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ABSTRACT BOOK

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The European Hematology Association (EHA) aims to promote excellence in clinical practice, research and education in European hematology.
EHA was founded in June 1992 and today – with over 3500 members from 100 countries – is a consolidated representative of European hematologists.

Our aim

- To become the official European representative of hematology and hematologists – especially where research, education and regulatory issues are concerned – and to become a conduit for European harmonization;
- To promote the creation of a highly attractive market for practitioners and researchers in Europe thus fostering the mobility of hematologists in and to Europe;
- To reach out and offer a platform to countries that wish to further develop excellence in hematology;
- To promote education, training and scientific research in hematology in Europe;
- To exchange and disseminate knowledge and scientific information in the field of hematology.

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- Dissemination of medical research, both basic and clinic, through the Haematologica/The Hematology Journal;
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- Providing postgraduate education through the annual congress, tutorials and workshops;
- Supporting junior basic and clinical researchers in the development of their careers through the EHA Fellowship Program.
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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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Haematologica/The Hematology Journal, as the official organ of the European Hematology Association (EHA), aims not only to serve the scientific community, but also to promote European cultural identity.

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Continuing Medical Education (CME) is a means for hematologists to maintain and develop professional knowledge and skills keeping up-to-date with latest developments within the field.

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BEST ABSTRACTS

BEST-001

DEVELOPMENT OF ALGORITHM FOR MANAGEMENT OF ACUTE EVENTS RELATED TO SICKLE CELL DISEASE AT THE EMERGENCY DEPARTMENT

Forni GL,[1] Balocco M,[1] Cremonesi P,[3] Finco G,[4] Graziadei G,[5] Perrotta S,[6] Polati E,[7] Rigano P,[8] Robello G,[1] Rosa A,[1] Russo G,[9] Sainati L,[10] Schweiger V,[7] Vassanelli A,[11] Bonomo P,[12] Olivieri O,[2] Cappellini MD,[5] De Franceschi L[2]

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Sickle Cell Disease (SCD) is a worldwide distributed hereditary red cell disorder characterized by the presence of pathological hemoglobin S (HbS). Under deoxygenation HbS polymerizes inside the red blood cell (RBCs) causing red cell membrane damage, generation of dense red cells and sickling. These RBCs tend to pile them resulting in slowing down blood flow in microcirculation with related ischemic/reperfusion organ damage. This process can affect any organ or apparatus with unpredictable clinical manifestations burdened with a high risk of mortality. Recently migration fluxes from areas of the world with high prevalence of hemoglobinopathies have spread this disease in all Italian regions. One of hallmarks of SCD is the acute vaso-occlusive crisis (VOC), which are most common cause of hospitalization of SCD patients. Thus, SCD patients are the most frequent user of Emergengy Departments (EDs) compared to other severe hemoglobinopathies, such as b-thalassemias. SCD patient with VOCs can come to the EDs with full-blown clinical manifestations acutely appeared, but often with a less painful symptoms The time spent by the appearance of the first signs of VOC is an important prognostic index quoad vitam too. The guidelines for the treatment of SCD of the British "Sickle Cell Society", updated to 2008, and the recent ENERCA 2013 and AIEOP recommendations, suggest: the administration of the first dose of an appropriate analgesic within 30 minutes, including the time spent in Triage, at the access to hospital. Therefore, it is necessary to ensure to SCD patients high priority access to the ED evaluation and subsequent treatment. Thus, we have developed an algorithm to manage SCD patient in first 3-12 hours since their arrival to the EDs. We propose to give to SCD patients with VOCs a yellow code (excluding cases with impairment of vital functions: red code). The flow-chart of the algorithm has been designed to access, clicking on the button, to the detailed description of the diagnostic and therapeutic steps (Figure 1). The algorithm has been generated by a multidisciplinary group to consent to the triage operators a fast and appropriate approach to the pediatrics and adults SCD patients. We tested the handiness and reliability of the algorithm with the triage operators and we utilized their feedback to improve the final version, which we propose to use as a friendly tool for treatment of SCD patients in EDs.

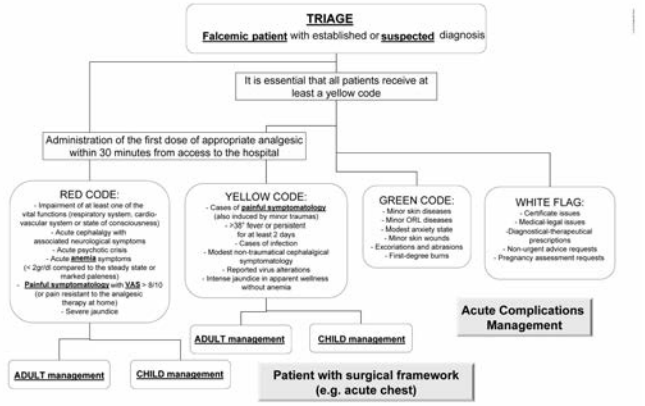

Figure 1.

BEST-002

ASSOCIATION BETWEEN MOLECULAR LESIONS AND SPECIFIC B-CELL RECEPTOR SUBSETS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Rossi D,[1] Spina V,[1] Bomben R,[2] Rasi S,[1] Dal-Bo M,[2] Bruscaggin A,[1] Rossi FM,[2] Monti S,[1] Ciardullo C,[1] Grossi A,[3] Zaja F,[4] Pozzato G,[5] Laurenti L,[6] Efremov DG,[7] Di-Raimondo F,[8] Marasca R,[9] Forconi F,[10] Del-Poeta G,[11] Gaidano G,[1] Gattei V[2]

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Background. Genetic lesions and B-cell receptor (BCR) signaling are both oncogenic drivers in chronic lymphocytic leukemia (CLL). However, little is known regarding the association between specific genetic aberrations and distinct stereotyped BCR subsets. Methods. Mutations (TP53, NOTCH1, SF3B1, BIRC3, MYD88), chromosomal abnormalities (del13q, +12, del11q, del17p, BIRC3 deletion), and BCR stereotypy were investigated in 1419 newly diagnosed CLL. Associations between genetic lesions and BCR features were assessed by non-parametric binomial

test and multiple hypothesis correction. Results. Two BCR subsets, namely subset 2 (IGHV3-21) and 8 (IGHV4-39), showed distinctive patterns of genetic alterations. Subset 2 CLL were significantly enriched in SF3B1 mutations (52% of cases; p<.001). Conversely, SF3B1 mutations occurred at low prevalence in IGHV3-21 CLL with heterogeneous BCR (13%; p=.002). Subset 2 CLL lacked TP53 abnormalities, thus pointing to SF3B1 as the main driver of progressiveness in this disease subset. Consistently, subset 2 CLL harboring SF3B1 mutations showed a higher probability of being treated at 5 years (67%) compared to subset 2 CLL with wild type SF3B1 (38%; p=.064) and compared to IGHV3-21 CLL with heterogeneous BCR (46%; p=.076). Subset 8 CLL were significantly enriched in +12 (87% of cases; p<.001) and NOTCH1 mutations (62% of cases). Conversely, +12 and NOTCH1 mutation prevalence was significantly lower in IGHV4-39 CLL with non-stereotyped BCR (27%; p=.003 and 8%; p=.006, respectively). The majority (62%) of subset 8 CLL had transformed to Richter syndrome (RS). All transformed cases carried both NOTCH1 mutations and +12, while this genetic association was never observed in non-transformed patients (p=.017). Conclusions. These data suggest that: i) synergy of SF3B1 mutations and subset 2 BCR configuration promotes disease progression in IGHV3-21 CLL; and ii) cooperation between NOTCH1 mutations, +12, and subset 8 BCR configuration primes RS transformation in IGHV4-39 CLL. Taken together, our observations provide a proof of concept that specific BCR configurations may contribute to clonal selection of specific genetic lesions influencing CLL outcome.

BEST-003

VASCULAR ENDOTHELIAL GROWTH FACTOR OVEREXPRESSION IN BONE MARROW CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROME: BIOLOGICAL AND CLINICAL EFFECTS

Invernizzi R,[1] Travaglino E,[1] Della Porta MG,[2] Malcovati L,[2] Gallì A,[2] Bastia R,[3] Bellistri F,[2] Quaglia F,[2] Boveri E,[3] Rosti V,[4] Cazzola M[2]

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Vascular endothelial growth factor (VEGF) is one of the most important angiogenic agents. In myelodysplastic syndrome (MDS), VEGF may have autocrine and paracrine regulatory effects on the hematopoietic system and contribute to disease progression. We analyzed by immunocytochemistry VEGF expression in bone marrow (BM) cells from 211 patients with MDS stratified according to IPSS criteria (134 low-risk and 77 high-risk patients), not previously treated, and 96 non hemopathic subjects. We also measured by an immunoassay VEGF BM plasma levels and the release of VEGF in the supernatants of cell cultures from representative MDS and control cases. Our aims were to evaluate whether abnormalities in VEGF expression were associated with relevant laboratory or clinical findings and to define their possible prognostic value; moreover, to investigate a possible correlation between VEGF expression levels and various biological parameters. VEGF was detected in most maturing myeloid cells from control samples (median 25%, IQR 14-44%). In MDS VEGF myeloid levels (median 42%, IQR 30-56%) were higher than those in controls (P<0.0001), and also many erythroblasts expressed VEGF. A few MDS CD34+ stem cells expressed VEGF, whereas it was not expressed by normal CD34+ cells. The release of VEGF was demonstrated in all samples; its levels were tendentially higher in the media conditioned by MDS mononuclear cells, especially from low-risk cases (median 56 pg/L, IQR 2-88), than in controls (median 9 pg/mL, IQR 0-23), and significantly higher in MDS BM plasma than in normal BM plasma (P=0.01). No significant relationship was detected between VEGF expression and circulating endothelial cells (CECs) or marrow microvessel density, whereas there was a positive correlation between microvessel density and CECs (P<0.001). In MDS a positive correlation between VEGF myeloid or erythroid expression and apoptotic rate (P=0.02 and P=0.04 respectively) was observed. In multivariate analysis including WHO subgroups and IPSS variables, myeloid VEGF levels above median values were independently associated with longer overall survival (P=0.03) and evolution-free survival (P=0.04). Our findings suggest that, in MDS, rather than stimulate angiogenesis, the production and release of VEGF may influence hematopoietic cell death and contribute to ineffective hematopoiesis, possibly by a paracrine induction of inflammatory pro-apoptotic cytokines, with a potential prognostic role.

BEST-004

RELATIONSHIP BETWEEN SKELETAL INVOLVEMENT, CYTOGENETIC FEATURES AND BONE MARROW PROFILES OF CYTOKINES AND CHEMOKINES IN PATIENTS WITH MONOCLONAL GAMMOPATHY

Dalla Palma B,[1] Guasco D,[1] Pedrazzoni M,[2] Bolzoni M,[1] Storti P,[1] Sammarelli G,[1] De Filippo M,[3] Ruffini L,[4] Aversa F,[1] Giuliani N[1]

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Osteolysis is the hallmark of multiple myeloma (MM). The pathophysiological role of RANKL, OPG, DKK-1, IL-3, IL-7, Activin A, CCL3 and CCL20 has been highlighted although the relationships between skeletal involvement and their bone marrow (BM) levels are not defined. A cohort of 350 patients with monoclonal gammopathy has been evaluated in this study including 58 patients with MGUS, 61 patients with smoldering MM (SMM) and 231 patients with symptomatic MM. A group of 31 healthy subjects was also included. MM patients showed significant higher BM median levels of RANKL, DKK-1, Activin A, and CCL20 than MGUS (p<0.05) and those of DKK-1 Activin A and CCL20 as compared to SMM (p<0.01). BM plasma levels of Activin A was higher in high-risk cytogenetics (t(4;14) and/or 17p-) patients as well as Activin A, DKK-1 CCL3 and CCL20 BM levels correlated with ISS staging (p<0.005). Regarding the bone status we found that 59%, 85% and 65% of MM patients were positive at the X-ray survey, magnetic resonance imaging (MRI) and positron emission tomography (PET)/computerized tomography (CT) scan, respectively. MM patients with high-risk cytogenetics showed higher positivity to the MRI scan than standard risk (100% *vs* 73%, p=0.05) whereas any significant relationship was not observed with the other cytogenetic abnormalities. Significant higher BM levels of DKK-1 and CCL20 (p<0.001) were found in MM patients with almost one osteolytic lesion as compared to those negative at the X-ray survey whereas Activin A (p=0.047), DKK-1 (p=0.004) and CCL20 (p<0.001) were significantly higher into the BM of patients with high bone disease (more than three lesions) as compared to those with low bone disease. MM patients with a positive MRI scan have higher BM levels of RANKL, Activin A, IL-3, DKK-1, CCL3 and CCL20 as compared to those negative (p<0.05) showing a relationship with the different patterns of infiltration; whereas only CCL20 levels significantly correlated with the presence of vertebral fractures (p=0.04). On the other hand, MM patients positive at the PET/CT scan have significant higher BM levels of CCL3 and CCL20 as compared to those negative (p<0.05). Finally by a logistic multivariate analysis we found than CCL20 levels were the only significant predictor of osteolysis (OR ratio: 1.03; p=0.014). Our study identifies which are the relationship between skeletal involvement and the profiles of BM cytokines and chemokines identifying CCL20 as a marker of MM bone disease.

BEST-005

VALUE OF FLOW CYTOMETRY IN THE DIAGNOSIS OF INDOLENT SYSTEMIC MASTOCYTOSIS

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Systemic Mastocytosis (SM) is characterized by infiltration of neoplastic MC in extracutaneous tissues, usually in bone marrow (BM). According to World Health Organization (WHO), SM diagnosis is defined by the presence of one major and one minor criterion or three minor criteria (see Table). However, the MC burden may be very low. Besides, in

indolent disease, the presenting symptoms are frequently anaphylactic reactions or unexplained osteoporosis without typical skin lesions. Hence, the real incidence of SM may have been significantly underestimated so far. The study aimed to investigate the value of flow-cytometry analysis to identify neoplastic MC, mainly in patients with indolent disease. From May 2007 to March 2013 we studied 227 patients (102 Females, 125 Males; median age of 53 years, ranging from 14 to 83 years) suspected for SM. They were referred at our Multidisciplinary Mastocytosis Outpatient Clinic, because of mediator-related symptoms or anaphylaxis (68.2%), Urticaria Pigmentosa (21.8%), unexplained osteoporosis (7%), hematological abnormalities (3%). Patients underwent complete BM examination. Moreover, highly sensitive immunophenotype and molecular assays were performed to identify the clonal CD25+ MC and the presence of D816V KIT mutation respectively. One hundred fifty-nine of 227 patients were diagnosed with clonal MC disorders (146 SM, 7 monoclonal MC activation syndrome and 6 SM with an associated hematologic non–mast-cell lineage disorder). The most sensitive diagnostic tools were molecular biology and flow cytometry that were positive in 158/159 and in 155/159 cases respectively. The abnormal MC infiltration was documented by cytology and histology only in 123 and 73 cases respectively. Serum tryptase level was greater than 20 ng/ml in 93/159 patients. According to WHO criteria, in our experience, the minor criteria allowed us to establish the diagnosis of SM in all but one patient, without need of histological demonstration of multifocal MC clustering (major WHO criterion). Our experience reveals that the real incidence of clonal MC disorders is probably greater than the estimated one. This result relies certainly on the multidisciplinary approach to MC disorders, but also on the use of highly sensitive tools to identify neoplastic MC. In this context flow cytometry seems to be more suitable than molecular biology as regards the operating speed and the lower cost of technology.

