin.[18]

In a large series of 379 patients with acute leukaemia, treatment for 20 patients who had one (n=16) or two (n= 4) VTE events was essentially based on the administration of enoxaparin 100 U/kg bid; in the case of a platelet count <50 x10^9/L or in the clinical suspicion of bleeding risk the dose was reduced to 100 U/kg qd or 50 U/kg bid. Alternatively, the patients received a continuous i.v. infusion of UFH to obtain aPTTs in the lower therapeutic range (1.5 times greater than the basal value). Secondary prophylaxis after acute VTE was based on the administration of enoxaparin 100 U/kg qd in the case of ongoing chemotherapy or VKA (INR between 2 and 3) otherwise. In general, the length of secondary prophylaxis was reported to be not longer than six months.[22]

The safety of therapeutic anticoagulation treatment for the management of thrombocytopaenic patients was also evaluated in two series of patients receiving HSCT.[23,24] In 10 patients with multiple myeloma who had received autologous HSCT, anticoagulation was required following pre-transplant CVC-related subclavian vein thrombosis (n= 8), pulmonary embolism two months prior to transplant (n= 1), or a history of acute intermittent atrial fibrillation that was complicated by an arterial embolus to the leg (n= 1). Beginning on the first day of high-dose chemotherapy, the 10 patients received therapeutic UFH (a 5000 U i.v. bolus followed by 1000 U per h) to maintain aPTTs between 50 and 70 seconds and were switched to VKA treatment when their conditions stabilised. UFH treatment was interrupted once the VKA administration produced a therapeutic INR >2 for two consecutive days. Heparinised patients received platelet transfusions to maintain counts >30x10^9/L. Three patients developed bleeding (haematuria, haematemesis, mucosal bleeding) that did not not require transfusion, and no thrombotic events occurred.[23]

In another series of 26 patients with HSCT who were given enoxaparin for the treatment of VTE, 21 patients had haematologic malignancies. There were 25 VTE events recorded (four patients had two events at different sites) and 11 cases had upper extremity CVC-related deep venous thrombosis. During periods of thrombocytopaenia (<55x10^9/L), enoxaparin administration was reduced to a median value of 49 U/kg/day (range 34-75) and was withdrawn in some instances when the platelet count fell below 20x10^9/L. Two major bleeding events (8%) occurred. The first consisted of a retroperitoneal bleed that developed during the enoxaparin transition from 235 U/kg/day to 60 U/kg/day when the platelet count dropped from 83 x10^9/L to 51 x10^9/L. A second retroperitoneal fatal haemorrhage occurred when the enoxaparin dose was switched from 46 U/kg/day to 115 U/kg/day at a platelet count of 52 x10^9/L.[24]

In an additional case of a 50 year-old woman with a mechanical valvular prosthesis who received an allogeneic HSCT for treatment of acute myeloid leukaemia, VKA treatment was substituted during the entire hospitalisation period with a continuous i.v. infusion of LMWH nadroparin calcium (30,000 anti-Xa U/day) to maintain anti-Xa activity within the therapeutic range of 0.5–1.0 U/ml. The platelet count was maintained above 52 x10^9/L by regular platelet transfusions, and the patient did not develop any haemorrhagic or thrombotic complications.[25]

In a large series of 452 children with haematological malignancies, 12 patients who received chemotherapy or bone marrow transplantation had pulmonary embolisms (recurrent events for three of the cases), and 14 of 17 events occurred in patients with CVC. Thrombolytic treatment was most often performed with urokinase at a loading dose of 2,000 to 4,560 U/kg as single bolus followed by 2,000 to 4,530 U/kg/h for 12 to 42 hours. Before the thrombolytic therapy was discontinued, UFH was initiated at a daily dose of 100 to 500 U/kg as a continuous infusion and was continued for 6 to 26 days. This treatment was effective for 15 of the 17 events, and there was no major bleeding in any of the cases. A detailed platelet count was only available from one patient, and this patient's count was <20 x10^9/L at the time of the embolic event and fibrinolysis [(26)].

The aforementioned data are insufficient for the production of evidence-based guidelines. Experts and the AIEOP (Associazione Italiana di Ematologia e Oncologia Pediatrica) have suggested that the first two weeks of treatment should consist of the administration of full- dose LMWH (anti-factor Xa level 0.5-1 U/ml), maintaining the platelet count above 50 x10^9/L. After the first two weeks, halving the dose is recommended if the platelet count is between 20 and 50 x10^9/L. If the platelet count is below 20 x10^9/L, it is advised that the LMWH therapy be discontinued until the platelet count recovers to greater than 20 x10^9/L.[27-29] Recent guidelines of the SISET (Società Italiana per lo Studio dell'E-

mostasi e della Trombosi) also suggested that in patients with haematological malignancies and VTE LMWH should be preferred over VKA either for the first six months or a longer time.[6]

There have been no published studies concerning the use of novel antithrombotic agents such fondaparinux, direct thrombin or factor Xa inhibitors, in patients with haematological malignancies or thrombocytopaenia. However, *in vitro* experiments that have been performed on plasma from children with ALL and antithrombin deficiency as a result of the administration of asparaginase have demonstrated that the direct thrombin inhibitor melagatran generates a consistent anticoagulant response that is independent of the antithrombin level. Therefore, this drug class may have important potential for use in this field.[30]

Use of inferior vena cava filters

For patients with acute proximal deep venous thrombosis where effective anticoagulation is contraindicated due to a high risk of bleeding, the placement of an inferior vena cava (IVC) filter is recommended[2] Practical approaches for using IVC filters have been reviewed.[31,32] The placement of a permanent filter may present a number of potential adverse effects, including recurrent deep venous thrombosis, thrombosis of the filter, migration, and fracture. Non-permanent filters are classified as temporary or retrievable devices. Temporary filters remain attached to a wire or catheter that can be removed through the skin, and the advantage of these filters is their ease of insertion and removal. However, the external fixation is a potential pathway for infection, which would render such devices highly inappropriate for neutropaenic patients. Furthermore, temporary filters are often difficult to manage and present frequent complications such as thrombosis or migration. In addition, the limited duration of use that can be achieved with temporary filters is insufficient to recover the platelet count after high-dose chemotherapy. However, retrievable filters can be removed after they become unnecessary, and they can also be left in place permanently if warranted. Thus if an IVC filter is given to a patient with acute VTE for whom anticoagulant therapy is temporarily contraindicated, there is the option to insert a retrievable filter that could be removed when it is safe to initiate anticoagulant therapy. However, in some cases retrievable filters cannot be removed for technical reasons, including if the filter is severely angled, if a thrombus is trapped in the filter or if the filter struts substantially penetrate the IVC wall, and there are very limited data describing the long-term safety of these devices when they are not removed. Finally, their use in patients with haematologic malignancies has not been specifically addressed, so special caution should be used when deciding to use a retrievable filter in this setting due to the risk of haematoma and infection at the insertion site.

Conclusions

VTE complications are not uncommon for patients with haematologic malignancies. Concomitant prolonged thrombocytopaenia challenges the use of anticoagulants for the prevention of thrombus extension and recurrence. There are limited published data concerning the management of VTE in this setting, although the use of LMWH appears to be safe and effective even for patients with severe thrombocytopaenia. A careful treatment approach for either platelet transfusion or LMWH dose modulation should aim to obtain a satisfactory balance between antithrombotic potential and haemorrhagic risk. However, although the reported results are encouraging, special caution should be adopted for the treatment of severely thrombocytopenic patients, and the judicious use of retrievable IVC filters may be an important and helpful tool.

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HODGKIN LYMPHOMA: THE BEST INITIAL THERAPEUTICAL STRATEGY

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During the last decades, survival of patients treated for classical Hodgkin lymphoma (HL) has improved substantially and, to date, the overall cure rate of this neoplasm is about 80-85%.[1] Improvement in the outcome is mostly due to the development of more active chemotherapy (CT) regimens, of a more accurate radiotherapy (RT) and of a rationale combination of these different treatment modalities. Unfortunately, however, the risk of treatment-related morbidity and mortality is still significant; therefore, modern therapies should attempt at maximizing the chance of cure, while minimizing late toxicity.

First-line therapy for early-stage disease

The significant risk of relapse after RT alone, even in favourable subgroups and the abandonment of staging laparotomy have substantially reduced the role of RT as the sole treatment in early stage Hodgkin lymphoma.[1] A number of randomized studies have analysed the results after a combined modality therapy including different chemotherapy schedules and different radiation fields and doses. In a four-arm randomized study (HD10 trial), the German Hodgkin Study Group (GHSG) has compared the efficacy of a different number of cycles of ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) followed by involved-field radiation therapy (IF-RT) at different doses. In patients belonging to the favourable prognostic subgroup (no bulk, less than 3 nodal sites, ESR < 30 with B symptoms and < 50 without symptoms), two courses of ABVD followed by IF-RT (30 or 20 Gy) were compared to four courses of ABVD followed by IF-RT (30 or 20 Gy). The final results of this study indicate that two cycles of ABVD followed by 20 Gy IF-RT is as effective as, and less toxic than four cycles of ABVD followed by 30 Gy IF-RT, and should therefore be considered the standard therapy for this category of patients.[2] In the unfavourable group (bulky disease, 3 or more nodal sites, VES ≥ 30 with B symptoms or ≥ 50 without B symptoms), four courses of ABVD followed by IF-RT (30 or 20 Gy) have been compared to four courses of standard-dose BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) + IF-RT (30 or 20 Gy). Moderate dose escalation using standard BEACOPP does not significantly improve outcome in early unfavourable Hodgkin lymphoma. Four cycles of ABVD followed by 30 Gy IF-RT should be considered the standard therapy in unfavourable early stage Hodgkin lymphoma [3]. Hodgkin lymphoma survivors who have been treated with chest RT are at increased risk of cardiovascular and/or pulmonary complications.[4] Late toxicity of RT may include coronary alterations, myocardial and pericardial fibrosis, valvular abnormalities, conduction disturbances, and pulmonary fibrosis, with restrictive syndrome; the risk of toxicity is significantly enhanced by the combination of RT with a CT regimen containing doxorubicin and bleomycin. In the attempt to avoid RT-related toxicity, randomized studies have compared CT alone to combined modality therapy in patients with non bulky early-stage Hodgkin lymphoma. In a study from the Memorial Hospital,[5] six courses of ABVD alone have been compared to six courses of ABVD followed by 36 Gy IF-RT; no significant differences in remission duration, freedom from progression (FFP) and overall survival (OS) were found between the two arms. A study from the National Cancer Institute of Canada and Eastern Cooperative Oncology Group[6] has compared ABVD alone (four to six courses) to a strategy including chemotherapy and radiation therapy and found no difference in OS; the 5-year FFP was slightly superior in patients given adjuvant RT; however, this advantage was counteracted by deaths from causes other than progression of Hodgkin lymphoma. No evidence has yet been obtained of the non-inferiority for CT alone compared to combined modality therapy. The on-going EORTC-GELA-IIL H10 trial is the first randomized intergroup trial on early treatment adaptation guided by the results of interim FDG-PET (after 2 cycles of ABVD), and aiming at a reduction of treatment burden. In the experimental arm, when according to interim FDG-PET, no viable tumor is left, adjuvant RT will be withhold, while when interim FDG-PET is positive, chemotherapy will be changed from ABVD to the more intense BEACOPP escalated for two cycles followed by IN-RT. :In the standard arm, adjuvant involved node RT will be administered after ABVD, irrespective of the results of interim PET, without CT intensification in interim PET positive patients.

First-line therapy for advanced-stage disease

The introduction of ABVD chemotherapy has represented a major advancement over the classical MOPP in the treatment of advanced-stage Hodgkin lymphoma.[7] In a large U.S. intergroup trial [8], ABVD has demonstrated to be superior to MOPP and to be equivalent to the alternating MOPP and ABVD regimens and has become the standard therapy for advanced Hodgkin lymphoma.

The BEACOPP regimen (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone) has been introduced by the GHSG in its baseline and dose-escalated variants, with a substantial increase of dose-intensity compared to ABVD. In the HD9 randomized trial,[9] alternating COPP and ABVD have been compared with baseline and dose-escalated BEACOPP. Radiotherapy (36 Gy) was administered on sites of bulky disease or on residual disease after chemotherapy. At 5 years, a significant superiority was demonstrated in freedom from treatment failure (FFTF) and OS for dose-escalated BEACOPP (87%) versus the baseline BEACOPP (76%) and the alternating COPP/ABVD (69%) program. Dose-escalated BEACOPP was associated with a significantly greater toxicity compared to baseline BEACOPP and COPP/ABVD. The 10-year up-date of the HD9 study confirms that BEACOPP escalated chemotherapy produced a significant improvement in long-term FFTF and OS compared to both COPP/ABVD and baseline BEACOPP and this advantage is particularly evident in the subset of poor prognosis patients, as defined by the International Prognostic Score.[10] Therefore, the GHSG advocated BEACOPP as the new standard of treatment for patients with advanced-stage Hodgkin lymphoma.

The choice of a preferred first-line treatment requires balancing the control of the disease with the occurrence of early and late treatment-related effects. To fully assess this balance, the treatment decision process should ideally take into account the outcome after a consistent second-line therapy, particularly when widely applicable and effective regimens exist. A recent study[11] assessed the long-term clinical outcome after initial therapy with BEACOPP as compared with ABVD in advanced dis-

ease. All patients with residual or progressing disease after the initial treatment went on to receive a salvage regimen consisting of reinduction standard-dose chemotherapy followed by high-dose consolidation therapy and autologous hematopoietic stem cell transplantation. The treatment with BEACOPP, as compared with ABVD resulted in a better initial tumor control, but the long term clinical outcome did not differ significantly between the two regimens. A parallel study[12] has suggested a superiority of BEACOPP over ABVD in term of FFP but not in term of OS: These studies reinforce the concept that ABVD should be considered the first choice in advanced Hodgkin lymphoma if the goal is cure with the least overall toxic effects, reserving rescue therapy with high-dose chemotherapy and autologous hematopoietic stem cell rescue for patients in whom the primary treatment fails. The utility of irradiation to areas of initial bulk in patients achieving complete remission with ABVD is still controversial.

FDG-PET scan can help

It is well accepted that an interim FDG-PET scan performed very early during treatment in advanced-stage Hodgkin lymphoma is an important prognostic factor. So far, the published data have shown that the negative predictive value for treatment outcome ranges from 97 and 100%, while the positive predictive value is about 80%; patients with a negative early FDG-PET had a 2-yr PFS of 95% versus 13% for early PET positive patients [13]. Several on-going clinical trials in advanced-stage HL (Table 1) are being conducted utilizing prospectively the high predictive value of PET to guide subsequent therapy after the initial two cycles of chemotherapy (ABVD or BEACOPP).

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URRENT ROLE AND FUTURE DEVELOPMENTS OF RADIOTHERAPY IN HODGKIN'S LYMPHOMA

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For a patient with Hodgkin's lymphoma (HL) in any stage, the primary goal of therapy is cure. In recent studies, the survival rates for patients with early stage disease has consistently been 90% or higher. Particularly in patients with such a good prognosis, in studies with a very long period of follow-up, the number who die from treatment-related complications exceeds the number who die from lymphoma. The frequency of late complications is dependent on the particular treatment used. Radiation-related cardiac disease (coronary artery disease, myocardial injury, valvular disease, pericardial fibrosis) and second malignancies (breast and lung cancer) may occur many years after thoracic irradiation. The risk of late complications after chemotherapy appears to be dependent on the type of drugs prescribed (alkylating agents, anthracycline, bleomycin) and their doses. Treatment strategies in HL changed therefore dramatically during the last recent years, with current clinical protocols now focusing, expecially in early stage HL, on minimizing the intensity of treatment to avoid late, potentially fatal toxic effects, without the risk of lowering the overall survival rates.

Early Stages (Favorable and Unfavorable)

For many decades the optimal and standard treatment for early stage HL was extended field radiotherapy (EF-RT), totally replaced right now from a combination of short-term chemotherapy with involved field radiotherapy (IF-RT). The evolution of effective treatments for early stage HL is best exemplified by the successive randomized trials of the German Hodgkin's Study Group (GHSG), as well discussed in a paper by Hans Theodor Eich and Rolf-Peter Müller in 2007.

The first protocol dealing with a radiotherapy-related endpoint was the HD4 trial, designed in early Eighties (1988–1994). The major aim of HD4 was to show whether the radiation dose to the non-involved lymphatic regions could be reduced while maintaining effective tumor control. This trial was conceived as an effort towards a further improvement of results obtained in 1962-1984 by the Stanford group in early stages with radiotherapy only, showing complete remission rates of 100% and recurrent free survival of 80% in stages IA, IIA and IIB without large mediastinal tumor, excellent results unconfirmed by other groups. In HD4, patients in stage I or II without risk factors (large mediastinal mass, extranodal extension, massive spleen involvement, > 3 lymph node areas, high ESR) were randomized between standard treatment consisting of 40 Gy EF-radiotherapy (arm A) and 30 Gy EF-radiotherapy plus additional 10 Gy to the IF (arm B). Staging laparotomy was obligatory in this protocol. The results showed no statistically significant differences in recurrent free survival (RFS) and overall survival (OS) between the two treatment arms, but the overall recurrence rate approached 20%, as reported by Stanford studies. Due to a sufficient salvage therapy (polichemotherapy), RFS after seven years came up to 80% and the overall survival was 93%. The pattern of relapses in this study showed interesting results, with the majority of recurrences documented outside high-dose radiation fields, probably due to errors in initial staging or in radiotherapy prescription. Due to the crucial importance of good quality radiotherapy in such studies, German Hodgkin Study Group promoted the creation of a task force for radiotherapy quality assurance, and for all patients enrolled in the study a treatment plan was given by the radiotherapy reference center based on the documentation of the disease extension on case report forms (CRF), and after completino of the EF-radiotherapy, simulation and verification films of every individual patient as well as the treatment data were analyzed by an expert panel. This retrospective quality control showed that deviations of radiation treatment portals and radiation doses from prospective treatment prescriptions were unfavourable prognostic factors for patients with early-stage HL.

Next research step of GHSG was a trial designed keeping the approach of low-dose EF of HD4 while trying to eradicate microscopic disease with chemotherapy and improving Relapse-Free Survival. In HD7 (1994–1998) patients were randomized between radiotherapy alone (30 Gy EF + 10 Gy IF) (arm A) or upfront 2 cycles ABVD followed by radiotherapy (30 Gy EF + 10 Gy IF) (arm B) for early stages PS IA, IIA, IIB without risk factors. Staging laparotomy was not obligatory and the spleen was irradiated with 36 Gy in both treatment arms. At 7 years there was no difference between treatment arms in terms of complete response rate

(arm A: 95%, arm B: 94%) or OS (arm A: 92%, arm B: 94%; p= 0,43). However, freedom from treatment failure (FFTF) was significantly different with 67% in arm A and 88% in arm B (p ≤ 0,0001). This was mainly due to significantly more relapses after EF-radiotherapy only (arm A: 22%; arm B: 3%).

HD10 trial (1998–2002) was designed eliminating the EF approach, including IF only and with the primary aim of reducing acute and long term toxicities while maintaining optimal tumor control. This trial also incorporated the results of the major studies published in the Nineties by North-American, European/French and Italian Groups, focusing on the role of chemotherapy and the "involved fields" concept. All these studies are briefly summarized in Table I, showing a complete equivalence for the brief chemotherapy + IF vs. EF alone or chemotherapy + EF approach. As well pointed out by HT Eich and RP Muller, the HD10 trial represents a very decisive step, since irradiation was performed as IF-radiotherapy in all treatment arms. The HD10 is the first trial designed to investigate the optimal intensity of chemotherapy and radiotherapy. All the treatment strategy is based upon a selection of patients with favorable prognostic factors, in which reduced treatment intensity should offer very good results in terms of disease control while reducing toxicity. Therefore patients in stages I or II without risk factors (no bulky disease, less than 4 involved sites, low ESR values) were randomized in a four-arm study between an IF-radiotherapy dose of 30 Gy versus 20 Gy and 2 versus 4 cycles of ABVD. To ensure that IF-radiotherapy was performed exactly according to the RT-prescriptions of the protocol, an extensive quality assurance program was performed. Results of HD10 were published in 2010: the 2 chemotherapy regimens did not differ significantly with respect to freedom from treatment failure (P=0.39) or overall survival (P=0.61). At 5 years, the rates of freedom from treatment failure were 93.0% (95% confidence interval [CI], 90.5 to 94.8) with the four-cycle ABVD regimen and 91.1% (95% CI, 88.3 to 93.2) with the two-cycle regimen. When the effects of 20-Gy and 30-Gy doses of radiation therapy were compared, there were also no significant differences in freedom from treatment failure or overall survival (P=0.61). HD10 demonstrated that treatment with two cycles of ABVD followed by 20 Gy of involved-field radiation therapy is as effective as, and less toxic (acute toxicity) than, four cycles of ABVD followed by 30 Gy of involved-field radiation therapy. A parallel but different study is ongoing in early stage favorable and unfavorable patients, designed by EORTC/GELA/IIL, comparing a treatment strategy based on interim (after 2 ABVD cycles) 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) on the introduction of an innovative radiotherapy concept, the so-called "Involved Nodes Radiation Therapy" (INRT). This trial is now closed and final results will be available in next 2 years. Two major trials investigating the role of chemotherapy alone (ABVD) were published some years ago, showing that CT alone is a feasible option for patients with non-bulky early-stage Hodgkin's lymphoma. An increased freedom from progression was shown for the combined-modality arms when compared with chemotherapy alone (86% vs. 81% and 93% vs. 87%, respectively), and since current recommended approaches to treating patient in relapse after primary therapy include autologous stem cell transplant, the current dilemma facing clinicians is whether all patients should be irradiated to prevent progression in 5% to 6% of cases or whether it is justified to withhold radiation, knowing that patients with progression will be referred to high-dose chemotherapy.

For patients with unfavorable early stage disease presentation (bulky disease, many involved sites, high ESR values), the treatment approach was similar but results should be evaluated separately; all major trials investigated a combination of at least 4 chemotherapy cycles (but in the majority of treatment arms 6 cycles) + EF or IF radiotherapy (EORTC H7U, EORTC/GELA H8U, EORTC/GELA H9U), showing that the IF approach is safe and equivalent to EF and that globally the results of combined modality therapy are lower than in favorable stages, with FFTF rates in the range 84-94%. In this setting a reduction in treatment intensity did not demonstrate any equivalence in terms of disease control. The recently published GHSG HD11 trial was designed to specifically investigate this issue. With a total of 1,395 patients included, BEACOPP was more effective than ABVD when followed by 20 Gy of IFRT (5-year FFTF difference, 5.7%; 95% CI, 0.1% to 11.3%), however, there was no difference between BEACOPP and ABVD when followed by 30 Gy of IFRT (5-year FFTF difference, 1.6%; 95% CI, -3.6% to 6. 9%). Similar results were observed for the radiotherapy question: after 4 cycles of BEACOPP, 20 Gy was not inferior to 30 Gy (5-year FFTF difference, -0.8%; 95% CI, -5.8% to 4.2%), whereas inferiority of 20 Gy cannot be excluded after four cycles of ABVD (5-year FFTF difference, -4.7%; 95% CI, -10.3% to 0.8%). At the moment, in unfavorable early stages HL, 4 ABVD followed by 30 Gy IF-RT certainly continues to represent a standard clinical approach.

Advanced-stage HL

Although the role of consolidation radiotherapy after chemotherapy remain controversial, irradiation is often added in patients with advanced stage HL who present with bulky disease or remain in uncertain complete remission after chemotherapy. This clinical choice is based upon the results of several retrospective and prospective studies indicating that RT could be beneficial in converting to complete remission patients in partial remission after chemotherapy (with results in terms of FFTF comparable to patients treated with chemotherapy alone and in complete remission). Different prospective phase III trials (including trial FIL HD0801) investigating the role of consolidative RT on bulky sites at presentation in chemo-sensitive patients with negative interim FDG-PET are ongoing, and will probably answer this question in next years. On the other side, according to the results of GHSG trials in advanced disease (HD12, HD15), consolidation radiotherapy could be safely omitted in patients with bulky disease achieving a CR status with functional imaging negativity at the end of chemotherapy.

Considerations on Radiotherapy and future developments

As briefly discussed, radiotherapy in early favorable and unfavorable presentations is an essential component of the standard treatment, as confirmed by a recent analysis by Cochrane Collaboration Group on the outcome of combined modality therapy vs. chemotherapy alone. Outside clinical trials, radiation volumes and doses could be adapted to prognostic factors. A low-dose involved field approach (20 Gy IF) can be considered the standard for favorable stage I-II patients after 2 cycles of ABVD chemotherapy. In unfavorable presentations, the standard dose should be at least 30 Gy, after 4 cycles of ABVD chemotherapy, or lower (20 Gy) only when BEACOPP chemotherapy regimen is employed.

In advanced disease, patients in CR after full-dose chemotherapy program may need RT consolidation only in case of bulky disease at presentation; at this regard, ongoing clinical trials could clarify this issue in the next years. Of course, IF-RT could be an option in case of focal persistent disease after full dose chemotherapy.

Current clinical research is investigating, once again, the possibility to omit irradiation in early stage disease, using as a new criteria to select highly chemosensitive patients the early negativity of FDG-PET scan (interim PET after the first two ABVD cycles). There are some ongoing studies evaluating this issue at international level, like the EORTC-GELA-IIL H10 trial. The latter study is also addressing a new radiation oncology question, such as the possibility of a further reduction of radiation fields compared to the classical involved field concept; this approach, limiting irradiation only to the single nodal station involved by the disease other than the whole anatomical region, is called Involved Nodal Radiotherapy.

A comparison between IF-RT and IN-RT is currently investigated in an ongoing GHSG trial, quite recently opened to accrual.

Below the INRT concept, the technological break-troughs in radiation oncology led to the employment of new treatment techniques such as Intensity Modulated Radiotherapy (IMRT) also in the field of hemato-

logical malignancies. The large fields of the past limited the radiation technique to simple opposed anterior and posterior fields, but smaller and better defined radiation volumes allow the utilization of more conformal radiation therapy, based on better imaging and advanced radiation delivery techniques. These recently introduced radiotherapy planning and delivery techniques already demonstrated better sparing of the heart, coronary arteries, lung and breast. The achievable dose reduction is surely protective for well known dose-related radiotherapy late effects such as radiation pneumonitis/fibrosis or coronary artery disease, with an open issue regarding the impact on secondary malignancies risk. As underlined by Joachim Yahalom in a recent paper, although it will take more years of careful follow-up of patients in randomized studies to display the full magnitude of risk tapering by current reduction of radiation fields and doses, recent data suggest that this likely to be the case, and current clinical protocols are including this new radiotherapy planning and delivery modalities as part of standard irradiation techniques.

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ROLE OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA

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Hodgkin lymphoma is not a common neoplasia, but it has had a deep impact on our knowledge of the treatment of malignancies. Today, Hodgkin lymphoma has a high cure rate when standard therapy is used. Unfortunately, some patients fail to attain an initial remission and approximately 30% of those, who achieve remission, relapse. Salvage chemotherapy followed by high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT) has become the first choice in relapsed/refractory patients. Alternative strategies such as allografting, new cytostatic drugs and biological agents with proven efficacy in preclinical model may improve the outcome of relapsed/refractory patients to ASCT.

Salvage chemotherapy treatments before ASCT

The ideal salvage regimen should produce: a) high response rate, b) acceptable hematologic and nonhematologic toxicity and c) not impair the ability to mobilize peripheral blood stem cell mobilization. There is no consensus on the gold-standard of salvage chemotherapy. Regimens such as IGEV, DHAP, ICE or GDP are reasonable options but no direct comparison among them is available. In our opinion, patients who are non responders to platinum and/or gemcitabin containing regimens and have stable disease, can safely proceed to ASCT without any kind of further previous chemotherapy. An alternative salvage regimen could be used in patients with progressive disease and/or larger volume disease but the results are generally worse. It has recommended to use a salvage chemotherapy with which clinicians are confortable that results in acceptable toxicity, high response rates and good stem cell mobilization.

Autologous Stem Cell Transplantation

At the end of '80th, the Italian teams pioneered ASCT in 50 patients with advanced Hodgkin lymphoma1. Soon after, a number of international Phase II clinical trials have confirmed the feasibility and the efficacy of this procedure alone. In all these retrospective studies, the results with ASCT appeared superior to those achieved with conventional salvage therapy alone. Unfortunately, the studies had a relatively small sample size and needed to be tested in controlled clinical trials to better clarify the role of ASCT. The first randomized trial comparing HDC/ASCT versus standard-dose salvage chemotherapy was carried out within the British National Lymphoma Investigation[2]. As known, the study was stopped earlier because of an event-free survival advantage for the HDC arm (53% versus 10%; p=0.025). This study showed that HDC produced a better response rate, but, it did not answer the crucial question if was better to utilize ASCT in early or delayed phases of disease. The second controlled study was organized by the German Hodgkin Study Group (GHSG) in cooperation with our Unit in Genoa[3]. They carried out a randomized trial in chemotherapy-sensitive patients comparing HDC (BEAM) vs conventional chemotherapy (two courses of Dexa-BEAM). At a median follow-up of 39 months, freedom from treatment failure was significantly better for the patients in the transplant arm (55% vs 34%, p=0.019) and, most importantly, the significant benefit from ASCT was confirmed in early and late first relapse. In UK and German trials, the overall survival did not differ in the two treatments. These results have been confirmed in other two case-control studies[4,5]. The conclusion of all these trials was that HDC/ASCT is a superior treatment for patients with relapsed or refractory Hodgkin lymphoma and this approach has become the standard therapy for these patients.