Table 1. World Health Organization (WHO) criteria for the diagnosis of SM

	Major Criterion	Minor Criteria			
	MC aggregates (>15 MC)	Abnormal MC cytology	D816V KIT mutation	CD25/CD2 expression	Serum Tryptase >20ng/ml
Clonal MC disease	73/159	123/159	158/159	155/159	93/159
No clonal MC disease	0/68	2/68	0/68	0/68	23/68

BEST-006

RITUXIMAB DOSE-DENSE CHEMOTHERAPY FOLLOWED BY INTENSIFIED HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (HDC+ASCT) REDUCES THE RISK OF PROGRESSION COMPARED TO STANDARD RITUXIMAB DOSE-DENSE CHEMOTHERAPY AS FIRST LINE TREATMENT IN YOUNG PATIENTS WITH HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): LONG TERM ANALYSIS OF PHASE III RANDOMIZED TRIAL DLCL04 OF THE FONDAZIONE ITALIANA LINFOMI (FIL)

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The prognosis of young DLBCL patients at high risk (age-adjusted, aa-IPI score 2-3) treated with standard RCHOP is still rather poor. FIL conducted a multicenter randomized phase III trial, with a 2x2 factorial design, aimed at investigating the benefit of intensification with Rituximab + High Dose Cytarabine + Mitoxantrone + Dexamethasone followed by BEAM and ASCT (RHDC+ASCT) after standard R-dose-dense chemotherapy delivered at two different level of dose (RCHOP14, RC14, and RMegaCHOP14, RMC14). Primary end-point was to increase 2-year Progression Free Survival (PFS) from 50% of the standard R-dose-dense arm to 65% in the RHDC+ASCT experimental arm. Inclusion criteria were: age 18-65; untreated DLBCL; aa-IPI 2 or 3. Patients were stratified according to aa-IPI and randomized at diagnosis to receive: RC14x8 cycles; RMC14 x 6; RC14x4 or R-MC14x4+RHDC+ASCT. From 2005 to 2010, 399 eligible patients were randomized: 199 to RHDC+ASCT and 200 to R-dose-dense. Histology was centrally reviewed. Clinical characteristics were: median age 49 (range 18-65); stage III/IV 29/65%; aa-IPI score 2/3 74/26%. In the RHDC+ASCT group, 151 patients (76%) completed the treatment and 177 (88%) in the R-dose-dense. Complete Remission (CR) was 76% in RHDC+ASCT *vs* 72% in R-dose-dense. Overall 26 patients (7%) had a partial remission and 64 (16%) did not respond. Treatment-related deaths occurred in 8 (4%) patients in RHDC+ASCT *vs* 5 (2.5%) in R-dose-dense. Grade III/IV extrahematological toxicities were reported in 85 patients (43%) in RHDC+ASCT *vs* 38 (19%) in R-dose-dense. With a median follow-up of 49 months, 3-year PFS for RHDC+ASCT *vs* R-dose-dense was 72% (95%CI:65-77) *vs* 62% (95%CI:55-69), p.04) and 3-year OS was 79% (95%CI:73-84) both in RHDC+ASCT and R-dose-dense, p .96. No difference in 3-year PFS was observed between RC14 and RMC14. PFS by aa-IPI was reported in Figure 1. In a Cox-model including the four arms and assuming RC14 as reference, the risk of relapse was significantly reduced in both ASCT arms with a major effect in RC14+RHDC+ASCT (HR=0.69). In conclusion, a short R-dose-dense chemotherapy followed by RHDC+ASCT significantly reduced the risk of progression compared to standard R-dose-dense therapy in young patients with high-risk DLBCL without adding significant toxicity. The dose intensification of RCHOP (RC14 *vs* RMC14) has no impact on the outcome and increase toxicity. The advantage of PFS for R-HDC+ASCT arm does not translate in an OS advantage.

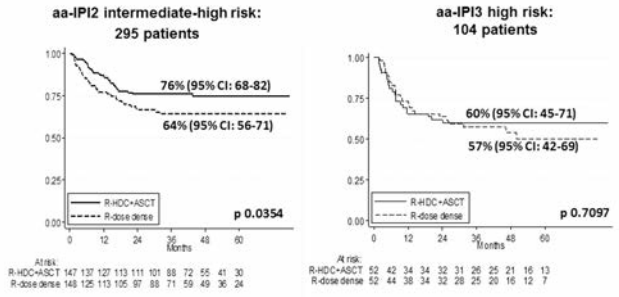

Figure 1. 3-years PFS: R-HDC+ASCT *vs* R-dose-dense in aa-IPI subgroups aa-IPI 2 and aa-IPI 3

BEST-007

SMO INHIBITOR SPECIFICALLY TARGETS THE HEDGEHOG PATHWAY AND REVERTS THE DRUG-RESISTANCE OF LEUKEMIC STEM CELLS

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Abnormal Hedgehog signaling is associated with human malignancies. Smo, a key player of that signaling, is the most suitable target to inhibit this pathway. To this aim several molecules, antagonists of Smo, have been synthesized, and some of them have started the phase I in clinical trials. Our hospital participated to one of these studies which investigated the oral administration of a new selective inhibitor of Smo (SMOi). To evaluate ex vivo SMOi efficacy and to identify new potential clinical biomarkers of responsiveness, we separated bone marrow CD34+ cells from 5 acute myeloid leukemia (AML), 1 myelofibrosis (MF), 2 blastic phases chronic myeloid leukemia (CML) patients treated with SMOi by immunomagnetic separation, and we analysed their gene expression

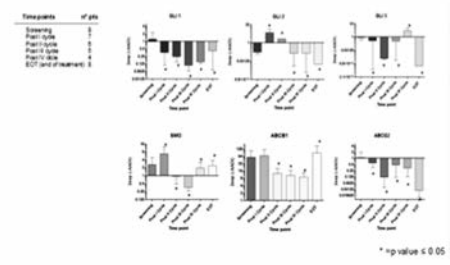
profile using Affimetrix HG-U133 Plus 2.0 platform. This analysis, showed differential expression after 28 days start of therapy (p-value ≤ 0.05) of 1,197 genes in CML patients and 589 genes in AML patients. This differential expression is related to Hedgehog pathway with a p-value = 0.003 in CML patients and with a p-value = 0.0002 in AML patients, suggesting that SMOi targets specifically this pathway. Among the genes differentially expressed we observed strong up-regulation of Gas1 and Kif27 genes, which may work as biomarkers of responsiveness of SMOi treatment in CML CD34+ cells whereas Hedgehog target genes (such as Smo, Gli1, Gli2, Gli3), Bcl2 and Abca2 were down-regulated, in both AML and CML CD34+ cells. It has been reported that Bcl-2 expression could be correlated with cancer therapy resistance and that Hedgehog signaling modulate ATP-binding (ABC) cassette transporters, whose expression has been correlated with chemoresistance. To support the ex vivo data we confirmed *in vitro* that SMOi specifically targets the Hh Pathway by Real Time PCR and Western Blot. We treated K562 cell line (Tirosin Kinase (TK)-resistant) with SMOi (10 M) in combination with Nilotinib, Imatinib, and Bosutinib (TKIs) (1 M). We confirmed *in vitro* that SMOi treatment down-regulate ABC transporters, Abcg2 and Abcb1 genes, and in combination TKIs could revert the chemoresistance mechanism in K562 TKIs-resistant cell line. The combination of SMOi with TKIs or conventional chemotherapy could represent a valid new therapeutic approach in these haematological malignancies. Work supported by European LeukemiaNet, FIRB 2008, AIRC, AIL, COFIN, University of Bologna and BolognAIL.

name	p-value
Development_Role of Activin A in cell differentiation and proliferation	0.002052003
Development_Ligand-independent activation of ESR1 and ESR2	0.002923441
Development_Hedgehog signaling	0.003441742
Regulation of lipid metabolism_Insulin signaling generic cascades	0.003722921

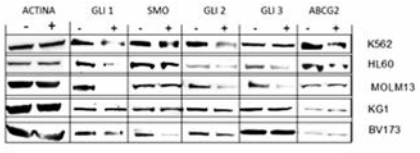
GeneGo MetaCore pathway analysis, report on CML patients

name	P-value
Development_Hedgehog signaling	0.000223512
Development_Regulation of epithelial-to-mesenchymal transition (EMT)	0.000324707
Neurophysiological process_GABA-A receptor life cycle	0.000718989
Proteolysis_Putative SUMO-1 pathway	0.001011649

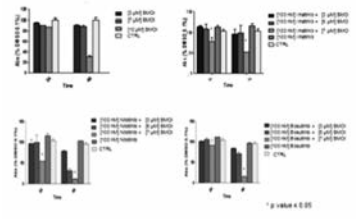
GeneGo MetaCore pathway analysis, report on AML patients



Real Time PCR analisys.



western blot analysis for Gli1, Smo, Gli2, Gli3, Abcg2 protein expression in Ph+ and AML cell lines,
after SMOi treatment. β-actin as housekeeping, or internal control.



Cell Proliferation Reagent WST-1 results on K562 TK-resistant cell line.
Red histogram= TKIs used as single agent. DMSO at 0.1% as a control

Figure 1.

BEST-008

QUALITY CONTROLS OF IMMUNE REGULATORY PROPERTIES OF EX-VIVO, GMP-GRADE EXPANDED MESENCHYMAL STROMAL CELLS FOR CLINICAL USE (EUROPEAN MULTI-CENTER STUDY CASCADE)

Bassi G,[1] Pacelli L,[1] Mènard C,[2] Dulong J,[2] Bezier I,[2] Zanoncello J,[1] Bifari F,[1] Ricciardi M,[1] Schrezenmeier H,[3] Sensebé L,[4] Tarte K,[2] Krampera M,[1] on behalf of CASCADE consortium (7th Framework Programme of European Commission: GA n° 223236)

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Aim of CASCADE is to standardize GMP-grade production and clinical use of Mesenchymal Stromal Cells (MSC). Immunological Unit is aimed at setting up and validating a standardized panel of functional assays to fully characterize the immunomodulatory properties of MSC obtained from bone marrow and adipose tissue through different GMP-grade expansion protocols (platelet lysate- *vs* fetal calf serum). Immune cells were isolated using indirect immunomagnetic depletion (purity >96%). MSC were expanded in the same medium used for production and harvested at 70% confluence. Primed MSC (pMSC) were obtained after 48h-treatment with rh-IFN and rh-TNF . MSC or pMSC were cocultured with T, B, NK cells for 4 or 6 days, and proliferation was evaluated by CFDA-SE dilution. T cells were stimulated with CD3 + CD28 antibodies; B cells were activated with CD40L, its enhancer, IL-2, CpG 2006, and anti-IgM/IgA/IgG; NK cells were activated with 100 U/ml rhIL-2. Cocultures were performed also with specific molecule inhibitors: L-1MT (IDO), snPP (HO-1), NS-398 (COX2), L-NMMA (iNOS) and anti- IFN antibody. For MSC immunogenicity assay, allogeneic T cell proliferation was evaluated at day 5 of culture; in addition, NK cells were activated for 2 days with rh-IL2, and MSC and pMSC were used as target cells. Inflammatory milieu significantly upregulated MHC class I and II, CD54, CD106, CD40, CD274, CD112, CD155 expression, and downregulated NKG2D ligands and mesenchymal markers (CD73, CD90, CD105). AT-derived MSC expressed less MHC class II, CD200 and CD106 molecules than BM-MSC. MSC coculture inhibited T and NK cell proliferation without inducing apoptosis, and this effect was greater in presence of primed MSC. Only primed MSC were capable of suppressing B cell proliferation. MSC inhibited apoptosis of resting T, B, and NK cells, while inflammatory priming increased their pro-survival activity. Activation of IDO and HO-1 was the main mechanism involved in MSC immune modulation. MSC never promoted allogeneic T cell proliferation; by contrast, IL-2-activated NK cells could efficiently recognize and kill allogenic unprimed MSC, while primed MSC became insensitive to NK cells. Some differences were observed depending on the origin and culture conditions of clinical-grade MSC. All the experimental protocols to assess MSC inhibitory effects on immune effector cells have been standardized and will be applied for the release of GMP-grade MSC produced inside the CASCADE Consortium.

BEST-009

MOLECULAR LESIONS OF SIGNALLING PATWHAY GENES IN INDOLENT B-CELL LYMPHOPROLIFERATIONS MIMICKING SPLENIC MARGINAL ZONE LYMPHOMA

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Background. Almost 60% cases of splenic marginal zone lymphoma (SMZL) harbor molecular lesions affecting signalling pathways involved in normal marginal zone (MZ) differentiation, including the NOTCH pathway, the NF-kB pathway, the toll-like receptor (TLR) pathway and the B-cell receptor (BCR) pathway. However, little is known regarding these lesions in other indolent B-cell lymphoproliferative disorders mimicking SMZL. Methods. Candidate gene mutations (NOTCH2, NOTCH1, BIRC3, TNFAIP3, TRAF3, IKBKB, MYD88, CD79A, CD79B, CARD11) were investigated by Sanger sequencing in 60 indolent B-cell lymphoproliferative disorders, including nodal marginal zone lymphoma (NMZL=32), monoclonal B-cell lymphocytosis showing a MZL-like phenotype and bone marrow morphology consistent with MZL (MZL-like MBL=17), and variant hairy cell leukemia (vHCL=11). In all cases, tumor representation was >50% to allow the detection of clonal lesions. All cases lacked the BRAF V600E mutation as assessed by ARMS PCR. Results. Overall, the genetics of NMZL and MZL-like MBL was consistent with that of SMZL, suggesting the involvement of a common oncogenic pathway in these disorders. Indeed, among NMZL, 56% (18/32) of cases where characterized by mutually exclusive genetic lesions affecting MZ differentiation genes, including NOTCH2 stabilizing mutations in 25% (8/32) of cases, TNFAIP3 disrupting mutations in 15% (5/32), MYD88 activating mutations in 12%, and NOTCH1, TRAF3 and BIRC3 mutations in 3% (1/32) of cases each. Among MZL-like MBL, 41% (7/17) of cases harbored mutually exclusive lesions of MZ genes, including MYD88 mutations in 29% (5/17) of cases, NOTCH2 mutations in 18% (3/17), and TNFAIP3 and CD79B mutations in 6% (1/17) of cases each. On the contrary, all cases (n=11) of vHCL lacked mutations of NOTCH, NF-kB, TLR or BCR genes, suggesting that none of these signaling pathways plays a relevant role in this disease. Conclusions. These data suggest that: i) SMZL, NMZL and MZL-like MBL share a similar genotype and are all promoted by the same molecular deregulation of MZ differentiation genes; and; ii) vHCL stands as a genetically different entity among indolent B-cell tumors mimicking SMZL. These data might help integrating the differential diagnosis of vHCL versus mimicking lymphoproliferations.

BEST-010

EFFICACY AND SAFETY OF THALIDOMIDE IN THE TREATMENT OF SEVERE RECURRENT EPISTAXIS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disease that leads to multiregional angiodysplasia. Recurrent severe epistaxis is the most common presentation, frequently leading to severe anemia. Multiple therapeutic approaches have been tried, but they are largely palliative. Since angiogenesis has been implicated in the pathogenesis of HHT, anti-angiogenic agents may be effective in its treatment. The aims of our ongoing, phase II, prospective, non-randomized study (ClinicalTrials.gov Identifier: NCT01485224) are to evaluate the effectiveness of thalidomide (thal) in reducing epistaxis and to identify the lowest effective dose in patients with HHT refractory to standard therapy. HHT patients with at least one episode of overt bleeding/week requiring at least one blood transfusion during the last three months and refractory to mini-invasive surgical procedures are enrolled. Thal is administered at a starting dose of 50 mg/day orally. In the event of no response, thal dosage is increased by 50 mg/day every 4 weeks until complete or partial response to a maximum dose of 200 mg/day. After response achievement, patients are treated for 16 additional weeks. Monthly follow-up is based on the epistaxis severity score and transfusion need, with adverse events being reported. Eighteen patients, 11 M and 7 F, aged 44-80 years (median 60), have been enrolled so far (median follow-up 36 weeks, range 2-72). Treatment was effective in all 17 evaluable patients. Nine cases responded within 4 weeks of starting the drug: cessation of nose bleeding was observed in one case, and reduction in the severity of epistaxis in 8 cases. Eight patients achieved partial response after 8 weeks of treatment. Thal significantly increased hemoglobin levels (P=0.04), decreased the transfusion need and improved the quality of life. Only nonserious, drug-related adverse effects were observed, including constipation and drowsiness. Eleven patients completed the treatment: with a median follow-up of 29 weeks, range 4-52, after the end of therapy, 6 cases remained stable without the loss of response, whereas 5 relapsed (median time to relapse 20 weeks). No correlation was found between genetic or clinical features and time to response or response duration. In conclusion, low-dose thal is safe and very effective for the therapy of epistaxis in HHT patients who did not benefit from other modalities of treatment, allowing for a rapid, often durable clinical improvement.

BEST-011

LONG TERM SURVIVAL (>10 YEARS) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS RECEIVING UP-FRONT AUTOLOGOUS STEM CELL TRANSPLANTATION: RESULTS FROM TWO PROSPECTIVE CLINICAL TRIALS

Zamagni E,[1] Di Raimondo F,[2] Patriarca F,[2] Tosi P,[2] Pezzi A,[1] Cellini C,[2] Tacchetti P,[1] Ronconi S,[2] Volpe S,[2] Pantani L,[1] Catalano L,[2] Fiacchini M,[1] Angelucci E,[2] Masini L,[2] Gozzetti A,[2] Galieni P,[2] Zannetti B,[1] Narni F,[2] Mancuso K,[1] Lazzaro A,[2] Brioli A,[1] Terragna C,[1] Califano C,[2] Ledda A,[2] Testoni N,[1]Cavo M[1]

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Survival of patients with MM has been extended with the introduction of autologous stem cell transplantation (ASCT) and more recently of highly effective novel agents. However, it is still the matter of debate whether a proportion of patients treated with ASCT can enjoy a long term survival, while sustaining prolonged high quality response. To identify the variables which were related to long-term survival, we performed a post-hoc analysis of two large prospective clinical trials of ASCT in newly diagnosed MM patients, the first one comparing single versus double ASCT (321 patients) and the second one incorporating thalidomide-dexamethasone (TD) into double ASCT (357 patients). Details of the studies were previously reported (Cavo M *et al*, JCO 2007 and JCO 2009). After a median follow-up of 61 months in the first study, CR was sustained for more than 5 and 10 years in 24% and 12% of the patients, respectively. On multivariate analysis, CR was the most important variable significantly extending PFS and OS; random assignment to double ASCT was an additional variable extending PFS After a median follow-up of 84 months in the second study, CR was sustained for more than 5 and 8 years in 42% and 9% of the patients, respectively. On multivariate analysis, achievement of CR, absence of t(4;14)±del(17p) and baseline high levels of hemoglobin were independent variables predicting for longer PFS and OS. Overall, 23% and 20% of patients in the first and second study were alive over 10 or 8 years, respectively. Long-term survivors showed a significantly prolonged CR duration(P<0.001)., PFS (P= 0.0000) and post-relapse OS (p<0.0001) in both the studies, as respect to the remaining patients. Sustaining a durable response for more than 42 months was favourably affecting survival after relapse on multivariate analysis. In a logistic regression analysis, independent factors predicting for long-term survival in both the trials appeared to be attainment of CR, sustaining the response for more than 42 months and application of double ASCT. In conclusion, approximately 20-25% of the patients undergoing up-front ASCT can achieve long term survival, with 33% of them remaining relapse free. Attainment of CR, sustaining a durable

response and application of double ASCT were the leading independent variables predicting for long-term OS. Prolonged survival after relapse was a contributing factor as well and was influenced by the sustenance of a durable response.

BEST-012

LOW-DOSE LENALIDOMIDE PLUS LOW DOSE CYTARABINE INDUCE COMPLETE REMISSION THAT CAN BE PREDICTED BY GENETIC PROFILING IN VERY ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS

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We designed a prospective phase II study to assess the efficacy of the concomitant administration of low-dose lenalidomide and low-dose cytarabine in 40 very elderly patients (median age 76 years) with acute myeloid leukemia (AML). Median white blood cell count at diagnosis was $3.2x10^9$/L (range: 0,7-46,8x10^9/L). 19/40 patients had an intermediate karyotype, 17/40 an unfavorable karyotype and 4/40 were not evaluable. Seventeen patients had a de novo AML, whereas 23 patients had a secondary AML. Patients received low-dose lenalidomide (10 mg/day orally, days 1-21) and low-dose cytarabine (20mg twice day subcutaneously, days 1-15) every 6 weeks, up to 6 cycles. To identify possible biomarkers associated to sensitivity/resistance, global gene and miRNA expression profiling (Affymetrix Transciptome 2.0) was performed on purified AML cells obtained from 15 patients. Induction-period mortality was 20%, with 8 deaths occurring during cycle 1. Overall CR rate was 38% among evaluable patients. The CR rate was significantly higher in patients presenting with bone marrow blasts <30% (p=0.04). Six out of 12 responding patients are still in CR after a median follow-up of 20 months (range: 6-33). Statistical analysis showed that responding patients had a longer median overall survival than non-responders (491 *vs* 64 days, P<0.0001). Interestingly, cytogenetic risk was not predictive of CR. Conversely, by studying the global miRNA and gene expression profile we identify a molecular signature, including 114 genes and 18 miRNA associated with the clinical response (CR *vs* no CR). Of note, based on the expression of 5 genes belonging to relevant functional categories such as angiogenesis, cell cycle regulation and immune response, we developed an algorithm to predict treatment response that was successfully validated in 15/15 (100%) tested cases.The combination of low-dose lenalidomide and low-dose cytarabine induce a high rate of complete remission that can be predicted by genetic profiling in a subset of very elderly AML patients with extremely poor-prognosis, not suitable for intensive chemotherapy. The study was registered at EMA with the EUDRACT no 2008-006790-33. Acknowledgements: Celgene is gratefully acknowledged for providing Lenalidomide for the patients. The study was supported in part by AIL Pesaro Onlus.

ORAL COMMUNICATIONS

Acute Myeloid Leukemia I

C001

CD44V6-TARGETED T CELLS MEDIATE POTENT ANTITUMOR EFFECTS AGAINST ACUTE MYELOID LEUKEMIA AND MULTIPLE MYELOMA

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Introduction. The recent and extraordinaire clinical successes of T cells redirected with chimeric antigen receptors (CARs) indicate this strategy as the new frontier for the immunotherapy of hematological malignancies. A widespread application of the strategy is however limited by the current lack of CARs for diseases different from B-cell tumors. The variant isoform 6 of CD44 (CD44v6) is expressed on acute myeloid leukemia (AML) and multiple myeloma (MM) cells, but not on hematopoietic stem cells (HSC). The possible involvement of CD44v6 in chemoresistance and relapse makes it an attractive target for disease eradication Aim. To develop a CAR strategy for safely eradicating AML and MM through the elimination of chemoresistant cells Results. We recently found that CD44v6 is crucially involved in stroma-induced chemoresistance and *in vivo* tumorigenicity in both AML and MM. We therefore constructed a novel CAR specific for CD44v6 including the CD28 endodomain (CDD4v6 CAR). To provide a safety switch in case of toxicity, the CD44v6 CAR was cloned in a LV carrying a bi-directional promoter for its co-expression with the HSV-tk suicide gene. After LV transduction, T cells were highly cytotoxic against autologous primary AML and MM cells, and could be ablated with the prodrug ganciclovir. In the presence of chemoresistance-inducing stroma, CD44v6-targeted T cells completely cleared tumor cells. Once infused in NSG mice, CD44v6-targeted T cells persisted long term and eradicated AML (THP1 cells and autologous primary cells) and MM xenografts (MM1.S cells). Interestingly, the eradicating effect was dependent on both the CD28 endodomain and on CD28 costimulation used for transduction (beads). As expected, CD44v6-targeted T cells were not cytotoxic to HSC, however they recognized mature monocytes, suggesting the need of a suicide gene for controlling late toxicities. Since the rapidity suicide-gene activation is critical to ensure the safety of potent effectors such as CAR-redirected T cells, we explored the novel inducible form of caspase 9 (iCasp9) as an alternative to HSV-tk and crucially demonstrated a much faster kinetics (hrs *vs* days). Conclusions. CD44v6-redirected T cells have the potential to selectively eliminate AML and MM cells that resist chemotherapy. Once the eradicating effect is achieved, CD44v6-targeted could be ablated through suicide-gene activation not to interfere with full hematopoietic reconstitution.

C002

NEWLY IDENTIFIED SMALL MOLECULES TARGETING THE N-TERMINAL PORTION OF NUCLEOPHOSMIN (NPM1) INDUCE APOPTOSIS, CELL CYCLE ARREST AND ABERRANT MITOSIS IN AML CELLS HARBOURING NPM1 MUTATION

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Treatment of AML is still challenging since current protocols cure only 30% of patients. Developing new drugs to target the product of tumor genetic lesions is an attractive therapeutic alternative expected to improve outcome. NPM1-mutated AML is a good candidate for such approach, since NPM1 mutation is an initiating genetic event that is consistently stable at relapse. NPM1 is a predominantly nucleolar protein with nuclear-cytoplasmic shuttling activity and multiple functions including ribosomagenesis, control of centrosome duplication, stability of tumor suppressors. NPM1 exerts most of its functions and builds the nucleolus as oligomer and for this purpose the N-terminal portion of the protein is critical. Starting from the crystal structure of the NPM1 oligomer, we have recently applied innovative computational modelling and analyses in order to dissect the mechanisms of oligomerization and identify and develop new small molecules able to interfere with it. Following these analyses, we indeed selected a series of candidate small molecules and tested these compounds *in vitro*. Interestingly, some of them showed relevant biological activities against human AML cells. In particular, with doses ranging from 0.2 to 5 μM (according to the compound), they were able to induce significant apoptosis in different AML cell lines. However, when lower doses of the compounds were tested, apoptosis appeared higher in OCI-AML3 and IMS-M2 (harbouring NPM1 mutation) than in U937 and OCI-AML2 cells (not harbouring NPM1 mutation and used as control), (Fig 1a).

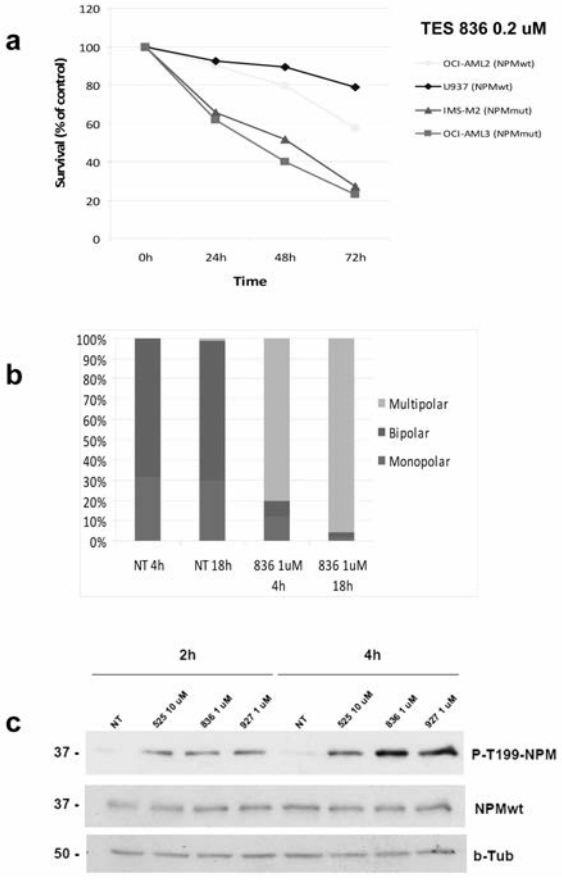

Figure 1.

Apoptosis was associated with p53 upregulation, caspases activation, G2-M cell cycle arrest. Moreover, aberrant mitosis with multipolar spindles were observed as early as at 4-8 hrs upon treatment, suggesting interference by these compounds with NPM1 control of mitotic spindle formation (Fig 1b). Interestingly, treatment induced early phosphorylation of NPM1 in multiple sites known to be involved in the regulation of NPM1 function in centrosome duplication control (Fig 1c). All these findings are in accordance with a functional inhibition of NPM1 being observed also in the NPM1 knock-out mouse model and indicate that NPM1 is druggable. Tuning the dose of compounds targeting NPM1 could allow obtaining a more selective effect on AML cells with NPM1 mutation which, because of both the haploinsufficiency and the cytoplasmic delocalization of NPM1, are already defective in at least some NPM1 normal functions.

CO03

FLT3-ITD+ ACUTE PROMYELOCYTIC LEUKEMIA (APL) PATIENTS SHOW A LONG-TERM UNFAVORABLE OUTCOME

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Although previous studies in APL have revealed that the FlT3-ITD mutation is associated with an elevated presenting WBC count, hypogranular variant (M3v) morphology and the short (bcr3) isoform of PML-RARA, the prognostic significance of FlT3 mutations in APL has not been firmly established. We report an update of these patients with a median follow-up of 9 years in which we observed that the presence at baseline of the FlT3-ITD mutation confers a very poor overall survival (OS). One hundred and forty-seven patients with newly diagnosed APL were observed and treated with the AIDA (73 patients) and AIDA2000 protocols (74 patients) at the Sapienza University of Rome during the period April 1993-October 2010. Diagnosis was initially established morphologically and subsequently confirmed in all cases by RT-PCR. The following clinical characteristics at diagnosis were analyzed according to the FlT3 status: age, sex, FAB classification, peripheral WBC and platelet count, hemoglobin, karyotype, PML/RAR isoform and relapse risk. For statistical analysis, the Wilcoxon-Mann-Whitney test was used to compare non-parametric series and the Fisher's exact test to compare categories. OS was estimated using the Kaplan-Meier method, whereas relapse-free considered as events relapse and death in CR. Thirty-three patients were identified as FlT3+, 19 were males (57%) and 14 were females; 27% had a variant type according to the FAB classification and 36% were classified as high-risk. Twenty-one FlT3+ patients (63%) presented the bcr3 transcript compared to 37/114 (32%) FlT3- patients (p=0.002). Eight FlT3+ patients (24%) experienced a differentiation syndrome compared to 14 (12%) in the negative cohort (p=0.02). After a median follow-up of 9 years (range 5-19), we could document a significant worse long-term outcome for FlT3+ patients: OS was 96% in the FlT3- cohort compared to 39% in the FlT3+ cohort (p=0.0001), relapse-free survival (RFS) 90% in FlT3- patients *vs* 30% in the FlT3+ ones (p=0.017) and disease-free survival (DFS) 64% *vs* 21% (p=0.001). While this study confirms that there is no difference in response to induction with the AIDA schedule in FlT3+ APL patients, the longest follow-up so fare reported has allowed to demonstrate the significantly worse long-term outcome for this subset of APL, in terms of OS, RFS and DFS. Further studies aimed at investigating whether other clonal mutations may play a role in this unfavorable subset of patients are needed.

CO04

IDENTIFICATION OF HUMAN ACUTE MYELOID LEUKEMIA CELL LINES CARRYING DISRUPTIVE BCOR MUTATIONS AS *IN VITRO* MODEL TO STUDY THE FUNCTIONAL ROLE OF BCOR IN LEUKEMOGENESIS

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Background. We recently identified, by whole-exome sequencing, somatic disruptive mutations of the X- linked transcriptional repressor BCOR (BCL6 co-repressor) as a frequently recurring event (17%) in the least genetically characterized subset of acute myeloid leukemias (AML), *i.e.* those carrying a normal karyotype and no mutations in typical AML oncogenes/tumor-suppressor genes (NPM1, FLT3, CEBPA, MLL, IDH1). The disruptive pattern of BCOR mutations (non-sense, frame-shifting or conserved splice-site mutations across the whole coding sequence), together with the decreased BCOR mRNA levels associated to them (suggestive of non-sense mediated mRNA decay) and to the absent or weak expression of truncated BCOR proteins, points to a previously unrecognized tumor-suppressive role of BCOR in AML pathogenesis. AIM. To identify AML cell lines carrying disruptive BCOR mutations as *in vitro* tools for dissecting the presently uncharacterized functional role of BCOR in leukemogenesis. Methods. We subjected genomic DNA of a broad panel of 77 AML cell lines to PCR and Sanger sequencing for all BCOR coding exons. BCOR gene expression was evaluated at the mRNA and protein level by RT-qPCR and Western blotting, respectively. Results. We report for the first time BCOR disruptive mutations in 5 AML cell lines (20%), including 5 non-sense mutation and 1 frame-shifting insertion, scattered throughout the coding sequence. These mutations all predict to introduce premature stop codons, and (with the exception of one non-sense mutation in the last coding exon) to potentially trigger non-sense mediated mRNA decay. Indeed, in comparison to 3 BCOR–unmutated cell lines, BCOR mRNA was found to be down-regulated (from 4.1 to 1.7 fold) in 4 of the 5 cell lines with disruptive BCOR mutations. Furthermore, BCOR full length protein was clearly expressed in the 3 BCOR–unmutated cell lines but not in any of the 5 cell lines carrying BCOR disruptive mutations, which on the contrary displayed shorter bands of variable intensity and of a molecular weight compatible with the truncated protein encoded by the respective mutation. Conclusions. We have identified 5 human AML cell lines that harbor disruptive BCOR mutations with genetic features and gene expression consequences similar to those found in AML patients, and that therefore represent ideal *in vitro* models where to study BCOR function in leukemogenesis.