In recent years, the ASCT techniques have been greatly improved, prognostic factors have been identified, and the optimal timing of ASCT has been evaluated. A new opportunity to further improve the ASCT procedure was to intensify the therapy with the use of augmented doses of mobilization regimens[6] or additional therapy after PBSC collection[7]. Both these techniques have been reported to be able to improve the outcome. The Cologne high-dose sequential (HDS) protocol utilized 2 cycles of conventional chemotherapy. The patients, who achieved a response, received high-dose cyclophosphamide with subsequent PBSC collection; soon after, high-dose methotrexate and etoposide and, finally, HDC (BEAM) and ASCT was given to the patients. The authors were able to demonstrate that HDS was feasible with acceptable toxicity[8]. Recently, the German Hodgkin Study Group and EBMT reported the HD-R2 trial, a randomized comparison of high-dose sequential therapy followed by ASCT vs standard chemotherapy (DHAP) followed by ASCT. The median follow-up was of 42 months and no significant difference in freedom from therapy failure, progression free survival or overall survival was observed until now[9]. In both randomized studies, the results in primary refractory patients were disappointing. Sweetenham et al. published a retrospective EBMT analysis in 175 patients who fail to enter remission with induction chemotherapy10. The actuarial 5-year PFS and overall survival was 32% and 36%, respectively; moreover, patients receiving more than one chemotherapy line before ASCT did worse in terms of overall survival and progression-free survival. Similar results were achieved by GELTAMO Cooperative Group and Fermè in France in 62 and 157 patients, respectively[11,12]. The results were better in the ABMTR analysis on 122 patients not responsive to first line therapy[13]. The reasons of these discrepancies are not clear. Unfortunate-

ly, patients with rapidly progressive disease, poor performance status, older age, and poor stem cell harvest were not included in these reports; moreover, it is possible that these different results were subject to significant selection of patients.

Can we improve the results after Autografting?

An intensification strategy has been tested with the use of double ASCT[14]. There are limited registry data to support such a strategy. In the GELA multicenter H96 trial the patients were assigned to a single or double ASCT on the basis of presence of risk factors. The patients with primary refractory disease or with at least 2 poor risk factors, received double ASCT; all others received a single ASCT[15]. The overall survival at 5 years was 46% in the poor risk patients with 6% transplant-related mortality. The IBMTR reported 21 Hodgkin lymphoma patients underwent a second ASCT.[16] No difference was found in outcome; the results were particularly worse in patients receiving the second ASCT within 12 months from the first ASCT. Transplant-related mortality was 11% with the double ASCT and 5-year PFS was 30%. These trials demonstrated to be feasible but should be tested prospectively and compared with a standard single ASCT. New drugs and novel treatment strategy, that are based on our understanding of the disease biology and signaling pathways, have been identified as promising new agents for the treatment of patients with relapsed Hodgkin lymphoma. The two leading monoclonal antibodies (SGN-35) and histone deacytilase inhibitors OLDH568 (Panobinostat) are being tested in prospective clinical trials. A new strategy to improve ASCT might be to incorporate these drugs with salvage regimens and as maintenance after stem cell transplantation. The new randomized trials with these new drugs (SGN-35, Panobinostat) are ongoing and hopefully can modify the number of relapses.

Prognosis after failure ASCT

The median overall survival for patients who progress or relapse after ASCT is 2 years or less. The GELTAMO cooperative group reported the long-term outcome in 175 patients who relapsed at a median time of 10 (range, 4-125) months after ASCT. One third and one fourth of them are alive and free of relapse at 3 years[17]. Adverse prognostic factors for progression-free survival were advanced stage at relapse and short time interval between ASCT and relapse less than 1 year. The patients who had both characteristics had 14% 3-year progression-free survival. Another analysis derived by Lymphoma Working Party of the EBMT database on 462 patients. After a median follow-up of 49 months, overall survival was 32% at 5-years. In multivariate analysis independent risk factors for overall survival were: advanced stage, poor performance status, age >50 years, early relapse. Overall survival was extremely low (12%) in patients with 2 or more risk factors while was high in 62% of patients with one factor only[18].

The role of functional imaging before ASCT.

Retrospective studies suggest that Gallium or FDG-PET after salvage chemotherapy and before HDC/ASCT can be predictive of poor outcome[19]. More recently, Moskowitz et al. have studied functional imaging after salvage chemotherapy reporting 75% 5-years event-free survival if functional imaging was negative and 31% for functional imaging-positive disease.[20] Castagna *et al.* in 24 patients who underwent FDG-PET after 2 cycles of salvage chemotherapy, reported 2-years PFS of 93% for PET-negative and 10% for PET-positive patients[21]. More recently, the role of PET has been analyzed in a group of 101 patients who received ASCT for Hodgkin lymphoma and non-Hodgkin lymphomas. Both FDG-PET after 2 courses of chemotherapy and clinical risk score were independent prognostic factors for progression-free survival after ASCT[22]; moreover a poor outcome after ASCT was identified by the Houston team when PET resulted positive before ASCT19. Despite these and other reports, PET scanning is still contradictory to predict outcome after ASCT. We need more robust results in order to determinate risk-adapted therapy outside of a clinical trial.

Allogeneic stem cell transplantation (AlloSCT)

Registry-based studies published in 1996 with myeloablative allografting gave disappointing results. AlloSCT was able to result in lower relapse rates but TRM exceeded 50% due to toxicity and infection complications. The explanation for this high mortality is uncertain but might include the selection of very high risk patients (many patients were allografted in advanced phase of the disease) combined with immunodeficiency, peculiar to Hodgkin lymphoma, leading to infectious complications. The use of low-dose preparative regimens, aimed at immunosuppression rather that tumor ablation, has decreased the early mortality rate in hematological neoplasias.[23,24] This new approach have become increasingly popular after it became apparent that much of the anticancer effect from allografting is due to the adoptive immunotherapy. The largest cohort of patients (n=285) treated with RIC was recently reported by the Lymphoma Working Party of the EBMT25. Patients have been highly pretreated with a median of 4 lines of therapy. The 100-day TRM was 12% but increased to 22% at 3 years. The best results were achieved in patients with chemosensitive disease. The development of chronic GVHD was associated with a higher TRM but a lower relapse rate. In a landmark analysis the development of GVHD by 9 months post-RIC was associated with lower relapse rate. Similar results were recently updated by MD Anderson Cancer Center in 58 patients with relapsed or refractory Hodgkin lymphoma.[19] More recently, the final results of a multicenter phase II prospective study on the role of RIC were presented at ASH201026. Ninety-two patients with an HLA identical sibling or a MUD were treated with two courses of salvage chemotherapy. Seventy-eight patients (85%), who showed no progression, were eligible to receive a RIC. All patients with refractory disease died of lymphoma. Chronic GVHD was associated with a lower relapse incidence and a better progression-free survival. Patients allografted in remission had a significantly better outcome.

Sureda et al. has performed under Lymphoma Working Party of the EBMT the only analysis which to compare outcomes after RIC or myeloablative conditioning[27]. Ninety-seven patients were allografted after RIC and 93 patients after myeloablative regimens. Non-relapse mortality was significantly decreased in the RIC group. Chronic GVHD significantly decreased the incidence of relapse, which translated into better progression-free survival and overall survival. The conclusion of this analysis was that the significative reduction of the transplant-related mortality after RIC was able to put in evidence the existence of a GVHL, which was able to improve the long-term outcome of relapsed and refractory Hodgkin lymphoma patients. The most direct evidence of a GVHL comes from Hodgkin lymphoma response to donor lymphocyte infusion. Peggs and associates described the results of RIC and donor lymphocyte infusion in patients with multiply relapses. The conditioning regimen included fludarabine, melphalan and alemtuzumab[28]. Patients who had less than a CR or progression at 3 months received donor lymphocyte infusion. All patients engrafted and the 4-year progression free survival rate was 39% with a nonrelapsed mortality at 2 years of 16%. Sixteen patients received donor lymphocyte infusion at 3 months because of lack of response or progression and 8 of them achieved complete remission.

Other small series in Hodgkin lymphoma confirmed these results and reported response rate of 44-54% following DLI administration.[24,28-31] All these data confirm the presence of a clinically effective GVHL effect.

Tandem ASCT and RIC

Autografting and myeloablative allografting each offers potential roles in the treatment of relapsed/refractory Hodgkin lymphoma. Each modality has its limitations: ASCT has become the standard therapy for these patients but the relapses remain the most important cause of treatment failure; allografting is conditioned by high-mortality risks mediated by immunosuppressive drugs and GVHD. The safety of allografting has improved with the use of RIC, but, since graft vs Hodgkin lymphoma responses might be insufficient when Hodgkin lymphoma is bulky and tumor growth is rapid, it was thought that intensive lymphoma cytoreduction prior to RIC may allow reaction to be exploited[31]. This tactic could provide the benefit of a conventional allograft but with a reduction in the typical acute toxicities and associate mortality of myeloablative therapy. Tandem ASCT/RIC was pioneered by the Genoa team in 10 patients with Hodgkin lymphoma and 5 patients with non-Hodgkin lymphoma[31]. Thirteen of the 15 patients were considered to have poor prognoses due to chemotherapy-refractory disease (n=6), relapse (n=8), or bulky disease at ASCT. The allogeneic conditioning consisted of fludarabine and cyclophosphamide with short-course of methotrexate and cyclosporine post-grafting for GVHD prophylaxis. Nine of 12 patients in partial remission after ASCT achieved complete remission after RICT, while 3 patients had progressive disease. Donor lymphocyte infusions were given to 7 patients, in 2 patients for progressive or persistent disease and in 5 patients for mixed chimerism and persistent disease. At the time of the analysis, 10 patients were alive, while

5 patients died either from progressive disease (n=2), progressive disease and GVHD (n=1), chronic GVHD (n=1) or Aspergillus infection (n=1). The severity of GVHD appeared tolerable with only 1 patient dying directly from complications of extensive chronic GVHD. Recently, we have updated our results together with those of Humanitas Institute of Milan[32]. A total of 27 patients underwent tandem ASCT/RIC. Twelve patients were chemosensitive and 15 chemorefractory at ASCT. The time interval between ASCT and RIC was 3 months. Chimerism studies indicated 100% donor-derived engraftment. Seven patients developed acute GVHD and 9 chronic GVHD (2 limited and 7 extensive). At the last follow up 17 patients (63%) were alive, 12 (70.6%) in CR and 5 (29.6%) with persistent disease. Ten patients expired (37%): 7 patients of disease progression, 1 patient of acute GVHD, 1 patient of extensive chronic GVHD and 1 patient of infection. Overall survival was 47 months at a median follow up of 46 months. These results suggest that GvLy may have a role on residual disease after ASCT. These results have been recently confirmed also by Gutterman et al. in 23 Hodgkin lymphoma patients. The U.K. Authors has employed a more complex approach with a combination of an intensive preparative regimen (BEAM) together with a profound T-cell depletion with Alemtuzumab as acute GVHD prophylaxis. They demonstrated that this approach was associated with sustained donor engraftment, high response rate, minimal TRD (7.6%), and a low incidence of extensive GVHD[33]. In conclusion, the tandem auto-allotransplant is a feasible approach in high-risk Hodgkin lymphoma and in the next future it should be evaluated on more patients, possibly in a randomized size.

Conclusions

Stem cell transplantation is an effective approach in relapsed/refractory patients with Hodgkin lymphoma. In these last three decades, the autografting techniques evolved. Today the patients receive cell collected from peripheral blood in combination with growth factors and a rapid return of normal blood cells is achieved. The procedure is generally safe and, in some instances, can be carried out as an outpatient basis.

The place of allografting in Hodgkin lymphoma remains a point for debate. Allografting has yielded lower relapse rates compared to ASCT, likely due to graft-versus-lymphoma effect. Long-term results of RIC demonstrate a progression free survival of 25-35% and an overall survival at 2-3 years of 35-60%. However, the long-term effects of acute and chronic graft-versus-host disease including both mortality and potentially serious morbidity represent a reason for caution in recommending this treatment approach. On this basis, we recommend RIC in the context of prospective clinical trials. In the next future we should try to reduce the relapse rates after RIC and prospectively compare RIC to both ASCT and conventional salvage therapy.

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EPIGENETIC THERAPY IN ACUTE PROMYELOCYTIC LEUKEMIA

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Epigenetic alterations are linked to the establishment and maintenance of the cancer phenotype. Importantly, those alterations are potentially reversible, since they do not involve genetic mutations in the underlying DNA sequence. Epigenetic therapies are based on the identification of drugs able to interfere with the activity of enzymes responsible for the epigenetic alterations occurring in tumour cells: new drugs have recently been approved for use in cancer patients, validating clinically this strategy. Unfortunately, clinical responses are not always consistent, and do not parallel closely the results observed in preclinical models.

The main reasons for this failure are: a) the use of epigenetic drugs in a modality of treatment similar to traditional chemotherapy, not based on a targeted approach; b) the lack of biomarkers for directing therapeutic choices, due at least in part to the lack of appropriate technologies for epigenome profiling of cancer samples; c) the use of inappropriate preclinical models, mainly based on cell lines where epigenetic drift has occurred as a consequence of long term in vitro culture.

Our work is based on the postulate that epigenetic therapies have to be directed against specific functional epigenetic alterations present in cancer cells. In order to identify those alterations, and investigate their mechanistical and biological consequences, we are developing novel technologies for the study of the epigenome, and adapting their use to preclinical models of leukemias based almost exclusively on the use of primary cells.

Acute Promyelocytic Leukemia (APL) represents a paradigm for cell transformation caused by the establishment of an aberrant epigenetic status leading to gene silencing.

Here, the balanced chromosomal translocation t(15;17) promotes the generation of the oncogenic PML/RARalpha fusion protein which behaves as an altered RARalpha, recruiting different chromatin modifying enzymes (as histonedeacetylases (HDACs) and DNA methyltransferases (DNMTs)) and inducing stable silencing of RARalpha target genes at physiological concentrations of retinoic acid. These transcriptional changes lead to myeloid differentiation block, that together with subsequent genetic lesions leads to the development of leukemia. Preclinical models of APL are among the best studied for epigenetic alterations in cancer. In this disease, HDACi and in particular the HDAC class I inhibitor valproic acid (VPA) was shown to be selectively active on leukemic blasts through the induction of apoptosis mediated by TRAIL/DR5 and FAS/FASL death receptor pathways (Insinga A. et al., 2005). Indeed, VPA induced rapid tumor regression and sharply prolonged survival. However, discontinuation of treatment was associated to an immediate relapse of the disease. These results suggest that VPA is able to act on the bulk of leukemic mass but has a little effect on leukemic stem cells (LIC), which represents the leukemic subpopulation responsible for leukemic growth and propagation.

For this reason, we focused our attention in studying whether distinct subpopulations within the leukemic mass show differential sensitivity to epigenetic drugs (i.e. LIC). To this purpose, we set up an in vivo leukemia transplantation mouse model (Figure. 1). We used leukemic cells derived from mGC-PML/RARA mice (Westervelt P. et al., 2003) which are Ly5.2, and transplanted them in the congenic strain C57BL/6J-Ly5.1. In this way it is possible, by facs analysis, to detect donor-derived APL blasts. This information allows us to determine in the best way when to start the epigenetic treatment. After epigenetic treatment the purified leukemic blasts (expressing the common leucocyte antigen Ly5.2) are intravenously inoculated into secondary recipients (Ly5.1+) at different dilutions in order to establish the effect of the in vivo administration of the drug on the LICs through their transplantation capacity.

To further investigate the interplay between PML-RAR and HDACs, and in particular to understand the different contribution to APL development played by the different HDACs from class I, we have performed a selective knock down of HDAC1, 2 and 3 through RNA interference. In detail, we generated several retroviral vectors containing shRNAs targeting different regions of the HDACs transcript and expressing GFP as a marker. As control, we used a hairpin, targeting the firefly luciferase gene (LUC), Using these vectors, we transduced in vitro lineage-negative (lin-) cells enriched in hematopoietic stem cells (HSCs) and progenitors from mCGPR/PR mice (Minucci S. et al, 2002), and analysed the phenotype of transduced cells in vitro and in vivo, upon transplanting knock-down cells into lethally irradiated syngenic mice. These studies are going and will provide a comprehensive analysis of the role of individual HDACs in the pathogenesis of APL.

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MOLECULAR PATHOGENESIS OF MYELOPROLIFERATIVE DISORDERS

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The Philadelphia-chromosome negative chronic myeloproliferative neoplasms (MPNs), according to the WHO 2008 classification, include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) [(1)]. These are clonal disorders that originate by a multipotent hematopoietic stem/ progenitor cell and manifest with abnormally increased proliferation of terminally differentiated myeloid cells. They are characterized by intriguing phenotypic overlapping, that led to the suggestion of a *continuum* among them; PV and ET can evolve to post-PV and post-ET myelofibrosis (MF) while all can transform to acute leukemia (AML).

Although the categorization of these disorders in only one family dates back to 1951, it was in 2005 only that the molecular basis of MPNs started to be appreciated following the discovery, by four groups almost simultaneously, of a point mutation in exon 14 of Janus kinase 2 gene, the JAK2V617F mutation. A number of subsequent studies then demonstrated that the V617F allele is harbored by more than 95% of patients with PV and about 60% of those with ET or PMF. The mutation is located in the JH2 domain of the protein, or pseudokinase domain, considered to be catalytically inactive but credited to exert a negative control role on the activity of the catalytic domain, JH1. As a consequence of the V617F mutation, this negative controlling role is lost. Recent data, however, partially challenged this theory by showing that JH2 has a dual catalytic activity by phosphorylating Ser523 and Tyr570 in the JH1 domain; in JAK2V617F mutated cells, the levels of phosphorylation of Tyr570 resulted significantly reduced. Thus, JAK2V617F mutation is a *gain-of-function* mutation in that it results in a constitutive and hypersensitive JAK/STAT signaling pathway, but actually it would originate from a loss/reduction of a normal function exerted by functional JH2 domain. Additional mutations in exon 12 of JAK2 (that contributes to JAH2 domain as well as) were described in a few patients with PV lacking the V617F allele, while mutations at codon 515 of MPL, the receptor for thrombopoietin, were subsequently described in 3-8% of patients with ET and PM. These mutation phenocopy the V617F allele, all resulting in activation of tyrosine kinase-dependent cellular signaling pathways that include at least the JAK-STAT, Akt/mTOR and ERK pathway. Noteworthy, evidence of activation of the JAK/STAT pathway is found also in patients who lack mutations in JAK2 or MPL, pointing to still unknown mutations in other genes linked to this pathway. Conceivably, inhibitors of JAK2 and JAK1 resulted equally active irrespective of the JAK2 mutational status and the disease being a PMF or a post-PV/post-ET MF. The identification of the JAK2 and MPL mutations promoted very rapidly the revision of the diagnostic criteria of MPNs by the WHO in 2008. Thus, genotyping for JAK2 and MPL deserves diagnostic relevance, while their role and importance as variables useful for prognostic assessment is less clear. Based on several retrospective, and a few prospective, studies, it appears that presence and/or high V617F mutated allele burden is likely associated with increased risk of thrombosis in ET and PV and a higher risk of evolution to post-PV and post-ET myelofibrosis; however, it does not matter at all for survival in ET or PV. In PMF, on the other hand, a low JAK2V617F allele burden has been shown to have negative prognostic relevance. Data for MPL mutations are still debated, but this mutation did not resulted in a discrete phenotype in ET and both PMF and post-PV/post-ET MF in at least two large series including almost 1000 patients.

Mutations in JAK2 and MPL point to dysregulation of tyrosine kinases as a pathogenetic lesion in MPNs, and this observation put the basis for the rapid development and clinical exploitation of small inhibitors of JAK2, with the hope to reproduce the successful history of the tyrosine kinase inhibitor Imatinib in chronic myelogenous leukemia. Indeed, involvement of tyrosine kinase is a recurrent theme in myeloid neoplasms, as exemplified by the constitutive activation of ABL in the BCR-ABL fusion protein in chronic myelogenous leukemia, the D816V (and other les frequent) mutation in the tyrosine kinase receptor c-KIT in mastocytosis, or the rearrangement of platelet-derived growth factor receptor- or - and fibroblast growth factor receptor in hypereosinophilic disorders. Additional mutations that might equally result in dysregulated tyrosine kinase-dependent signaling have discovered in LNK, that encodes for a member of a family of adaptor proteins involved in the negative regulation of JAK/STAT signaling, or in CBL. The latter encodes for a multifunctional adapter protein with ubiquitin ligase activity that negatively regulates activity of JAK2, MPL, and several other tyrosine kinases and receptors by inducing proteosomal degradation through ubiquitination in endosomes. Mutations in CBL are seen rarely in MPNs (approsimately 6% of PMF), while they are definitely more common in juvenile myelomonocytic leukemia and classic chronic myelomonocytic leukemia in the adult. It is unclear whether they produce distinct phenotype and/or have prognostic relevance at all.

High-throughput genomic approaches have more recently resulted in the identification of an unforeseen large group of mutations that affect proteins involved in the epigenetic regulation of transcription, and include TET2, ASXL1 and EZH2 [2]. These mutations occur in a wider spectrum of myeloid malignancies than classic MPNs only, such as myelodysplastic syndromes (MDS), MDS/MPNs and acute myeloid leukemias (AMLs), suggesting that these mutations might contribute a common genomic hit in myeloid malignancies. Abnormalities in other genes involved in epigenetic regulation, such as IDH1 and IDH2 and DNMT3A, have been detected preferentially in association with leukemic transformation of chronic MPNs as well as in de-novo leukemias.

TET2, that stands for Ten-Eleven-Translocation-2, is involved in the 5-methylcytosine hydroxylation resulting in the generation of 5-hydroxymethylcytosine (hmC) in the DNA; hmC has been found enriched in actively transcribed genes. Mutations consist of small insertions, deletions and nonsense mutations, all expected to result in a loss-of-function of the protein, and missense mutations affecting catalytically active regions. Most TET2 alterations are heterozygous, suggesting that TET2 haploinsufficiency may be a mechanism sufficient for transformation. Tet+/- mice showed a myeloproliferative phenotype with splenomegaly, extramedullary hematopoiesis and marked expansion of monocytic compartment. TET2 mutations have been reported in approximately 14% of MPNs; the greatest frequency was found in CMML (50%). Sequential analysis of TET2 mutation occurrence during MPN progression has shown that TET2 mutations may precede or follow JAK2V617F mutation or occur at the time of disease transformation to AML. Inhibition of TET2 catalytic activity is also driven by the neomorphic IDH1/2 mutant proteins.

EZH2 encodes for the catalytic component of the polycomb repressive complex 2 (PRC2), the PcG Enhancer of Zeste Homolog 2. PRC2 is involved in the methylation of histone H3 at lysine 27 (H3K27me3) that is associated with inactive chromatin. EZH2 also associates with DNA-methyltransferases regulating DNA methylation. Both monoallelic and biallelic mutations of EZH2 have been reported in 5-10% of patients with PMF; they are scattered over the gene and include missense, nonsense and premature stop codons resulting in loss of function. Conversely, an activating Tyr641missense mutation has been identified in lymphomas, overall suggesting that EZH2 may behave as tumor suppressor or oncogene depending on the cellular context. A recent study showed that EZH2 mutated PMF patients had shorter overall survival and leukemia free survival compared to wild-type subjects [5]; therefore, EZH2 might help in the prognostic stratification of PMF patients complementing IPSS score categorization.

ASXL1 encodes the Additional SeX combs–Like protein-1 , one of the 3 mammalian homologs of Drosophila Additional Sex Comb (Asx) gene. All mammalian ASXL proteins have conserved sequence features: the amino-terminal ASX homology (ASXH) region, which contains 2 of the 3 putative nuclear receptor (NR) box domains, and a carboxy-terminal plant homeo-domain (PHD) finger. It is a member of the Enhancer of Trithorax and Polycomb (ETP) family that includes proteins involved in both the maintenance of activation and silencing of gene expression by modifying chromatin configuration, depending on the cell context. Frameshift mutations, nonsense mutations, and large 20q11 deletions of ASXL1 have been

described in 10-15% of MPNs and MDSs, 40% of CMML. There is a mutational hot-spot in exon 12; mutations at this level disrupt the protein downstream of the ASXH domain with loss of the PHD domain. Embryonic/perinatal lethality was produced by germline targeted disruption of Asxl1 with no evidence of myelodysplastic or myeloproliferative disorder in the few surviving mice. In MDSs, ASXL1 mutation have adverse correlation; data in MPNs are still largely inconclusive. Thus, from a putative single-gene driven (JAK2) disease, the MPNs have become an attractive arena for novel mutational discoveries. There is no question that the JAK2 and MPL mutations have a central role in diagnostic approach to these disorders, due to their frequency and feasibility of mutational analysis, and that this is not the case for most other genes whose mutations are confined to relatively small percentage of patients and are technically consuming being scattered over many exons. This mutational plethora has also made MPNs a complex field of investigation to delucidate the number and sequence of pathogenetic events. Several lines of evidences indicate that mutations in tyrosine kinase (such as JAK2V617F and similar) are not sufficient for disease initiation and progression; rather, mutated JAK2V617F can provide a proliferative advantage to progenitors during differentiation, allowing a clonal dominance in late stages of differentiation, but it does not appear to target the stem cell [4]. On the other hand, abnormalities of proteins involved in epigenetic regulation might be of outmost relevance by presumably targeting pivotal mechanisms involved in stem cell fate [5]. Furthermore, intriguing recent data stand for a link to exist between mutated tyrosine kinases and epigenetic regulators, since JAK2V617F has been shown to localize nuclear and displace the repressor heterochromatic protein HP1 from chromatin by phosphorylating histone H3 at Tyr41[6]; on turn, this would affect the methylosome by disrupting the association between protein arginine methyltransferase 5 (PRMT5) and its cofactor MEP50 following uncontrolled PRMT5 phosphorylation[7].

This growing molecular complexity of MPNs will certainly reflect also in the possibility to find novel abnormalities that could be targeted for innovative therapies, that could include more specific and potent (and less toxic) inhibitors of JAK2 and/or other pathways such as Akt/mTOR [8], agents (such as demethylating and inhibitors of histone deacethylase) that act on proteins involved in complexes regulating gene expressions, alone but more efficiently (perhaps) in combination. Finally, it will be key to understand the molecular events that transform a chronic and relatively benign MPN into an acute leukemia; the recent identification of an excess of abnormalities in Ikaros [9] and p53 [10] at this stage represent novel fundamental observations.

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FROM BENCH TO BEDSIDE. A NEW APPROACH FOR GENETIC DIAGNOSIS OF ACUTE LYMPHOID LEUKEMIA.

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New genomic technologies have greatly enriched our knowledge of the molecular events underlying the genesis and/or evolution of acute leukemia. With on-going discoveries of genetic lesions multiple molecular events have emerged as co-operating in different pathogenic pathways in acute lymphoblastic leukemia (ALL). Leukemogenesis of T-cell ALL (T-ALL) in children and adults depends upon recurrent lesions affecting oncogenes and tumor suppressor genes which are implicated in cell proliferation and/or survival (LCK, PTEN, NF1, PTPN2, JAK1, and ABL1), self-renewal (NOTCH1 and FBXW7), cell differentiation (TLX1, TLX3, HOX genes, MLL, LYL1, TAL1/2 and LMO1/2), and cell cycle control (CDKN2A/CDKN2B, CCND2, RB1).[1] Gene expression is deregulated by chromosome translocations that frequently juxtapose T-cell receptor (TCR) promoter or enhancer sequences next to oncogenes which are then aberrantly expressed in developing thymocytes. It is also deregulated by cryptic rearrangements, such as ABL1 abnormalities and C-MYB tandem duplication and by gain- and loss- of-function mutations.[2] Moreover aberrant expression of HOX11, TAL1, LMO1, LMO2, HOX11L2, MYB, or HOXA is associated with a distinctive expression profile.[3-5] The genetic characterization of B-cell ALL (B-ALL) has made great progress with the advent of the SNP array. It has revealed cryptic genetic lesions of oncosuppressor genes, such as EBF and IKZF1, and/or cryptic chromosome rearrangements, i.e., t(X;14)(p22;q32), t(Y;14)(p11;q32), del(X)(p22.33p22.33), or del(Y)(p11.32p11.32), all resulting in a deregulated CRLF2 expression.[6] Intrachromosomal amplification of chromosome 21 (IAMP21) was identified in a distinct subgroup of pediatric B-ALL with poor outcome.[7] IAMP21 behaves as primary event probably because of chromosome 21 instability and other abnormalities, such as IKZF1, CDKN2A/B, and ETV6 deletions occur as secondary events. In addition, in B-ALL gene sequencing has detected loss-of-function mutations of transcription factors such as PAX5, that regulate early B cell differentiation, and gain-of-function mutations that constitutively activate tyrosin-kinases, such as JAK1 and JAK2, and stimulate cell proliferation.[6] Although the challenge at present is to translate all this biological information into routine clinical practice the number of advanced tests that need to be performed to fully characterize genetic subgroups in ALL constitute a major obstacle. To overcome this limitation, we set up and validated a combined interphase fluorescence in situ hybridization assay (CI-FISH) using genomic clones (http://www.ncbi.nlm.nih.gov; http://genome.ucsc.edu) that were designed to pick up heterogeneous chromosome rearrangements such as losses, gains and structural changes of genes that are specifically associated with T-ALL and B-ALL. In either break-apart tests or combined split FISH tests clones were directly labelled for double- or triple- colour assays. To optimise use of biological material each slide was spotted with 8 hybridization areas that were delimited by a round 10 mm coverslip stick with rubber cement to avoid cross-contamination. For each individual patient that has been tested to date this cost-effective, flexible and time saving assay has provided in-depth genomic information and is now being proposed as a useful diagnostic tool.[7]

CI-FISH in T-ALL

CI-FISH assay, which can investigate new discoveries of rearranged genes emerging in T-ALL, sofar includes DNA probes for 40 oncogenes and tumour suppressor genes.[8] When applied in 35 adults and 41 children it classified 65% of adults and 82% of children according to well-established genetic groups. CI-FISH reliably detected cryptic aberrations and elucidated preferential and forbidden associations of multiple genetic events. For instance, deletion of 6q16/GRIK2 was strongly associated with TAL1/TAL2 and/or LMO1/LMO2 rearrangements. Remarkably, it revealed new cytogenetic mechanisms underlying known rearrange-

ments as well as previously unknown genomic abnormalities. In about 4% of adult patients it detected a recurrent fusion gene involving both NUP214 and SET at 9q34.[8] Finally, in our latest report, CI-FISH identified SQTM1, at 5q35 as partner of NUP214 in an adult patient confirming NUP214 is "promiscuous" gene that undergoes recombination with diverse partners in T-ALL.[9]

CI-FISH in B-ALL

The specific CI-FISH assay for B-ALL, which set up is in progress, carries by now 26 genomic probes for genes involved in structural and numerical aberrations and for the most frequent numerical aberrations. In a preliminary study of 11 patients, focussing on feasibility, it provided information on 7/11 in whom karyotyping had failed. it reliably detected chromosome translocations and/or genomic losses in more than 90%, even unravelling cryptic losses or gains in the 4 patients with successful karyotyping. Hyperdiploid karyotypes were found in 20% of cases and multiple genetic lesions were found in 7 patients. The most frequent were deletion of CDKN2A/B/9p21 (44%) and IGH-translocations (40%). A marker of poor prognosis i.e. loss of IKZF1/7p11, was observed in 2 patients. Interestingly, a rare IGH-CEBPA rearrangement was detected with its underlying translocation t(14;19)(q32;q13) in one patient. Another rare abnormality was loss of EBF1/5q33 in another one.