CO05

A TRIB2-RELATED GENE SIGNATURE IS ASSOCIATED WITH ADULT MINIMALLY DIFFERENTIATED ACUTE MYELOID LEUKEMIA

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In humans, TRIB2 has been found up-regulated in T cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML) with mixed myeloid/lymphoid phenotype, associated with activating mutations of NOTCH1 gene of which TRIB2 is a direct target. We speculated that TRIB2 could participate in AML pathogenesis in the hematopoietic stem cells during early stages of differentiation. TRIB2 gene expression was measured by qRT-PCR on total RNA extracted from bone marrow aspirate of de novo AML patients. Bone marrow aspirates from 35 adult de novo AML patients were analyzed: 15 AML-M0, 13 AML-M2, 5 AML-M3, and 3 AML-M5b Four healthy samples were used as reference. Expression profiles were generated from CEL files (data file created by Affymetrix DNA microarray image analysis software) downloaded

from GEO database. Selected datasets were GSE12662, GSE17061, GSE14834 and GSE18239. All analysis were performed using dChip software starting from CEL files. All CEL files were normalized and "Model-based expression" was performed. The qRT-PCR analysis for TRIB2 showed a significant overexpression of the TRIB2 gene in the AML-M0 group as compared to the references data (p=0.002). The AML-M2, AML-M3, and AML-M5 FAB subgroups did not show any consistent differences from the reference. NOTCH1 activating mutations were investigated by direct sequencing of its HD and PEST domain (exons 26, 27, 34). Data from analysis did not revealed any mutation in our samples suggesting that TRIB2 overexpression was independent from NOTCH1 misactivation. The comparative analysis between AML and ALL on available microarray data from GEO database was performed and because of the great heterogeneity of the TRIB2 expression we grouped samples using the descriptive statistics on all values for "High" and "Low" expression of TRIB2. The comparison between "High" and "Low" TRIB2 expression detected 523 genes differentially expressed. Than clustering software returned some overlapping clusters as "High" TRIB2 expressing samples (p=0.007), T-ALL (p=0.008), B-ALL (p=0.004) and AML-M0 samples (p=0.008). Trough TRIB2 expression analysis we have found that TRIB2 is over-expressed in AML-M0 compared to the other AML subtypes independently of NOTCH1 mutational status. Microarray data analysis comparing samples on the base of the TRIB2 expression led to the selection of a TRIB2-related gene signature which for the first time, molecularly, highlights the similarities between AML-M0 subtype and ALL.

CO06

CHARACTERIZATION OF HEMATOPOIETIC STEM AND PROGENITOR CELL ADHESION TO SURROGATE MICROENVIRONMENTS

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Hematopoietic stem and progenitor cells (HSPCs) reside in particular and specialized microenvironments, called stem cell niches. Interaction of HSPC with its niche is fundamental for cell proliferation, differentiation and quiescence. An alteration of these mechanisms plays a key role in numerous hematological malignancies, as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Our goal was to study and characterize the HSPC adhesion to bone marrow extracellular matrix proteins. Afterwards, we intended to distinguish between the different subsets of stem and progenitor cells and describe their ability to attach to the substrates. Using an high-throughput microscope, we established a novel and robust adhesion assay, functional even when just a low number of cells is available. With multicolor flowcytometry (FACS), we investigated the different subpopulations of normal CD34+ cells and characterized their adhesion preferences and affinity to the different substrates. Over 80% of plated CD34+ cells adhere to fibronectin, about 70% to VCAM-1 and in a reduced number to laminin, osteopontin or collagen-1 (11%-19%). Inhibiting alpha-4 integrin, but not alpha-5, the quote of adherent cells could be effectively reduced. With FACS, we noticed that megakaryocyte-erythroid progenitors (MEPs) were the most adherent population to each substrate (about 3 fold increase), followed by common myeloid progenitors (CMPs) and hematopoietic stem cells (HSCs). Granulocyte-macrophage progenitors (GMPs) resulted to stick less to all the studied matrix proteins. Then, in the adherent fraction, the expression levels of CD34 were increased compared to the non adherent fraction and the contrary was shown for CD45RA, suggesting that the differentiation grade could influence the adhesion properties. Next, we showed that CXCR4, a key chemokyne receptor involved in HSPCs' homing and differentiation, and CD44, a glycoprotein required for migration, cells interactions and adhesion, are expressed specially by GMPs. The HSCs, compared to the other subpopulations, showed higher levels of alpha-2 integrin; MEPs express more alpha-4 integrin. Herein, a serious heterogeneity in the adhesion processes between the subsets of CD34+ cells is shown: GMPs adhere less than other subpopulations, but express more CXCR4. These data, if confirmed in analogous studies with AML cells, could provide new sensitive tools for targeted therapies.

Myeloma and Monoclonal Gammopathies I

CO07

EFFICACY AND SAFETY OF THREE SUBCUTANEOUS BORTEZOMIB COMBINATIONS IN ELDERLY, NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: INITIAL RESULTS FROM A PHASE II COMMUNITY-BASED STUDY

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Bortezomib-Melphalan-Prednisone (VMP) is the standard treatment for newly diagnosed elderly multiple myeloma (MM) patients. Weekly and subcutaneous bortezomib administration was associated with a significant reduction in adverse events (AEs), such as peripheral neuropathy, without affecting efficacy. Frail elderly patients are more susceptible to AEs, thus suggesting the need for an adapted-dose strategy. To assess the safety and the efficacy of 3 reduced-dose intensity subcutaneous (sc) bortezomib-based treatments in elderly, frail, newly diagnosed MM patients unsuitable for protocol with standard inclusion criteria. Induction treatment included nine 28-day cycles of bortezomib 1.3 mg/m^2 sc days 1, 8, 15, 22, oral prednisone 50 mg every other day (VP) or VP plus oral ciclophosphamide 50 mg every other day (VCP) or oral melphalan 2 mg every other day (VMP), followed by maintenance with sc bortezomib every 2 weeks until progression. Primary endpoint was overall response rate (ORR); secondary endpoints were safety, progression free survival (PFS), and overall survival (OS). Overall, 152 patients were enrolled in the study, including 51 patients in the VP, 51 in the VCP and 50 in the VMP group. Median age was 78 years. Patients were defined as frail (60%) or unfit (24%) or fit (16%) according to age, Charlson comorbidity index, Activity of Daily Living score (ADL) and Instrumental Activity of Daily Living score (IADL). All three induction regimens exhibited substantial activity, with an ORR of 60% in the VP, 60% in the VCP, and 70% in the VMP group (p=0.67). After a median follow-up of 10 months no significant difference in PFS and OS was observed between the 3 groups: 1-year PFS was 72% (VP), 78% (VCP), 67 % (VMP), p=0.74 and 1-year OS was 84% (VP), 93 % (VCP), 87% (VMP), p=0.48. In frail and unfit patients all 3 induction regimens showed comparable outcomes. Hematologic grade >3 AEs occurred in less than 10% pts in all groups. Non-hematologic grade >3 AEs were comparable in the 3 groups and were mainly infective (12%), cardiovascular (8%), and neurologic (7%), including 5% of peripheral neuropathy. Discontinuation rate due to AEs was 14% in the VP, 16% in the VCP and 22% in the VMP group (p=0.30, VP *vs* VMP). In elderly, frail, newly diagnosed MM patients, the doublet VP showed similar activity compared with 3-drug combinations. Longer follow-up is needed to assess long-term outcomes.

CO08

BENDAMUSTINE, BORTEZOMIB AND DEXAMETHASONE (BVD) EXERT A SUBSTANTIAL ACTIVITY WITH A MANAGEABLE TOXICITY IN PATIENTS WITH RELAPSED-REFRACTORY MULTIPLE MYELOMA

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Bendamustine, a bi-functional alkylating agent with a purine-like benzimidazole ring, has been shown activity in patients with Multiple Myeloma (MM), particularly when combined with other agents. Based on *in vitro* data showing that bortezomib enhances the sensitivity of MM cells to bendamustine, we evaluated the efficacy and toxicity of the combination bendamustine (70 mg/m^2 days 1, 8), bortezomib (1.3 mg/m^2 days 1, 4, 8, 11) and dexamethasone (20 mg days 1-2, 4-5, 8-9, 11-12) (BVD) administered every 28 days for 6 cycles and then every 56 days for further 6 cycles. Patients with relapsed/refractory MM and measurable disease were enrolled in this multicenter, phase II study provided they had no more than 4 prior lines of therapy and were not refractory to bortezomib. Response rate, according to IMWG criteria, was the primary end-point. From March 2011 to June 2012, 75 patients were included. Median age was 68 years (range 41-85), 26.5% had ISS stage 3, 20% IgA myeloma, 20% adverse cytogenetics and 9% renal failure. Median lines of prior therapies were 2 (1-4), 57% of patients had received thalidomide, 54.5% lenalidomide and 46.5% bortezomib. Moreover, 69% had been treated with alkylators and 44% had been undergone autologous stem cell transplant. After 4 cycles of BVD a response ≥ PR was documented in 71.5% of evaluable patients (n=70) (CR: 16%, VGPR: 18.5%, PR:37%) while 20% achieved SD and 8.5% had PD. Median time to response was 1.2 months (range 0.9-1.4). After a median follow-up of 12 months, median TTP and PFS were 16.5 and 15.5 months, respectively. OS was 78% at one-year. Grade 3-4 adverse events occurred in 55% of patients leading to therapy reduction in 20% and to protocol discontinuation in 10.5% of patients. The most frequent severe adverse events were thrombocytopenia (30.5%), neutropenia (18.5%), infections (12%), peripheral neuropathy (8%) and cardiac toxicity (3.5%). Compared with younger, patients aged > 70 years had a significantly higher incidence of grade 3-4 thrombocytopenia (22% *vs* 37%; p=0.042) and severe infections (7 *vs* 19%; p=0.047) and consequently a higher rate of therapy reduction (9% *vs* 34.5%; p=0.007) and therapy discontinuation (7% *vs* 15.5%; p=0.043). Moreover, 4/5 early deaths occurred in patients aged more than 70 years. BVD combination is a feasible and effective regimen in relapsed-refractory MM patients. However, adapted therapy and adequate antibiotic prophylaxis are warranted in older patient.

CO09

HIGH COPY NUMBER ALTERATION (CNA) LEVEL AND THE DEREGULATED EXPRESSION OF GENES INVOLVED IN CELL CYCLE REGULATION BOTH CHARACTERIZE NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS CARRYING AMPLIFIED MDM4 AND/OR DELETED TP53

Terragna C,[1] Martello M,[1]Pantani L,[1] Patriarca F,[2] Zamagni E,[1] Galli M,[3] Tacchetti P,[1] Petrucci MT,[4] Crippa C,[5] Bringhen S,[6] Brioli A,[1] Offidani M,[7] Zannetti BA,[1] Borsi E,[1] Dico F,[1] Testoni N,1 Marzocchi G,[1] Mancuso K,[1] Martinelli G,[1] Cavo M[1]

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The p53 pathway silencing might pass through changes in the expression level or activation of p53 itself, regulated by several specific inhibitors and/or activators. In MM, TP53 del on chr 17p13 represents one of the genomic aberration most significantly associated to poor outcome. One of the most potent inhibitor of p53 is MDM4, which is critical for control of p53 activity and is often amplified in several types of tumors, as well as in MM. Aim of the study was to investigate the frequency and the prognostic role of TP53 del and/or MDM4 amp in newly diagnosed MM pts treated with bortezomib-thalidomide-dexamethasone (VTD) as induction therapy before, and as consolidation after the double ASCT. The rationale relied upon the hypothesis that both of these chromosomal aberrations might contribute to impaired p53 function. Eighty-nine pts were analyzed at diagnosis by unpaired analysis of copy number alterations (CNA) (Affymetrix 6.0 SNP array), gene expression profile (GEP) (Affymetrix U133 Plus2.0 array) and Real-time PCR. The CNA analysis showed a 482 Kb minimal deleted region on chr17p13, including TP53, in 19/89 pts (10%) and a 1.1 Mb minimal amplified region on chr1q32.1 including MDM4 in 27/89 pts (30,3%). Pts were stratified into two subgroups according to the presence or absence of amp MDM4 and/or del TP53 (group A, 34 pts, or 38%; group B, 55 pts, or 62%). Baseline clinical characteristics were homogeneous, whereas groups A and B were clearly imbalanced with respect to the genomic background (165 *vs* 103 CNAs, p = 0.03). An overall deregulation of pathways related to cell cycle, DNA damage repair, cell adhesion and cytoskeleton remodeling was shown by GEP (627 differentially expressed probes-set, FDR<0.05). The rate of complete and/or near complete response after VTD induction therapy was 38% in group A and 20% in group B; the presence of TP53 del and/or MDM4 amp correlated with shortened median TTP (40.13m *vs* nr, p=0.0015) and OS (66.1m *vs* nr, p=0.0006). The poorer impact was retained also in the presence of MDM4 amp without TP53 del (TTP: 41m *vs* nr, p=0.01). In conclusion, a high number of CNAs and the deregulation of genes involved in cell cycle control was shown in pts carrying amp MDM4 and/or del TP53. This might account for the worse outcome of this group of pts and suggest that the p53 pathway involvement in MM might be wider than expected, possibly due to the activation negative regulators of p53. Supported by Ateneo RFO grants (M.C.) BolognAIL.

CO010

CONCLUSIVE ANALYSIS OF CLINICAL AND MOLECULAR RESULTS. FROM RV-PCL-PI-350 TRIAL, THE FIRST PROSPECTIVE STUDY OF A NOVEL AGENT (LENALIDOMIDE) IN PRIMARY PLASMA CELL LEUKEMIA

Musto P,[1] Neri A,[2] Simeon V,[1] Martorelli MC,[1] Todoerti K,[1] Mosca L,[2] Agnelli L,[2] Fabris S,[2] Lionetti M,[2] De Luca L,[1] Tassone P,[3] Mina R,[4] Petrucci MT,[5] Cascavilla N,[5] Di Raimondo F,[5] Caravita T,[5] Morabito F,[5] Offidani M,[5] Olivieri A,[5] Nobile F,[5] Filardi N,[5] Benevolo G,[5] Levi A,[5] Falcone A,[5] Cavalli M,[5] Pietrantuono G,[1] Villani O,[1] Guariglia R,[1] D'Arena G,[1] Mansueto G,[1] Bringhen S,[4] Omedè P,[4] Boccadoro M,[4] Palumbo A[4]

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We updated the first prospective study of initial treatment in primary plasma cell leukemia (PPCL), based on a combination of lenalidomide (25 mg/d for 21 days) and weekly oral dexamethasone (40 mg) for each 28-day cycle (Ld). The primary end-point was response rate after 4 cycles; secondary end-points were PFS, OS, safety, feasibility/efficacy of autologous stem cell transplantation (SCT) after Ld and molecular studies. Twenty-three newly diagnosed PPCL were enrolled and a total of 95 induction cycles were given (median 4, range 1-8). Treatment discontinuation was needed in 4 patients (pts) because of severe non-hematological adverse events. On ITT analysis, ORR was 74 %, with 39% of pts achieving at least very good partial response (VGPR). Of the 15 pts who received the 4 planned Ld cycles (per-protocol analysis, 65.2% of ITT population), 14 responded (ORR 93.3%; VGPR or better: 59.9%). Maintenance therapy with lenalidomide alone (10 mg/d), planned for pts not eligible for SCT, was given after 8 LD cycles in 5 responders (29 cycles: median 7, range 1-12). Twelve out of 15 eligible pts (80%) received SCT (9 responding after 4 Ld, 3 after salvage therapy). No patient failed to collect peripheral blood stem cells. After a median follow-up of 34 months, PFS and OS were respectively 14 and 28 months (Figure). PFS was 27 months in transplanted pts *vs* 2 months in those not receiving SCT frontline. In the same populations, OS was not reached *vs* 12 months, respectively. At multivariate analysis, SCT and response to Ld affected PFS, while OS was influenced only by SCT. Genomic abnormalities were identified in all cases tested by FISH (23) and high resolution mapping array (17), with prevalence of t(11;14) (40%) and t(14;16) (31%), and numerical alterations at 1p (38%), 1q (48%), 6q (29%), 8p (42%), 13q (74%), 14q (71%), 16q (53%), and 17p (35%). We also identified a biallelic deletion in 8p21.2 encompassing the PPP2R2A

gene, as well as TP53 (4/17) and BRAF (1/15) mutations. Finally, specific miRNA and gene expression signatures were found significantly associated with response and survival. In conclusion, Ld is a feasible first line regimen in PPCL, able to induce a high response rate and to improve PFS and OS in patients receiving consolidation with SCT. The extended genomic characterization of this prospective series revealed molecular lesions that could contribute to define the prognosis of single patients and to identify new potential therapeutic targets.

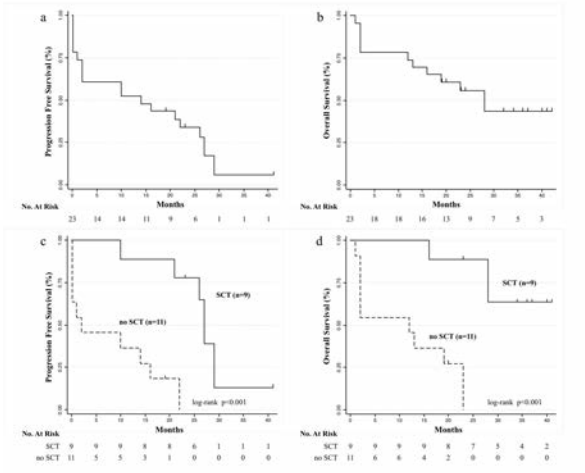

Figure 1.

CO011

PHASE II STUDY OF EFFICACY AND SAFETY OF THE COMBINATION CARFILZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (CCD) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS: PRELIMINARY RESULTS

Cerrato C,[1] Bringhen S,[1] Cavallo F,[1] Petrucci MT,[2] De Paoli L,[2] Siniscalchi A,[2] Genuardi M,[1] Villani O,[2] Offidani M,[2] Magarotto V,[1] Benevolo G,[2] Corradini P,[2] Ruggeri M,[1] Pantani L,[2] Conticello C,[2] Finsinger P,[2] Sonneveld P,[3] Boccadoro M,[1] Palumbo A[1]

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Background. The three-drug regimens VMP and MPT are now considered the standards of care for newly diagnosed elderly MM patients, reporting 41% of at least very good partial response rate (≥VGPR), but with a discontinuation rate due to adverse events (AEs) of 35%. Carfilzomib is a novel, highly selective proteasome inhibitor that was tested alone and in combination with other drugs first in relapsed/refractory MM patients; it is now being evaluated in newly diagnosed elderly (≥65 years) MM patients. Aims. In this study, efficacy and safety of the combination carfilzomib-cyclophosphamide-dexamethasone (CCd) in newly diagnosed elderly MM patients were evaluated. Methods Patients received oral cyclophosphamide (300 mg/m^2 on days 1,8,15), oral dexamethasone (40 mg on days 1,8,15,22) and iv carfilzomib (20 mg/m^2 infused over approximately 10 minutes on days 1,2, and 36 mg/m^2 over a 30 minutes period on days 8,9,15,16; cycle 1; 36 mg/m^2 on days 1,2,8,9,15,16; cycles 2-9) for 9 28-day cycles, followed by maintenance with iv carfilzomib (36 mg/m^2 on days 1,2,15,16) every 28 days until disease progression or toxicity. Results. Fifty-eight patients were enrolled: median age was 71 years, 40% had ISS stage III and 35% had unfavorable cytogenetics by FISH profile (4;14) or t(14;16) or del17p]. After a median follow-up of 8 months, 93% of patients achieved at least a partial response (≥PR), 68% ≥VGPR, 46% at least a complete response (≥CR/near-CR), including 12% stringent-CR. After 9 cycles of therapy, response rates improved to 100% ≥PR, 77% ≥VGPR, 53% ≥CR/nCR, including 23% stringent-CR. The 1-year progression free survival (PFS) was 85% and the 1-year overall survival (OS) was 86%. Grade 3-4 hematologic AEs included neutropenia (15%) and thrombocytopenia (5%). Grade 3-4 non-hematologic AEs were infections (7%), cardiac (4%), constitutional (4%), renal (4%) and gastrointestinal complications (2%). Six patients (11%) discontinued treatment due to AEs and 9 patients (16%) required carfilzomib dose reductions. No differences in response rate and safety between patients younger and older than 75 years were observed. Conclusions Carfilzomib-cyclophosphamide-dexamethasone for newly diagnosed elderly MM patients showed encouraging results in terms of efficacy; of note, the combination was well tolerated with a discontinuation rate due to AEs of 11%.

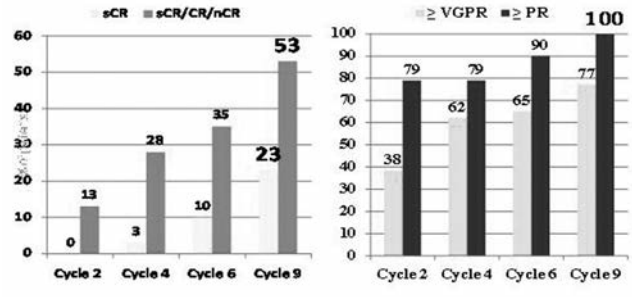

Figure 1. Response rates by treatment duration

CO012

PROGNOSTIC RELEVANCE OF 18-FDG PET/CT IN MULTIPLE MYELOMA PATIENTS TREATED WITH ALLOGENEIC TRANSPLANTATION

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[1]Clinica Ematologica, AOU Udine, Italy; [2]Istituto Seragnoli, Università di Bologna, Italy; [3]Clinica Ematologica, Istituto Tumori di Milano, Italy; [4]Clinica Ematologica, AOU Giovanni Battista di Torino, Italy; [5]Istituto di Medicina Nucleare, AOU di Udine, Italy; [6]Istituto di Statistica,Università di Udine Italy

F-18-FDG PET/CT has been reported to be useful for screening of myelomatous lesions in MM at diagnosis and for monitoring response to therapy and prognosis after auto-SCT. Aim of the study was to prospectively evaluate the prognostic significance of PET/CT in MM patients (pts) who received allo-SCT. Pts were studied with PET/CT before and within 6 months after allo-SCT. The number, the maximum SUV and the location (medullary or extramedullary) of focal lesions (FL) were recorded and PET/CT was considered positive if it showed at least 1 FL. The outcome of PET/CT positive and negative patients was compared in term of PFS and OS after allo- SCT. Multivariate analyses were performed to identify baseline and post treatment prognostic factors significantly affecting PFS and OS. A total of 67 pts, median age of 51 years were analyzed . At diagnosis 85% were in stage III and 69% of evaluable pts had unfavourable FISH karyotype. All pts received upfront auto-SCT,followed by allo-SCT within 3 months in 27 cases, while other 40 cases performed allo-SCT after failure of previous auto-SCT. Median time between diagnosis and allo-SCT was 36 months (8-128). Conditioning was at reduced intensity in 88%.Fifty-five per cent were transplanted from unrelated donors. Two-year PFS and OS were 45% and 69%.Before allo-SCT , 34/54 pts (63%) had a positive PET/CT and 6 pts (11%) had extramedullary disease (EMD). In univariate analysis positive PET/CT before allo-SCT significantly shortened PFS (HR 2.20; p=0.027),but did not influence OS. Moreover, EMD detected with PET/CT before allo-SCT significantly shortened both PFS and OS(HR 3.30;p=0.009 for PFS and HR 3.54; p=0.013 for OS). Six months after ASCT, PET/CT remained positive in 32 out 59 pts (54%).In univariate analysis persistence of positive PET-CT and EMD after allo-SCT were significantly associated with poor OS (HR 2.40; p=0.029 and HR 4.00; p=0.002, respectively)(Figures 1-2). In multivariate analysis only EMD after allo-SCT was significantly associated with poor PFS (HR 9.53; p=0.001), while the variables significantly associated with poor OS were allo-SCT at relapse (HR 4.06; p=0.001), persistence of EMD after allo-SCT (HR 6.42; p=0.001)and < VGPR after allo-SCT (HR 3.02; p=0.004). This study shows that the persistence of EMD by PET-CT after allo-SCT

was an independent prognostic factor for poor PFS and OS, suggesting that PET/CT is an useful tool for monitoring response after allo-SCT.

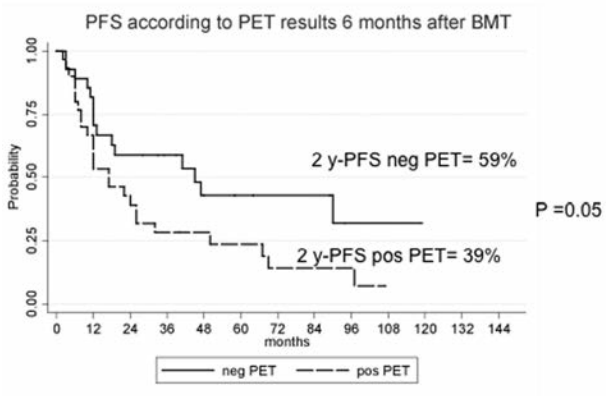

Figure 1.

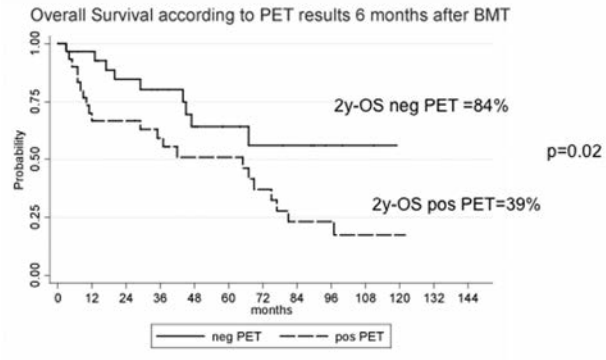

Figure 2.

Non-Hodgkin's Lymphoma I

CO013

THE ROLE OF INTERIM-PET AND FINAL-PET IN THE OUTCOME OF PERIPHERAL T-CELL LYMPHOMA TREATED AT THE DIAGNOSIS WITH CHOP

Pellegrini C, Broccoli A, Gandolfi L, Casadei B, Stefoni V, Derenzini E, Quirini F, Tschon M, Papadopulos F, Narducci R, Stefani G, Maglie R, Argnani L, Zinzani PL

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Role of interim- and final-PET in peripheral T-cell lymphoma (PTCL) is quite unknown. To determine predictive value of PET on overall survival (OS), we evaluated interim-PET (i-PET) and final-PET (f-PET) in PTCL patients treated in first-line with 6 CHOP-21 courses. From September 2003 to July 2010 we diagnosed and treated in our institution 34 advanced stage PTCL patients (15 females and 19 males). The median age at diagnosis was 46 years (range, 21-81 years); 9 patients were in stage III, and 25 in stage IV. According to the histologic subtype there were 11 PTCL-nos, 6 AILT, 9 ALCL Alk+, 6 ALCL Alk-, and 2 NK/T nasal type patients. Four patients had bulky disease; eight patients had bone marrow involvement, 15 patients had 1 extranodal involvement and 10 had more than 2 extranodal sites. All patients underwent initial staging PET/CT; i-PET was performed after 3 cycles of CHOP-21 and the median time from the end of third course to i-PET was 14 days (range, 7- 18 days). f-PET scans were performed 35 days (range, 30- 45 days) after the end of therapy. 19/27 i-PET negative patients had also a negative f-PET, whereas 8/27 had a positive final one; 6/7 i-PET positive patients had also a positive f-PET, whereas only 1 patients had a positive f-PET. With a median follow-up of 71 months (range, 5.8-120.9 months), 17/19 (89.5%) patients with i-PET negative are in continuous CR (CCR) and only 1/7 (14.2%) patient with i-PET positive is still in CCR. Estimated OS plotted according to i-PET results showed 78.6% for negative patients and 21.4% for positive patients at 88.7 months (p=0.02); estimated OS plotted according to f-PET results reported 93.7% for negative patients and 21.4% for positive patients (p<0.0001). In conclusion, our results demonstrate that positive i-PET is predictive of a worse outcome in PTCL and this significant statistical difference between the two curves could be clinical informative. The f-PET also seems to represent a significant step forward in the prediction of survival for these patients. Larger and prospective studies and harmonization of PET reading criteria are needed.

CO014

THE LYMPHOCYTES TO MONOCYTES RATIO IDENTIFIES A PATIENT SUBGROUP AMONG HIGH RISK DLBCL THAT MAY BENEFIT FROM UPFRONT INTENSIVE TREATMENT WITH AUTOGRAFT

Barbui AM,[1] Masciulli A,[2] Scarano M,[2] Delaini F,[1] Rossi A,[1] Ladetto M,[3] Corradini P,[4] Di Nicola M,[5] Patty C,[6] Mulè A,[6] Zanni M,[3] Zoli V,[7] Billio A,[8] Gallamini A,[9] Di Raimondo F,[10] Ferreri AJM,[11] Benedetti F,[12] Leoni P,[13] Semenzato G,[14] Frezzato M,[15] Flenghi L,[16] Marchioli R,[2] Barbui T,[1] Gianni AM,[5] Tarella C,[17] Cortelazzo S,[8] Rambaldi A[1]

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Background. At diagnosis, a peripheral blood lymphocytes to monocytes ratio (LMR) lower than 2.6, identifies a group of DLBCL patients

with a poor prognosis when treated with R-CHOP (Rambaldi *et al*, ASH 2012). No data are available for patients treated upfront with Rituximab containing high dose sequential chemotherapy programs (R-HDS) and autologous stem cell transplantation (AST) Aims. To investigate whether LMR ratio may identify a high-risk patient subgroup that benefits from a primary high-dose program with ASCT Methods. We analysed LMR ratio at diagnosis in a series of DLBCL patients enrolled into a trial comparing R-CHOP 14 with R-HDS associated with AST (R-HDS 0305, Clinical Trials.gov.number NCT00355199 by GITIL). Patients characteristics: DLBCL without CNS involvement, with an age between 18–60 years and an High IPI (stage > II B-bulk with ECOG-PS=0-3 and age adjusted IPI (aaIPI) 2–3 or age 61–65 years with ECOG-PS = 0–2 and IPI > 3). R-CHOP 14 (8 cycles) or R-HDS regimen and AST were carried out as previously reported(Tarella C *et al*, Leukemia 2007) Results. LMR data were collected in 216 evaluable DLBCL patients enrolled into this trial. We identified two groups of patients according to baseline LMR: 144 patients (67%) had a low LMR (<2.6), while 72 patients (33%) had a high (>2.6) LMR. The two groups were comparable for age, gender, stage, ECOG, extranodal sites, bone marrow infiltration while high LDH level was associated with a low LMR (p= 0.009). In multivariate analysis OS and EFS corrected by age, gender, stage, ECOG, LDH, extranodal sites, BM involvement and treatment arm, resulted significantly improved by RHDS and AST only in the low LMR group with an hazard ratio (HR) of 0.41 (95% IC 0.2-0.81), p=0.011. In the same patient population with a low LMR, a high ECOG was associated with a two times higher risk of events (HR 2.55, 95% IC 1.26-5.16, p=0.009). After a median observation of 35.4 months (0.3–89.2), the OS and EFS of patients treated by RCHOP 14 or R-HDS and AST were 71% versus 86% (p=0.022) and 63% versus 83% (p=0.008) respectively. Conclusion: The analysis performed among high-risk DLBCL patients enrolled in a prospective, randomized study, confirms the negative impact of a low LMR at diagnosis in patients treated with R-CHOP. R-HDS and AST improved OS and EFS and overcome the prognostic value of LMR.