CI-FISH potentialities and limits

In the future, it is to be hoped CI-FISH will be used in diagnostic laboratories as a surrogate of diverse and most sophisticated technologies. Since CI-FISH successfully explores gene fusions, non-fusion producing chromosome rearrangements, and promiscuous genes extensive investigations with RT-PCR and real-time PCR may be avoided in many patients. As far as regards its detection of genomic imbalances, very preliminary data from our ongoing study tend to show CI-FISH is as sensitive as CGH and array-CGH but not as sensitive as SNPs. Interestingly, CI-FISH provided a result with fewer leukaemic cells in each sample than SNPs. In all our investigations one limitation has emerged with CI-FISH. It provides no information about gene mutations so must needs be flanked by gene sequencing in order to obtain a full genomic identity card.

Conclusion

This brief report has shown that CI-FISH offers several advantages. It helps identify specific known genomic events and unravel unknown lesions as well as simultaneous recurrent events, thus delineating critical leukemogenic pathways in each individual case. As it has the potential to obtain from a few cells full genomic information, except for details of mutations, it constitutes a major step forward in providing an individual genetic diagnosis in T- and B- ALL. This is essential if we are to stratify patients genetically into prognostic groups, use the most appropriate molecular markers for disease monitoring and develop molecularly targeted therapies.

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HEMOSTATIC EMERGENCY IN HEMATOLOGICAL DISEASES

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Hemostatic emergency leading to hemorrhage and thrombosis may be defined as an acute illness that poses an immediate risk of severe debilitating complications. Clinical result of management will depend strongly on the rapidity of making diagnosis and availability of resources. Aim of this paper, is to review some selected conditions potentially associated with life threatening severe thrombosis and bleeding focusing on Antiphospholipid syndrome (APA), Heparin-induced thrombocytopenia (HIT), acute leukaemia (AL) and myeloproliferative neoplasms (MPN).

Emergencies in antibody-mediated bleeding and thrombosis

This area of medicine is currently receiving a great deal of attention. The pathophysiological mechanisms by which antibodies may induce hemostatic disturbancies are still incompletely clarified, although many interesting proposals have been put forward. In this review two paradigmatic clinical syndromes of antibody mediated thrombosis will be considered.

The antiphospholipid syndrome. Antiphospholipid (aPL) antibodies are a wide and heterogeneous group of immunoglobulins that include, among the others, Lupus anticoagulants (LAs) and anticardiolipin (aCL) antibodies (reviewed in 1). LAs are acquired inhibitors of coagulation, firstly described in patients with systemic lupus eythematosus (SLE), which prolong the phospholipid-dependent coagulation reactions. Despite this 'in vitro' behaviour, LAs are not usually associated with bleeding complications.

Investigators made clear that LAs and aCL antibodies do not recognize anionic phospholipids, as long believed, but plasma proteins bound to suitable anionic (not necessarily, phospholipid) surfaces. Among them, beta2-glycoprotein I (beta2-GPI), and prothrombin (PT) are the most common. Interestingly, a pathogenetic scenario for antiphospholipid-mediated thrombosis based on parallelism with HIT has been proposed.[2]

Clinical features and predictors of thrombotic risk. The clinical importance of aPL derives from their association with a syndrome of vascular thrombosis and complications of pregnancy, named "Antiphospholipid syndrome" (APS). Deep vein thrombosis and pulmonary emboli are the most common venous events while the arterial site of cerebral circulation is the most commonly affected.[3] The most severe clinical presentation is the catastrophic syndrome, an emergency that should be managed in conjunction with many specialists including those of intensive care unit.[4] Patients with aPL at presentation might be divided into two groups: the first - the asymptomatic subjects - has a low risk of vascular complications and needs only careful observation; the second - patients with previous thrombosis and/or recurrent abortion - needs active therapy. A considerable number of studies have been performed in the attempt to establish the role of the different aPL antibodies as risk factors of arterial and/or venous thrombosis in theAPS. This analysis suggests that LAs are the strongest risk factor of thromboembolic events for aPL-positive patients,[5] particularly when associated with increased concentration of both aCL and anti-beta2-GPI antibodies (so called "triple positivity")[6]

Management. The optimal treatment of patients to prevent recurrent thrombosis was first evaluated in retrospective studies.[7,8] These studies showed that high-intensity warfarin therapy (INR>3) was significantly more effective than standard-intensity warfarin (INR 2.0-3.0) or aspirin alone in preventing recurrent vascular events (recurrence rates 1.3%,

23% and 18% per year respectively. However, fatal cerebral or uncontrollable bleeding was reported during anticoagulation. Intracerebral hemorrhage in these patients with warfarin-associated coagulopathy in whom a concomitant thrombocytopenia is frequently observed is a typical life-threatening condition that requires emergent management. [9]

Two randomized clinical trials examined the question whether high-intensity anticoagulation (INR 3.1 to 4) is superior to moderate intensity (international normalized ratio [INR] 2-3) for secondary prophylaxis in patients who had an initial thrombotic event and satisfy the laboratory criteria for APS. Both studies found that high-intensity anticoagulation was not superior to moderate-intensity therapy. The role of the emerging novel anticoagulants in preventing thrombotic recurrence in the context of APS awaits to be determined in formal clinical trials.[10,11]

The catastrophic syndrome. The "catastrophic APS", is defined as a life threatening condition (mortality rate 50%) characterized by multiple vascular occlusive events, usually affecting small vessels, presenting over a short period of time, and laboratory confirmation of the presence of antiphosphoplipid antibodies.[4] The clinical picture may be triggered by several factors including infections, trauma, surgery and the withdrawal of oral anticoagulation and mainly depend on two factors: (a) organs affected by the thrombotic event and the extent of the thrombosis; and (b) manifestations of the systemic inflammatory response syndrome (SIRS), which are presumed to be due to excessive cytokine release from affected and necrotic tissues. Specific therapies, according to the CAPS Registry, [12] are anticoagulation (AC) plus corticosteroid (CS) followed by AC plus CS plus plasma exchange (PE) and/or intravenous immunoglobulin (IVIG). The highest recovery rate was obtained by the combination of AC plus CS plus PE (77.8%), followed by AC plus CS plus PE and/or IVIG. Distinguishing APS from other prothrombotic and thrombocytopenic conditions may be difficult and differential diagnosis is posed among other microangiopathic syndromes including thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS) and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, as well as Heparin Induced Thrombocytopenia (HIT).

Heparin-induced thrombocytopenia (HIT). HIT is an important adverse reaction of heparin therapy that can have catastrophic consequences if overlooked or misdiagnosed. This is an autoimmune disease mediated by an antibody that causes platelet activation in the presence of heparin.[13] The antigenic target of this antibody is a multimolecolar complex formed by platelet factor 4 (PF4) and heparin. Binding of heparin-PF4-antibody complexes to the platelet surface leads to tight occupancy of the platelet Fc RII receptors by the IgG Fc-moiety. Strong platelet activation results, with further release of PF4; a vicious circle is activated, ultimately leading to thrombosis.

Diagnostic and clinical aspects. Clinical diagnosis of HIT is usually made on the following criteria: a) a fall in platelet count >50% of basal, typically occurring after 5 to 10 days of heparin use. Importantly, HIT can begin more rapidly (within 2 to 18 hours after the start of heparin) in patients who had already received heparin within the previous 100 days; b) exclusion of other causes of thrombocytopenia; c) possible contemporaneous occurrence of a new thromboembolic complication; d) resolution of thrombocytopenia after cessation of heparin; this last criterion, however, can be applied only retrospectively. Whenever possible, the clinical suspicion of HIT should be confirmed with a specific laboratory test, such as an ELISA measuring the PF4-heparin complexes, now commercially available. Both venous and arterial thrombosis may complicate HIT. Deep venous thrombosis and pulmonary embolism are the most frequent events. Other unusual venous thrombotic complications include warfarin-induced venous limb gangrene, cerebral sinus thrombosis and adrenal haemorrhagic infarction secondary to adrenal vein thrombosis. Arterial thrombosis commonly involves the large arteries of the lower limbs, leading to acute ischemia. Other complications that involve arteries include acute cerebrovascular accidents and myocardial infarction.

Management. Discontinuation of heparin therapy has long been the cornerstone of management of HIT, but this step alone is not enough even for patients with isolated thrombocytopenia. The risk of thrombosis is 10% at 2 days, 40% at 7 days and 50% at 30 days despite stopping heparin. Thus, administration of another rapidly acting anticoagulant is recommended until the platelet count is restored. The major treatment options include danaparoid, hirudin and argatroban.[14] In addition, several observational studies support the use of Fondaparinux which does not usually promote antibody binding to PF4, due to absent cross-reactivity.[15] Warfarin alone, LMWH and platelet transfusions are contraindicated in the acute phase of HIT.

Bleeding and thrombosis emergency in haematological malignancy

Bleeding and thrombosis are major risk factors for early death in acute leukaemia and are cause of severe morbidity in patients with chronic myeloproliferative neoplasms (MPN) and other hematologic neoplasms including lymphomas and multiple myeloma. The frequency of these events depends on the interference with the hemoststic system of cancer cells and may be enhanced by chemotherapy and other specific drugs.

Acute leukaemia. Patients with acute leukemia often present with a hypercoagulable state or with evidence for chronic disseminated intravascular coagulation, even in the absence of active thrombosis and/or bleeding. Leukemic cell procoagulant properties, cytotoxic therapies, and concomitant infections are major determinants of clotting activation in acute leukemia. Clinical manifestations range from localized venous or arterial thrombosis to diffuse life-threatening bleeding and still account for an excess of mortality in acute promyelocytic leukaemia (APL).[16]

How to reduce early hemorrhagic death in APL. All-trans retinoic acid has greatly improved the management of acute promyelocytic leukemia, but has not significantly changed the rate of early hemorrhagic deaths and may actually promote thrombosis.[17] Experts suggest to start ATRA in emergency rooms in patients with suspected new diagnoses of APL in order to be effective in avoiding long delays so characteristic of the hospital course of many patients. Very early treatment with ATRA at the first suspicion of the diagnosis may be one of the few ways in which the early death rate resulting from bleeding, often in the central nervous system (CNS), lung, or gastrointestinal tract, and representing approximately 50% to 60% of early deaths, can be decreased. Although no guidelines are available for prophylaxis or treatment of thrombosis, extrapolation can be made from existing guidelines for management of patients with other malignancies. However, prolonged periods of treatment-induced thrombocytopenia in patients with acute leukemia, require a more judicious application of standard anticoagulant approaches. The use of the newer anticoagulants will require careful assessment of hemorrhagic risk in groups of high risk patients but may be justified under special circumstances.

Acute lymphoblastic leukaemia. In acute lymphoblastic leukemia, susceptibility of patients to thrombotic complications is more frequent than bleeding.The results of meta-analysis performed by pooling available information on thrombotic complications in children and adults with acute lymphoblastic leukaemia documented a major role for therapy-related factors both in children including .L-Asparaginase along with the use of anthracyclines.[18]

Myeloproliferative Neoplasms (MPN). Major causes of morbidity and mortality in polycythemia vera (PV) and essential thrombocythemia (ET) are represented by thrombosis and bleeding, progression to myelofibrosis and transformation to acute leukemia. The most frequent types of major thrombosis include stroke, transient ischemic attack, myocardial infarction, peripheral arterial thrombosis and deep venous thrombosis. Special clinical conditions leading to emergent hemostastic situations include thrombosis occurring in unusual sites, such as hepatic, portal and mesenteric veins, cerebral venous occlusion and spontaneous or drug induced severe bleeding complications. Practical suggestions to guide clinical decisions in these settings remain largely empirical, but recently developed guidelines based on experts' consensus may help to tackle these problems.[19]

Abdominal vein thrombosis, including extrahepatic portal vein occlusion, Budd-Chiari syndrome and mesenteric vein thrombosis, is characteristically encountered in MPNs. Diagnosis may be difficult because symptoms (abdominal pain, hepatomegaly, ascites) are aspecific. Doppler ultrasonography, CT scan and MRI are usually required to achieve a diagnosis. In these thrombosis, MPN may not be clinically obvious because concurrent hypersplenism, occult gastrointestinal bleeding, hemodilution can mask blood count abnormality. Of signifi-

cant diagnostic help is the determination of JAK2 V617F mutation found in about 45% of Budd-Chiari syndrome and 34% of portal vein thrombosis.[20] Splanchnic vein thrombosis requires full-dose heparinization, despite the high risk of gastrointestinal bleeding, followed by long-life oral anticoagulation with PT INR range 2.0-3.0.[21] In Budd-Chiari syndrome trans-jugular intrahepatic portosystemic shunt, angioplasty with or without stenting, surgical shunts, up to liver transplantation should be considered in the most severe cases. Joint management with liver team and follow up varices, and warning about pregnancy is recommended by ELN experts. For those patients with thrombocytosis, Hydroxyurea (HU) should be used to restore counts to 400 x109/L or less as soon as possible.

Severe bleeding. In recent large prospective studies enrolling high-risk patients, mostly treated with HU plus aspirin, reported rates of major bleeding of respectively 0.8 and 0.9 events per 100 persons per year in PV and ET.[22] The main sites affected are gastrointestinal tract. Intracranial bleeding occurs rarely but can be severe and potentially fatal, requiring hospital admission. Intra-articular, retroperitoneal and deep intramuscular hematomas, like those seen in hemophilia, are distinctly unusual.

Hemorrhagic symptoms are more frequent in patients with platelet counts in excess of 1,000-1,500x10^9/L and this may be related to an acquired deficiency of von Willebrand factor (AvWS). Normalization of the platelet count is accompanied by restoration of a normal plasma vWF multimeric distribution and regression of the hemorrhagic tendency. A practical consequence of these observations is the recommendation to consider cytoreductive therapy to ET patients whose platelet count is over 1,500x10^9/L. Qualitative platelet abnormalities in PV and ET have long been investigated. Unfortunately, there is a disappointing lack of clinical correlation with hemostatic complications and these abnormalities do not have any relevant role in identifying patients at risk of bleeding. Serious bleeding may be triggered by simultaneous antithrombotic therapy with anticoagulants or antiplatelet agents. Intracerebral hemorrhage is of major concern and requires emergent management.[23] The value of rFVIIa in patients with cerebral hemorrhage is under scrutiny in clinical trials in which it was shown to reduce growth of bleeding if administered within 4 hours of onset.[9]

Aspirin and other anticoagulants should be used with great caution in patients with previous history of hemorrhagic events or with anatomical conditions with a high bleeding risk (e.g. gastric ulcers or esophageal varices secondary to abdominal vein thrombosis and portal hypertension). The combination of aspirin with anagrelide can increase the risk of bleeding, as shown in the primary thrombocythemia 1 (PT1) clinical trial.[24]

In severe life threatening conditions, platelet apheresis, anti-fibrinolytic agents, such as tranexamic acid, may be considered. Platelet transfusions have been rarely used although the defective platelet function in MPDs may represent a rationale for their indication. The utility of recombinant factor VII has been reported in MPN patients with uncontrolled life-threatening bleeding, but further study are needed. Occasional patients may present with a simultaneous occurrence of both bleeding and thrombosis: in this difficult cases, treatment should be based on the prevalent clinical symptoms and tailored on individual basis.

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INHERITED PLATELET DEFECTS

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Inherited thrombocytopenias

Knowledge of hereditary thrombocytopenias is greatly advanced since the beginning of the century, because several new disorders have been discovered and some of them were unexpectedly found to be among the most frequent forms of hereditary thrombocytopenia. This is the case of MYH9-RD [(1)] and THC2 [(2,3)]. Moreover, a better understanding of the pathogenetic mechanisms of many forms allowed to realize that diseases previously considered separate entities derive from mutations of the same gene and are indeed different clinical expressions of a single disorder. For instance, identification of MYH9 as the gene whose mutations cause May-Hegglin anomaly [(4)] allowed to recognize that Sebastian platelet syndrome, Epstein syndrome and Fechtner syndrome derive from mutations of the same gene and describe overlapping disorders. Similarly, Montreal platelet syndrome disappeared from the list of inherited thrombocytopenias since it has been shown that subjects with this diagnosis were actually affected by von Willebrand Diseases 2B [(5)]. The same fate occurred to Mediterranean macrothrombocytopenia after demonstration that some patients carried the genetic defects of STSL (6) or had a mild form of BSS [(7)]. Table 1 describes inherited thrombocytopenias and Figure 1 reports their relative frequency in our case series.

Although requiring an update to include some new forms, the algorithm proposed by the Italian Platelet Study Group may be useful for diagnostic purposes [(8)].

Hematopoietic stem cell transplantation cured several patients with very severe forms (CAMT, WAS, BSS), but recent demonstration that an oral thrombopoietin mimetic increased platelet count in patients with MYH9-RD opened new therapeutic perspectives [(9)].

Inherited defects of platelet function

Most of the genetic alterations that are responsible for thrombocytopenia also cause a functional defect of platelets. Generally it is mild and contributes only marginally to the hemorrhagic diathesis, as in MYH9-RD, dominant monoallelic BSS, THC2 and GPS. In contrast, in recessive biallelic BSS the functional defect is severe and represents a major determinant of bleeding.

In other diseases, the qualitative defect of platelets is not associated with thrombocytopenia. Due to the shortage of standardized techniques for the study of platelet function, these disorders are difficult to diagnose whenever other defects do not add to platelet dysfunction. Thus, a limited number of cases have been reported and only a few forms are well defined both in terms of phenotype and genotype. They are reported in Table 2.

A diagnostic algorithm for diagnosing these disorders in children has been recently proposed [10].

Hematopoietic stem cell transplantation has been successfully used for children with severe diseases such as CHS and GT. Supportive treatment is used in the other cases.

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ATYPICAL THROMBOSIS IN HEMATOLOGY

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Thrombo-Embolic Events (TEE) are important causes of morbidity and mortality in the general population: they can occur also in patients with Hematology problems as concomitant disorders. TEE can be observed during venous thromboses such as Deep Vein Thrombosis (DVT) with or without Pulmonary Embolism (PE) as well as during arterial thromboses such as Acute Myocardial Infarction, Stroke and Peripheral Artery thromboses. While arterial thromboses are associated with atherosclerotic lesions and become symptomatic especially when they

are located in the terminal arteries, venous thromboses typically involves the lower extremity circulation. Only rarely venous thrombosis with or without embolism (VTE) are localized in atypical sites, such as the cerebral (CVT) and splanchnic veins (SVT). These Atypical Thrombosis (ATH) are the most frightening manifestations because of their high mortality rate. A third site of these rare ATH is the deep system of the extremities that, as for the lower extremity, can be complicated by embolism and post-thrombotic syndrome. ATH can also be associated with several hematologic disorders and often can be underestimated until manifestations of TEE occur. Nevertheless, ATH must be prevented diagnosed and treated as soon as possible, because they complicate the prognosis of the patients with: a) Hemolytic Anemias (HA) such as Sickle Cell Disease (SCD), Thalassemia (THA), and Paroxysmal Nocturnal Hemoglobinuria (PNH); b) Myelo-Proliferative Neoplasm (MPN) such as Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Primary Myelo-Fibrosis (PMF); c) Lympho-Proliferative Disorders (LPD) such as Acute Lymphoblastic Leukemia (ALL), Hodgkin Disease (HD) and Non Hodgkin Lymphomas (NHL) and Multiple Myeloma (MM). In this brief report, the pathophysiology, clinical features and the management of these ATH associated with hematologic disorders will be described.

Atypical thromboses (ATH) associated with Hemolytic Anemias (HA). An increased incidence of thrombosis has been reported in different HA, particularly in sickle cell disease (SCD), thalassemia (THA) and paroxysmal nocturnal hemoglobinuria (PNH). Although HA have different patophysiology, hemolysis per se, whatever the cause, seems to be a pro-coagulant condition. Several mechanisms can be involved, including abnormal red blood cell (RBC) properties, increased plasma concentration of microparticles, release of cell-free hemoglobin and RBC arginase resulting in impaired nitric oxide (NO) bioavailability, increased blood concentration of oxidants and endothelial dysfunction. The major clinical consequence is an increased tendency to develop VTE although the clinical sequelae of hemolysis may include a variety of symptoms caused by NO depletion as a consequence of increased cell-free plasma hemoglobin. Hemoglobinemia, which is a feature shared by most of the HA, has also been suggested as a possible contributing factor to explain the clinical manifestations in thrombotic microangiopathies (TMA), characterized by microangiopathic HA and thrombocytopenia. Among these conditions, SCD and THA syndromes as well as PNH will be discussed in more detail.

Sickle Cell Disease (SCD) and Thalassemia (THA). SCD and THA represent the most frequent genetic disorders worldwide. Although they have different patho-physiologies, patients with both diseases share many clinical manifestations, including thrombotic complications. Stroke, caused by large-vessel obstruction with superimposed thrombosis, is one of the major complications in SCD. Pulmonary hypertension (PAH) is a life-threatening complication in SCD, documented in approximately 30% of SCD patients who were screened. Autopsy series showed evidence of new and old thrombi in the pulmonary vasculature in up to 75% of SCD patients at the time of death. Several clinical series describe the occurrence not only of Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE) but also of the atypical Portal Vein Thromboses (PVT) in patients with beta-Thalassemia Major (THA-M) and beta-Thalassemia Intermedia (THA-I). In a survey of more than 8,000 patients with THA in the Mediterranean region and Iran, the overall prevalence of thrombotic events was 1.65%, distinguished in 0.9% in THA-M and 4% in THA-I. Although less frequent than in SCD, stroke has also been reported in THA-M from Greece and Italy with a prevalence of 20% and 2%, respectively. Important issues related to the thromboses associated with SCD, THA and other HA are the rate of transfusion and the absence of spleen. Regular transfusions were thought to reduce the risk of thromboses in THA. On the other end, it has been suggested that the absence of the spleen in a variety of HA may contribute to an increased propensity to thrombotic complications, especially ATH such as PVT and Budd Chiari Syndrome (BCS). Although data on the relationship between splenectomy and thromboses are scant, moderate thrombocytosis with activated platelets has been reported in older children and adult as the consequence of splenectomy.

Paroxysmal Nocturnal Hemoglobinuria (PNH). PNH is an acquired genetic X-linked disease clinically characterized by a severe HA, VTE and blood cytopenias. The molecular basis of PNH is a somatic mutation of the PIGA gene which encodes a subunit of N-acettylglucosamine phosphatidylinositol transferase, essential for the synthesis of the phosphatidyl–inositol (GPI) that serves as the membrane anchor for different cellular proteins. Intravascular hemolysis is the primary clinical manifestation of the classical PNH form, due to deficiency of CD55 and CD59 proteins responsible for controlling the activity of plasma complement. Although a TEE as the presenting manifestation of PNH is uncommon (5%), thrombosis remains the leading cause of death in these patients. One of the major reasons of the high rate (25%) of fatal thromboses in PNH is due to these ATH: Hepatic Vein Thromboses (HVT), Mesenteric Vein Thromboses (MVT) and Cerebral Vein Thromboses (CVT) were significant predictors of thrombosis-related mortality. HVT leading to Budd-Chiari Syndrome (BCS) appears to be the most frequent thrombotic complications in PNH, accounting for the majority of death. CVT and sinus thromboses were also very frequent. The most remarkable feature of VTE in PNH is its unpredictability and the possibility of an occurrence even years after diagnosis. Why about 50% of the patients with PNH do not develop VTE is unknown. Inherited factors may play a role: VTE is more common in patients who have FV Leiden. VTE in PNH are less frequent in Asian population than those in European ancestry but more common in African Americans. A proven thrombosis requires anticoagulant prophylaxis, to be continued as long as the patient has PNH. Normally this is accomplished with oral anticoagulants although subcutaneous heparin has been used in few cases.

Atypical thromboses associated with Myelo-Proliferative Neoplasm (MPN). Thrombosis is the presenting manifestation in about 20% of Philadelphia-negative MPN and is the leading cause of mortality of these disorders. Diagnostic criteria of these MPN classified as Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Primary Myelo-Fibrosis (MF) have been recently revised according to the discovery that the protein tyrosine kinase JAK2 is mutated (V617F) in >90% of patients with PV and approximately 60% of patients with ET and PMF. Because the current therapy of PV and ET is aimed at lowering the risk of thromboses, the risk classification system in MPN is shaped according to the thrombotic risk. Patients with PV and ET should be defined as high risk if age is greater than 60 years or there is a history of previous thrombosis. Risk stratification in PMF should start with the International Prognostic Scoring System (IPSS) for newly diagnosed patients and dynamic IPSS for patients being seen during their disease course, with the addition of cytogenetic evaluation and transfusion status. Leukocytes and JAK2V517F allele burden have been associated with the high risk of TEE. Patients with MPN can show both arterial and venous events. However there is an high frequency of ATH, such as Splanchnic Vein Thromboses (SVT). The most clinically relevant of SVT are Extra-hepatic PVT, BCS or Hepatic Vein Thrombosis (HVT) and Mesenteric Vein Thrombosis (MVT). Imaging techniques (Doppler US, CT, and MRI) facilitate early diagnosis, which is often difficult because the most frequent symptom (severe upper abdominal pain) is not specific. The geographic epidemiology of SVT is peculiar, PVT being more prevalent in Western counties and HVT in Asia. MPN are the leading cause of SVT not associated with cirrhosis, accounting for approximately 50% of BCS and 25% of PVT. However the diagnosis of MPN associated with SVT is muddled by the frequent concomitant presence of portal hypertension and hyperspenism, which may mask any increase in blood cell counts. The search for JAK2V617F together with the other JAK2 and MPL mutations is therefore useful in the diagnostic workup of SVT because it may help to unravel hidden MPN. In fact JAK2V617F mutation was detected in 40-60% of patients with BCS and in 30-40% of those with PVT also in patients not fulfilling the diagnostic criteria of MPN.

Atypical thromboses associated with Lympho-Proliferative Disorders (LPD): the link between cancer and thrombosis is well recognized and the occurrence of thrombotic complications in cancer patients has important implications, including need for chronic anticoagulation with the association risk of bleeding, possible delays in delivering chemotherapy, a high risk of recurrent thrombosis along with a decreased quality of life. Solid tumors increase the risk of VTE with an incidence as high as 50% in autopsied findings. However, recent surveys in large cohorts of patients with hematological malignancies (HM) have indicated that TEE are at least as common in HM, if not more frequent, than in solid tumors. According to recent retrospective analyses performed in one of the largest cohorts of hospitalized cancer patients, acute and chronic Limpho-Proliferative Disorders (LPD) showed one of the relatively highest rate (%) of TEE, since Acute Lymphoblastic Leukemia (ALL), Hodgkin Disease (HD) and Non Hodgkin Lymphomas (NHL) and Multiple Myeloma (MM) showed TEE in 4.39%, 5.01%, and 5.29%, respectively. These HM with relatively high rates of TEE are also characterized by ATH, such as CVT and SVT.

*Acute Lymphoblastic Leukemia (*ALL) is more frequent in children than in adult, indeed 2/3 of all cases occur at pediatric age. The rate of thrombosis in 1,752 children from 17 prospective studies was 5.2% and the risk varies depending on several factors. Most TEE occurred in the induction phase of therapy: lower doses of asparaginase for long periods were associated with the highest incidence of thromboses as were anthracycline and prednisone. The presence of central line catheters and of thrombophilic genetic abnormalities also appeared to be frequently associated with thrombosis. As far as ATH, Cerebral Venous Thrombosis (CVT) together with Cerebral Infarction (CI) and Stroke are the most frequent (53.8%) in ALL.

Hodgkin Disease (HD) and Non-Hodgkin Lymphoma (NHL): Incidence rate of thrombosis in HD-NHL vary between 3% and 13% when systemic forms of VTE are considered and reach up to 60% in the case of primary CVT: Therefore, these ATH play a major role in the prognosis of these patients. According to the results of a recent meta-analysis including 29 cohorts, 18,018 patients and 1,149 events, the global incidence ratio (IR) of arterial and venous thrombosis was 6.4% with values of IR of 5.3% and 1.1% for venous versus arterial TEE, respectively. The IR was higher for NHL than for HD (6.5% versus 4.7%) and within NHL the TEE occur more frequently in patients with high-grade disease. A relatively highly frequent localization is the atypical site of CVT.

Multiple Myeloma (MM): patients with MM are at increased risk of venous and arterial thrombosis. The pathogenesis remains unclear, but probably involves several factors such as activation of procoagulant factors, acquired activated protein C resistance, and inflammation. In addition to general risk factors for VTE, such as older age, immobility, surgery, and inherited thrombophilia, there are some MM-specific and treatment-related factors that contribute to the increased risk. The risk of VTE is particularly high when patients are treated with thalidomide or lenalidomide in combination with dexamethasone or multi-agent chemotherapy. Thromboprophylaxis should be given in these settings. Which agent is the most appropriate is still a matter of debate, but the results of clinical trials ponted out on the major role of aspirin and low molecular weight heparin more than warfarin. ATH can also be observed in these MM patients, including CVT and SVT.

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THE ROLE OF STEM CELL TRANSPLANT IN CHRONIC MYELOID LEUKEMIA IN TKI ERA

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The treatment of chronic myeloid leukemia (CML) dramatically changed in the last decade. From 1980s to 2000, allogeneic hematopoietic transplant (HSCT) was considered the only treatment for CML. With the arrival of Imatinib (IM) in CML scenario [1], HSCT become the second line treatment, and, recently with the second generation TKi development, third line treatment [2-3]. So HSCT is now reserved for patients with insufficient response to TKi or for advanced phases of disease. The use of IM and other TKi has postponed allogeneic transplant for most of patients and many studies show that TKi use before transplant procedure, does not result in worse outcomes [4-5]. IM and other TKi, expecially in advanced phases, stabilized a bridge to transplant also by a second chronic phase (CP) [6]. It has been showed that achieving a response to IM was associated with a better outcome post HSCT, patients with advanced phases of disease who achieved a cytogenetic response (CyR) on TKi treatment had significantly less toxicity, improved overall survival (OS) and leukemia-free survival (LFS) and less transplant related mortality (TRM) [7]. National comprehensive cancer network (NCCN) and European Leukemia Net (ELN) guidelines recommend consideration of allo-HSCT in patients with suboptimal or failure of response to IM or other TKis. This includes patients who fail to achieve the hematologic response after 3 months of treatment, a CyR by 6 months, an Major Cytogenetic Response (MCyR) by 12 months, a complete cytogenetic response (CCyR) by 18 months, or those who have lost their response anytime. Patients in CML-accelerated phase(AP) or –blastic phase (BP) should receive a TKi to obtain a second CP before transplant to improve the outcome. The 8-year follow up from the IRIS trial confirmed an OS 85% for the IM group, and only one case of disease progression in year 8 after the third year post-achievement of CCyR [8]. At present, there is no role for allogeneic transplant for CML-CP as first line of therapy. In selected cases of patient with high Sokal risk at diagnosis, the option of allo-HSCT could be considered. It has been recently demonstrated that three factors-CyR to IM, Sokal Score and recurrent neutropenia on IM can predict response to second generation of TKi in patients who IM failed (Hammersmith Score HS) [9]. Patients with a high HS could be candidates for allo-SCT, particularly if they have a low EBMT score too. About the management of post-transplant relapse of CML and the use of TKi after that treatment, recently many studies conclude that TKI therapy is capable to induce durable molecular response for CML relapsing after HSCT, both in chronic and advanced phases. Few studies still have examined successfully the role of second generation TKis in the management of CML relapse post-transplant, even though the data are still limited [10]. Thus, different and news approaches are needed for those CML patients being selected for HSCT to improve transplant outcomes. The introduction of conditioning with reduced intensity allow to eligible most patients to the transplant procedure. It is important to pay attention at the correct timing to perform the allo-SCT, the right indications to do that and the treatment before and post-transplant for these patients.

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NEW DRUGS AND STEM CELL TRANSPLANTATION IN MDS: BEFORE, DURING AND AFTER.

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The availability of new drugs in myelodisplastic syndromes (MDS) raises new questions on their use in combination with allogeneic stem cell transplantation (alloSCT). Demethylating agents (DMTi's) including azacytidine and decitabine have been adopted in high risk MDS and results are now available showing that these agents cannot be considered curative despite the fact that these agents are capable of inducing remission either partial or complete including cytogenetic remission, reduce the need of transfusional support and azacitidine was able to prolong survival compared to best supportive care in the recent AZA001 study [1]. Thus in a new algorithm of high risk MDS (MDS with intermediate 2 or high risk according to the International Prognostic Scoring System [IPSS]) treatment options include DMTi's agents (azacitidine and decitabine), intensive chemotherapy (ICT), and allogeneic stem-cell transplantation (alloSCT). The use of the DMTi's agents has transformed the approach to this patients population, in particular older individuals, for whom ICT and alloSCT are not an option. Although these results represent an important advance for patients with MDS, 40% to 50% of patients did not respond to therapy (ie, primary treatment failures), and most responders experienced disease progression within 2 years of response (ie, secondary treatment failures). In lower-risk MDS, treatment strategies are used sequentially and usually include observation in patients with low risk and no transfusion dependency, growth factors, and lenalidomide for patients with alteration of chromosome 5 and anemia. The use of a DMTi's agents is less understood in this group of patients. AlloSCT is usually reserved for patients with lower-risk MDS closer to the time of transformation.

Critical to further development of DMTi's therapies are the identification of molecular/cytogenetic factors that may predict or enhance response although prognostic factors for both response and survival with azacitidine in high risk MDS have been identified by Itzykson et al [2] . Previous low-dose cytosine arabinoside treatment (P = .009), bone marrow blasts > 15% (P = .004), and abnormal karyotype (P = .03) independently predicted lower response rates. Complex karyotype predicted shorter responses (P = .0003). Performance status ≥ 2, intermediate- and poor-risk cytogenetics, presence of circulating blasts, and red blood cell transfusion dependency ≥ 4 units/8 weeks (all P < 10(-4)) independently predicted poorer overall survival (OS). DMTi's agents are being investigated before alloSCT to reduce the tumor burden and particularly the marrow blast percentage as a means to reduce the risk of relapse after transplantation. DMTi's agents could be especially attractive in patients with unfavorable karyotype, where ICT yields low response rates. Azacitidine and decitabine are also being evaluated after alloSCT to reduce relapse after transplantation[3]. Azacitidine has also been used in higher-risk MDS or in AML after MDS, after having achieved CR or PR with ICT, with disease-free survival rates being at least similar to those obtained with consolidation chemotherapy, but with less myelosuppression..

Because ICT can increase the risk of death or prevent patients proceeding to HCT because of toxicities, DMTi's are an alternative for patients to control the disease while they await HCT. There are currently no data on the proportion of patients that are treated with DMTi that are eligible to proceed to HCT. At this time, there are several trials (EBMT, GITMO etc) currently exploring the combination of azacitidine treatment followed by alloSCT. These trials are in their recruiting phase and results are not available. Preliminary retrospective data of post-transplant outcomes on patients receiving prior to alloSCT 5-azacitadine or decitabine are instead available. Based on a retrospective analysis from the Lee Moffit Cancer Research Center, Field et al [4] reported 54 patients with MDS or CMML receiving pre-HCT 5-azacitidine (n=30) or no 5-azacitidine (n=24) and outcomes were similar. One-year OS and relapse-free survival were 47% and 41% for azacitidine patients and 60% and 51% for patients not receiving azacitidine. There was a trend toward decreased early relapse in patients receiving azacitidine. Cogle et al examined 8 patients receiving pre-HCT 5-azacitidine and reported full donor chimerism in both granulocyte and lymphocyte subsets [5]. Two groups have described pre-HCT decitabine effect on HCT. Lubbert et al described 15 patients with MDS or AML and De Padua et al reported on 17 patients with MDS who received pre-transplant decitabine prior to HCT [6-7]. Both groups found no increased toxicity or adverse effects on HCT outcomes. Use of pre-HCT DMTi with unrelated donors did not affect outcomes in a subsequent experience from Field et al [8].

A different approach is to combine DMTi's agents after alloSCT in order to prevent or control relapse after transplantation. In fact the use of non myeloablative regimen for alloSCT in MDS, despite a reduction of transplant related mortality, has been associated to an increase risk of relapse. The rationale for the use of DMTi's relies on several observations. Azacitidine and decitabine may cause phenotypic modification of leukemic cells (including increased expression of major histocompatibility complex class I and human leukocyte antigen [HLA]-DR) and induction of expression of cancer antigens that could potentially enhance the graft-versus-leukemia effect.[3] After an initial report from MD Anderson in patients with acute myeloid leukemia (AML) relapsing after alloSCT and treated with low dose azacitidine , this strategy has been reported to be active and well tolerated also in MDS patients after alloSCT as soon as day 30 after transplantation as maintenance in a phase 1 study from the same group. Azacitidine was given for 1 to 4 30-day cycles. In each cycle, the drug was administered subcutaneously for 5 days, starting on the sixth week after HSCT at 1 of 5 dose levels (8, 16, 24, 32, or 40 mg/m2). The authors demonstrated that it was possible to administer azacitidine early after allogeneic HSCT to the majority of a group of high-risk AML/MDS patients. Approximately 60% of this cohort of heavily pretreated patients was able to receive at least 1 cycle of the drug . This trial also suggested that this treatment may prolong EFS and OS, and that more cycles may be associated with greater benefit considering that longer exposure may be important with DMTi's agents. Interestingly toxicities encountered when azacitidine was given shortly after alloSCT were low, mainly hematological and no increase of graft versus host disease (GVHD) was reported while it was hypothesized that DNA hypomethylating agents could magnify the graft-versus-leukemia effect of allogeneic HSCT by increasing the immunogenicity of cancer cells through increased expression of tumor antigens [3]. Azacitidine and decitabine may also induce increased FoxP3 expression and regulatory T lymphocyte generation, which could conceivably influence GVHD incidence. The experience from the John Hopkins Hospital has also been reported retrospectively on 10 patients relapsing after alloSCT and treated with azacitidine. Six out of 10 patients receiving azacitidine at standard dose achieved complete remission that was associated with complete or near loss of host chimerism. Five of these patients were alive and disease free at a median of almost 2 years [9]. It should be noted that some of these patients were treated also with donor lymphocyte infusions. Platzbecker et al recently reported the results of a prospective single center phase II clinical trial (RELAZA) with pre-emptive azacitidine treatment using minimal residual disease (MRD) detection by cytofluorimetric assay of CD34+ donor chimerism in order to prevent or delay relapse after alloSCT. Patients who experienced a drop in CD34+ donor chimerism to values below 80% without concurrent hematologic relapse (that is, patients with <5% bone marrow blasts as obtained at that time point) entered the treatment phase and were offered treatment with azacitidine. In this trial standard dose azacitidine for 4 cycles was planned. Twenty out of 59 patients were enrolled in the treatment phase. A total of 16 patients (80%) responded with either increasing CD34+ donor chimerism to 80% (n=10; 50%) or stabilization (n=6; 30%) in the absence of relapse. Stabilized patients and those with a later drop of CD34+ donor chimerism to <80% after initial response were eligible for subsequent azacitidine cycles. Again no exacerbation of GVHD occurred and myelosuppression was the main cause for dose reduction or treatment delay [10]. A recent work based on the compilation of four data sets, including three clinical trials and the French AZA compassionate use program reports the outcome of patients experiencing azacitidine failure [11]. The median OS of 5.6 months for high-risk MDS confirmed the poor outcome of these patients. The results of our multivariate model showed that simple clinical and biologic characteristics, including age, sex, cytogenetics, initial bone marrow blast count before AZA, and initial response to AZA, can predict the outcome after failure of AZA treatment. Conventional treatment, such as BSC or cytotoxic drugs, appeared to be of little benefit for such patients. Same results have been reported by the MD Anderson group after failure of decitabine treatment. Interestingly the median overall survival for patients submitted to alloSCT was 17 months for patients failing azacitidine and the median survival was not reached in patients transplanted with stable disease after azacitidine. Thus as stated by the authors allogeneic transplantation remained the option with the best outcome, with long-term survival in a substantial proportion of patients even if

some patients underwent transplantation with progressive disease.

Both combination strategy and new drugs should be explored in order to reduce tumor burden prior to alloSCT.. These include the addition of histone deacetylase (HDAC) inhibitors and tumor necrosis factor inhibitors, among others. In vitro, the combination of an HDAC inhibitor with either azacitidine or decitabine results in synergistic antileukemia activity. In early clinical studies with the HDAC inhibitor valproic acid, faster and increased response rates have been documented. Preliminary data combining azacitidine with lenalidomide in high risk MDS showed a overall response rate of 67% and complete remission rate of 44% [12]. Examples of investigational agents being investigated include clofarabine, sapacitabine, topoisomerase I inhibitors, and a compound known as ON1910. On average, response rates are approximately 30%, with responses documented in patients who have experienced treatment failure with prior DMTi's -based therapy. Finally considering the introduction of new agents in MDS we should also consider the availability of new iron chelators. Iron overload caused by repeated RBC transfusions is clearly associated with liver and cardiac failure in thalassemia major, and iron-chelating agents have demonstrated a beneficial impact on survival in those patients. In MDS, the deleterious effect of iron overload and the beneficial role of chelating agents in multitransfused patients are more controversial. Many consensus statement have been published including the one from GITMO which combines the indications to the old and the new iron chelators for MDS patients candidates to alloSCT [13] and it will be discussed in detail.

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MULTIPLE MYELOMA. NEW DRUGS AND TRANSPLANT: BEFORE, DURING AND AFTER

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Multiple myeloma is a fatal plasma cell malignancy. New insights into its biology have identified mechanisms that have become molecular targets of so-called "new drugs" such as thalidomide, lenalidomide and bortezomib. The central role of high-dose therapy followed by autologous peripheral cell transplantation (AHCT), which remains a standard for younger and/or medically fit patients, has consequently evolved. Currently, cyto-reductive induction therapies before AHCT have incorporated several combinations of both the immunomodulatory derivatives thalidomide or, more recently, lenalidomide, and theproteasome inhibitor bortezomib. Though optimal combinations and treatment duration are a matter of debate, the use of these induction combinations has led to high response rates. Following the cyto-reduction obtained pre-transplant, AHCT can further improve depth of response. Patients younger than 65 years of age without relevant co-morbidities that contraindicate high-dose therapy are ideal candidates for AHCT. Though not all studies have uniformly reported the same advantages, randomized trials have demonstrated superior response rate and overall survival in patients treated with high dose therapy compared with conventional chemotherapy. [1] Whether single and double AHCT have similar outcomes remain to be determined.

Before the introduction of new drugs, the combination vincristine-doxorubicin-dexamethasone (VAD) and dexamethasone alone had been used for many years as pre-transplant induction therapy. So called VAD-based regimen often became reference treatments in phase III study where toxicity and efficacy of new induction treatments, which incorporated new drugs as single agents, were first explored. Initially, a prospective phase III study compared thalidomide and dexamethasone (TD) with VAD and showed higher response rates after induction in the TD arm. However, the benefit was not confirmed 6 months after autologous transplant, since very good partial response rates were almost identical. [2] Two randomized trials demonstrated that TD was better than high dose dexamethasone (HD) in terms of higher response rates and prolonged time to progression in patients treated with TD, but this did not translate into overall survival improvement. [3,4] Main toxicities related to thalidomide were deep vein thrombosis and peripheral neuropathy. In the light of these trials, the Food and Drug Administration (FDA) granted approval for TD for the treatment of newly diagnosed multiple myeloma. In 2 parallel German-Dutch phase III multi-centre trials compared standard VAD regimen with TD plus doxorubicin (TAD): higher very good partial response rates after induction and after AHCT were observed with TAD.[5] In a phase III study, the combination of bortezomib and dexametasone (VD) was compared with VAD as induction therapy before single or double AHCT. Importantly, in both arms, lenalidomide was given as consolidation/maintenance after AHCT. Response rates of at least very good partial response were significantly higher in the VD arm than in the VAD. An advantage was also maintained after the first and the second AHCT. However, the progression free survival did not reach statistical significance between the two arms.[6] Lenalidomide with high-dose dexametasone (RD) was compared with lenalidomide and low-dose dexamethasone (Rd) in a prospective control trial which included either eligible or ineligible patients for AHCT. Though response rate was significantly higher with RD compared with Rd, toxicity and early mortality were higher with RD. A landmark analysis showed that the 3-year overall survival of patients who received AHCT after RD or Rd was 92% whereas in patients who did not receive AHCT was 79%.[7]

The encouraging results of induction therapies with new drugs as single agents and preclinical findings, which showed that immunomodulatory drugs could increase the anti-myeloma activity of bortezomib, formed the rational for combination therapies. Results of a phase III study of bortezomib-thalidomide-dexametasone (VTD) versus TD as induction therapy before and consolidation therapy after double AHCT have recently been reported. [8] After three 21-day induction cycles, VTD was superior to TD in terms of response rates. Higher response rates in the VTD arm were also observed after two AHCT and subsequent consolidation therapy. The estimated 3-year PFS for the VTD group of patients was significantly longer than for those assigned to TD and double AHCT, 68% vs 56% respectively. Longer follow up is needed to possibly confirm a long-term survival advantage. In Total Therapy 3,

VTD combined with cisplatin, doxorubicin, cyclophosphamide, and etoposide was given as induction therapy before and consolidation after double AHCT, while maintenance therapy with VTD was continued for one year after AHCT. Total Therapy 3 significantly improved 2-year EFS and duration of complete remission as compared to Total Therapy 2 which associated TD with double AHCT. [9] A triplet combination of lenalidomide-bortezomib-dexamethasone (RVD) has been explored in small series of newly diagnosed patients. [10,11] A phase I-II study on a series of 66 patients, who included eligible and ineligible patients for AHCT, received up to 8 cycles of RVD. Moreover, RVD maintenance was allowed in responding patients. After the first 4 cycles, the rates of at least near-complete remission and very good partial remission were 6% and 11%. Importantly, in about two thirds of the patients, deeper response was observed after the forth cycle and a further improvement was also reported during maintenance.

The most common toxicities associated with thalidomide include constipation, somnolence, and peripheral neuropathy, frequently sensory or sensory-motor. Dose reduction or drug discontinuation commonly improves symptoms. By contrast, its analogue lenalidomide induces neutropenia and thrombocytopenia and only rarely peripheral neuropathy. Major clinical challenge of up-front treatment with these agents is the risk of thromboembolic complications. Prophylaxis guidelines have recently been proposed by the International Myeloma Working Group. [12] Peripheral neuropathy, primarily sensory, which may seriously impair quality of life, remains an important side effect during bortezomib treatment. To decrease incidence and severity, dose reduction, given on a twice-weekly basis, or once-weekly administration at a higher dose have been proposed.

Overall, the proven clinical efficacy of these new agents, that target not only malignant plasma cells but also the myeloma microenvironment, does not currently allow to reach a cure. This provides the clinical rationale for using them in sequential approaches as induction before and as consolidation/maintenance after AHCT with the goal of converting the disease into a chronic phase that prolongs survival and improves quality of life. Consolidation treatment is designed to improve response following AHCT. Increase in response rates have been reported with the use of both bortezomib and lenalidomide. Moreover, some studies reported molecular remissions in a subset of patients. [13] Maintenance treatment as a means to prolong response duration and extend overall survival remains controversial. Recently, two independent phase III study indeed showed a longer progression free survival in patients treated with lenalidomide maintenance rather than placebo after single or double AHST. 14,15 However, concern has been risen by an unexpected incidence of second primary malignancies during lenalidomide treatment.

In summery, the incorporation of "new drugs" during induction therapy has practically become standard treatment in younger myeloma patients eligible for AHCT. Consolidation/maintenance therapy has resulted in longer progression free survival in several studies. However, convincing evidence that this will translate into a benefit in overall survival is lacking. Therefore, much longer follow up is needed to address this issue. At present, whether the recent advancements in myeloma treatment will eventually allow a cure remains unanswered.

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DIAGNOSTIC WORKUP FOR CLINICAL AND PROGNOSTIC ASSESSMENT OF ACUTE LEUKEMIA

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In the era of crescent understanding of molecular pathogenesis of hemopoietic neoplasms, the diagnostic workup of acute leukemia requires a coordinated intervention of specialized laboratories combining solid expertise in the field of morphologic, immunophenotypical and molecular cytogenetic analysis. Indeed the new World Health Organization (WHO) classification defined different clinical entities mainly on the basis of a combination of phenotypic and genetic findings and the widespread application of this scheme allowed for a reproducible identification of distinct disease subsets and for a more effective stratification of the patients into different risk groups.1 This internationally accepted classification provided scientists with a "common language" permitting the design of international trials testing the efficacy of risk-adapted treatment strategies as well as new drugs targeting specific genetic lesions. The severity of acute leukemia and the importance of a prompt administration of specific treatment makes it mandatory for the diagnostic workup to be rapid, efficient and reliable in assigning the correct nosological and prognostic category.

Basic diagnostic and prognostic assessment

Physical examination, automated blood count with evaluation of the peripheral blood smear and standard biochemistry have a central role in raising the suspicion of acute leukemia. Once leukemia is confirmed by morphologic assessment of bone marrow aspiration, the first information on prognosis derive from collection of simple parameters, such as age, performance status, existence of prior myelodysplasia or previous cytotoxic therapy, high white blood cell count or clinical-laboratory emergencies like tumour lysis syndrome (TLS) and disseminated intravascular coagulation (DIC) as shown in Table 1.[2]

Morphology

Cytomorphologic examination of a stained blood and bone marrow film has a key role in the diagnosis of acute leukemia and in the recog-

nition of specific WHO nosological entities (i.e acute myeloid leukemia with myelodysplasia-related changes (AML-MRC), acute myeloid leukemia-not otherwise specified (AML-NOS)). Rapid recognition of acute promyelocytic leukemia and Burkitt leukemia (acute lymphocytic leukemia-L3 according to the former FAB classification) can change the prognosis of the patient because it allows prompt administration of specific therapy and management of clinical emergencies frequently associated with these leukemia-subtypes (TLS, DIC). Accurate enumeration of blast cells is essential in the diagnostic process. In the WHO scheme, a threshold of 20% or more blasts in the PB or BM is required for diagnosis of AML. In some cases associated with specific genetic abnormalities, however, the diagnosis of AML may be made regardless of the blast count in the PB or BM: AML with t(8;21)(q22;q22), inv(16)(p13.1q22) or t(16;16)(p13.1;q22) and APL with t(15;17)(q22;q12).[3]

The International Working Group on Morphology of Myelodysplastic Syndrome proposed that the sufficient number of cells that should be counted to give a precise blast percentage is at least 200 leukocytes on blood smear and at least 500 nucleated cells on marrow smear, including at least 100 nonerythroid cells.[4] The panel also agreed on a set of recommendations for the morphological definition of myeloid blast cells, normal and dysplastic promyelocytes, monocytes and their precursors, as shown in Table 2.[4,5] Abnormal promyelocytes and promonocytes, but not immature monocytes (former abnormal monocytes), are counted as blast equivalents respectively in acute promyelocytic leukemia and in AML with monocytic or myelomonocytic differentiation.[2]

The new WHO classification renamed the subgroup "acute myeloid leukemia (AML) with multilineage dysplasia" as "AML with myelodysplasia-related changes (AML-MRC)".[3] Patients are assigned to this category if they have 20% or more blasts in the peripheral blood (PB) or bone marrow (BM) and (1) they have a history of documented MDS or MDS/MPN, or (2) have specific myelodysplasia-related cytogenetic abnormalities, or (3) exhibit dysplasia in at least 50% of cells in 2 or more myeloid lineages.[1] Recent observations highlighted that multilineage displasia alone shows no independent clinical effect in AML whereas myelodysplasia-related cytogenetics and a history of previous MDS or MDS/MPN demonstrated to be biologically and prognostically highly relevant, thus justifying the significantly worse overall survival, progression free survival and complete remission rate of patients with AML-MRC compared with cases of AML-not otherwise specified (AML-NOS).[6,7]

Patients with therapy-related AML (alkylating agents, radiation therapy, topoisomerase II inhibitors) have a poor outcome, significantly worse compared to cases of de novo-AML with the same genetic abnormalities.[2] According to a recent study[8] this applies also to patients with therapy-related core binding factor-AML (CBF-AML), thus contrasting with earlier reports. In contrast to myeloid leukemias, there is no official consesus about the threshold for the percentage of blasts required to establish a diagnosis of lymphoblastic leukemia.[1] In general, anyway, the diagnosis should be avoided in cases with fewer than 20% blasts.[1] Morphologic characteristics of lymphoid blast cells are detailed in the WHO classification ot tumours of hematopoietic and lymphoid tissues.[1]

Cytochemistry and immunophenotyping

These techniques are used to determine lineage involvement and maturation stage of blast cells and the diagnostic role of these investigations retains its significance for the diagnosis of AML-NOS, particularly AML with minimal differentiation, and acute leukemias of ambiguous lineage.[2] AML with minimal differentiation shows no evidence of myeloid differentiation, neither morphologic nor cytochemical; the myeloid nature of the blasts is demonstrated by immunological markers so that flow cytometry is essential to distinguish this disease from acute lymphoblastic leukaemia.[1] Acute leukemias of ambiguous lineage comprise those cases that show no evidence of lineage differentiation or express markers of more than one lineage. The criteria for assigning specific lineages to the blasts are both immunological and cytochemical, as shown in Table 3.[2,3] Flow cytometry determination of blast count should not replace cytomorphologic evaluation, because CD34 is not always present on the surface of leukemic blasts and hemodilution or processing artefacts can produce misleading results.[2,3] Conversely, detection of leukemia-associated aberrant immunophenotypes allows for measurement of minimal residual disease during follow-up.[2]

Cytogenetic and molecular genetic analysis

At present, conventional cytogenetics is a fundamental diagnostic and prognostic tool for the assessment of a patient with acute leukemia.

According to the new WHO classification a number of recurrent chromosomal abnormalities subclassifies both acute myeloid leukemia and B lymphoblastic leukaemia/lymphoma (B-ALL). With respect to the former category "AML with recurrent genetic abnormalities" three new cytogenetically defined entities are added: AML with t(6;9)(p23;q34), AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) and AML with t(1;22)(p13;q13) and the former group of AML with 11q23 (MLL) abnormalities has been redefined as AML with t(9;11)(p22;q23) to focus on the most frequent MLL abnormality and best characterized clinical entitiy.[3] Chromosomal aberrations that define the new category of "B lymphoblastic leukaemia/lymphoma with recurring genetic abnormalities" are either balanced translocations and abnormalities involving chromosome number and are associated with distinctive clinical or phenotypic properties and prognostic implications.[1]

Furthermore, as previously mentioned, a number of cytogenetic lesions are considered sufficient to establish the WHO diagnosis of "AML with myelodysplasia-related changes" when 20% or more blasts are present (see Table 4).[2,3]

The karyotype of leukemic cells is the strongest prognostic factor in AML. Younger adults with AML are commonly categorized into 3 risk groups according to cytogenetic profile: favorable, intermediate or adverse (see Table 5).[5]

Complex karyotype, defined as the presence of 3 or more chromosome abnormalities, has been consistently associated with a very poor outcome.[2] It's nevertheless noteworthy that among patients with t(15;17)(q22;q21), t(8;21)(q22;q22) and inv(16)(p13q22)/ t(16;16)(p13;q22), treated with appropriate therapeutic protocols, the presence of additional cytogenetic abnormalities (irrespective of the nature or complexity) has no impact on prognosis.[3,10] Cases harboring these abnormalities have therefore been excluded from the definition of complex karyotype.[2] According to the new WHO classification, cases with other recurring genetic abnormalities should also be excluded because they constitute separate entities.[3]

A recent study of multivariable analysis conducted on a large patients cohort showed that several cytogenetic abnormalities were independent predictors of a poor prognosis, some of them being too infrequent to be considered previously.[10] These included abn(3q) (excluding t(3;5)(q25;q34)), inv(3)(q21q26)/ t(3;3)(q21;q26), add(5q)/del(5q), -5, -7, add(7q)/del(7q), t(6;11)(q27;q23), t(10;11)(p11□13;q23), other t(11q23) (excluding t(9;11)(p21□22;q23) and t(11;19)(q23;p13)), t(9;22)(q34;q11), -17 and abn(17p).[10] Karyotype complexity has been demonstrated to have little impact on outcome in patients already having at least one of the independent adverse-risk abnormalities above identified.[10] From these observation it can be inferred that complex karyotype provides the most significant prognostic information in patients lacking any of the independent favorable-risk or adverse-risk associated abnormalities.[10]

Recently, a new cytogenetic category has been proposed, that is, the monosomal karyotype (MK), defined by the presence of one single monosomy (excluding isolated loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core-binding factor-AML).[2] Patients with monosomal karyotype has been reported to define a subset of acute myeloid leukemia patients with an extremely poor prognosis.[11] A detailed analysis of a large number of patients with AML highlighted that the proportion of patients with MK increased with age and the ninety-eight percent of MK cases were within the unfavorable cytogenetic risk category.[11] Significantly, complete remission rate and overall survival in patients with unfavorable cytogenetics and MK were poorer compared with cases harboring unfavorable cytogenetics but not MK.[11]

Genetic abnormalities do not account entirely for the outcome in ALL but they still provide indispensable prognostic information.[12] In general, the Philadelphia chromosome, t(4;11) and hypodiploidy (<44 chromosomes per leukemic cell) confer a poor outcome, whereas hyperdiploidy (>50 chromosomes) and TEL-AML1 fusion are associated with a better prognosis.[12] Age affects strongly the prognostic significance of these abnormalities, with children performing always better compared to adult patients with the same genetic lesions.[12] The reasons for such an observation are still not clear.[12]

Conventional karyotypic analyses of patients with Ph-positive acute lymphoblastic leukemia showed molecular genetic heterogeneity within the group, with Ph chromosome as the sole abnormality in 30% of patients, associated with other aberrancies in 65% and absent (detectable only by fluorescence in situ hybridization or quantitative

reverse transcriptase-polymerase chain reaction analysis) in 5%.[13] Anyway, in the context of intensive, TKI-based chemotherapy, outcome of patients with Ph+-ALL has been demonstrated to be independent from the degree of karyotypic complexity.[13]

If cytogenetic analysis fails or needs to be confirmed or a faster result is required, fluorescence in situ hybridization (FISH) is an option to detect suspected gene rearrangements. This is particularly important for patients with suspected acute promyelocytic leukemia (APL) because the efficacy of differentiation treatment based on all-trans retinoic acid and/or arsenic trioxide is strictly dependent on the presence of the PML/RARA fusion product, so that genetic confirmation of this specific lesion is mandatory in every case of morphologic diagnosis of APL.[14] Although highly specific, conventional cytogenetic analysis, by definition, fails to detect cases where the PML-RARA fusion results from cryptic rearrangements. It is otherwise potentially useful in the characterization of rarer molecular subtypes of APL including those with t(11;17)(q23;q21), t(11;17)(q13;q21), and t(5;17)(q35;q21), leading to PLZF-RARA, NuMA-RARA and NPM1-RARA fusions, respectively.[14] Fluorescence in situ hybridization using probes that target the RARA-PML fusion gene is highly specific and sensitive. However, depending on the type of probe, FISH analysis can fail in the presence of nonreciprocal rearrangements where RARA-PML is deleted or where PML-RARA results from an insertion.[14] Furthermore, FISH provides no information about the isoform of PML/RARA, which is required for molecular monitoring of minimal residual disease.[14]

An interesting and rapid technique, highly specific for presence of an underlying PML-RARα fusion protein, is immunostaining with anti-PML monoclonal antibodies on dry smears of bone marrow or pheripheral blood.[14] A microspeckled staining pattern (>30 nuclear dots) in the nuclei of leukemic cells indicated the presence of the fusion protein.[14] In normal cells and blasts from other subtypes of leukemia (including APL molecular variants, eg, PLZF-RARA and NPM1-RARA) a different PML staining pattern is observed (<20 nuclear dots/nucleus).[14]

RT-PCR represents an option to detect suspected rearrangements (eg. the recurring gene fusions) if chromosome morphology is of poor quality or if the marrow morphology is typical but the suspected cytogenetic abnormality can't be detected.[2] At present, RT-PCR represents the "gold standard" approach for confirming a diagnosis of APL and for reliable molecular monitoring of MRD through precise identification of PML breakpoint location.[14]

With the advent of molecular diagnostics, somatically acquired mutations with prognostic significance have been identified in several genes, particularly in AML, therefore a marrow and/or blood specimen should routinely be taken for molecular diagnostics.[2]

Within the largest AML subgroub, that is, the cytogenetically normal-AML (CN-AML) prognostic significance has consistently been shown for mutations in the NPM1, CEBPA, and FLT3 genes, alone or in combination in younger adult patients.[2,3,9] KIT mutation appears to attenuate the favorable prognostic significance of CBF rearrangements in AML.[9] The importance of these observations is highlighted by two provisional entities added in the recent WHO classification (AML with mutated NPM1 and AML with mutated CEBPA) and the proposal of a risk-stratification of AML based on both cytogenetic and molecular criteria (See Table 3).[9] There is a growing list of genetic abnormalities that are being investigated for their prognostic significance in AML (among others, TET2, IDH1, IDH2, WT1, RAS, TP53) but specific testing of these lesions is still investigational and far to be included in clinical practice.[2,9]

Conclusions

Reviewing the diagnostic workup of acute leukemia underscores the importance of the immediate clinical recognition of the severity of disease and of a clever integration of clinical and morphologic data to rapidly guide subsequent investigations on the basis of a possible nosological suspect (Figure 1). A paradigmatic example, in this context, is represented by the need of resolute identification of the underlying genetic lesion in cases of morphologic diagnosis of APL, a fudamental step for the selection of the appropriate treatment which, as in many other subtypes of acute leukema, must be started very rapidly.