CO015

ANEMIA IN LYMPHOMAS: THE IL-6-HEPCIDIN AXIS IN HODGKIN AND DIFFUSE LARGE B CELL NON-HODGKIN LYMPHOMA

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[1]Institute of Hematology; [2]Department of Laboratory Medicine (Clinical Chemistry/441), Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Anemia is a presenting symptom in approximately 40% of patients with Hodgkin's lymphoma (HL) and about 30% of patients with diffuse large-B cell lymphoma (DLBCL), and is associated with unfavourable patients' characteristics. Mechanisms that contribute to development of anemia may differ according to lymphoma type. We were interested to compare the role of the IL-6-hepcidin axis to the pathogenesis of anemia in HL and DLBCL. We studied 118 patients with lymphoma (65 HL, 53 DLBCL; median age 47 years; 64 females, 54 males), diagnosed at the Institute of Hematology of the UCSC. Plasma samples were analyzed for hepcidin levels using a combination of weak cation exchange chromatography and time-of-flight mass spectrometry (TOF MS). At diagnosis, anemia with Hb <12 g/dL was present in 30 of 65 (46%) of patients with HL, and 30 of 53 (57%) of patients with DLBCL. Hepcidin plasma levels were significantly higher in patients compared to controls in both HL and DLBCL (p=0.001, p=0.006, respectively), and hepcidin levels were higher in patients with more aggressive disease characteristics as advanced stage disease (P=.03), B symptoms (P=.0005), elevated LDH levels (p=0.01), IPS score≥3 (P=.005) in HL, and age-adjusted IPI score > 1 (p=0.01) in DLBCL, and in males (p=0.002). Hepcidin levels showed a weak, but significant inverse correlation with hemoglobin levels in anemic patients (r=-0.29, p=0.02), while there was no correlation in non-anemic patients. Analysing separately HL and DLBCL, the association between hepcidin and haemoglobin levels in anemic patients was only observed in HL, but not in DLBCL arguing for a stronger impact of elevated hepcidin on development of anemia in HL. Hepcidin levels strongly correlated to ferritin (r=0.7, p=<0.0001) and inversely correlated to iron-binding capacity (r=-0.41, p=0.001) and iron (r=-0.23, p=0.04). Hepcidin levels were significantly correlated with IL-6 (r=0.5; p<.0001). IL-6 inversely correlated to haemoglobin values (r=-0.42, p<0.0001). In a multivariate logistic regression analysis including IL-6 and hepcidin, and correcting for age, gender, lymphoma type and bone marrow infiltration, IL-6 levels were the only factor associated to anemia (p=0.008). Our findings suggest that the IL-6-hepcidin axis is active in both HL and DLBCL, resulting into iron-restriction, but that hepcidin-independent mechanisms play the major role in the IL-6 induced development of anemia, in particular in patients with DLBCL.

CO016

BRIEF CHEMOIMMUNOTHERAPY RITUXIMAB, BENDAMUSTINE, MITOXANTRONE (R-BM) FOLLOWED BY RITUXIMAB CONSOLIDATION IN ELDERLY PATIENTS WITH UNTREATED ADVANCED STAGE FOLLICULAR LYMPHOMA (FL): RESULTS OF A PROSPECTIVE PHASE II STUDY BY FONDAZIONE ITALIANA LINFOMI (FIL)

Boccomini C, Ladetto M, Rigacci L, Arcaini L, Evangelista A, Lobetti-Bodoni C, Volpetti S, Chiappella A, Chiarenza A, Freilone R, Corradini P, Franceschetti S, Rusconi C, Stelitano C, Nicolosi M, Puccini B, Bolis S, Zaccaria A, Liberati AM, Tucci A, Baldini L, Pulsoni A, Balzarotti M, Vitolo U

SC Ematologia, Città della Salute e della Scienza di Torino, Italy, on the behalf of Fondazione Italiana Linfomi (FIL)

Introduction. To investigate safety and efficacy of a brief R-BM regimen for treatment of elderly FL patients (pts). Patients and methods: 76 pts (age 65-80) were enrolled (Sept 2009-Nov 2011). Inclusion criteria were: advanced or stage II disease requiring treatment; "FIT" pts according to comprehensive geriatric assessment. Treatment plan was: 4 monthly courses of R-BM (375 mg/sqm Rituximab day 1, 90 mg/sqm Bendamustine days 1-2, 8 mg/sqm Mitoxantrone day 1) followed by 4 weekly Rituximab consolidation. Polymerase chain reaction (PCR) for BCL2/IgH rearrangement was performed on bone marrow samples at diagnosis and during treatment. Results. Median age was 71 (range 65-79); 29 males; according to stage: 15% II, 30% III and 55% IV; 47% had BM involvement, 20% B symptoms and 21% had ≥2 comorbidities; FLIPI score were: 8% low, 32% intermediate and 60% high risk. PCR analysis for BCL2/IgH rearrangement was carried out in 57 pts at diagnosis: 39 were Bcl-2 positive. Seventy (92%) pts completed the planned treatment (67/70 in the planned time) while six pts did not because of: 1 progressive disease, 4 adverse events (2 haematological toxicities with prolonged neutropenia; 1 CMV colitis and 1 for infection and concomitant worsening of pre-existing oral pemfigo) and 1 worsening of performance status. Overall response to treatment was 92%: 76% complete remission (CR), 16% partial remission (PR) and 8% stable (SD) or progressive disease; 29 (71%) of the 41 pts in PR/SD after R-BM converted to CR following further Rituximab consolidation. At a median follow-up of 19 months, 2-yrs PFS was 80%. Twenty-three (60%) of 39 Bcl-2 rearranged pts at diagnosis were evaluable after treatment: PCR negativity was achieved in 22/23 (96%) pts at the end of treatment and 18 (82%) were also in CR. A total of 577 courses were given: the most frequent CTC grade 3-4 toxicity was neutropenia, in 18% of the courses. Extra-haematological toxicities, all resolved, were: 8 neutropenic fevers, 8 grade 3-infections (4 due to bacterial agents), 1 NSTEMI, 1 massive pulmonary embolism. Two deaths were recorded: one pneumonia with worsening of pre-existing pemfigo and one patient with hepatic metastasis of occult carcinoma diagnosed at final restaging after completion of therapy. Conclusions: A brief course of chemo-immunotherapy R-BM followed by Rituximab consolidation is safe and effective with a high clinical and molecular remission rate in elderly patients with untreated advanced FL.

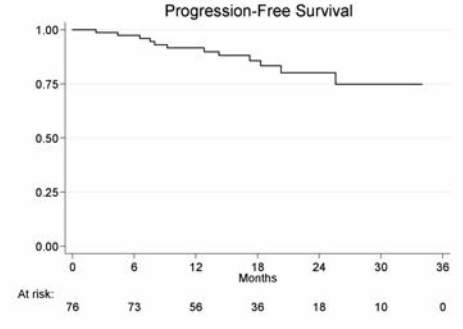

Figure 1.

CO017

FLUDARABINE-MITOXANTRONE-RITUXIMAB REGIMEN IN UNTREATED INTERMEDIATE/HIGH-RISK FOLLICULAR NON-HODGKIN'S LYMPHOMA: EXPERIENCE ON 142 PATIENTS

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There is no international consensus on optimal frontline chemotherapy regimen for advanced stage follicular lymphoma patients, or a clear definition of cure for this disease. Aim of this study was to test the degree of effectiveness and the safety of the regimen containing fludarabine, mitoxantrone and rituximab (FMR) in a subset of poor prognosis follicular lymphoma patients with particular focus on the long-term disease free survival. An observational retrospective study was conducted on 142 intermediate/high-risk follicular lymphoma patients treated in first-line with 6-cycles FMR regimen. From September 2000 to March 2010 in our institution, patients aged 18 years or older with biopsy-proven, bidimensionally measurable, stage III or IV untreated indolent follicular lymphoma expressing the CD20 antigen underwent FMR regimen. The prognostic value of PET was also investigated in a 56-patients subset. Overall response rate was 95.5% with 88% of complete responses: 18% of patients had disease relapse, yielding an estimated 12-year disease-free survival of 72% (median follow up 48 months). All cases showed the lymphoma recurrence within 40 months: after this timing the disease-free survival curve presented a plateau. Overall survival was 73% at 12 years. Post-treatment PET positivity remained a highly significant predictor of disease progression with a 5-year progression-free survival rate of 42% in PET-positive versus 75.5% in PET-negative patients (P= 0.0024). The FMR regimen was globally well tolerated and reversible haematological toxicities were the most common adverse events. Six patients developed secondary malignancies; in particular, only 1 (0.7%) of them was an haematological neoplasia after 8 months from end of treatment. The observed high rate of complete responses following the use of FMR regimen in intermediate/high-risk patients seems to be the first step to improve disease-free survival. Our study could be the starting point to consider disease-free survival as a potential alternative endpoint of future clinical trial on follicular lymphoma patients.

CO018

FREQUENCY AND CLINICAL OUTCOME OF PRIMARY REFRACTORY DISEASE IN NON-HODGKIN'S LYMPHOMA: A RETROSPECTIVE SURVEY ON 3,492 NEWLY DIAGNOSED PATIENTS UNDERGOING FIRST-LINE CHEMOTHERAPY

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Introduction. Non-Hodgkin's lymphoma (NHL) is a malignancy usually sensitive to chemotherapy. However, a variable group of patients shows refractory disease, *i.e.* poor or absent response to induction therapy. The present study was undertaken to define on a large series of NHL: i. the overall rate of refractory patients; ii. the main clinical factors associated with refractory disease; iii. the long-term outcome of refractory *vs* responsive NHL. Patients and Methods. Data have been collected on 3,492 patients, referred at the University Hematology of Torino (S. Giovanni B. and Mauriziano Hospitals) (865 cases) and the Hematology Division of Bergamo (2,627 cases), between 1984 and 2012. There were 46% female patients, 53% were aged < 60 yrs, B-cell NHL were 92%; main histological subtypes were Diffuse Large Cell (50.5%) and Follicular (18.8%) Lymphoma. There were 64.5% patients with advanced-stage disease, 31% had an intermediate-high IPI score. Overall, 42% received conventional therapy supplemented with rituximab. The criteria to identify primary refractory NHL were: stable or progressive disease (fully refractory) or transient response with disease progression within 6 months (early relapse). Results. Among 3.175 patients analysed for their primary response, 699 (22%) were refractory (12% fully refractory, 10% early relapse). The overall incidence of refractory NHL was similar in Torino and Bergamo Centers. The rate of refractoriness was 41.8% in the small T-cell subgroup. Besides T-cell histology, the following factors had the highest association with treatment response: i. intermediate-high IPI score (32.3% refractory patients); ii. female gender, with a markedly lower incidence (19.1%); iii. rituximab addition, that cut the incidence of refractoriness to 13.6% *vs* 28.6% for patients treated without rituximab. At present, 2,029 (58.1%) patients are alive, the overall survival (OS) was significantly poorer for fully refractory (median survival: 0.8 yrs) compared to early relapse patients (2.01 yrs) (p<0.001); both these subgroups had a poorer OS compared to responsive patients (median survival: 18.9 yrs) (see Figure 1). Conclusions: i. overall, 22% of NHL patients displayed primary refractory disease; ii. the introduction of rituximab has markedly reduced the risk of refractory disease; iii. a high rate of refractory disease is observed with T-subtypes; iv. patients responsive to first-line therapy have a very prolonged life expectanc

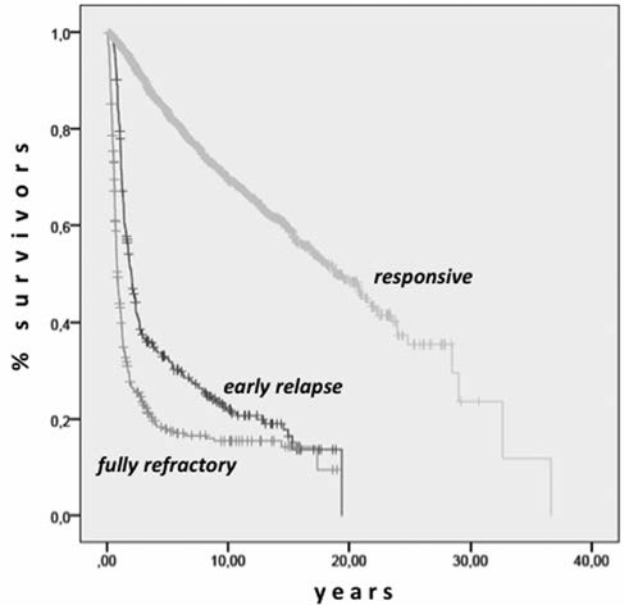

Figure 1. Overall survival of 2,476 responsive, 371 fully refractory and 304 early relapse NHL patients following first-line therapy

Anemias and Hemoglobinopathies

CO019

TRANSCRANIAL COLOR DOPPLER ULTRASONOGRAPHY IN ADULT PATIENTS WITH SICKLE CELL DISEASE

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Background. Sickle Cell Disease (SCD) is one of the most common severe monogenic inherited disorder worldwide; during the last years, the prevalence of the disease in Italy is increased due to the immigration of patients from endemic areas. Clinical manifestations are consequent to ischemic events due to haemolysis and vaso-occlusion caused by haemoglobin (Hb) polymerization. One of the most important and serious complication of the disease is stroke. The risk of stroke in patients with SCD is much higher than in the general population, with incidence of 11% by 16 years old and of 24% by 45 years old. In the Stroke Prevention Trial in Sickle Cell Anemia (STOP Trial) validity of the TransCranial Doppler and threshold velocity ≥200 cm/sec were demonstrated to be useful to identify paediatric patients with SCD at risk of stroke, however little is known about the adult patients. Aim. to standardize the Doppler parameters in adult patients with SCD, using Transcranial Color Doppler (TCCD) with the correction of the angle of insonation. Material and Methods. we enrolled 52 outpatients followed at Tertiary Centre for Hereditary Anaemia in Milan, affected by SCD (15 with Sickle Cell Anaemia, SCA; 26 Sickle -Thalassemia, HbS-Thal and 11 haemoglobinopathy HbS/HbC, SC disease), over the age of 16 years and 25 control subjects, matched for sex, ethnicity and age. We evaluated blood cell count, Hb fractions and TCCD parameters (systolic and end diastolic peak, resistance and pulsatility index in the middle (MCA), anterior (ACA), posterior (PCA) cerebral arteries, carotid siphon (SIPH), vertebral and basilar arteries) in both patients and controls. Results. mean Hb values of the overall patients and controls were 9.9±1.5 and 14.8±1 g/dl ($p<0.01$); in SCA, HbS-Thal and SC were respectively 9.1±1, 9.5±1.4 and 11.9±1 g/dl. In all controls TCCD was normal. Adults with SCD had a higher peak-systolic velocity (120.1±19.16 cm/sec) compared to controls (109.2 cm/sec ±14.7; $p<0.05$), particularly SCA patients (126.32±15.18 cm/sec; $p<0.01$), according with lower Hb values, higher HbS and worse clinical features. The statistically significant difference in peak-systolic velocity was confirmed in MCA, ACA, ICA, PCA and SIPH between the patient group and the control group ($p<0.05$). Conclusion: peak-systolic velocities in adults are lower than those observed in children, confirming that the speeds disclose an age-related decline, however are higher than in healthy controls.

CO020

IRON COMPARTIMENTALISATION IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA (PNH) PATIENTS DURING ANTI-C5 TREATMENT

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Paroxysmal Nocturnal Haemoglobinuria (PNH) is known to lead to iron deficiency because of iron urinary loss secondary to intravascular hemolysis. Eculizumab has been shown effective in the control of intravascular hemolysis; however, its long-term effects on iron homeostasis have not been elucidated yet. We investigated iron compartmentalisation in patients with hemolytic PNH, looking for changes occurring during eculizumab treatment, possibly requiring specific therapeutic interventions. We studied iron distribution in 20 hemolytic PNH patients: two untreated, 14 on eculizumab and 4 analysed before and during eculizumab treatment. Biochemical testing including iron parameters and markers of intravascular hemolisis were combined with magnetic resonance imaging to assess calculated iron content in kidney, liver, spleen and heart. Patients free from eculizumab had overt intravascular haemolysis with normal/low serum ferritin levels, and showed an homogeneous pattern of iron compartmentalization with renal cortex siderosis and absence of hepatosplenic iron deposition. Patients on eculizumab (median follow up 50 months) tended to normalize their renal siderosis, with the exception of those experiencing residual intravascular hemolysis. Most patients increased their iron content in the liver and in the spleen, eventually developing objective iron overload, as demonstrated by increased ferritin levels and transferrin saturation. Moderate to severe iron overload was usually associated with residual blood transfusions, or with residual anemia due to extravascular hemolysis. Two transfusion-dependent patients received iron chelation, whereas in the majority of cases iron overload was considered subclinical and iron chelation was not started. This conservative approach is supported by long-term re-assessment, which demonstrated that iron content may slightly increase over time in the liver without any clinical consequence, and in any case did not affect the heart. We demonstrate that iron homeostasis undergo to dramatic changes during effective anti-complement treatment. Indeed, on eculizumab, the inhibition of intravascular hemolysis prevents iron deficiency typical of PNH. Furthermore, many patients become prone to develop iron overload, especially in presence of residual transfusional needs, or of remarkable extravascular hemolysis. Whether this overload requires regular iron chelation is still unclear, but it suggests that iron homeostasis should be assessed in all PNH patients on eculizumab.