The central role of laboratory investigations in this process raises some practical issues in daily clinical practice that can be resumed by the question: "Is it more convenient the centralization of diagnostic testing in large laboratories or is it preferable to develop specialized lab technology and expertise at single centres?"

On the one hand, it is undoubtly profitable that a direct communication could occur between the doctor who visits the patient, hopefully the same that formulates the morphologic diagnosis, and the biologists or

technicians responsible for the subsequent laboratory investigations. On the other hand, this close interaction would require many of the required technologies to be present in efficient and specialized labs at the same hospital structure, a situation which would probably determine loss of efficiency due to the low number of tests performed.

While the basic morphologic, clinical and immunophenotypic assessment need to be available at each centre, large and efficient cooperative groups may offer a unique opportunity for centralization of more sophisticated cytogenetic and molecular genetic testing in reference laboratories, each highly specialized in a particular field of investigation and all interconnected in an efficient diagnostic network.[15,16]

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ACUTE LEUKEMIA: UPDATE ON DIAGNOSIS, RISK STRATIFICATION AND MANAGEMENT ACCORDING TO PATHOBIOLOGICAL FEATURES.

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Abstract

Acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) are heterogeneous diseases characterized by large differences in prognosis. Balancing risks and benefits of different treatment approaches in accordance with the individual prognostic profile of patients is actually the basic principle of therapy in both AML and ALL1. Current treatment strategies are based on prognostic factors, and on clinical and biological features, studied at diagnosis, that contribute to therapy stratification. Chemotherapy sensibility, evaluated by Minimal Residual Disease (MRD) detection, is also an useful prognostic value, and should be used to take decisions about post-consolidation therapy, particularly allogeneic transplantation.

Acute Myeloid Leukemia

Fifty years ago the diagnosis of acute myeloid leukemia (AML) was based solely on microscopic evaluation. Initially AML was considered one monolithic disease for which "one size fits all" chemotherapy was applied. Although therapeutic advances have lagged over the past two decades, there has recently been an explosion in new prognostic factors in AML, which is driving our understanding of disease biology and also provided useful information predicting the likelihood of any given patient achieving and maintaining remission following conventional chemotherapy, so that the optimum treatment approach can be selected for individual patients.

It has been widely adopted that acute myeloid leukemia (AML) is a clinically heterogeneous disease, with marked differences in survival following intensive chemotherapy based on age, blast cell morphology, and cytogenetic abnormalities. Defining the individual genetic abnormalities or combinations of markers that provide significant independent prognostic information and establishing their respective relationships to other pretreatment characteristics that impact on outcome, such as age and presenting white blood cell count, presents a major ongoing challenge. Moreover, there is increasing evidence that risk of relapse and overall survival can be predicted by assessment of kinetics and depth of response following frontline therapy and monitoring of the leukemic burden using molecular or immunological approaches to minimal residual disease (MRD) detection. These advances present the exciting prospect that panels of pre-treatment parameters affording independent prognostic information can be integrated with precise measurement of treatment response using MRD technologies to provide greater refinement in risk-adapted management of AML[2].

Diagnosis and Prognosis of AML

1. Cytogenetic Analysis

Refinements in Cytogenetics and prognosis in AML Cytogenetics remain the most important disease-related prognostic factor in AML, and are well-characterized as "favorable," intermediate risk, and adverse (Table 1), that deserve risk-guided treatment strategies. It also allows the identification of cytogenetic entities that deserve targeted treatments (all transretinoic acid [ATRA] and arsenic trioxide in t(15;17)(PML-RARalpha+) acute promyelocytic leukemia and Tyrosine Kinase inibitors in Ph1 t(9;22), BCR-ABL–positive leukemia) .

An important recent observation is the existence within the adverse cytogenetic risk AML of the so-called "monosomal karyotype" (MK+), characterized by the presence of an autosomal monosomy in conjunction

with at least one other autosomal monosomy or structural abnormality. Patients with MK+ have a dismal prognosis, with < 5% long-term survival[3] .

2. Gene Mutations in CN-AML

AML without favorable and particular unfavorable cytogenetic aberrations is classified as intermediate prognosis. The intermediate-risk cytogenetic AML includes cytogenetically normal (CN) and AML with other cytogenetic abnormalities and accounts for approximately 60% of all AML patients. It represent the largest and most heterogeneous group of patients, and, as outlined below, there have been great efforts to identify those in this group who are at higher risk and who may be candidates for allogeneic HCT. Gene mutations studies can help identify genetic lesions that escape cytogenetic detection(e.g., gene mutations, gene expression abnormalities) associated with treatment failure. Recently a number of occult gene mutations have been described that have significant prognostic impact and allows the further dissection of AML into molecular subtypes with distinctive prognosis (Table 1). For instance, mutations in FLT3,NPM1, and CEBPA all carry variable prognostic value. Recently, IDH1 and IDH2, DNMT3A, BCOR mutations were identified. Nevertheless, the prognostic value of these mutations appears to be controversial, and has been hampered by a lack of consensus regarding the likely outcome of such patients4.

Mutations of FLT3, an important class III receptor tyrosine kinase normally expressed in early bone marrow progenitors, are among the most prevalent genetics lesions in AML and are consistently associated with significantly worse survival. The majority of mutations are tandem duplications (ITDs) that lead to in-frame insertions within the juxtamembrane region of the receptor. Less frequent are mutations involving the region encoding the activation loop (tyrosine kinase domain, TKD), which most commonly affect codons aspartate 835(D835) and isoleucine 836 (I836). At the present targeted therapy with FLT3 inhibitors are making in progress.

A major advance in understanding the molecular basis of AML was the discovery by Falini and colleagues that a third of cases, including approximately 50% with normal karyotype, harbor heterozygous mutations in the carboxyterminof the nucleolar phosphoprotein, nucleophosmin (NPM1), associated with a comparatively favorable prognosis, if not associated with FLT3-mutations. Clinically favorable genotypes of AML, for example, involve mutations in the genes of the transcription factor CEBPA (CCAAT enhancer bindingfactor alpha) or nucleophosmin-1 (NPM1) whereas unfavorable genotypes may include those with partial tandem duplications of the MLL gene (MLL-PTD), internal tandem duplications of the gene of fms-like tyrosine kinase3 (FLT3-ITDs) and mutations in Wilms' tumor 1 gene(WT1). While NPM1, FLT3, MLL-PTD, CEPBα are all mutations noted in CN-AML, additional genetic abnormalities are noted in the form of over-expression. Aberrantly expressed genes (e.g. BAALC, ERG, EVI1, miR-181a) will likely become useful in refining molecular risk in CN_AML Furthermore, as these molecular markers are not mutually exclusive, the prognostic impact of the different combinations of mutated and/or aberrantly expressed genes present within the same patient should be carefully evaluated to construct a molecular-risk score for practicing haematologists. These findings emerge from a field of intense scientific activity. There is little question that more genetic markers with clinical value will be discovered. These genetic events perturb diverse cellular pathways and functions, and they often confer a profound impact upon the clinical phenotype of the disease and treatment response.

Minimal Residual Disease and prognosis

There is increasing evidence that risk of relapse and overall survival can be predicted by assessment of kinetics and depth of response following front-line therapy. Therefore is important monitoring of the leukemic burden using molecular or immunological approaches to MRD detection.

A number of studies have highlighted the potential of MRD monitoring by real-time quantitative polymerase chain reaction (RQ-PCR) to detect leukemia-specific targets (ie, fusion gene transcripts such as PML-RARA,CBFB-MYH11,AML/ETO1,or mutations such as that in NPM1) that would reveal those patients at highest risk of relapse and provide an opportunity for early treatment intervention. However, approximately half of AML patients lack a suitable leukemia-specific target. Alternative approaches involves use of flow cytometry to identify and monitor leukemia-associated aberrant phenotypes; it has the advantage of wide applicability, but it is technically demanding. Another approach involves using RQ-PCR assays to detect transcripts that are highly over expressed in AML blasts relative to normal PB and BM, with most of the attention being focused on the Wilms tumor(WT1) gene[5].

Acute Lymphoblastic Leukemia

Survival in adult acute ALL has slightly improved in recent decades. Risk oriented and targeted therapy are currently evaluated to obtain improvement of results. Recognition of the biologic heterogeneity of ALL, development of protocols that include optimized chemotherapy combinations employed in the successful treatment of children with ALL, and utilization of risk-adapted therapy are currently used. Apart from age and wbc at diagnosis , that are the most important clinical risk factors, it is crucial to consider biological characteristic at diagnosis because, together with the kinetic and depth of leukemic cell clearance, they lead to better risk-adapted therapeutic strategies. Tyrosine Kinase Inibitors are now being used , with or without chemotherapy in the induction treatment of bcr/abl+ ALL.

Prognostic factor and risk stratification

ALL are stratified as standard risk, high and very high risk on the bases of clinical and biological features at diagnosis, according to genetics and immunophenotyping analysis (Tab 2.)

1. Immunophenotype

Similar to acute myeloid leukemia, immunophenotypic evaluation of ALL is essential to confirm the diagnosis and perform further subclassification. It enables rapid identification, quantification, and characterization of leukemic blasts, permitting accurate and timely diagnosis. Besides, it permits the detection of leukemic blasts after therapy at a level lower than that achievable by conventional microscopic examination. Flow cytometric detection of minimal residual disease is among the powerful indipendent prognostic factors in patients affected by ALL and may provide an opportunity for more precise risk-adapted therapies.

EGIL classification6 was proposed to establish guidelines for the characterization of acute leukemias based on marker expression and pro-

vide a uniform basis for the diagnosis of the various types of these hemopoietic malignancies. Assessment of the blast cell maturation stage has proven to be of unequivocal clinical and prognostic value as for B lineage, like T lineage (TCP and BCP). Specific phenotypic characterization of ALL blast cells is usually performed using lineage associated markers such as CD19 for B-cells and CD7 for T blasts in all tube combinations.

Mature B-type ALL (monotypic surface immunoglobulin positive SIg) is the leukemic counterpart of Burkitt Lymphoma and need of a different therapeutical approach. CD20 antigen is expressed in a minority of BCP-ALL, and is a prognostically adverse factor. In TCP-ALL, the prognosis is worse for pro-, pre- and mature-T subtypes (CD1a-, CD3-/CD3) compared with the CD1a- cortical/thymic phenotype, and usually for CD56 and probably CD13 cases.

2. Cytogenetics and molecular genetics

Cytogenetic analyses in ALL have revealed a great number of non-random chromosome abnormalities. In many instances, molecular studies of these abnormalities identified specific genes implicated in the process of leukemogenesis. The more common chromosome aberrations have been associated with specific laboratory and clinical characteristics, and are now being used as diagnostic and prognostic markers guiding the clinician in selecting the most effective therapies. In acute lymphoblastic leukemia of B-cell origin it is possible to define homogeneous subgroups with different prognoses related to associated chromosomal abnormalities. In BCP ALL, a CD10-negative pro-B phenotype is HR especially when associated with t(4;11)/abn 11q23. Ph-negative common ALL (CD10+) with WBC lower than 30 per 109/L is standard risk (SR).

The Philadelphia chromosome remains the most frequent and clinically significant abnormality in adult ALL, with an incidence ranging from 15 to 50% in older patients with B-lineage ALL.

Most patients fall within an intermediate risk (IR) group comprising the normal diploid subset plus hyperdiploidy and several random chromosomal abnormalities. Alteration such as del(6q) and t(1;19) may constitute an IR/high-risk (HR) group. Patients with t(9;22)/Ph chromosome or BCR-ABL1 rearrangements, t(4;11) or MLL rearrangements at 11q23, -7, low hypodiploidy/near triploidy fall into the very HR cytogenetic category, with a disease-free survival (DFS) rate lower than 25%. Molecular genetics identifies specific gene translocations (eg, BCR-ABL1 in Ph ALL, MLL rearrangements in t[4;11] ALL) and integrates the cytogenetic analysis when it fails or documents a normal karyotype. In t(4;11) ALL, FLT3 mutation is frequently detected. In TCP ALL, over expression of HOX11L2 and ERG would impart a bad outlook, whereas overexpression of TLX1, combined low ERG and BAALC expression, and mutations of NOTCH1 and FBXW[7] would be favorable markers. An early T/myeloid stem cell variant with mutant CEBPA expression and other distinct gene lesions represents a new adverse subset. Recently genetic alteration of IKAROS were shown in BCP-ALL conferring poor outcome. Genome-wide analysis identifies specific gene signatures which may be prognostically relevant when associated with drug resistance. The polymorphism of genes metabolizing thiopurines, methotrexate, and cytarabine has been associated with variable treatment response7.

Minimal residual disease (MRD)

In ALL treatment response is increasingly evaluated with minimal residual disease (MRD). Different methods are available to detect and monitor MRD, including multicolor flow cytometry, and polymerase chain reaction (PCR) assays, especially real time quantitative PCR. PCR techniques take advantage of either fusion transcripts resulting from chromosome abnormalities (eg, BCR-ABL, MLLAF4, TEL-AML1) or patient-specific junctional regions of rearranged immunoglobulin and T-cell receptor genes. MRD can be evaluated at fixed time points during induction and consolidation therapy using cytofluorometry or patient-specific molecular probes. There is a close positive association between rapid MRD signal reduction (which is proof of chemosensitivity) and the duration of CR, independently of the applied treatments. In contrast, patients with persistent or recurrent MRD almost always relapse[8]. 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 postconsolidation 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. Assays based on polymerase chain reaction or flow cytometry can detect one ALL cell among 10,000 to 100,000 normal cells in clinical samples. The vast majority of cases have antigen-receptor gene rearrangements and leukemia immunophenotypes for MRD monitoring; about half of the cases currently have suitable gene fusions.

Developing therapeutic options

Better definition of subset-specific characteristics will direct future development of molecularly targeted therapies. The incorporation of molecularly targeted therapy using the ABL tyrosine kinase inhibitor, imatinib mesylate, has begun to change the therapeutic landscape and outcome for high-risk Ph+ ALL. Patients who have the Philadelphia (Ph) chromosome and associated BCR-ABL oncogene have a particularly poor prognosis. At the present, imatinib administered or not in combination with chemotherapy is the first-line treatment for adults with Ph+ ALL, with an overall goal of treatment of allo-HSCT in eligible patients. Imatinib has significantly improved the prognosis of Ph+ ALL and is associated with good tolerability. However, despite its clear benefits, many patients with Ph+ ALL who are ineligible for allo-HSCT do not experience durable remission with imatinib and resistance frequently occurs. Currently, one of the main goals of treatment in Ph+ ALL is for eligible patients to undergo allogeneic haematopoietic stem cell transplantation (allo-HSCT) at the earliest opportunity, with the option of post transplant Imatinib therapy[9].

In the CD20+ BCP ALL the anti CD20 monoclonal antibody Rituximab, added to induction chemotherapy, improves disease free survival and overall survival, even in high risk patients and in allo-HCST setting. The allogeneic BMT is unquestionably superior to chemotherapy alone or autologous HSCT to reduce the relapse risk. However the curative potential of HSCT must be balanced against the disadvantages (mortality of 20% to 30%, morbidity, late complications, reduced quality of life) and assessed in relation to the improved outcome by chemotherapy regimens. The evaluation of MRD improves significantly the definition of risk class. SR patients who are confirmed MRD, treated with chemotherapy, obtain excellent results, and would be saved from allo-SCT. This policy aims to reduce TRM by identifying patients who have a real chance of cure without.

Patients in first remission with persistent o recurrent high levels of MRD after induction therapy have a high risk of relapse and have to be treated with alternative approach. Unfortunately even the allo-SCT results are unsatisfactory in this setting, with a DFS < 40%. Blinatumomab, a bispecific anti CD3 and anti CD19 monoclonal antibody, is extremamely effective in chemotherapy refractory MRD and clinical studies are making in progress in the pretrasplant setting[10]. Up to now, the most promising agents like a bridge to allo-SCT appear to be clofarabine in the BCP ALL or nelarabine in TCP ALL.

Conclusions

Progress in the understanding of the biology and pathogenesis of adult acute leukaemia patients has helped to improve outcome and prognosis. Based on chromosomal banding analyses, cytogenetic abnormalities are identified in ~55% of adult AML and in ~ 60%–80% of ALL patients. Molecular analyses reveal recurrent genetic markers in 85% of patients with normal karyotype AML, and in 60%–80% of patients with ALL. These genetically based subtypes are associated with diverse biological characteristics and distinct clinical profiles. Investigations are focusing on refinement of the basic treatment stratagem of induction, consolidation, and maintenance, expansion of risk-based, subgroup-oriented therapies; assessment of minimal residual disease, its impact on disease recurrence, and its practical implications in clinical practice and the development of new drugs based on a better understanding of disease biology. Emerging data has identified molecular markers that provide additional prognostic information to better classify these patients into those with a more favorable prognosis and those with an unfavorable prognosis who may require more aggressive or investigational therapies.

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IMMUNOPHENOTYPING IN DIAGNOSING ACUTE MYELOID LEUKEMIA

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The recent WHO classification stressed the importance of the molecular, genetic and clinical classification of acute leukemias, introducing new entities recognized on the basis of these informations[1]. However, morphology and immunophenotypic analysis still remain crucial for diagnosis and monitoring response to therapy. They represent the first diagnostic approaches to these diseases and allow to quickly define definitive or differential diagnosis and guide in a specific and restricted way the choice of molecular and genetic investigations to be performed. Although there are immunophenotypic aspects characteristic or highly suggestive of a specific diagnosis, immunophenotyping should not be used alone, but always integrated with clinical, morphological, genetic and molecular data[2]. In particular, a comparison should be done with a stained smear of the sample to ensure that the population of interest is present and that is the same as analyzed by flow cytometry. As in acute myeloid leukemia (AML) immunophenotyping significance is less stringent than in acute and chronic lymphoproliferative syndromes, it is essential to identify those subtypes that can be morphologically confused with acute lymphoid leukemia (ALL), such as the forms with minimal differentiation, the form with megakaryocytic differentiation and the pure erythroid leukemia. The immunophenotyping is also essential for the diagnosis of the rare acute undifferentiated leukemia (or stem cell acute leukemia) and mixed phenotype acute leukemia (MPAL) and to evaluate the monocytic differentiation in cases of negative specific cytochemical reactions[3]. Other objectives are the demonstration of immunological typing of heterogeneity of the leukemic population, the recognition of special patterns to predict phenotypes such as cytogenetic translocations (8,21) and (15,17), the search for aberrant phenotypes or leukemia-associated immunophenotypes (LAIP), useful for monitoring of minimal residual disease (MRD) and identification of prognostic markers of outcome.

The immunophenotypic analysis must identify and quantify the blast and abnormal cells, distinguish them from immature cells normally present in the bone, determining the lineage to differentiate between AML and ALL and allow the subtype classification.[3] Myeloblasts can be identified and distinguished from the more mature myeloid cells by their physical characteristics (low side scatter or SSC), weak expression of CD45, expression of markers of immaturity such as CD34 and CD117, and lack of maturation of myeloid markers such as CD11b, CD16 and CD153. The neoplastic myeloblasts can be distinguished from normal cells and normal immature myeloid and monocytic blast for the presence of phenotypic aberrations such as the expression of lymphoid markers, the overexpression (ie, CD34 or CD33) or absence of antigens (ie HLA-DR) or asynchronous antigen expression (ie, CD34+, CD15+), not consistent with the normal maturative process[4]. The quantification of blasts using flow cytometric assessment of CD34 + cells cannot replace the manual counting microscopy, which remains the gold standard despite inherent imprecision and inaccuracy in case of poor quality of smears or the presence of cells with morphological atypia difficult to classify. Flow cytometric counts lower than manual counts may be due to a lower number of bone marrow spicules and greater dilution of the marrow blood sample used for flow cytometric analysis than that used for the morphological examination, the lack of expression of CD34 on blast cells and CD117 and the necessary inclusion, according to new classification criteria, morphology of blasts in the count as the equivalent promonocytes. Flow cytometric counts higher than manual counts may be due to the removal of erythroid precursors by sample lysis, the difficulty in morphological blast recognition or elevate number of disrupted cells in the sample, the expression of CD117 on some maturing myeloid cells and mast cells and the presence of hypogranular mature myeloid cells going to fall into the "blast window" in the plot CD45/SSC3. The multiparameter flow cytometry (3 or more colors) is the method of choice for determining the blast lineage as well as for detecting aberrant antigenic profiles than may prove useful for disease monitoring[5]. Generally neoplastic myeloblasts express antigens characteristic of granulocytic and monocytic differentiation such as CD13, CD33, CD15, CD64, CD117, myeloperoxidase (MPO) and frequently lymphoid antigens such as CD19, CD7, CD56, CD2. The recognition of the myeloid lineage can be difficult if only a few blasts express antigens of line or antigens associated to other cell lines. Lineage heterogeneity characterizes MPAL, bilinear leukemia (2 populations with different lineage of blasts) or biphenotypic leukemia (expression of lineage antigens as a single population of blasts). According to the specifications of the WHO guidelines, which replace the diagnostic system to score points by the EGIL classification[6], the MPO and the expression of two or more monocytic antigens (CD11c, CD64, CD14, nonspecific esterases, lysozyme) indicate myeloid lineage, surface or cytoplasmic CD3 T-lymphoid lineage and CD19 strong or weak, respectively, associated with strong expression of one or two of CD10, CD22 and cytoplasmic CD79a B-lymphoid lineage. The MPAL group encompasses acute BCR-ABL1-positive leukemia, usually with myeloid and B lymphoid blast cells. This diagnosis should not be made in patients affected by chronic myeloid leukemia. At the myeloid lineage also belongs a nosographic entity known as "blastic lymphoma NK-cell" and previously placed by the WHO in NK precursors neoplasms or in the literature also referred to as "CD4+/CD56 + hematodermic tumor." This form is currently classified by WHO as "blastic plasmacytoid dendritic cell neoplasm" to emphasize the derivation of this neoplasm from a specialized subset of dendritic cells, the plasmacytoid dendritic cells.

The samples used for the initial diagnosis is the peripheral blood and bone marrow aspirates, which must be collected before starting therapy[5]. The analysis should be performed as soon as possible to have a high cell viability. The treatment of the sample as the first step involves erythrocyte lysis followed by resuspension in cell culture medium and cell count. This allows to check the number of cells that must be submitted for staining (ideally from 500,000 to 1 million per tube, but only 50,000 if the population is predominant in the sample). The choice of the various antibodies and fluorochromes can be critical. The criteria for selection of antibody combinations or panels are varied and depend on previous experience and preference. The strategies for selecting a panel of antibodies has been, or enlarged, all-inclusive, or a initial screening panel followed by a panel of specific focus or a custom panel based on clinical and morphological data. The use of panels including many colors (8-10 fluorescence) improves the sensitivity in recognizing immunophenotypic small aberrations in the determination of MRD in samples with low cellularity, and they can be analyzed within 1 or tubes[2]. The analytical approach should include the gating strategy and definition of quantitative criteria of positivity. The gating system aims to isolate blast cells from normal bone marrow cells even if they belong to the same hemopoietic line. The immunological gate using SSC/CD45 is optimal because these molecules are expressed with characteristic intensity in normal myeloid and lymphoid populations, but abnormally expressed in pathological blast cells. With the SSC/CD45 panel, myeloblasts form a round/oval cluster, the so-called "blast gate", with dim CD45+ and SSC low; monoblasts acquiring slightly more CD45 and SSC tend to expand the area occupied by normal monocytes; lymphoblasts tend to have a flattened distribution longitudinally along the CD45 axis. In determining the lineage is also impor-

tant to assess the association of the blasts with their normal counterparts ripening. An initial screening based on the SSC/CD45 panel, could provide a correct diagnosis in 94% of AML and ALL, using selected panels of antibodies that would allow more specific and narrow-saving cost7. Today there is not a general consensus in defining the cut-off of positivity of a marker in a leukemia population. For many markers is used the cutoff criterion of 20% (EGIL), while for others selected antigens, such as cCD3, MPO, TdT, CD34, CD117, the best cut-off is 10%[8] Results expressed as a percentage, however, can lead to a reduced quantification of abnormal population and give less information on the co-expression of antigens on the leukemic population. In reporting results, beside describing the gating strategies and the monoclonal used, it is recommended to describe also the intensity of expression (weak, moderate, high) and the mode of distribution (homogeneous, inhomogeneous, partial) of the antigens on the blast population. It should also be given the percentage of the various cell populations studied, both pathological and normal, and reported for the evaluation of the LAIP for MRD evaluation[2]. For a more comprehensive clinical significance, the morphological and cytochemical data should be added to the immunophenotipic data into a single integrated report. Problems and pitfalls may occur, depending to the nature of random sampling bone marrow or to the presence of fibrosis that can lead to negative results even in the presence of bone marrow, or to artifacts, non-specific links, errors of interpretation due to narrow panels or inadequate gates.

Immunophenotyping and the WHO classification

Among the several monoclonal antibodies available for flow cytometry studies, some are widely used and well characterized and are the core of most immunophenotypic panels. However, few of these markers are lineage specific. Most have a more complex reaction, a different sensitivity and show a non-specific distribution between the different subtypes of AML. This does not allow to recognize a classification of different immunological types of AML as stringent as in ALL, although some proposals have been advanced[2].

AML with recurrent genetic abnormalities. Some of these forms show abnormal phenotypic characteristics, although not specific, that if these can be addressed promptly in search of the genetic lesion suspected. AML with t(8;21) shows strong expression of CD34, MPO, HLA-DR, CD13 and weak expression of CD33. The expression of the B-lineage markers CD19 and CD79a is frequent. It can be positive CD56, whose presence is associated with worse prognosis. The coexpression of CD34, CD19 and CD56 is strongly predictive cytogenetic abnormality. AML with inv(16) or t(16, 16), characterized by bone marrow eosinophylia, have different blast populations: immature blasts CD34+ and CD117+, with markers associate with granulocyte maturation (CD15, CD13, CD33, MPO) and monocytic lineage (CD64, CD14, CD36, CD4, CD11b, CD11c)[9]. Characteristic though not specific is the expression of CD2. AML with t(15;17) in the hypergranular form has weak or absent expression of CD34 and HLA-DR, consistent and bright expression of CD33 and heterogeneous expression of CD13. CD117 and CD64 are usually positive, whereas CD15, CD66b, CD66c, CD11a, CD11c and CD65 are usually negative. CD56 positivity is associated with poor prognosis. The microgranular variant is frequently positive for CD34 and CD2. ATRA treatment promotes the upregulation of several markers, but not CD11a, with production of a typical asynchronous phenotype (CD11a-, CD11b+, CD15+, CD45RO+). Therapy with arsenic trioxide produces instead the expression of CD66c, which seems related to the apoptotic process[10]. Most of AML with NPM1 mutant are CD34- and CD33+ bright; typically they also express CD13, CD117 and CD123, while in half of cases express HLA-DR9. A particular subset of these forms has an unusual phenotype, with strong expression of CD33 and MPO, negativity for CD34 and HLA-DR and weak expression of CD117 and CD64. This group must be distinguished from AML (CD34-, HLA-DR and MPO+) with NPM1 mutation and FLT3-ITD associated with "cuplike" nuclear invaginations[2].

AML not otherwise specified. Although the correlation is not very tight, there is also a different pattern of reactivity of different antigens among the various categories, with preferential expression of early markers in the forms without maturation, of later markers in the forms with maturation and monocytic antigens in forms with monocytic differentiation. Forms with minimal differentiation express antigens normally present on myeloblasts (CD34, HLA-DR, CD117, CD38), panmyeloid antigens such as CD13 and/or CD33, and sometimes the CD7, and lack of mature myeloid and monocytic antigens such as CD15, CD64 , CD14, CD11b, which can be express in forms with and without maturation. The monocytic forms in addition to monocytic maturation markers show stronger expression of CD45 and SSC. Myelomonocytic form is characterized by the presence of two distinct populations, with or without monocytic differentiation. A phenotype CD34-, CD13-, and CD33+ characterizes the monoblastic form. The AML with more immature erythroid differentiation express not lineage-specific markers as HLA-DR, CD36 and CD71, while the more mature forms express specific markers such as glycophorin (CD235a). Megakaryocytic forms express markers strongly associated with megakaryocytic differentiation, such as the platelet protein CD41 and CD61, and less frequently CD42. Artifacts related to the adesion of platelets to blasts should be excluded.

Unique categories of AML. The therapy-related AML, the AML with myelodyplasia-related changes and myeloid sarcoma frequently have morphological and flow cytometric monocytic or myelomonocytic features. Myeloid leukemia associated with Down syndrome often show phenotypic characteristics with megakaryocytic expression of CD36, CD41 and CD61. Blastic plasmacytoid dendritic cell neoplasm, that frequently involves the skin, presents a typical phenotype HLA-DR positive and CD34-CD117 negative with CD4, CD45RA, CD56, CD123 bright[2].

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MOLECULAR BIOLOGY IN ACUTE MYELOID LEUKEMIA AT DIAGNOSIS

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Even if the initial diagnostic approach to Acute Myeloid Leukemia (AML) still remains based on morphological examination of peripheral blood (PB) smear, bone marrow (BM) aspirate and trephine biopsy, the revised WHO 2008 classification of myeloid neoplasms and acute leukemia reflects the feature that an increasing number of AML may be categorized based upon the results of either cytogenetic or molecular genetic abnormalities, defining distinct clinico-pathological entities (Table 1) [1]. From a clinical perspective, it has become evident that specific chromosomal abnormalities and molecular genetic changes are among the most important prognostic markers and therefore they may

be used for stratification of patients with AML to risk-adapted therapeutic strategies. After having excluded acute promyelocytic leukemia, cytogenetics remain the most important disease-related prognostic factor in predicting response to induction chemotherapy and survival of AML patients. Based on karyotype of leukemic cells at diagnosis, AML patients are assigned to either favorable or intermediate or high risk subgroup (Table 2) [2]. Molecular examinations should be performed on either PB or BM samples, in combination with cytogenetic analyses, in order to investigate the presence of specific fusion transcripts, namely RUNX1-RUNX1T1 and CBFB-MYH11, especially in cases with M2 and M4eo, respectively, which identify Core Binding Factor (CBF) AML. These latter distinct entities, comprising 15-20% of patients, are characterized by good prognosis and a long term disease-free survival of approximately 60% [3]. On the other hand, those patients with intermediate prognosis cytogenetics, comprising patients with cytogenetically normal (CN) AML, which account for approximately 40-50% of all the newly diagnosed cases, represent the largest and more heterogeneous group of AML patients. In recent years, somatically acquired gene mutations and deregulated expression of genes and non-coding RNAs have been identified, more frequently in CN-AML patients, providing insights into the mechanisms of leukemogenesis and potentially unraveling the molecular and clinical heterogeneity within these patients. In particular, gene mutations studies can help to identify genetic lesions associated with biologic aggressiveness and treatment failure, leading to discrimination of patients who may be candidates for alternative therapies, including hematopoietic cell transplantation (HCT) (Table 3).

Gene mutations in AML

NPM1 mutations

Nucleophosmin (NPM1) gene mutations, resulting in cytoplasmic delocalization of nucleophosmin (NPMc+), are the most common genetic alteration in adult AML, being detected in about 30% of total cases (50-60% of CN-AML cases) (4). Interestingly, all the 50 NPM1 mutations so far identified, invariably heterozygous, result in a shift of the reading frame leading to common changes at the C-terminus end of NPM1 protein. Of note, NPM1 protein wild-type shuttles across cytoplasm and nucleoplasm as well as between nucleoplasm and nucleolus, regulating ribosome biogenesis, genomic stability, DNA repair and centrosome duplication. Mutation-related changes at the C-terminus prevent or decrease NPM1 binding to the nucleolus, leading to aberrant NPM1 cytoplasmic accumulation in AML cells. NPM1 mutations/NPMc+ are specific for AML, usually de novo, frequently with M4-M5 blast cell morphology according to FAB classification and lacking expression of CD34. Of interest, NPM1 mutations are mutually exclusive with other recurrent cytogenetic abnormalities observed in AML. In patients under 60 years of age, NPM1 mutations, in the absence of FLT3-internal tandem duplications (ITD), consistently predict a favourable prognosis, with 60% overall survival at 5 years. Unfortunately, FLT3-ITD mutation is frequently observed in NPMc+ AML (40% of cases), with a significantly worsened prognosis (4). Conversely, the prognosis of NPMc+ AML is not influenced by additional cytogenetic abnormalities, in the absence of FLT3-ITD. NPM1 mutations also have favourable prognostic impact in older patients with CN-AML, especially those aged >70 years. AML with mutated NPM1 is actually recognized as a provisional entity in the WHO 2008 classification of myeloid neoplasms and acute leukemia, because of its unique molecular, immunophenotypic and prognostic features [1].

CEBPA mutations

CEBPA encodes for CCAAT/enhancer binding protein-alpha, a granulocytic differentiation factor important in the regulation of myeloid progenitors. Mutations in CEBPA occur in approximately 5% to 10% of de novo AML and is most common in CN-AML (10-18% of these latter cases) and in cases with 9q deletion. Two major types of heterozygous CEBPA mutations, namely nonsense mutations affecting the N-terminal region and in-frame mutations in the C-terminal basic region-leucine zipper domain, have been identified in AML. In approximately two thirds of cases, C- and N-terminal mutations are biallelic mutations (also called "double mutations"), with the majority (approximately 90%) of them resulting heterozygous (C-terminal on one allele combined with N-terminal mutation on the other one). In the absence of FLT3-ITD, a CEBPA mutation confers significantly better prognosis in patients with CN-AML, with approximately 60% long-term survival, particularly if biallelic mutations are documented. AML with biallelic mutated CEBPA is actually recognized as a provisional entity in the WHO 2008 classification [1, 5-7].

Table 1. WHO 2008 classification of acute myeloid leukemia

Acute myeloid leukemia with recurrent genetic abnormalities:
AML with t(8;21)(q22;q22); RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11*
APL with t(15;17)(q22;q12), PML-RARA* ^
AML with t(9;11)(p22;q23); MLLT3-MLL°
AML with t(6;9)(p23;q34); DEK-NUP214
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
Provisional entity: AML with mutated NPM1
Provisional entity: AML with mutated CEBPA
Acute myeloid leukemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukemia, not otherwise specified (NOS):
Acute myeloid leukemia with minimal differentiation
Acute myeloid leukemia without maturation
Acute myeloid leukemia with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemia
Pure erythroid leukemia
Erythroleukemia, erythroid/myeloid
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute myelosclerosis)
Myeloid sarcoma (syn.: extramedullary myeloid tumor; granulocytic sarcoma; chloroma)

* For these entities, the genetic abnormality is sufficient for the diagnosis of AML in the appropriate setting, regardless of the blast percentage in either PB or BM.

^ RARA rearrangements with other partner genes are recognized separately.
° Other translocations involving MLL should be reported accordingly: for example, AML with t(6;11)(q27;q23); MLLT4-MLL; AML with t(11;19)(q23;p13.3); MLL-MLLT1; AML with t(11;19)(q23;p13.1); MLL-ELL; AML with t(10;11)(p12;q23); MLLT10-MLL.

FLT3 mutations

FLT3 is an important class III receptor tyrosine kinase normally expressed in early bone marrow progenitors. Mutations that result in the constitutive activation of FLT3 have been identified in two functional domains of the receptor, the juxtamembrane (JM) domain and the tyrosine kinase domain (TKD). FLT3-ITD are found in approximately 20-25% of unselected cases of AML and mainly cluster in the JM domain. However, it has recently been shown that approximately 30% of ITDs do not insert in the JM but in the TK1 domain of the receptor. Patients with FLT3-ITD often present with high white blood cell count and, typically, with CN-AML. The presence of this genetic lesion correlates with shorter survival and high risk of relapse, when patients are treated with conventional chemotherapy. There is also evidence that outcome is related to the ratio of mutated versus wild-type allele in that a high burden of mutated allele predicts for inferior survival. Even if data from prospective trials are lacking, there is growing evidence that allogeneic HCT may represent an attractive therapeutic option in these patients at high risk of relapse. Furthermore, randomized phase III trials evaluating FLT3 inhibitors in combination with chemotherapy as frontline treatment are ongoing. On the other hand, the activation loop in the carboxyterminal lobe of the TKD is affected by point mutations, small insertions, or deletions, mainly observed at codons 835 and 836, in 5% to 10% of cases of AML. Contrary to FLT3-ITD mutations, the prognostic significance of these latter abnormalities remains controversial [5-7].

NPM1, CEBPA, and FLT3 mutations are recommended to be analyzed in clinical trials and in routine practice at least in patients with CN-AML who will receive treatment other than low-dose chemotherapy or best supportive care [5-7]. Falini and colleagues have proposed a possible diagnostic algorithm for patients with AML: NPM1 mutations could be studied first either by molecular or by immunohistochemical analyses or by a combination of both, reserving all other studies, except for investigations directed to Flt3-ITD, to AML cases with wild-type NPM1. This approach could rationalize cytogenetic and molecular studies in AML at diagnosis. Furthermore, immunohistochemistry could be used as a first-line screening to restrict mutational analysis of NPM1 gene to cases showing aberrant cytoplasmic expression of NPM1 [4]. Combining cytogenetic and molecular investigations at diagnosis, approximately 60-65% of AML cases may thus be categorized, based upon either chromo-

somal or genetic abnormalities. In the remaining cases, especially in patients with CN-AML, without NPM1, CEBPA or Flt3 mutations, the combination of new diagnostic and prognostic molecular markers should be further investigated [5, 6]. Whether NPM1 mutations could be incorporated into prognostic scores for elderly patients remains to be further assessed [8].

Table 2. Revised MRC prognostic classification based on cytogenetic abnormalities

Favorable risk group (irrespective of additional cytogenetic abnormalities)
t(15;17)(q22;q21)
t(8;21)(q22;q22)
inv(16)(p13q22)/t(16;16)(p13;q22)
Intermediate risk group
Entities not classified as favorable or adverse
Adverse risk group (excluding cases with cytogenetic lesions associated with good prognosis)
abn(3q) [excluding t(3;5)(q21 25;q31 35)]*
inv(3)(q21q26)/t(3;3)(q21;q26)
add(5q), del(5q), −5
−7, add(7q)/del(7q)
t(6;11)(q27;q23)
t(10;11)(p11 13;q23)
t(v; 11)(v;q23), with MLL rearranged [excluding t(9;11)(p22;q23) and t(11;19)(q23;p13)]°
t(9;22)(q34;q11)
−17/abn(17p)
Complex (≥ 4 unrelated abnormalities)

* The outcome of t(3;5), associated with the formation of NPM1-MLF1 fusion transcript, did not differ significantly from patients with normal karyotype.

° The presence of t(9;11)(p22;q23) correlates with intermediate risk, whereas the prognostic significance of t(11;19)(q23;p13) is debated, since in some series it correlates with poor outcome.

KIT mutations

KIT, also known as CD117 or stem cell factor receptor, is a member of the class III receptor tyrosine kinase family. KIT mutations, mainly D816V mutation, are found in 20% to 40% of cases of CBF-AML, prevalently in cases with inv[16] and are rare in other AML subsets. In most studies, KIT mutations have been associated with inferior outcome, with significantly higher incidence of relapse and lower survival. Of note, KIT is not only mutated, but is also expressed at significantly higher levels in CBF-AML compared with other AML subgroups. KIT mutation testing appears to be prognostically relevant and should be investigated in CBF-AML. However, to date, there are no data supporting the use of KIT mutational status to guide therapy. Despite these limitations, some authors suggest that patients with KIT mutations may benefit from allogeneic HCT. Clinical trials are currently ongoing, in order to evaluate a potential role of KIT inhibitors in CBF-AML [5, 6].

RAS mutations

Mutations in NRAS and KRAS occur in approximately 10% and 5% of unselected AML patients, respectively. In more detail, NRAS mutations are found in 9-14% of CN-AML, in up to 40% of CBF-AML and in 25-30% of AML with inv(3), while KRAS mutations are found in 5-17% of CBF-AML. RAS mutations are only rarely observed in association with Flt3-ITD. No prognostic significance have been shown for RAS mutations in AML patients. Notwithstanding, recent observations have suggested that in patients with CN-AML, RAS mutations may predict sensitivity to high-dose cytarabine consolidation [5, 6].

MLL mutations

Several different Mixed lineage leukemia/mixed lymphoma leukemia (MLL) gene lesions may be observed in AML patients. The overall outcome for patients with chromosomal translocations involving 11q23 is significantly worse than for those with CN-AML. However, the chromosomal partner is reported to have an important relevance upon prognosis. The t(9;11)(p22;q23), which leads to the MLLT3-MLL fusion transcript, has a relatively favorable outcome (intermediate risk AML). A similar outcome has recently been observed with t(11;19)(q23;p13). By contrast, AML cases with translocations involving other fusion partners predict a very poor survival. In addition to chromosomal translocations, further MLL abnormalities may be observed on molecular analyses in AML patients. In details, Partial tandem duplications (PTD) of MLL are found in 5% to 11% of patients with CN-AML and more frequently in those with AML with trisomy 11 (up to 90% of cases). MLL-PTD have been shown to contribute to leukemogenesis through DNA hypermethylation and epigenetic silencing of tumor suppressor genes. MLL-PTD have been associated with inferior CR duration and relapse-free survival, although more recent studies showed no prognostic impact in patients with CN-AML intensively treated with either autologous HSCT or four cycles of consolidations [5-7].

IDH1 and IDH2 mutations

IDH1 and IDH2 encode cytoplasmic/peroxisomal isocitrate dehydrogenase 1 (IDH1) and mitochondrial isocitrate dehydrogenase 2 (IDH2), respectively, which represent enzymatic activities catalyzing oxidative decarboxylation of isocitrate to alpha-ketoglutarate, generating NADPH from NADP+, involved in cellular defense of oxidative damage. Mutations of IDH1 and IDH2 were first reported in gliomas and were described only more recently in AML. Mutations are commonly located at residue 132 (IDH1) and residues 140 and 172 (IDH2), leading to a neomorphic enzyme activity and to accumulation of a putative oncogenic metabolite. Of interest, metabolic enzymes, such as IDH proteins, represent a new class of mutated proteins in leukemogenesis [5, 6]. The aggregate frequency of these two mutations in AML is relatively high, approximately 15% to 20% of all patients with AML and 25% to 30% of patients with CN-AML, harbouring either IDH1or IDH2 mutations. Initial studies from larger and homogeneous cohorts of patients indicate that IDH1 and possibly also IDH2 R140 mutations are significantly associated with NPM1 mutations and predict worse outcome for patients with mutated NPM1 without FLT3-ITD. Of interest, similarly to NPM1 and Flt3-ITD, IDH mutations have recently been observed in AML with cup-like nuclei, a rare and distinct morphological subtype of AML (9). Interestingly, the distinct R172 IDH2 mutation is rarely associated with any other known prognostic mutations and seems to confer lower probability of achieving CR after remission induction chemotherapy and possibly also inferior outcome [6].

WT1 mutations

Wilm's tumor 1 (WT1) gene, a zinc-finger-motif containing transcription factor, is over-expressed in leukemias and may be considered as a surrogate to monitor minimal residual disease (MRD). A failure to reduce WT1 transcript levels below a threshold limit by the end of consolidation predicts an increased relapse risk in AML patients. Furthermore, WT1 mutations may be documented in 10% to 13% of CN-AML. Their prognostic significance is controversial, even if most published studies reported a negative prognostic impact. However, post remission therapy with high-dose cytarabine may potentially abrogate the poor prog-

nosis associated with WT1 mutations. Of further note, in one study, WT1 single nucleotide polymorphism rs16754 located in the proximity of the WT1 mutational hotspot was found to be associated with favorable outcome in CN-AML [5, 6].

RUNX1 mutations

RUNX1 is deregulated in AML either by chromosomal translocations or by mutations clustering in the Runt domain of the gene. RUNX1 mutations, which are documented in 5-13% of AML patients, have been associated with undifferentiated (M0) morphology, and cluster with specific chromosome aberrations, such as trisomy 21 and trisomy 13. RUNX1 mutations are also associated with MLL-PTD, whereas an inverse correlation with NPM1 and CEBPA mutations has been reported. The prognostic significance is still under investigation, but preliminary data indicate a correlation with lower CR rate and shorter survival [5, 6].

TET2 mutations

TET2 belongs to a 3-member family of highly conserved genes, whose precise protein function still remains unknown, but possibly involved in epigenetic regulation. TET2 mutations are found in a wide spectrum of myeloid neoplasms, namely myelodysplastic syndromes (20%), myeloproliferative neoplasms (12%), secondary AML (25%) [5, 6]. In a recent study, among 486 adult patients with primary AML, TET2 mutation occurred in 13% of the cases, but its exact incidence in de novo AML is yet to be defined. TET2 mutation was an unfavorable prognostic factor in patients with intermediate-risk cytogenetics, and its negative impact was further enhanced when the mutation was combined with FLT3-ITD, NPM1-wildtype or other unfavorable genotypes. TET2 mutation appeared to be unstable during disease evolution, rendering it an unsuitable marker for MRD evaluation [10].

DNMT3A mutations

DNMT3A encodes DNA methyltransferases, that catalyze the addition of a methyl group to the cytosine residue of CpG dinucleotides. A recent study documented that DNMT3A mutations are frequent in patients with AML (22% of total cases), prevalently in the group of patients with intermediate risk cytogenetic profile (33% of the cases). Despite the fact that DNMT3A mutations neither cause genomic instability, nor alter total 5-methylcytosine content or global patterns of methylation, nor dramatically alter gene expression, it has been suggested that DNMT3A mutations may be involved in the pathogenesis of AML. DNMT3A mutations are associated with poor event-free and overall survival, independently of age and presence of FLT3 or NPM1 mutations and regardless of the type of mutation or genetic location [11].

Other gene mutations

Mutations in other genes, namely TP53, ASXL1, JAK2 and CBL, occur at a very low frequency in AML patients, and are also not specific for AML. They have so far been less studied and their prognostic significance remains to be further evaluated [5, 6].

Altered gene expression in AML

In addition to structural genetic lesions, changes in expression of specific genes seem to impact prognosis within some molecular subsets of AML patients.

BAALC expression

The Brain and Acute Leukemia, Cytoplasmic (BAALC) gene was originally identified to have higher expression in AML with trisomy 8. Subsequently, a wide range of expression was also documented in CN-AML. High BAALC expression has been associated with significantly lower CR rates and inferior survival, although BAALC expression also frequently correlated with other adverse molecular prognostic features, such as Flt3-ITD, the absence of NPM1 mutations and high ERG expression. Allogeneic HCT may have a potential benefit in this adverse prognostic subgroup [5, 6].

MN1 expression

Meningioma 1 (MN1) gene over-expression is associated with poor response to induction chemotherapy, higher relapse rate and dismal overall survival. Conversely, low MN1 expression was correlated with better response to ATRA in elderly patients with non-promyelocytic AML. In younger patients with AML, higher MN1 expression significantly correlated with unmutated NPM1 and increased BAALC expression [5, 6].

ERG expression

ERG, a member of the ETS family of transcription factors, is highly expressed in patients with CN-AML, complex karyotype, and megakaryoblastic AML and is associated with an increased risk of relapse and shorter survival. Interestingly, high ERG expression levels also impacted outcome of otherwise low molecular risk CN-AML, such as cases with mutated NPM1 in the absence of FLT3-ITD. The expression levels of ERG, BAALC and MN1 often correlate each other, with similar prognostic significance [5, 6].

EVI1 expression

EVI1 protein is involved in regulation of transcription factors critical for hematopoiesis (GATA 1, GATA2) and in epigenetic regulation. Deregulated expression of EVI1 is found in AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2), leading to the fusion transcript EVI1-RPN1. Furthermore, EVI1 over-expression is documented in approximately 10% of unselected AML, more frequently in association with monosomy 7 and t(11q23)/MLL rearrangements. Deregulated EVI1 expression correlates with low CR rate and inferior survival, especially in cytogenetic intermediate-risk AML. Allogeneic HCT may improve survival in patients with high EVI1 expression [5, 6].

Gene Expression Profiling (GEP) in AML

Novel approaches in genomics, such as investigating the expression levels of thousands of genes in parallel, using DNA microarray technology, open possibilities to further refine the studies on AML. To date, GEP is becoming well established in AML and has already been proven to be valuable in diagnosing different cytogenetic subtypes, discovering novel AML subclasses, and predicting clinical outcome. Recently, GEP studies in AML showed a remarkable level of concordance in findings, which may ultimately lead to an increasingly refined molecular taxonomy. While many challenges remain to be overcome, a combination of GEP with other microarray-based applications, high-throughput mutational analyses and proteomic approaches will not only significantly contribute to the classification and therapeutic decision making of AML, but also give important pathogenetic insights [5, 6].

In conclusion, even if morphological analysis remains the milestone for diagnosis and may potentially predict at least some of the cytogenetic and molecular abnormalities that may be encountered in AML, a modern approach aims to increase the number of AML cases that may be categorized based upon the results of either cytogenetic or molecular lesions (1, 3). Notwithstanding, while cytogenetic analysis remains mandatory in the routine diagnostic work-up to provide a correct risk stratification in both younger and elderly patients, to date, only a few number of the molecular markers described above has been validated and has to be firmly considered in the clinical practice as prognostic elements to guide risk-adapted therapeutic strategy of AML patients (5-7). The biological and clinical significance of new molecular biomarkers should be further investigated, especially in the heterogeneous setting of CN-AML, in future perspective cooperative clinical trials, in order to develop modern algorithms for molecular diagnosis, prognostic stratification and subsequent monitoring of MRD.

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HISTIOCYTOSES AND FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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The term "Histiocytosis" describes a group of disorders characterized by accumulation and/or proliferation of mononuclear-phagocytes, comprising dendritic cells and macrophages. They originate from the bone marrow and spread over the body to contribute to immune system function and homeostasis. The histiocytic disorders are classified into two main groups: dendritic cell-related disorders, the most frequent of which is Langerhans cell histiocytosis (LCH), and macrophage-related disorders, represented by Hemophagocytic LymphoHistiocytosis (HLH).

Langerhans cell histiocytosis (LCH). LCH is a rare disease; its age-adjusted incidence rate has been reported between 5 and 6 cases per million per year. Although about two thirds of the patients present when less than 5 years, LCH may affect any age group, from the newborn to the elderly [1]. For several reasons the disease is more familiar to pediatricians than to adult physicians and so most of the available information concerning clinical features, pathogenesis, and treatment outcome derives from the pediatric experience. Only a few reports are available describing series of patients in whom LCH was diagnosed during adulthood. Furthermore, most reports are based on single-specialty experiences, which favour biased description of the patients. The gold standard for diagnosis rests on histology: a lesion that is morphologically appropriate for LCH (cytoplasm rich pale histiocytes interspersed with inflammatory cells, eosinophils and lymphocytes), in which lesional cells demonstrate CD1 and Langerin (CD207) positivity. In current practice, electron microscopy for Birbeck granules is now largely obsolete. It is important to note that pathological examination of LCH lesional tissue does not discriminate between lesions that remain localized from those that disseminate.

The pathogenesis of LCH remains elusive. Many attempts to document a specific viral pathogen have been inconclusive. Evidence of clonality of lesional cells was considered by some authors as proof for malignancy of LCH, which was however, not accepted by the majority of investigators in the light of lack of histologic evidence of malignancy, lack of genetic aberrations, and frequent spontaneous regression of the disease. The recent demonstration of mutations in the BRAF oncogene in many cases of LCH seems once again to favor a diagnosis of malignancy. On purely clinical grounds, LCH behaves as a non-familial disorder. Yet, familial clustering has been observed in 1% of cases. Furthermore, concordance for the disease in over 80% of identical twin pairs, versus less than 15% of fraternal twins, suggests a genetic component in the pathogenesis of LCH [2]. Analysis of chromosomes from peripheral blood lymphocytes of patients with LCH, or even of cells from lesional tissue, failed to document specific, recurrent chromosomal aberrations. Evidence of chromosomal instability in patients with active disease might be related to the presence of a viral pathogen [3]. Recent studies showed that cultures from peripheral blood cells of patients with LCH may provide a model for the lesional granuloma and that IL-17 appears to play a primary role in its pathogenesis [4]. Whether this may provide additional novel insights into the pathogenesis or even disclose novel avenues for non-chemotherapy driven therapeutic approaches, remains to be clarified.

Lichtenstein, recognizing that the entities of eosinophilic granuloma, Hand-Schuller-Christian and (Abt-)Letterer-Siwe diseases were only apparently different and independent and that they shared some clinical and pathologic features, suggested encompassing them all under the term "Histiocytosis X". This was a major advance in understanding that LCH is an unique nosologic entity with heterogeneous clinical manifestations. The historical categories had the advantage of describing to physicians the more frequent patterns of clinical manifestation, which may vary from a single bone involvement to wide-spread disease involving any organ, with very different prognoses. A single, small osteolytic lesion in a non-weight bearing bone usually has limited clinical implications, while massive involvement of vital organs may be immediately life-threatening. The first responsibility of the attending physician when diagnosing LCH is to define whether the disease is restricted to one site or tissue (unifocal single system), involves many different sites within one tissue type (multifocal single system, usually skin or bone) or whether it involves different tissues or organs (multisystem).

Painful swelling is the most frequent cause for consultation in patients with localized LCH. The skull, long bones, and then the flat bones are most frequently involved, while hands and feet are usually spared. Bone involvement was observed in 80-100% of cases in large series. Plain radiography, the first-line approach for detection of bone lesions, usually shows single or multiple irregularly marginated lytic lesions, sometimes with swelling of adjacent soft tissue; peripheral sclerosis suggests initial healing, and periosteal thickening may mimic malignancy in some cases. At the time of diagnosis, combined use of CT and MR may better define the lytic lesion and the surrounding soft tissue alteration. Radioisotope scanning has been largely used to screen for subclinical, potentially involved sites; recently FDGP-PET scanning is being explored as more sensitive for diagnosis and follow-up. Clinical features and possible complications of the osteolytic lesions depend on the bone involved: involvement of the ear and mastoid bone may mimic mastoiditis; periorbital involvement may lead to proptosis and mimic orbital sarcomas; vertebral involvement may result in vertebra plana, while an associated soft tissue mass may result in paraplegia.

Skin involvement is observed in over one third of children with LCH; scalp and diaper areas are more frequently affected, but any area may be involved. The lesions may appear as reddish papules that progress and ulcerate, or depigmentate and heal. Skin is reported as the only affected site in about 10% of cases, especially in male infants, and in such cases spontaneous regression is frequent, but early progression to involve "risk" organs may occur and these patients need to be closely observed. In some cases isolated skin involvement may be misdiagnosed as seborrhoic dermatitis thus delaying the diagnosis. Interestingly, skin involvement rarely appears in follow-up if not present at diagnosis.

Lymphnodes may be enlarged in less than 10% of cases at presentation, as part of disseminated disease or as regional nodes to affected skin or bone. The cervical nodes are most frequently involved, and their enlargement may be massive. Occasionally nodal involvement may be the only clinical manifestation, sometime with a recurrent course. Although hepatomegaly is very common in patients with disseminated disease, only a minority show altered liver function with evidence of reduced protein synthesis (hypoalbuminemia, ascites) or enzyme function (hyperbilirubinemia). Failure to rapidly achieve disease control may lead to progression, from mild cholestasis to portal infiltration, and sclerosing cholangitis. At this stage, progression of liver damage may be independent of the disease activity, with possible evolution toward end-stage dysfunction requiring liver transplant. Splenomegaly is observed in about 5% of patients at diagnosis. In the course of refractory disease, this may contribute to cytopenia. Lung involvement represents one major difference between LCH manifestations in children and adults. While only a minority of children, usually with disseminated disease, are involved, pulmonary isolated LCH represents over 80% of the cases in adults, usually young cigarette smokers. The radiological picture consists of diffuse, interstitial infiltration with nodules, which may evolve into cysts. Rupture of superficial cysts may cause spontaneous pneumothorax, occasionally representing the first manifestation of the disease. The clinical course may be difficult to predict, with some subjects remaining stable over many years while others progress to fibrosis and pneumatization, finallly independent of active LCH, thus requiring lung transplantation.

Diabetes Insipidus (DI) presenting as polyuria and polydipsia, sometime as massive as 6-8 liters of daily water intake, should suggest possible LCH in children and adults. MRI scan may show loss of the posterior pituitary bright signal, and thickening of the pituitary stalk. Once the central origin of DI has been established, a thorough diagnostic work-up for possibly silent LCH localizations is mandatory. DI may present as the first manifestation of LCH or it may occur later, within months or even many years, more often in patients with brain or cranio-facial lesions. Patients with DI may progress to multiple pituitary hormone deficiencies; the first usually is Growth Hormone deficiency with a median latency of about one year, and thyroid and gonadal hormone deficiency may follow. Cerebral masses, beyond the hypophyseal-pituitary, may be occa-

sionally observed. A minority of patients, most often with preceding DI, develop progressive neurodegeneration with ataxia, coordination disturbances,and cranial nerve and neuropsychological defects during the disease course. MRI scan shows a characteristic pattern of demyelination starting from the cerebellum, usually bilateral and symmetric. This picture is unfortunately expected to progress both clinically and radiologically, and the prognosis in these cases remains very poor due to lack of effective therapies. Although anemia is common in patients with multisystem disease, this only reflects persistent inflammation, while thrombocytopenia represents a hallmark of aggressive disease. Cytopenia usually occurs in the absence of morphologic bone marrow infiltration, thus making marrow aspirate not necessary for disease staging.