CO021

AUTOIMMUNE HEMOLYTIC ANEMIA: CLINICAL SEVERITY AND LABORATORY HETEROGENEITY IN 157 PATIENTS

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Autoimmune hemolytic anemia (AIHA) is a greatly heterogeneous disease classically divided in warm (WAIHA), cold (CHD), and mixed forms, based on the thermal and isotype characteristics of the anti-RBC antibody (IgG, IgM or both, respectively); in addition, atypical forms include DAT (direct antiglobulin test)-negative, IgA-positive, and warm IgM cases, which are often difficult to diagnose with consequent delay in treatment. We retrospectively evaluated 157 AIHA patients followed for a median of 26 months (range 6-271) from 1978 until September 2012, to investigate the prevalence of the different forms of AIHA, the severity of anemia, the number of therapy lines required, and the corresponding responses. Results showed that 40% of cases were WAIHA, 32% CHD, 19% mixed forms and 9% atypical (12 DAT negative and 1 DAT positive for IgA only). Considering the severity of anemia at onset 33% of cases had Hb levels<6 g/dL, 34% Hb 6-8 g/dL, 18% Hb 8-10 g/dL, and 15% Hb>10 g/dL; interestingly, reticulocytopenia (<100.000 mmc) was more frequently observed in cases with severe onset (14/52, 27%); the more severe AIHAs were mainly mixed (18/30, 60% $p=0.001$) and atypical (6/13, 46%) forms, whereas only a small fraction of CHD was characterized by a severe onset (8/51, 16% $p=0.002$). As regard therapy, 45% of cases were treated with I line steroid therapy only (mostly WAIHA), 23% with 2 lines, 10% with 3, and 6% with 4 or more lines, including splenectomy (20 cases, mostly mixed forms, $p=0.001$), cytotoxic drugs (23 patients), or rituximab (33 cases, mostly mixed and atypical forms, $p=0.009$); 16% of cases have never been treated, mainly CHD. Transfusions were performed in 65 cases, plasma-exchange in 3 (all with Hb<6 g/dL), and erythropoietin administered in 6 cases. Of note, the presence of an Hb value lower than 6 g/dL at onset was a risk factor for the requirement of 3 or more lines of therapy (odds ratio 3.148, CI 95% 1.312-7.552). Response rates to steroid therapy were similar in warm, cold, mixed and atypical AIHAs (on average 70%). Responses to rituximab were similar in cold and other AIHA forms (70-80%). Splenectomy, was ineffective in the 2 CHD who underwent surgery, whereas response rates were 63% in WAIHA and 80% in mixed and atypical cases. In conclusion, AIHAs showed a marked clinical heterogeneity, 1/3 of cases with a severe onset. These cases are frequently mixed or atypical forms and refractory to different therapies.

CO022

ANTI-COMPLEMENT TREATMENT FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH): UNMET CLINICAL NEED WITH CURRENT THERAPIES AND EFFICACY OF NOVEL C3-TARGETED INHIBITORS *IN VITRO*

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PNH erythrocytes suffer from uncontrolled complement activation and subsequent lysis because of the lack of the complement regulators CD55 and CD59. The anti-C5 eculizumab has proven effective in controlling intravascular hemolysis *in vivo*, but persistent upstream C3 activation may limit hematological benefit because of C3-mediated extravascular hemolysis. Thus, there is a substantial unmet clinical need, which could be satisfied by upstream inhibition at the level of C3. Here we describe the preclinical development of two novel classes of C3 inhibitors. Candidate agents were tested in an *in vitro* model which assesses hemolysis of and C3 opsonization on PNH erythrocytes; furthermore, systemic administration in non-human primates was used to investigate pharmacokinetic (PK) and pharmacodynamic (PD) properties. Mini-factor H (FH) is an engineered 43kDa protein that combines an increased affinity for the opsonins C3b, iC3b and C3d with the regulatory activities of FH, resulting in a potent and selective inhibition of the complement alternative pathway. Cp40 and its pegylated derivative PEG-Cp40 are analogs of the peptidic inhibitor compstatin, which binds to C3 and its activated fragment C3b, preventing the initiation, amplification and terminal damage of the complement cascade via all its major pathways. Mini-FH showed a dose-dependent inhibition of hemolysis, with full inhibition at 0.1 μM. CP40 and PEG-Cp40 also demonstrated a dose-dependent inhibition of hemolysis, with full inhibition at 6 μM. All these compounds completely abrogated complement activation, thus preventing surface deposition of C3 fragments on PNH erythrocytes. When injected to non-human primates, either intravenously or subcutaneously, Cp40 and PEG-Cp40 resulted in complete inhibition of plasma complement activity, without any adverse event. Even if pegylation increased plasma half-life of Cp40, single subcutaneous injection of Cp40 sustained complement inhibition for 48 hours, and currently represents the preferable alternative for further development in humans. We demonstrate that a C3-targeted complement inhibition (either with large FH-derived proteins or with small peptidic inhibitors) efficiently prevents hemolysis and C3-opsonization of PNH erythrocytes *in vitro*, likely preventing both intravascular and C3-mediated extravascular hemolysis *in vivo*. Preliminary PK and PD data in baboons confirm the feasibility and safety of this approach, strongly supporting future studies in humans.

CO023

ANAEMIA IN PATIENTS UNDERGOING ELECTIVE SURGERY: WHY TACKLING THIS ISSUE?

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It is well known that anaemia in elective surgical patients is predictive of the number of allogenic red blood cells (RBCs) transfusion requirement and more interestingly it is implicated in postoperative outcomes. Anaemia is known to be a significant risk factor for morbidity, mortality and hospital length stay in patients undergoing elective surgery. Moreover, allogenic RBCs transfusion has been reported to be an independent and addictive risk factor for adverse events like morbidity and mortality for all causes. To our knowledge conclusive date on prevalence of pre-operatory anemia are not known; it ranges from 5% to 75% according to different reports. The most frequent causes of anaemia in these patients are nutritious deficiency of iron and vitamins, concomitant chronic inflammatory conditions and renal impairment. The aim of our study was to evaluate the prevalence of anemia in a large cohort of patients undergoing elective surgery. From June 2011 to December 2012, an observational study on elective surgical patients in San Paolo Hospital has been driven. 4267 patients have been enrolled, 2483 men (58.2%), with a median age of 60 years (range, 9-94) and 1784 women (41.8%), with a median age of 54 years (range, 15-97). The 74.7% of patients were undergoing major surgery, which has a potentially higher risk of RBCs transfusion. Anaemia has been defined according to Beutler's criteria. The overall prevalence of anaemia was 20.6% (n=881); in details, the 21.5% (n=533) of men and the 19.5% of women (n=348) were anemic. The majority of patients (55.3%) showed a normocytic anaemia, which was more frequent among women (n=200, 57.5%; median Hb=11.7 g/dL, range 9-12.1) than in men (n=287, 53.8%, median Hb=12.8 g/dL, range 8.5-13.6). The elderly population (more than 60 years) was the 48% (n=2052) of the study population. The 61.5% of these were men, and 38.5% were women. The prevalence of anaemia was 30.1% (n=618) in the overall elderly population, 36% in the male group (n=454) and 20.5% in the female one (n=164). (Table 1) In conclusion, the prevalence of anaemia in our population of patients undergoing elective surgery was high and increased in the elderly and more fragile population. These results may have important consequences, since simple interventions before surgery (iron, vitamin and/or erythropoietin supplementation) may reduce this prevalence and allogenic RBCs transfusion improving postoperative outcomes.

Table 1.

ANEMIC POPULATION (n=881)	MEN	WOMEN
n	533 (21.5%)	348 (19.5%)
Median age [range]	60.5 [14-96]	56 (14-96
Median Hb g/dL [range]	12.6 [7.9-13.6]	11.5 [7.9-12.1]
n, MCV<80 fL	89 (16.7%)	103 (29.6%)
n, MCV normo	287 (53.8%)	200 (57.5%)
n, MCV>94 fL	157 (29.5%)	45 (12.9%)
Elderly anemic population (n=618)		
n	454 (36%)	164 (20.5%)
Median age [range]	73 [60-94]	74 [60-96]
Median Hb g/dL [range]	12.7 [8.4-13.6]	11.6 [7.9-12.1]
n, MCV<80 fL	58 (12.8%)	35 (21.1%)
n, MCV normo	250 (55.1%)	93 (56.7%)
n, MCV>94 fL	146 (32.1%)	36 (21.2%)

CO024

LONG-TERM HEALTH-RELATED QUALITY OF LIFE IN PATIENTS TRANSPLANTED FOR BETA-THALASSEMIA COMPARED TO GENERAL POPULATION NORMS

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The main objective of this study was to investigate whether long-term surviving patients transplanted for beta-thalassemia major have a different health-related quality-of-life (HRQoL) compared to healthy individuals of the general population. A total of 109 ex-thalassemia patients who underwent hematopoietic stem cell transplantation (HSCT) in the 1980s and 90s were enrolled in the study. The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) and the Functional Assessment of Cancer Therapy–Bone Marrow Transplant (FACT-BMT) scale, were administered to assess generic HRQoL profiles. Comparisons were adjusted for possible key confounders of HRQoL. Further adjustment was performed for patient age at HSCT and the presence or absence of graft versus host disease (GvHD). Socio

demographic and clinical variables were also analyzed. The median age of our cohort was 34 years, (range 21-48). Median age at HSCT was 12 years (range 1-36) with a median follow up of 22.8 years (range 11.7-30.3). The results showed that HRQoL profiles of transplanted patients were very similar to those reported by the general population. Clinically meaningful differences were only observed for the general health (GH) scale (-8.9; 95% CI, -15.0 to -2.7, p=0.005). Patients who developed acute or chronic GvHD as well as patients transplanted above the age of 15 years had significantly worse outcomes. Conversely, HRQoL of patients without GvHD was similar to that of the control population, with even better scores for mental health (5.3; 95% CI, 1.6 to 9; p=0.006). Also patients with comorbidities (p=0.0031) or living alone (p=0.0263) reported worse HRQoL outcomes. Education level, employment status, marital status, living arrangements and birth rate yielded results compatible with normal living patterns. In many areas, the long-term HRQoL of patients transplanted for thalassemia was comparable with general population norms, thus indicating a return to normal life style. Nevertheless, GvHD remains an impairing factor and, whenever possible, HSCT should be performed in pediatric age.

Allogeneic Transplantation

C0025

IN VIVO T CELL DEPLETION WITH ANTITHYMOCYTE GLOBULIN OR ALEMTUZUMAB FOR UNRELATED DONOR STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING: RESULTS OF A MULTICENTER RANDOMIZED PHASE II CLINICAL TRIAL (THE GLOBAL STUDY) FROM THE GRUPPO ITALIANO TRAPIANTO DI MIDOLLO OSSEO (GITMO)

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Background and Aim. We recently reported the clinical outcome of high risk patients undergoing allogeneic stem cell transplantation (HSCT) from unrelated donors (UD) after a reduced intensity conditioning (RIC) based on Antithymocyte Globulin (ATG) or Alemtuzumab (Alem) *in vivo* T cell depletion for GVHD prophylaxis (Rambaldi *et al.*: Leukemia 2012). Since both these programs proved to be well tolerated and reasonably effective we compared them in a prospective randomized Phase II clinical trial. Study design and Patients From March 2005 to September 2008, 245 patients were registered into this study at time of UD search activation. All of them were unfit for conventional transplants due to age or advanced disease. 112 patients underwent HSCT (median age 58 years, range 17-67) and were randomized to program A, (Melphalan 30 mg/m^2, Fludarabine 90 mg/m^2, TBI 200cGy and Alem 80 mg) (n= 58) or program B, (Thiotepa 10 mg/Kg, Cyclophosphamide 100 mg/Kg, Melphalan 30 mg/m^2 and ATG 7,5 mg/Kg) (n= 54). Results. The median time of neutrophil and platelet engraftment was similar in the 2 study arms (18 *vs* 16 days and 18 *vs* 18 days, respectively) but graft failure and graft rejection occurred most likely in arm A (9 *vs* 1, p 0.036). At day +100 the incidence of acute GVHD (> grade II) was slightly higher in the ATG arm (37% *vs* 26%, p 0.224) with a higher incidence of late occurring acute GVHD in the Alem arm, most likely because of DLI. The incidence of chronic GVHD was similar (40% and 41%). The immunologic reconstitution was slower in arm A but no significant difference was observed in the incidence of infections. With a median follow up of 18 months (range 1-39), at 2 years, the non-relapse mortality (36% *vs* 35%) and the relapse rate (34% *vs* 25%, p 0.294) do not differ in the 2 arms. Similarly, no significant difference was observed in terms of event free survival (29% *vs* 41%, p 0.295) and overall survival (OS) (28% *vs* 46%, p 0,206) (Figure 1). A significant decrease of the risk of death was associated with the incidence of chronic GVHD (p<0.001). Conclusion. A better engraftment and immune reconstitution as well as a trend for a lower relapse rate was observed in patients receiving an ATG based RIC. Chronic GVHD confirms its protective role on the risk of death confirming that an accurate dosing of *in vivo* T cell depleting agents is crucial for the long term clinical outcome after HSCT.

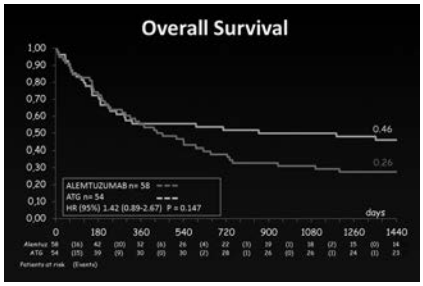

Figure 1.

CO026

UNMANIPULATED HAPLOIDENTICAL TRANSPLANTS COMPARED WITH OTHER ALTERNATIVE DONORS AND MATCHED SIBLING GRAFTS: A SINGLE CENTER EXPERIENCE IN 488 PATIENTS

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Background. There are currently at least 4 possible donor types for an allogeneic transplant: HLA identical siblings (SIBS), unrelated donors (UD), unrelated cord blood (UCB) and HLA haploidentical family donors (HAPLO). Aim of the study. The aim of the present study was to compare disease free survival (DFS) of HAPLO transplants, with SIBS, UD and UCB grafts, in a single Center. Patients. We therefore compared 92 HAPLO, with 176 SIBS, 291 UD and 105 UCB grafts. All 488 patients had hematologic malignancies, receiving a first allogeneic transplant (SCT) in a single Center, between jan-2006 and feb-2012. Patients were stratified for disease phase as early (first or second remission) (n=265) or advanced (beyond second remission) (n=223). The diagnosis was acute leukemia (n=275), lymphoma (n=79), myelofibrosis (n=51), myelodisplastic syndrome (n=94), other hematologic malignancies (n=19). The conditioning regimen was classified as myeloablative (MAC) (n=337) or reduced intensity (RIC) (n=151). Graft *vs* host disease (GvHD) prophylaxis was as follows: HAPLO transplants received unmanipulated marrow and post transplant cyclophosphamide (PT-CY) with cyclosporine (CsA) and mycophenolate (MMF), SIBS received CsA and methotrexate (MTX), UDs received CsA +MTX + antithymocyte globulin (ATG) and UCB received CsA+MMF+ATG. The 4 groups were comparable for disease phase (p=0.2), age (p=0.2), and conditioning regimen intensity (p=0.4), with the exception of UCB who received more frequently a MAC regimen. Results. Acute GvHD grade II-IV , was less frequent in UCB and HAPLOs (14%) as compared to SIBS and UDS (30%) (p=0.0003). Chronic GvHD was less frequent in HAPLOs ((12% *vs* 24% of other donor types). The cumulative incidence (CI) of transplant related mortality (TRM) was significantly lower in SIBS and HAPLOS (17%) as compared to UDs and UCB (34%) (p=0.0002). The CI of relapse was comparable in all 4 groups (26-36%, p=0.2). The 3 year actuarial DFS ,of all patients including early and advanced disease, is respectively 41% , 39%, 36%, 43% (logrank, p=0.6) . In multivariate Cox analysis, advanced disease was the only negative predictor of disease free survival (RR 2.2; p<0.0001). In conclusion, we find that unmanipulated HAPLO transplants, with PT-CY, have outcome comparable to UD and MSD grafts, in patients with hematologic malignancies. Advanced disease is the only adverse factor for disease free survival.