Table 1. Revised Diagnostic Guidelines for Hemophagocytic Lymphohistiocytosis (HLH) 3)

The diagnosis of HLH can be established if either 1 or 2 below are fulfilled:
1. A molecular diagnosis consistent with HLH
2. Clinical and laboratory criteria for HLH fulfilled (5/8 criteria below):
   - Fever
   - Splenomegaly
   - Cytopenia (affecting ≥2 of 3 lineages in peripheral blood):
   - Haemoglobin <9 g/dl (in infants <4 weeks: Hb <10 g/dl)
   - Platelets $<100\times10^9$/L
   - Neutrophils $<1.0\times10^9$/L
   - Hypertriglyceridemia and/or hypofibrinogenemia:
   - Fasting triglycerides ≥3.0 mmol/L
   - Fibrinogen ≤1.5 g/L
   - Haemophagocytosis in bone marrow or spleen or lymph nodes
   - Low or absent NK cell activity
   - Ferritin ≥500 g/L
   - Soluble CD25 (ie, soluble IL-2 receptor) ≥2400 U/mL

Supportive evidence are cerebral symptoms with moderate pleocytosis and/or elevated protein, elevated transaminases and bilirubin, LDH.

The natural course of solitary LCH lesions is usually one of spontaneous healing, supporting observation alone in patients with localized disease outside vital organs. By contrast, the natural history of patients with multisystem ("Letterer-Siwe") disease is usually fatal. Thus, the need for specific treatment depends on the number and type of involved sites. To address this issue, as in many other rare disorders, international, cooperative efforts were needed to allow accumulation of sufficient uniformly diagnosed and treated cases. To date, the Histiocyte Society has completed 3 prospective pediatric clinical trials, LCH-I to LCH-III, which defined the combination of vinblastine and steroids as the standard of treatment for patients with multifocal bone and multisystem LCH [5]. Patients who achieve at least partial disease control within 6 weeks, followed by complete disease resolution, are not at risk for fatal outcome, but rather for late sequelae, such as hormone deficiencies or bone deformities. By contrast, multisystem patients, usually young babies, who present with liver dysfunction, splenomegaly or thrombocytopenia and who fail to achieve disease control by 6 weeks, are at significant risk of death, most often due to liver failure or infectious complications. As a consequence, while in patients with single osteolytic lesions or skin-only disease a wait-and-see strategy should be applied, unless there is a risk for deformity of a weight-bearing bone, patients with multifocal bone disease deserve treatment aimed at prevention of the cascade of bone reactivations over the following months or even years. In patients with multisystem, risk organ involvement, aggressive chemotherapy is necessary to prevent a rapidly fatal outcome. For poor responders, treatment intensification with chemotherapy (cytarabine and cladribine) or sometimes even hematopoietic stem cell transplantation (HSCT) is warranted. Radiotherapy, which has been widely employed in the past, has a very limited role today, especially in children, due to inherent toxicity and risk of cancer. With the exception of sporadic reports, DI is considered as a non-reversible event, only amenable to replacement therapy.

Due to the lack of large, prospective trial, progress in the treatment of LCH in adults lags behind that in children. Although the combination of vinblastin and steroids has been the starting point for treatment of multisystem disease, compliance to vinblastine in adults has been jeopardized by someway higher toxicity, at least in some patients. Thus, the interest in the use of cladribine grew over the last decade. Local therapy is still considered an appealing option for bone disease. The treatment of pulmonary isolated disease still represents an unanswered challenge.

The quality of survival for patients with localized disease or disseminated disease that responds to treatment remains dependent on the morbidity generated by the disease or treatment itself. Bone disease may result in deformities in the affected bone or associated abnormalities like tooth loss or deafness. Significant bone deformities may be seen in patients previously treated with radiotherapy. The most common permanent consequence is diabetes insipidus, but other hormonal deficiencies such as growth and thyroid may occur. The neuropsychological conquences of CNS disease may be severe.

Hemophagocytic Lymphohistiocytosis

Familial Hemophagocytic lymphohistiocytosis (FHL) is a genetically heterogeneous disorder characterised by a hyperinflammatory syndrome with fever, hepatosplenomegaly, cytopenia, liver dysfuntion and sometimes CNS involvement. Bone marrow aspiration is usually performed early, enabling the identification of hemophagocytosis by activated macrophages. The absence of hemophagocytosis does not, however, exclude the diagnosis. Differential diagnosis of HLH may be difficult and diagnostic guidelines for HLH have been established by the Histiocyte Society (Table 1). In particular, demonstration of frequent association with common pathogens, together with evidence of impaired natural killer cytotoxic activity, provided the rationale for considering HLH as a selective immune deficiency. The pathogenic mechanisms of FHL are based on insufficient control of viral infections by cellular cytotoxicity. As a result, an excessive cytokine production induces lymphocyte overstimulation and limited killing of the dendritic, antigen presenting cell. In this vicious loop, the patient is unable to get rid of the target, often a common viral pathogen such as EBV or CMV. The disease manifestations are due to excessive inflammatory response caused by hypersecretion of proinflammatory cytokines such as interferon-γ (IFNγ), tumor necrosis factor α (TNFα), interleukin (IL)-6, IL-10 and macrophage-colony-stimulating factor (M-CSF). These mediators are secreted by activated T-lymphocytes and macrophages that infiltrate all tissues, and lead to tissue necrosis and organ failure. In spite of the excessive expansion and activation of cytotoxic cells, patients with FHL have severe impairment of the cytotoxic function of NK cells and CTLs. NK cells and CTLs kill their targets through cytolytic granules containing perforin and granzyme. Upon contact between the effector killer cell and the target, an immunological synapse is formed and cytolytic granules traffic to the contact site, dock and fuse with the plasma membrane and release their contents. All known genetic defects in FHL appear to be involved in this process.

In most cases the natural course of FHL is rapidly fatal within a few weeks,[6] unless appropriate treatment, including corticosteroids, cyclosporine, etoposide, or anti-thymocyte globuline, can obtain transient disease control. Yet, due to disease reactivation, rapidly fatal outcome follows, unless HSCT is offered [7]. Prompt identification of such patients is an on-going challenge for the attending physician. The very high transplant–related mortality associated with HSCT for both LCH and HLH has led to the current trials of reduced intensity conditioning for these diseases.

The identification of the causative gene(s) for Familial Hemophagocytic Lymphohistiocytosis (FHL) has been a long process [8]. In 1999 mutations in the perforin-1 gene (PRF1) were described as the cause of FHL-2. Perforin is a pore-forming protein with a mechanism of trans-membrane channel formation. PRF1 mutations induce a complete or partial reduction granule-mediated cytotoxicity by NK and T cells. Patients with FHL2

have different ethnic origins, with cases documented from all continents. Age at disease onset is usually very young, median 3 months, but with a wide range reaching the third decade. Overall, only 40% of the cases of FHL are type 2. Mutations of UNC13D have been associated with FHL3. It encodes the Munc13-4 protein, a critical effector of the exocytosis of cytotoxic granules including those containing perforin and granzyme. Munc13-4 deficiency results in defective cellular cytotoxicity and a clinical picture very similar to that of FHL2. Data on genotype/phenotype correlations showed that ethnic-specific mutations were not identified, that CNS involvement is more frequent than in FHL2, and that age at diagnosis is significantly higher than in FHL2. The combination of the classical clinical picture in association with defective granule release assay suggests FHL3. Most reported patients with FHL4 have been of Turkish/Kurdish descent. FHL4 is due to deficiency of syntaxin 11, a protein contributing to cytotoxic cell degranulation. In 2009, FHL5 was described in patients with mutations in the gene encoding syntaxin-binding protein-2 (STXBP2 or Munc18-2), involved in the regulation of vesicle transport to the plasma membrane. A few additional genetic conditions may have a clinical picture completely overlapping HLH (Table 2). Chédiak-Higashi syndrome (CHS), Griscelli syndrome type II (GSII), and X-linked lymphoproliferative syndromes type 1 (XLP) and 2 (XIAP) are immune deficiencies with distinctive clinical features in which the development of HLH is sporadic, though frequent. HLH is often the presenting symptom but may also occur later during the course of disease. Patients with CHS show partial albinism and frequent pyogenic infections. Their white blood cells exhibit decreased chemotaxis and characteristic giant inclusion bodies (lysosomes). Patients with GSII also have hypopigmentation and various degrees of neutrophil dysfunction but lack the giant granules. XLP is mainly characterized by a constitutional defect of a specific effective immune response to Epstein-Barr virus (EBV). Following exposure to EBV these subjects are prone to develop HLH; if they survive, they may develop lymphomas or dysgammaglobulinemia.

The clinical approach to the diagnosis of HLH has changed over the years. Initially the diagnosis was based on the identification of a clinical and biochemical constellation of signs and symptoms, supported by very young age and family history. The identification of NK activity defect in FHL became the first milestone in assigning a functional defect to these patients. Yet, NK cell cytotoxicity assay is not easily accessible for most clinicians, thus remaining a confirmative assay, restricted to reference laboratories. Identification of the genetic defects allowed investigation of novel tools for rapid screening of FHL. NK cells, and CTLs of patients with PRF1 mutations lack intracellular perforin detected by flow-cytometry, thus providing a reliable and rapid identification of patients with FHL-2 [9]. CD-107a (LAMP-1) lines the cytotoxic granule and is expressed on the surface of the NK/T cell after granule exocytosis. Lack of surface CD107a expression represents a rapid tool for identification of patients with degranulation defects as in FHL3, FHL4, and FHL5 [10], in contrast to healthy control subjects or perforin-deficient NK cells. The above diagnostic tools are extremely useful in the diagnostic approach to a patients with HLH. The aim is to discriminate those patients who have a genetic defect and thus deserve aggressive therapy followed by HSCT, from those in whom HLH may develop as a temporary complication of an infectious disease (viral or visceral Leishmaniasis), or other underlying conditions such as malignancy, in whom anti-HLH therapy may also be life-saving but who do not usually require HSCT. Based on recent report of patients with FHL diagnosed during adulthood, the diagnosis of HLH should be intrduced in the difefrential diagnosis of febrile cytopenia by adult hematologists. The term "macrophage activation syndrome" (MAS) has also been used to describe patients with rheumatologic diseases who develop a clinical syndrome indistinguishable from FHL.

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CONGENITAL ERYTHROCYTOSIS

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The term erythrocytosis defines a group of disorders characterised by an increase in circulating red blood cells (RBCs). Both erythrocytosis and polycythemia are used interchangeably to describe a condition in which the hematocrit (Hct) exceeds the upper limit of normal. The term erythrocytosis is more accurate when only the red cell lineage is involved while the term polycythemia can be confused with polycythemia vera (PV), a clonal stem cell disorder resulting in excessive production of red cells, neutrophils and platelets. Erythrocytosis is suspected in patients with an abnormally high Hct, hemoglobin (Hb) concentration or RBC count. All of these measurements are concentrations and are therefore dependent on plasma volume as well as circulating red cell mass (RCM). The RCM is defined as increased if it is greater than 125% above that expected for sex and body mass. If this occurs, then the subject has an absolute erythrocytosis. The presence of an absolute erythrocytosis is reflected in the fact that the Hb and Hct are also increased. The various parameters do not completely reflect each other as other factors may affect the measurements; however, an Hct of 0.60 or greater is always associated with an increased RCM. An Hb above 18.5 g/dL in a male or 16.5 g/dL in a female or an Hct above 0.52 in a male or 0.48 in a female suggests that there is an erythrocytosis. It may be necessary to carry out a RCM to establish unequivocally that an absolute erythrocytosis is present.

Erythrocytosis can be further classified as relative, where the RCM is normal and the plasma volume is decreased or absolute, where the RCM exceeds the normal upper limit.

Erythrocytosis may be classified as primary, secondary or idiopathic (Table 1). In primary erythrocytosis there is an increase in circulating RBC numbers caused by a molecular defect in the erythroid lineage. Secondary erythrocytosis can arise due to appropriate changes such as adaptation to high altitude, or inappropriate changes such as aberrantly increased circulating levels of erythropoietin (Epo). The term idiopathic erythrocytosis denotes a heterogeneous group of rare disorders of unknown etiology in which the cause may be either primary or secondary. Similar to anemias, the treatment of patients with erythrocytosis depends on the severity and cause of the disorder. Some patients have a relatively benign disease whilst others require venesection or cytoreductive therapy. McMullin et al. have provided guidelines for diagnosis, investigation and management of polycythemia and erythrocytosis.

Measurement of plasma Epo levels provides an important insight into the potential regulatory pathways which may be disrupted in the development of the erythrocytosis. The erythrocytosIs can also be classified using serum Epo levels. Erythrocytosis with an inappropriately normal or elevated Epo level can be associated with abnormalities in the oxy-

gen sensing pathway or abnormal hemoglobin oxygen affinity while those with low Epo levels may be linked to dysregulated Epo signaling and/or hypersensitivity of erythroid precursors.

Congenital erythrocytosis exhibits both autosomal dominant and recessive Mendelian transmission. Online Mendelian Inheritance in Man (OMIM; website: http://www.ncbi. nlm.nih.gov/sites/entrez?db5OMIM) data base administrated by the National Center for Biotechnology Information (NCBI) recognizes four categories of congenital erythrocytosis (ECYT1-4, Table 2)

Erythrocytosis with low Epo levels

Polycythemia vera (PV) is an acquired disorder leading to clonal expansion of a single hematopoietic stem cell containing an acquired JAK2 mutation characterized by hyperplasia of three bone marrow lineages. PV is excluded, and hence, will not be discussed here.

The cytokine Epo binds to the Epo receptor on the surface of the erythroid cell. When this occurs a phosphorylation cascade is initiated. Janus Kinase2 (JAK2) is autophosphorylated and then phosphorylates tyrosines in the cytoplasmic region of the Epo receptor. These tyrosines then act as a docking site for the signal transducer and activator of transcription factor 5 (STAT5) protein, which homodimerizes and translocates to the nucleus, where it initiates gene transcription, proliferation of erythroid precursors and ultimately the production of red cells. This process is regulated by a mechanism to turn off red cell production. About 30 minutes after Epo binds, the phosphatase SHP1 is recruited to the receptor and dephosphorylates the receptor and JAK2. The receptor then goes on to be ubiquitinated and degraded in the proteasome.

A number of mutations have been described in the Epo receptor that results in a truncation of the protein so that the JAK2-binding site is preserved but the SHP1-binding site is lost. These mutations result in a receptor that is "switched on" to stimulate red cell production but has no "switch off" mechanism. Thus they result in continued red cell production and erythrocytosis in the presence of low Epo levels, as the receptor only needs an initial signal to start red cell production. The known mutations result in the loss of between 57 and 127 amino acids from the receptor. At least 15 different mutations have been described in single families. They are present in the heterozygous state and are dominantly inherited. This group of familial erythrocytosis is designated ECYT1 by OMIM (Table 2).

In general, patients with truncating EpoR mutations have a benign condition that does not progress to leukemia and splenomegaly is absent. Instead, there is a propensity to severe cardiovascular problems. In the original Finnish family harboring the first known EpoR mutation, no obvious erythrocytosis-related complications were observed, but some cardiovascular disorders at advanced age were noted.

Erythrocytosis with high Epo levels

A number of relatively common clinical conditions are associated with an elevated Hct secondary to an appropriately elevated serum Epo in the setting of chronic tissue hypoxia. These include chronic respiratory diseases associated with hypoxia, right-to-left circulatory shunting, post-renal transplant and residence at high altitude levels.

One area of concern is the potential and actual misuse of recombinant human Epo to enhance athletic performance.

Erythrocytosis as a paraneoplastic syndrome has been associated with a range of malignancies including renal cell carcinoma, hepatoma, cerebellar hemangioblastoma, pheochromocytomas, ovarian tumors and Wilm's tumor and can be attributed to Epo production by the tumor or by normal parenchyma in response to hypoxia-induced tumor growth.

The oxygen sensing pathway modulates Epo expression, which is produced by the kidney in response to a deceased oxygen supply to the tissues. At the transcriptional level, Epo synthesis is regulated by the hypoxia inducible factor (HIF), which binds to a hypoxia response element (HRE) in the 3' region of the EPO gene. The HIF transcription complex is a heterodimer composed of one alpha and one beta subunit. The beta subunit, also known as ARNT, is able to associate with all three alpha subunits giving rise to HIF-1, HIF-2, and HIF-3 isoforms. Both ARNT and the alpha subunits are constitutively expressed but the alpha subunit is degraded by the proteasome in the presence of oxygen. Consequently, the HIF complex is unable to form and the expression of HIF target genes is maintained at a low level.

A family of prolyl hydroxylase domain (PHD) enzyme acts as the oxygen sensor in the HIF pathway and is constitutively active in normoxia. The PHD family, of which there are three members (PHD1, PHD2, and PHD3), are able to hydroxylate prolines located in the oxygen dependent degradation (ODD) domain of the alpha subunits. These prolines are present in the LXXLAP motif situated in the ODD region. The von Hippel Lindau (VHL) protein is then able to bind to HIFalpha and the assembly of an E3 ligase complex occurs. Once, HIFalpha is ubiquitinylated, it is recognized by the proteasome for degradation. Any decline in available oxygen results in decreased activity of the PHD enzymes and a reduction in hydroxylated HIFalpha. Hence, less VHL binds to the alpha subunit and more escapes proteasomal degradation. Heterodimerization of HIFalpha with its partner, ARNT, occurs and elevated expression of target genes ensues.

Recent studies of patients with inherited erythrocytosis from the Chuvash population in the Upper Volga region of Russia have identified an abnormality in the oxygen sensing pathway. A genome-wide screen of this ethnically homogeneous population localized a region on chromosome 3 associated with the disease. This led to identification of a C to T transition at nucleotide 598 of the VHL gene leading to an arginine to tryptophan change at amino acid 200 (R200W). Affected individuals are homozygous and carriers are heterozygous. R200W leads to a reduction in the interaction between VHL and HIF-1alpha , decreasing the levels of ubiquitinated HIF-1alpha , leading to more active HIF dimer and an increase in the expression of hypoxia inducible downstream genes, including Epo.

Table 1. Causes of an erythrocytosis

Primary Erythrocytosis
- *Congenital*
  - Erythropoietin (EPO) receptor mutations
- *Acquired*
  - Polycythemia vera (including JAK2 exon 12 mutations)

Secondary erythrocytosis
- *Congenital*
  - Defects of the oxygen sensing pathway
    - VHL gene mutation (Chuvash erythrocytosis)
    - PHD2 mutations
    - HIF-2α mutations
  - Other congenital defects
    - High oxygen-affinity hemoglobin
    - Bisphosphoglycerate mutase deficiency
- *Acquired*
  - EPO-mediated
    - Central hypoxia
      - Chronic lung disease
      - Right-to-left cardiopulmonary vascular shunts
      - Carbon monoxide poisoning
      - Smoker's erythrocytosis
      - Hypoventilation syndromes including obstructive sleep apnea
      - High-altitude
    - Local hypoxia
      - Renal artery stenosis
      - End-stage renal disease
      - Hydronephrosis
      - Renal cysts (polycystic kidney disease)
      - Post-renal transplant erythrocytosis
    - Pathologic EPO production
      - Tumors
      - Cerebellar hemangioblastoma
      - Meningioma
      - Parathyroid carcinoma/adenomas
      - Hepatocellular carcinoma
      - Renal cell cancer
      - Pheochromocytoma
      - Uterine leiomyomas
    - Drug associated
      - Erythropoietin administration
      - Androgen administration

Idiopathic erythrocytosis

Matched-cohort and case-control analyses have shown that VHL R200W homozygosity is associated with lower peripheral blood pressures, varicose veins, pulmonary hypertension, vertebral hemangiomas, lower white blood cell and platelet counts, and elevated serum concentrations of vascular endothelial growth factor, plasminogen activator inhibitor-1 and endothelin-1. These studies have also shown associations with arterial and venous thrombosis, major bleeding episodes, cere-

bral vascular events, and premature mortality. Spinocerebellar hemangioblastomas, renal carcinomas, and pheochromocytomas typical of classical VHL tumor predisposition syndrome have not been found, and no increased risk of cancer has been demonstrated. Retrospective analyses among patients with Chuvash polycythemia have not shown benefit for therapy with phlebotomy or aspirin, but these and other modes of therapy should be studied prospectively.

The R200W mutation is not peculiar to Chuvashia and has been detected in other ethnic groups worldwide. This same mutation is also endemic to the southern Italian Island of Ischia. Haplotype analysis suggests that, apart from one case in Turkey, all reported cases of the R200W mutation are linked to the same haplotype supporting a common origin. In erythrocytosis individuals, several other different mutations have been described, which can be present either singly or in the compound heterozygous state. Together, all individuals with VHL associated erythrocytosis belong to the second group of familial erythrocytosis disorders designated as ECYT2 and VHL mutations are the most commonly identified defect of the oxygen sensing pathway thus far (Table 2).

However, a substantial number of patients with congenital erythrocytosis with normal or elevated Epo do not have VHL mutations but may have other defects in oxygen-dependent gene regulation.

The activity of the PHD family of enzymes is strongly influenced by available oxygen and they show variable tissue distribution, with the PHD2 isoform exhibiting the most abundant distribution and the more profound affect on HIFalpha regulation. Screening all PHD isoforms in a family with erythrocytosis detected a missense mutation, Pro317Arg, in PHD2. Pro-317 is located in the active site of PHD2 and close to an iron chelating residue. In vitro assays confirmed reduced prolyl

hydroxylase activity of the Pro317Arg mutant. Further mutations in PHD2 have been reported and three of these mutations, located at amino acids 202, 281, and 377, cause full or partial deletion of the C-terminal portion of PHD2. The missense mutation, Arg371His, although adjacent to another iron chelating residue, is distant from Pro-317 but in terms of the tertiary structure is situated close to Pro-317 and the active site. Functional studies reveal the importance of this residue as loss of Arg-371 impacted upon the activity of PHD2. Thus it can be inferred that both Pro-317 and Arg-371 interact with HIFalpha and accordingly define the binding grove for the alpha subunit. Erythrocytosis associated-PHD2 mutations reduce hydroxylation of HIFalpha and support PHD2 being the dominant isoform that hydroxylates HIFalpha. Furthermore, the function of PHD2 cannot be compensated by either PHD1 or PHD3. Although both VHL and PHD2 mutations result in a loss of function, in contrast to VHL, all PHD2 mutations are present in the heterozygous state and in the familial cases are dominantly inherited. Consequently, PHD2-associated erythrocytosis has been designated as a separate group known as ECYT3 (Table 2).

The central axis of the oxygen sensing pathway is PHD-VHL-HIF and defects in two of these proteins are associated with erythrocytosis which raises the question whether HIF would be a cause of erythrocytosis. As previously indicated, there are three isoforms of HIF and there has been much debate over which isoform controlled Epo production. Several seminal studies in mice concluded that Epo synthesis was modulated by HIF-2. Previous investigation of the ODD region of HIF-1alpha uncovered a polymorphism, Pro582Ser, that did not correlate with the erythrocytosis phenotype. Several recent studies of the homologous region in the second isoform have established a further category of familial erythrocytosis designated ECYT4 that is associated with a gain-of function of mutation in HIF-2alpha (Table 2).

The first HIF-2alpha mutation, Gly537Trp, was reported in three affected members from the same family over three generations. The index case presented at the age of 23 years with serum Epo above the normal range. The dominantly inherited heterozygous mutation was located close to the site of prolyl hydroxylation at Pro-531 in the ODD domain. Functional studies revealed that the HIF-2alpha Gly537Trp exhibited significantly reduced hydroxylation as a consequence of poor binding to PHD2. The association with VHL was also strongly impaired and in normoxia HIF- 2alpha was able to escape proteasomal degradation. Target genes, such as vascular endothelial growth factor were upregulated in the cell model. Together these data supported HIF-2 being the major regulator of Epo. This has been borne out by the discovery of three further heterozygous mutations at Met-535 and Gly-537 in similar erythrocytosis patients. At Met-535, changes to valine and isoleucine have been detected and analysis of HIF-2 target genes in mononuclear cells from the individual with the Met535Ile mutation confirmed elevated expression of HIF-2 target genes. Most cases of HIF-2alpha associated erythrocytosis present at an early age, commonly in their twenties but also in teenagers and even in an 11-year-old girl. The serum Epo level was consistently well above the normal range. The phenotype of HIF-2alpha associated familial erythrocytosis appears to be more severe with thrombotic events in several instances and pulmonary arterial hypertension. In the older generations, deaths resulted from pulmonary embolism and mesenteric infarction. Although none of the triad of tumors associated with VHL syndrome has been reported to date.

Congenital erythrocytosis are extremely rare but also include a wide range (>100) of hemoglobin variants (http://globin.cse.psu.edu). The mutations are localized to regions of the hemoglobin molecule associated with oxygen transport leading to an increased affinity for oxygen and a characteristically abnormal "left-shifted" oxygen equilibrium curve. The RCM is increased to respond to the resulting tissue hypoxia.

In addition, the conversion of 1,3 bisphosphoglycerate (BPG) to 2,3 BPG is catalyzed by the enzyme bisphosphoglycerate mutase. Deficiencies in the red cell enzyme BPG mutase leading to low levels of 2,3 BPG may cause erythrocytosis. Reduced levels of 2,3 BPG, which binds to the central cavity of the hemoglobin molecule, lead to a failure to convert to a low oxygen affinity state. These enzyme deficiencies may be inherited in autosomal dominant or recessive traits depending on the severity of the deficiency. In patients without cyanosis, determining the hemoglobin oxygen dissociation P50 will help to distinguish between conditions that increase the affinity of hemoglobin for oxygen and those that are likely characterized by disordered hypoxia sensing. Since equipment for measuring hemoglobin oxygen dissociation is no longer widely available, there is a simple formula that permits the value of P50 to be estimated from venous blood gases.

Finally, once an erythrocytosis has been established identification of the cause is the next focus. This should start with a comprehensive history and examination with exploration for secondary causes such as chronic respiratory disease. In the patient who has an erythrocytosis and does not have PV or other obvious cause, checking Epo levels is an initial way to guide further investigation. Those with an Epo level below the normal range are likely to have an abnormality of the Epo-signalling pathway, and this pathway should be investigated first. Those with an inappropriately normal (as if the Hb is elevated the normal physiological response is a decreased Epo level) or elevated Epo are likely to have an abnormality of the oxygen sensing pathway; therefore, it is logical to look for defects in these pathways. There remain a group of patients in whom no cause has yet been identified for the erythrocytosis therefore with idiopathic erythrocytosis.

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ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN INDOLENT LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Patients affected by lymphomas or CLL relapsing after two lines of therapy, or autografting or with refractory disease are often candidates for reduced intensity conditioning (RIC) regimens followed by allo-SCT. All the published series reported a 2 year PFS of 50-60%, 40% and 30% in indolent lymphomas or CLL, aggressive and Hodgkin Lymphoma (HL), respectively. The long-term efficacy and toxicity of this strategy is still unknown. 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=68), including follicular lymphoma (FL, n=29), chronic lymphocytic leukemia (CLL, n=35), other (n=4); aggressive (HG-NHL, n=87), including B-cell phenotype (n=43); T-cell phenotype (n=28), mantle cell lymphoma (MCL, n=16)] and HL (n=39); 133 (68%) of 194 patients (pts) had chemosensitive disease and 100 (52%) of 194 failed previous autologous SCT. Median ages was 50 years (range, 20-63).

At last follow-up (median 60 months, range 15-113), 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 OS and PFS were 62% and 70% for LG-NHL, 61% and 59% for HG-NHL, and 42% and 19% for HL, respectively. Disease status before allo-SCT significantly influenced long-term 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], but not in LG-NHL [65% versus 56% (p=0.43)]. Although, the 5-year PFS was significantly different between FL and CLL (85% versus 58%, p=0.04), the 5-year OS was not (71% versus 56%, p=0.13). The OS and PFS were not significantly different between aggressive lymphoma of B- and T-cell origin [5 year OS: 67% versus 55% (p= 0.51); 5 year PFS: 63% versus 57% (p=0.45)], respectively. Overall, 30 pts died of NRM with a 5-year cumulative incidence of 15%. The incidence of acute and chronic GVHD wer e 34% and 55%, respectively. Interestingly, only 27 of 116 (23%) pts are still receiving immune suppressive therapy. The incidence of second tumors was 2% in the pts surviving more than 6 months after allo-SCT (n=2 alive, n=2 death). In conclusion, our long-term data with a median 5-year follow-up shows that: (i) pts with relapsed lymphomas or CLL can achieve long-term remission and are probably cured; (ii) non-relapse mortality is rather low; (iii) disease status before transplant is a critical determinant.

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RICHTER SYNDROME: MOLECULAR PREDICTORS AND GENETIC BASIS OF CHEMOREFRACTORINESS

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Definition of Richter syndrome. Over time, a fraction of chronic lymphocytic leukemia (CLL) develop Richter syndrome (RS), representing the clinico-pathologic transformation of CLL to an aggressive lymphoma, most commonly diffuse large B-cell lymphoma (DLBCL). Histologic documentation is mandatory for diagnosing RS, that otherwise can only be clinically suspected, but not proven. The clinical definition of RS maintains a certain degree of heterogeneity, and may be molecularly distinguished into at least two biologically different entities: i) transformation of CLL to a clonally related DLBCL; ii) development of a DLBCL that is unrelated to the CLL clone. Transformation of CLL to a clonally related DLBCL is by large the most frequent of the two conditions, accounting for approximately 80-90% of all RS cases. In clonally related RS, the pathogenetic link between CLL and the emerging DLBCL clone is apparently obvious, and is substantiated by gain of novel molecular lesions at a certain time point of the natural history of the CLL clone. On the other hand, the mechanisms underlying the development of a DLBCL clonally unrelated to CLL cells are not fully understood. With the advent of novel immunotherapies for CLL, and especially after the introduction of alemtuzumab, occasional CLL patients have been reported to develop clinically aggressive lymphomas characterized by Epstein-Barr virus (EBV) infection. These cases of alemtuzumab-associated aggressive lymphomas are clinically and biologically distinct from RS, and should be considered as a novel type of immunodeficiency-related lymphoma developing after T-cell depleting therapies in patients already immunocompromised because of the underlying disease and/or because of previous chemotherapy.

Pathology of Richter syndrome. Classically, RS is pathologically represented by DLBCL. The disease involves most frequently the lymph nodes, but extra-nodal localizations are also not uncommon. In some patients, transformation from CLL to RS does not occur simultaneously at all sites, but, at a certain time point, might be restricted to one single lesion, either nodal or extra-nodal. From a practical standpoint, this knowledge mandates that any biopsy aimed at exploring whether RS has occurred should be directed at the index lesion, i.e. the lesion displaying the largest diameter by imaging. The PET characteristics of the lesion, in particular the standardized uptake value (SUV), may also guide the choice of the site to be biopsied, since sites affected by RS are expected to have SUVs overlapping those of de novo DLBCL. Based on the Hans classifier of de novo DLBCL, approximately 90% of RS belong to the post-germinal center phenotype. The proportion of RS with a post-germinal center phenotype differs substantially from that detected in de novo DLBCL. CD20 is generally expressed by RS cells, and represents an important target for immunotherapy with anti-CD20 monoclonal antibodies, including rituximab.

Clinical epidemiology of Richter syndrome. Since RS diagnosis requires

pathological assessment, heterogeneity in biopsy policies among institutions represents a substantial factor influencing the incidence of RS observed in different CLL series. The hematologist's perception of RS as a rare complication of CLL may be due, at least in part, to under-recognition of RS in the current clinical practice. We reported on the incidence of RS in a consecutive series of CLL who, according to our institution policy, homogeneously underwent biopsy of the index lesion during CLL follow-up or at CLL diagnosis. The strict application of these homogeneous criteria to a consecutive CLL series revealed that the cumulative incidence of RS at 5 and 10 years excedes 10% and 15%, respectively. RS is generally regarded as a very late event in the clinical history of CLL. Data from consecutive series, however, document that a significant fraction of RS cases may occur relatively early after CLL diagnosis. In some cases, the diagnosis of RS and CLL are concomitant. In one recent study, median time to RS transformation was 23.0 months from CLL diagnosis. Also, RS was not observed after 82.4 months of follow-up, and the rate of transformation remained at 16.2% from that timepoint onward. Data from this and other series suggest that, in a fraction of patients, predisposition to RS transformation may be related to biological characteristics that are intrinsic to the CLL clone already at the time of CLL diagnosis. Novel molecular data support this hypothesis and document that cells with the molecular characteristics of the RS clone that will subsequently develop are already identifiable at the time of CLL diagnosis. This notion prompts the need of the early identification of CLL patients who are at risk of subsequent RS development.

Predictors of Richter syndrome development. Early recognition of RS may be clinically useful in order to avoid the exposure of patients to multiple lines of therapy that, being targeted to CLL progression, are of little efficacy on the transformed clone. This notion prompts the need for a close monitoring of CLL patients harboring clinical and/or biological risk factors of RS development. Recent advancements in the field have disclosed a number of risk factors, both clinical and biological, that might be helpful for RS prediction. Several of the predictors identified to date are specific for CLL transformation to RS, and are not related to other markers predicting CLL clinical progression, documenting that RS transformation and CLL progression without histological transformation are distinct events both clinically and biologically (Fig. 1 and Table 1). Of the biological risk factors that have been tested, the following have been scored as independent predictors of RS development: i) absence of del13q14 ; ii) usage of specific IGHV genes, namely IGHV4-39; iii) usage of a stereotyped BCR; iv) expression of CD38 (Fig. 2); v) genetic background of the host; and vi) telomere length. Among clinical predictors, lymph node size >3 cm, a parameter of solid disease, was the sole clinical risk factor of RS selected by two models of multivariate analysis. Conversely, parameters of leukemic disease, namely lymphocyte count, advanced Rai stage, splenomegaly, and percentage and pattern of bone marrow involvement, were not scored as independent clinical risk factors of RS. As expected, parameters of leukemic disease did predict for short time to progression without transformation, further reinforcing the notion that RS transformation and CLL progression according to NCI guidelines are distinct events in the natural history of CLL (Table 1).

As stated above, FISH karyotype at CLL diagnosis predicts RS. In particular, absence of del13q14 confers and independent risk of RS development. Conceivably, pathogenetic differences between CLL with and without del13q14 underlie the different predisposition of these two CLL subsets toward RS transformation. A sizeable fraction of CLL (30%) show a high degree of structural similarity in the BCR because of homology of the expressed heavy chain complementarity determining region 3 (VH CDR3). This phenomenon is known as stereotyped BCR and strongly suggests the role of antigen stimulation in disease development. Recently, we have shown that carrying a stereotyped BCR at CLL diagnosis may help in the identification of patients at risk of RS transformation. Comparing the immunogenetic features of 69 cases of RS with those of 714 cases of CLL, the prevalence of stereotyped VH CDR3 was significantly higher in RS than in non-transformed CLL (49.3% vs 21.3%). Of note, applying an actuarial approach to this cohort, stereotyped VH CDR3 at the time of CLL diagnosis was revealed to be an independent predictor of RS transformation. Among CLL carrying a stereotyped BCR, patients utilizing the IGHV4-39 gene in a stereotyped fashion (so called BCR "subset 8") had the highest risk of RS transformation (17-fold higher than cases without a stereotyped BCR; 5-year risk 68% vs 4%). In multivariate analysis, usage of IGHV4-39 along with stereotyped VH CDR3 was an independent risk factor of RS development. This observation, beside being of clinical interest, points to the role of antigen stimulation in RS development. The role of antigen stimulation in favoring RS development is further sustained by the observation that the BCR expressed by CLL belonging to subset 8 is characterized by the highest affinity for non-muscle myosin II, an autoantigen that might be involved in CLL promotion.

Both CD38 expression and lymph node size have been shown to be independent predictors of RS. From a pathogenetic standpoint, this observation suggests that molecular circuits in CLL lymph nodes may be important for determining the risk of RS in individual patients, and may directly contribute to CLL transformation to DLBCL. The lymph node may thus provide an optimal microenvironment for proliferation and blastic transformation of CD38 expressing CLL cells. In fact, interactions with other cells and cytokines that take place in the lymph node may provide adequate stimulation for CD38 up-regulation and signalling in CLL cells. Intriguingly, in vitro activation of CD38 signalling induces transformation of CLL cells to plasmablasts that are reminiscent of the blasts appearing during RS evolution.

Expression of CD38 is regulated at multiple levels. The 5' end of intron 1 of the CD38 gene is involved in the induction of CD38 expression by transcription factors. Within the same region maps a well-characterized single nucleotide polymorphism (SNP), leading to a C>G variation at position 184 (CD38 rs6449182). The presence of the minor G allele, either in a heterozygous or in a homozygous condition, strongly associates with RS. Compared to CC homozygotes, GG homozygote CLL patients had a 30.6% increase in the relative risk of developing RS, whereas GC heterozygotes showed an intermediate probability of 12.4 %. At 5 years, GG homozygotes showed an increase in probability of developing RS that approximately doubled that associated with GC heterozygotes. These observations document that the host genetic background may be of relevance in predicting RS development among individuals affected by CLL.

Analysis of a large number of SNPs has revealed that also the genetic background of the LRP4 gene (rs2306029) is an important predictor of RS transformation. In particular, patients that are homozygotes for the minor allele of LRP4 rs2306029 display a significantly higher 5-year cumulative probability of transformation to aggressive lymphoma compared to patients carrying the CT/CC genotypes that contain the wild type allele. LRP4 encodes for a protein involved in the Wnt/beta-catenin signaling pathway, that is known to be activated in CLL cells.

When assessed at the time of diagnosis, telomere length of CLL cells has also been shown to be an independent predictor of RS transformation. As already demonstrated for other neoplasms, telomere length recapitulates the cell proliferative history, and short telomere length denotes an aggressive clinical phenotype and chemo-refractoriness.

Prognosis of Richter syndrome. Prognosis of RS is generally considered highly unfavourable because of chemorefractoriness. However, survival of RS is not uniform, ranging from few weeks to 15 years, and may be predicted on clinical grounds by the RS score. Remarkably, risk factors that are relevant to the International Prognostic Index, namely number of extranodal sites of disease, age and stage, were not relevant to the RS score, confirming the notion that RS and de novo DLBCL are very different diseases. In contrast, survival prognostication in RS takes advantage of parameters reflecting marrow failure, such as thrombocytopenia,

and parameters reflecting immune system exhaustion and selective pressure to chemorefractory clones, such as number of prior lines of treatment. Recent data from our group indicate that, in addition to the clinical risk factors included in the RS score, survival post-transformation of RS may also be predicted by the tumor genotype and by the clonal relationship of the RS phase with the pre-existing CLL clone.

Figure 1.Clinical and molecular risk factors that, at the time of CLL diagnosis, predict RS transformation. At the time of CLL diagnosis, several risk factors predict an increased risk of RS transformation, including: expression of CD38, lymph node size, telomere length, absence of del13q14, immunogenetic features of the CLL clone, GG/CG genotypes of the CD38 rs6449182 SNP, and TT genotype of the LRP4 rs2306029 SNP.

Figure 2. Prediction of RS transformation according to CD38 expression at CLL diagnosis. Kaplan-Meier curves showing the value of CD38 in predicting RS transformation in the CLL series (n=360) from the Division of Hematology of the Amedeo Avogadro University of Eastern Piedmont (years 2000-2010).

Molecular pathogenesis and clinico-molecular correlates of Richter syndrome. The molecular mechanisms leading to transformation of CLL to RS are largely unknown. In classic RS represented by DLBCL, immunogenotypic studies have revealed that the majority (approximately 80%), though not the totality, of RS cases are clonally related to the pre-existing CLL clone. Cases of RS that are clonally related to the pre-existing CLL phase conceivably stem from the progressive accumulation of molecular lesions, either genetic and/or epigenetic, that favor the emergence and predominance of a large cell population causing clinical aggressiveness. In all available RS series, a fraction (10-20%) of RS cases is not related to the pre-existing CLL clone. Accumulation of molecular lesions in the CLL clone cannot be considered as a relevant factor in the development of clonally unrelated RS. Rather, these RS cases may be favoured by alterations of the host genetic background and immunologic function, or by derangements in the lymph node microenvironment induced by CLL cells. Until recently, the scarcity of biologic information about RS has hampered the identification of molecular predictors of RS outcome. We recently addressed this issue by performing a comprehensive molecular characterization of 86 pathologically-proven RS. TP53 disruption (47.1%) was the most frequent genetic lesion. By multivariate analysis and model validation, TP53 disruption was selected as an independent predictor of RS survival (HR: 2.27; p=.004), along with achievement of CR after RS treatment (HR 0.21; p<.001) and ECOG PS >1 (HR: 4.58; p<.001). By recursive-partitioning analysis, TP53 status, response to RS treatment and ECOG PS were used to build an algorithm for stratifying RS survival. Patients presenting with ECOG PS >1 had short survival, irrespective of TP53 status and type of response to RS treatment (median: 7.8 months). Patients presenting with ECOG PS <1, but harboring TP53 disruption or not achieving CR after RS treatment, had an intermediate survival (median: 24.6 months). Patients presenting with ECOG PS <1, no TP53 disruption, and achieving CR after RS treatment displayed a long survival (70% at 5 years). RS was clonally related to CLL in 50/63 (79.3%) assessable pairs, and clonally unrelated in 13/63 (20.6%). Compared to clonally related RS, clonally unrelated RS harbored less frequently TP53 disruption (23.1% vs 60.0%; p=.018) and stereotyped VH CDR3 (7.6% vs 50.0%; p=.009), and were characterized by a significantly longer survival (62.5 months; vs 14.2 months; p=.017). These data document that TP53 disruption, a well known marker of chemorefractoriness in lymphoid malignancies, is one of the major factors affecting RS survival. This observation provides the rationale for testing pre-transplant induction treatments with agents circumventing TP53 disruption. Also, our results document that clonally unrelated RS is clinically and biologically distinct from clonally related RS and should be considered as a secondary DLBCL arising de novo in the context of CLL. Therefore, the diagnosis of RS should be restricted to clonally related cases.

In order to further elucidate the pathogenesis of RS, we recently embarked on a whole exome sequencing program aiming at identifying novel mutations of the RS coding genome. This analysis has revealed the frequent involvement of mutations of the NOTCH1 proto-oncogene in RS. The frequency of NOTCH1 mutations in RS (31.0%) is significantly higher than in CLL at diagnosis. NOTCH1 mutations in RS are mostly represented by a recurrent two bp frameshift deletion (ΔCT7544-7545, P2515fs), and are predicted to cause NOTCH1 impaired degradation through the truncation of the C-terminal PEST domain. In order to establish the timing of acquisition of NOTCH1 mutations during the clinical history of RS patients, we investigated paired sequential samples collected at the time of CLL diagnosis. The data suggest that clonally represented mutations of RS might already be present years before RS transformation, and that the clone harboring the mutation is progressively selected during the CLL clinical history ending into RS transformation. The comparison of NOTCH1 activation in RS with the distribution of other genetic alterations that are recurrently associated with this disease revealed a potentially important pathogenetic pathway. In fact, in RS, NOTCH1 mutations are largely mutually exclusive with MYC oncogenic activation, that occur in ~20% of RS cases. This finding is consistent with the observation that NOTCH1 directly stimulates MYC transcription and suggests that activation of oncogenic MYC may be one common final pathway selected for RS tumorigenesis. Combining NOTCH1 mutations and c-MYC activation, 50% of RS might eventually have a genetic lesion deregulating the c-MYC pathway. Conversely, both NOTCH1 mutational activation and c-MYC deregulation often co-exist with inactivation of the TP53 tumor suppressor gene, an event that is commonly observed in association with c-MYC activation in tumors, and that may be selected to prevent the apoptotic effects and the response to genomic instability, both induced by c-MYC overexpression.

The role of EBV infection has been suggested by some studies as a potentially relevant factor for RS pathogenesis. The observation that the overwhelming majority of RS do not carry EBV infection in the malignant cells, however, does not favor this hypothesis. The presence of EBV sequences has been documented in some, though not all, cases of RS originating in patients previously treated with fludarabine for their pre-existent CLL. EBV infection in these cases is thought to be related to the immune deregulation caused by purine analogues and/or alemtuzumab.

Concluding remarks. Advancements during the last few years have revitalized the interest on Richter syndrome, and our ability to predict the development of this clinical condition has grown. A limited but

growing number of molecular and phenotypic markers have been identified that may facilitate the identification of CLL patients who are at risk of RS development at some point during the natural history of their disease. The availability of biological predictors of RS transformation suggests the adoption of a close surveillance and of a dedicated biopsy policy for patients who are judged to be at high risk. A close surveillance and an aggressive biopsy policy may anticipate the timing of RS recognition, that is clinically relevant since late diagnosis is an adverse factor in RS prognosis post-transformation. A combined effort of candidate gene studies and genome wide technologies have clarified the molecular pathogenesis of RS to a certain extent, and have identified novel biological prognosticators for this disease. Elucidation of the genetic complexity of RS might also be possibly useful for the future design of modalities of target therapy against this disorder.

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GENETIC TERAPY FOR BETA-TALASSEMIA: STATE OF THE ART AND CHALLENGES IT FACES IN HUMAN TRIALS

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Haemoglobinopathies are the most prevalent inherited disorders worldwide, and constitute a severe health and economic burden to patients and families at risk. Among these, beta-thalassemia is the most frequent monogenic disease with a global birth-rate incidence of 40,000/year, mainly in the Mediterranean, Middle East and Southern Asia countries [(1)]. Beta-thalassemia is caused by many different mutations in the beta-globin gene cluster, which ultimately result in reduced or complete absence of adult hemoglobin (HbA) production. The degree of imbalance in the alfa- and beta-globin protein, which is directly linked to the clinical severity of beta-thalassemia, hinders precursor maturation and results in ineffective erythropoiesis. Patient with markedly reduced or no beta-globin expression relies on routine red cell transfusion therapy for survival; chronic transfusion, while controlling the anemia does not correct ineffective erythropoiesis, and exacerbates iron accumulation in vital organs.

Advances in chelation therapy, including the recent availability of oral chelators, have improved the quality of the life and longevity in these patients, however iron overload, particularly in the heart, remain the major cause of death.[2] Moreover, higher incidence of new complications, as hepatocarcinoma have been describing probably as a result of the improvement in thalassemia outcomes.[3]

However, availability of economic resources has limited the optimal treatment of this disease worldwide. Medical treatment (transfusion and chelation), without even considering the associated complications, requires expertise and is very expensive as estimated in a recent study (an average cost of 25,000 euro per year per patient);[4] in addition, proper medical therapy for thalassemia requires the support of an increasing number of advanced technologies (eg, cardiac magnetic resonance imaging) that have to be provided by an increasing number of treatment centers. Considering that the very large majority of patients live today in non-industrialized countries, in which these effective therapies are improving, but are still severely curtailed, the problem of relative costs between a lifelong medical therapies versus a therapy intended to cure is crucial. The only currently conceivable means to cure beta-thalassemia is to provide the affected subject with hematopoietic stem cells (HSCs) containing functional beta-globin genes. More than 3000 allogeneic bone marrow transplantations (BMT) have been performed in thalassemia worldwide in the last 30 years using bone marrow, umbilical cord blood, or mobilized peripheral blood as a source of HSCs.

Transplantation outcomes today are much improved compared with the 1980s and 1990s, with more than 90% of patients surviving transplantation and with thalassemia-free survival ranging from 66% to 80% probably because of the reduced toxicity regimen. As recently reviewed by Angelucci E., 2010[5] the results of HSC transplant are remarkable if a matched sibling donor is available and the transplants are performed in well transfused and chelated children with good organ status, while transplantations using matched unrelated donors or in patients with later stage disease have been problematic.[5]

Availability of matched HSCs and immunological complications, including graft-versus-host disease have limited BMT to a minority of thalassemia patients. Therefore, for subjects lacking a matched donor, globin gene transfer in autologous hematopoietic stem cells offers the potential of curative stem cell transplantation without the risk of GVHD and without requiring immune suppression to prevent graft rejection.

Globin gene transfer as curative treatment.

The aim of globin gene transfer is to restore the capability of the thalassemic subject's own HSCs to generate red blood cells containing hemoglobin level sufficient to reverse the ineffective erythropoiesis and to correct the inherited anemia. In summary the proposed treatment is based on the administration of autologous CD34+ hematopoietic cells transduced ex-vivo with a vector encoding the normal beta-globin gene. After extensive tests for transduction efficiency, sterility, and other release criteria, CD34+ hematopoietic cells are returned to the donor after conditioning. From previous intensive preclinical studies and, more recently from the first clinical trial, we have learned that the immediate

challenges of the field are to optimize gene transfer and engraftment of a high proportion of genetically modified HSCs and to improve current vector design in order to achieve effective and safe level of expression (Figure 1).

Vector design to improve efficacy and safety of globin gene therapy

Earlier studies on globin gene regulation and gamma-retroviral globin gene-encoding vectors provided critical insights into elements required for the high-level regulated expression of globin genes. Gamma-retroviral vectors were limited by their size and unstable transmission to incorporate large elements of the beta locus control region (LCR) critical for adequate expression and were prone to position effects too.

In the mid-1990s, lentiviral (LV) vectors, derived from HIV, emerge as an effective system to mediate gene transfer and high-level expression of globin genes. They were capable to transmit globin regulatory elements and the coding sequences of the beta-globin gene without enabling the stability.

In a pioneering study, Sadelain and collaborators demonstrated phenotypic correction of thalassemia mice with an average increase of hemoglobin by 3 to 4 g/dL per vector copy with the TNS9 vector.[6] Several other groups later confirmed these results in various models of beta-thalassemia and sickle cell anemia, and based on them have been developed globin-LV vectors that are entering or already entered the stage of clinical trials (Figure 2).

Despite the great success reached by globin-LV vectors in these preclinical models it was evident that the anemia was rescue only when a large amount (approximately 80%) of HSCs were genetically modified with multiple copies per cell.

These conditions are hard to be applied in human clinical trials since human hematopoietic cells are more resistant to LV transduction than the mouse counterpart, and a high level of chimerism requires significant amount of pre-transplant chemotherapy conditioning for cytoreduction of endogenous HSCs. Moreover, safety concern suggests that the optimal vector copy number, to whom a clinical protocol should aim, should be close to 1 copy per cell.

The suboptimal expression of globin-LVs was essentially a consequence of position effects that negatively influenced the vector transcription even in the presence of large and complex set of endogenous β-globin regulatory elements. Therefore, subsequent studies by different groups, including our, focused on strategies to achieve higher and more consistent globin expression at a lower copy for effective correction of thalassemia major. To summarize many reports, two main strategies were adopted: inclusion of larger elements of the LCR, and flanking the vector with chromatin insulators or other genetic elements that create a functional domain within the vector sequences not influenced by the site of integration into transcriptional silent heterochromatin.

The latter offer a potential solution to generate vectors that are both more effective and safe in that insulator elements can function both as barrier elements to dampen position effects and enhancer blockers to prevent nearby genes from interacting with one another. For these reasons many studies have included the prototypic insulator element from chicken beta-globin gene locus (1.2 kb cHS4 sequence), in both globin gamma-RV and LV vectors and demonstrated that when flanking the vector in a "double-copy" configuration may reduce, position effects providing a more consistent/uniform expression, and therefore lead to superior genetic correction. Moreover, more recently, has been suggested that cHS4-insulated vectors have a lower propensity to perturb nearby gene expression.

Unfortunately the caveat is that LV vectors currying the full-length (1.2 kb) cHS4 element exhibit marked reductions in infectious titers, which is another aspect very important in hematopoietic stem cell gene transfer; in fact sharp, decline in titers limits the large-scale production of vector for human trials. Smaller core elements of cHS4 were therefore explored, but the core does not retain the full insulator activity.

Thus, many laboratories, including our own, are now focusing on identifying functional "insulator" elements that will not dramatically affect vector titer (Reviewed in Emery DW, 2011).[7]

Relatively little has been reported to date on the use of chromatin insulators other than cHS4 in the contest of retroviral vectors. Some reports involve an insulator element derived from sea urchin arylsulfatase gene locus termed Ars1 capable to reduce the transgene silencing but it does not display enhancer-blocking insulator activity.

Our group has focused on characterizing a new element from the early histone repeating unit of the sea urchin Paracentrotus lividus, termed sns5 (462bp long) that behaves as a classical chromatin insulator in the autologous and heterologous system and exhibits both enhancer-blocking and boundary functions in erythroid milieu.[8,9]

More recently we studied the effect of sns5 insulator in the contest of the globin-LV vector TNS9.3 (a modified version of TNS9) that has been approved for entering a phase1 clinical trial.[10] We found that insertion of this element in the "double-copy" configuration improved the likelihood of vector expression, both in-vitro experiments and in the mouse model of thalassemia, at levels even higher than cHS4 element with minimal effect on TNT9.3 LV titer (Figure 3).

The beta-globin gene expression was improved 2.5 fold at both mRNA and protein level and was sustained in long-term mouse chimera (6-14 and 40 weeks after BMT) (Figure 4). Moreover clonal in vivo assays (in single copy vector spleen colonies from a secondary recipient mouse) showed a reduction in the variability of beta-globin gene without gain in mRNA. All of these results make it a potential ideal candidate for improving both the expression and the safety of retroviral vectors.

Although maintaining high transgene expression is important to the success of clinical gene therapy, problems of vector-mediated genotoxicity have taken central stage. A safety concern is the risk of insertional mutagenesis with randomly integrating viral vectors.

Insertional activation of the LMO2 oncogene by the viral LTR enhancer has been shown in humans, in the context of gene therapy for X-linked severe combined immunodeficiency syndrome (X-SCID1) with gamma-retroviral vectors, despite a very high degree of success with

gene transfer. Recently, in a similarly successful trial for chronic granulomatous disease (CGD), insertional activation of MDS-EVI-1 by the viral LTR enhancer resulted in myelodysplastic syndrome in two patients.

In the contest of globin gene transfer, the absence of viral enhancers (SIN vectors) and the design of lineage and differentiation stage-restricted vectors represents one major step in reducing the risk of trans-activating oncogenes; nevertheless Hargrove et al., 2008[11] showed direct evidence that globin LV vectors can cause insertional deregulation of cellular genes in primary, clonal, murine, beta-thalassemia erythroid cells in which the globin locus control region was active.

Globin Gene Transfer Clinical Trials

HSCs gene transfer to treat beta-thalassemia is now becoming a therapeutic reality. While different group, including our, are planning to begin gene transfer clinical trials in the United States and in Europe in the near future, one trial for patients with β-thalassemia and SCD using the globin lentiviral vectors LV-betaT87Q is underway in France[13] (referred vectors showed in Figure 2). In this trial, the first patient, with transfusion-dependent β-thalassemia major, received ex-vivo transduced autologous CD34+ cells following myeloablative pre-transplant conditioning with high-dose busulfan. Unfortunately, the patient had prolonged post-transplant cytopenia and received frozen backup CD34+ cells for hematopoietic rescue and continued to have thalassemia. The second patient, a 19-year-old male with HbE/β0-thalassemia, also received autologous, vector-transduced CD34+ cells (~4 x 10^6 cells per kg) following myeloablative dose of busulfan in June 2007. The patient had hematological reconstitution about five weeks post-transplant and, in mid-2008, become transfusion independent with a stable hemoglobin level above 9 g/dL (Figure 4).[12]

Interestingly, the ~9 g/dL of hemoglobin, obtained after autologous, vector-transduced CD34+ cells transplantation, were composed for one third of endogenous HbE, one third composed of vector-encoded β-globin, and, somewhat surprisingly, one third composed of HbF. Regarding this last observation, it is notable that several beta-thalassemia and sickle cell patients who underwent allogeneic transplantation but subsequently rejected the donor grafts have been reported to have therapeutic levels of HbF following reconstitution with endogenous HSCs. Thus, it appears that the vector-encoded β-globin and "reactivation" of HbF expression both contributed to the therapeutic effectiveness in this case.

Despite the clinical improvement of the patient, the therapeutic effect was associated with the emergence of a dominant erythroid clone with insertion of the vector in the HMGA2 gene locus, in fact the patient showed approximately 10% gene marked hematopoiesis, with a large percentage due to this clone. The potential clinical relevance of alteration of HMGA2 expression by the vector integration is highlighted by the fact that this gene has been implicated as a potential oncogene in a variety of settings. So far, the expansion of the dominant clone has resulted in a therapeutic benefit for 3 years following gene transfer, the amount of this particular clonal population has been stable during this time, no perturbation of hematopoiesis has been observed and the patient is doing well [12].

However, the clonal dominance seen in the patient from this trial suggests that the cHS4 core alone is unable to shield the HMGA2 gene from the LCR enhancer. Caution regarding the insertional mutagenesis capability of any randomly integrating vector imposes that identification of more potent insulators is necessary at least until other strategies (homologous recombination and induced pluripotent stem cell technology) will become translatable to the clinic.

HSC source, ex vivo cell manipulation and transduction conditions will be important components in determining efficient HSC gene transfer and subsequent engraftment.

In the initial trials for hemoglobin disorders, the patient population for enrollment will likely be older than for the other trials aforementioned (X-SCID1 and CGD), which have enrolled young children. Some evidence suggests that the primitive repopulating cells of young patients are more amenable to gene transfer than those of adults. Therefore, determining the most optimal HSC source for gene transfer and transplantation in the context of hemoglobin disorders will likely be important in achieving success. Although steady state bone marrow CD34+ cells were used as the source of HSCs for gene transfer in the first globin gene transfer trial [12], others group, including our own, are evaluating whether cytokine mobilization of peripheral blood CD34+ cells in patients with β-thalassemia might be safe and effective as an alternative way to collect HSCs.

In collaboration with Boulad F and Sadelain M we have been conducting studies to assess the safety and efficacy of G-CSF mobilization in 5 adult subject with beta-talassemia major. The result indicates that CD34+ cells can be safely collected using G-CSF and that the yield in adult thalassemia subject is within the required cell doses.

The aim of efficient and safe transduction process is to modify a large number of HSCs with a low copy-vector per cell; HSCs have evolved unique mechanisms for self-preservation, including resistance to viral infection. Unfortunately, this characteristic may impede the ability to achieve high levels of gene transfer mediated by HIV-based lentiviral vectors. This is a main issue for gene therapy efforts being undertaken for beta-thalassemia. Particularly, the study of beta-thalassemia patients that underwent allogeneic stem cell transplantation and developed stable, long-term mixed chimerism suggests that HSC gene transfer levels of greater than 25% will be needed for a robust therapeutic effect in such patients. However, available pre-clinical and clinical trial lentiviral gene transfer studies suggest that improvements are needed to achieve this goal. Recently, our group-undertaken studies to investigate the optimal condition to expanded ex-vivo human HSCs collected from cord blood and from apheresis after G-CSF mobilization and to transduce them with high efficiency. Preliminary results showed that 4 to 6 fold expansion is achieved without loss of clonogenic potential and that expanded cells are much more permissive to LV vectors.

On the patient side, the degree and type of myeloablation for patients with beta-thalassemia and SCD that will allow sufficient engraftment of genetically modified HSCs remains undefined. The adrenoleukodystrofy (ALD) and the beta-thalassemia trials used full myeloablation with busulfan to achieve engraftment with 10% to 20% gene modified HSCs; a higher efficiency HSC gene transfer would perhaps allow the degree of myeloablation to be reduced. Finally, it is not yet known whether any disease-specific factors of the hemoglobin disorders will affect the many aspects of the treatment process negatively. Despite these uncertainties, researchers in the field remain optimistic that, with continued efforts, HSC gene transfer to treat β-thalassemia and SCD is likely to become a useful therapeutic option for patients with these diseases.

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