CO027

ALLOGENIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH POLYCYTHAEMIA OR ESSENTIAL THROMBOCYTHEMIA TRANSFORMED TO MYELOFIBROSIS OR ACUTE MYELOID LEUKEMIA: REPORT FROM THE MPN SUBCOMMITTEE OF THE CHRONIC MALIGNANCIES WORKING PARTY OF THE EUROPEAN GROUP FOR BLOOD AND MARROW TRANSPLANTATION

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Background.The clinical course of Polycythemia Vera (PV) and Essential Thrombocythemia (ET) is potentially associated with long-term severe complications, such as evolution to myelofibrosis (MF) or acute myeloid leukemia (AML). Once transformation develops, current medical treatments are of limited efficacy. Allogeneic haematopoietic stem cell transplantation (alloHSCT) is currently the only potentially curative treatment for advanced PV or ET, but, since it is associated with a significant mortality and morbidity, the need for and timing of alloHSCT is still under debate. Patients and Methods. We analysed 250 consecutive patients (male/female 137/113) with an initial diagnosis of PV (n=120) or ET (n=130), who underwent alloHSCT due to progression to MF (n=193) or AML (n=57) and reported to European Group for Blood and Marrow Transplantation (EBMT) registry between 1994 and 2010. The median age was 56 years (range 22-75) and 52% of patients had an interval from diagnosis to transplant of 10 years or more. Of all 250 transplants, 80 were performed after a standard myeloablative and 170 after a reduced intensity conditioning regimen. The preferred stem cell source was the peripheral blood which was used in 229 patients (92%). Donors were HLA related matched (n=115) or mismatched (n=2), or unrelated matched (n=124) or mismatched (n=9). GvHD prophylaxis was based on cyclosporine A in 179 cases either alone (n=8) or combined with methotrexate (n=84) or mofetyl mycophenolate (n=87). Results. With a median follow-up from alloHSCT of 13 months, 3 years overall survival (OS) and relapse incidence (RI) were 55% and 32%, respectively. In the univariate analysis, the main parameters that negatively affected post-alloHSCT outcomes were older age (>55 years), a diagnosis at transplant of AML and donor type (mismatched and unrelated *vs* related). The 3 years cumulative incidence of non relapse mortality (NRM) was 28%, significantly higher in older patients (>55years, 35% *vs* 20%, p=0.032), in mismatched and unrelated compared to related donor (49% and 34% *vs* 18%) and in diagnosis of AML compared to MF (29% *vs* 27%, p=0.045). Acute GvHD grade 2-4 was present in 27% and extensive chronic GvHD in 37% of patients. Conclusions. This large retrospective study confirms that alloHSCT is potentially curative for end-stage PV/ET patients progressing to MF or AML. Relapse and NRM remain unsolved problems for which innovative treatment approaches need to be urgently assessed.

Table 1. Univariate analysis for the main clinical outcomes evaluated at 36 months after transplant

Risk factor	N	OS (%) at 3-yr	P	RI (%) at 3-yr	P	NRM (%) at 3-yr	P
Overall	250	55		32		28	
Age							
<55	114	65	-	27	-	20	-
≥55	136	47	**0.015**	39	**0.047**	35	**0.032**
Diagnosis at TRX							
AML	57	28	-	53	-	29	-
MF	193	62	**<0.001**	28	**0.001**	27	**0.045**
Donor type							
Related	115	65	-	35	-	18	-
Unrelated	124	50	0.085	30	0.562	34	**0.034**
Mismatched	11	30	0.390	35	0.775	49	0.342
Initial diagnosis							
PV	120	60	-	34	-	28	-
ET	130	51	0.272	31	0.437	28	0.876
V617FJak2 mutation							
Absent	11	36	-	36	-	36	-
Present	96	58	0.072	39	0.483	19	0.094
Unknown	193	55	0.168	27	0.174	33	0.365
Interval diagnosis-TRX							
≤ 10 years	119	51	-	34	-	27	-
>10 years	131	59	0.327	31	0.454	29	0.870
Year of TRX							
≤ 2005	54	61	-	21	-	28	-
2005-2007	68	51	0.382	39	0.094	29	0.899
≥ 2008	128	64	0.693	28	0.120	23	0.863
Disease status at TRX							
CR	23	63	0.652	39	0.200	7	0.178
Relapse/progression	90	59	0.991	35	0.479	22	0.704
Untreated	65	61	-	31	-	25	-
Other	59	47	0.181	36	0.768	36	0.454
Conditioning regimen							
Myeloablative	80	56	-	16	-	30	-
Reduced intensity	170	55	0.231	40	0.097	27	0.396
CMV status							
-/-	54	70	-	22	-	13	-
-/+	27	55	0.335	41	0.219	13	0.862
+/-	51	55	0.137	19	0.659	35	0.081
+/+	94	62	0.564	27	0.503	26	0.223
T cell depletion							
No	86	57	-	24	-	28	-
Yes	162	56	0.614	38	0.123	27	0.824

CO028

THE POLICY OF ROME TRANSPLANT NETWORK (RTN) FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) IN PATIENTS WITH HIGH RISK HAEMATOLOGICAL MALIGNANCIES

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Pts with high-risk hematological malignancy eligible for HSCT should proceed timely to transplant. All retrospective studies comparing transplants from volunteer unrelated donor (VUD), cord blood (CBU) and haploidentical related donor (HRD) have shown no substantial differences in terms of final outcome. Based on this statement, the RTN transplant strategy, by changing the concept of "donor versus no donor" with that of "transplant versus no transplant", has as first aim the identification of a suitable donor in order to perform HSCT in adequate timing. RTN policy consider the use of a unique conditioning regimen based on the MAC or RIC version of Thiotepa, Busilvex and Fludarabine combination regardless of the disease and stem cell source. In order to start timely a simultaneous search for VUD and CBU, RTN strategy includes an early familiar HLA typing, including parents, followed by high resolution HLA of pts lacking an HLA identical sibling. HRD was contemporary considered. Preliminary search is always performed to assess the potential of VUD's identification in order to lead the subsequent search strategy. Selection criteria for VUD consist of at least 8/8 HLA matching. Single CBU was selected according to cell dose with HLA matching (4/6HLA loci: TNC>3.5x10^7/kg and CD34+>2x10^5/kg; 5/6HLA loci: TNC>2.5x10^7/kg and CD34+>1x10^5/kg). Pts for whom an unrelated VUD/CBU was not available in adequate timing proceed towards an haplo. All pts received MAC or RIC according to age and Sorror Index, while GVHD prophylaxis depends on the type of transplant. In 6 years, 731 pts have been candidates to an HSCT. HLA identical sibling donor was available in 232 (34%), while the alternative donor's search was activated for 448 pts. Of 448 pts, 13 were too early at time of analysis to be evaluated, 33 failed the identification of an alternative donor (8%) and 47 (12%) lost the eligibility during the search. Finally,382/415 evaluable pts (92%), lacking an HLA identical sibling, identified an alternative donor and 335/382 (88%) underwent an alternative HSCT (149VUD, 64CBT, 118Haplo).For all 731 candidates to HSCT, the eligibility was confirmed for 680, a suitable donor was identified for 618 of them (91%) and an HSCT was performed for 567 (83%) of the 680 eligible pts. Our analysis shows that by adopting the RTN policy of widespread donor search and multiple transplant options, the allogeneic HSCT can be offered as potential therapy to a large majority of pts.

CO029

INCIDENCE AND RISK FACTORS FOR LOSS OF MISMATCHED HLA AT LEUKEMIA RELAPSE AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Disease relapse after allogeneic Hematopoietic Stem Cell Transplantation (HSCT) remains a crucial unsolved issue. We and others have shown that genomic loss of patient-specific mismatched HLA is a frequent mechanism by which residual leukemic cells can evade donor T cell-mediated immune control and determine relapse after haploidentical and unrelated donor HSCT, but the actual incidence and risk factors for these peculiar relapses are to date unknown. We retrospectively evaluated 230 consecutive transplants performed over the last ten years in our institution. All donors were partially HLA mismatched (mismatched related donor (MMRD): 170; volunteer unrelated: 60). All patients were affected by high-risk myeloid malignancies (Acute Myeloid Leukemia (AML): 179; Myelodysplastic Syndrome (MDS): 27; Myeloproliferative Neoplasms: 17; Others: 7), and received the infusion of donor T cells, either as part of the graft or as add-backs. A total of 83 relapses occurred, and 21 (25%) were due to leukemic variants with genomic loss of the mismatched HLA. HLA loss occurred predominantly in patients with AML (n=19) and in all cases after MMRD HSCT. Putative risk factors for HLA loss were thus addressed in the relevant subgroup of MMRD HSCTs (170 transplants, 66 relapses: 21 HLA loss and 45 classical). HLA loss relapses occurred later than their "classical" counterparts (median time to relapse: 307 *vs* 86 days; p<0.0001), suggesting the existence of a long equilibrium between leukemic cells and donor T cells before the outgrowth of immune escape variants. None of the disease-related factors tested correlated significantly with HLA loss. Conversely, several transplat-related factors displayed strong association, comprising the infusion of an unmanipulated T-replete graft (Chi2=6.36; p=0.01) and the occurrence of Graft-versus-Host Disease (GvHD), either acute (HR:4.67, CI 95%: 1.53-14.22; p=0.007) or chronic (HR: 1.71; CI 95%: 0.68-4.28; p=0.01), in line with the hypothesis that selection of these leukemic variants may be prompted by a strong alloreactive T cell pressure. Interestingly, HLA loss occurred more frequently in patients that displayed the HLA-C*04 allele in the mismatched haplotype (Chi2= 8.07; p=0.04). In conclusion, our data demonstrate that loss of the mismatched HLA is a frequent mechanism of relapse for patients with high-risk myeloid malignancies, and is tightly linked to donor-versus-host T cell alloreactivity.

CO030

CIRCULATING ENDOTHELIAL CELLS ENUMERATION IS AN HELPFUL TOOL IN THE DIAGNOSIS OF AGVHD IN PATIENTS UNDERGOING ALLOGENEIC STEM CELLS TRANSPLANTATION

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AlloBMT can be burdened by life-threatening complications, being GVHD the major cause of morbility and mortality. Clinical and physiopathological evidences showed that vascular endothelium could be a target of GVHD in very early phase; therefore markers of endothelial damage are warranted as valuable support in GVHD diagnosis. We conducted a study with primary endpoint to identify and count circulating endothelial cells (CEC) in peripheral blood of patients undergoing alloBMT as a function of endothelial damage. The CellSearch System® is used to capture and enumerate CEC. Enriched and stained cells are dispensed into a MagNest® cartridge that is scanned and individual images of cells are scored as CEC, based on CD146+, CD105+, DAPI+ and CD45- phenotype. Patients undergoing alloBMT were tested before (T1), after the conditioning regimen (T2), at engraftment (T3), at GVHD onset (T4) and at 1-2 weeks after steroids treatment (T5). Ten healthy subjects served as controls. We enrolled 40 patients with hematologic neoplastic diseases (7 HD, 13 AML, 5 ALL, 8 MM, 3 CLL, 1 NHL, 1 CML, 2 SAA) undergoing alloBMT from either HLA-matched familial (n=12) or unrelated donor (n=28). aGVHD (grade I-IV) manifested in 19/39 patients. No clinical and transplant characteristics differences were present between patients with and without GVHD. The median CEC/ml pre-alloBMT was 20 (n=40, range 4-718), in comparison to a value of 2 (range 1-14) in the 10 healthy subjects. At time of engraftment CEC/ml were 47 (range 16-148) in patients with GVHD and 92 (range 23-276) in patients without GVHD (P=0,006). This difference remained significant in multivariate analysis by logistic regression model (OR 0,97, 95% C.I. 0,96-0,99; P=0,02). At GVHD onset, the relative increase of CEC counts (T4 *vs* T3) was 44% (range, -43 - 569%) in GVHD patients versus 0% (range, -49 – 2%) in patients without GVHD (P=0,003), being confirmed in multivariate analysis (OR 1,04, 95% C.I. 1,0-1,08; P=0,04). Circulating

endothelial cells can represent a promising marker to monitor endothelial damage in patients undergoing alloBMT. The confirmation of the clinical utility of CEC counts in a larger series of patients, together with the use of a semi-automatic, standardized and reproducible technology, will allow a valuable help in the diagnostic definition of GVHD in early phase, and moreover could be a valid complement in the prognostic stratification of patients candidates to alloBMT.

Acute Lymphocytic Leukemia

CO031

HIGH CURE RATES IN BURKITT LEUKEMIA AND LYMPHOMA: NILG STUDY OF THE GERMAN SHORT INTENSIVE RITUXIMAB-CHEMOTHERAPY PROGRAM

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Introduction. Burkitt lymphoma (BL) and B-cell acute lymphoblastic leukemia (B-ALL) are very aggressive malignancies with poor prognosis unless treated with highly specific intensive programs. The German Multicenter Study Group for Adult ALL piloted a short intensive rituximab (R)-chemotherapy program that improved outcome compared to the prior regimen. The Northern Italy Leukemia Group adopted the same protocol to treat 105 consecutive, unselected adult patients (pts.) with BL and B-ALL. Aim. We evaluate the long-term results of a prospective clinical study enrolling more than 100 adult pts. with BL/B-ALL. Patients: One-hundred five pts. were treated (median age 47 years, range 17-78); 48% had B-ALL, 25% were older than 60 years, 37% exhibited an ECOG performance status (PS) >1, and 14% were HIV-positive. Depending on extent of disease, treatment consisted of six-eight rituximab infusions and four-six intensive chemotherapy courses (attenuated in patients aged >55 years) with high-dose methotrexate, fractionated ifosfamide/cyclophosphamide, other drugs in rotation, and intrathecal chemoprophylaxis.

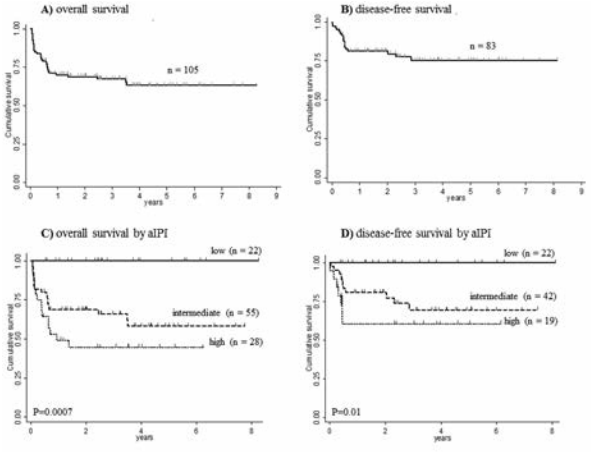

Figure 1.

Results. Eighty-three pts. (79%) achieved CR, 8 were refractory and 14 died of complications. All early deaths occurred in pts. aged > 40 years, correlated with stage III-IV BL or B-ALL (n=13), PS ≥1 (n=10), and were mainly caused by infections. After a median f-up of 23.8 months (range 0.7-99), 65 pts. (61%) were alive in 1st CR, 19 died of complications, and 19 had refractory/recurrent disease. The 3-year overall and disease-free survival were 67% and 75%, respectively, ranging from 100% to 45% for OS (P=0.000) and from 100% to 60% for DFS (P=0.01) in pts. with low, intermediate and high adapted international prognostic index. In multivariate analysis, only age (< *vs* >60 years) and PS (0-1 *vs* >1)

retained prognostic significance, identifying 3 risk groups with OS and DFS probabilities of 88% and 87.5%, 57% (P=0.0000) and 70.5%, 20% and 28.5% (P=0.0001), respectively. Relapse rate was only 7% in pts. treated with an intercycle interval <25 days. Conclusion. This regimen achieved 100% curability in pts. with low adapted international prognostic index (21% of total), and very close to 90% in pts. aged ≤60 years with PS 0-1 (48% of total). Rapid Burkitt lymphoma/leukemia diagnosis with prompt patient referral to prevent clinical deterioration, and careful supervision of treatment without chemotherapy delay can achieve outstanding therapeutic results.

CO032

PONATINIB IS SAFE AND ACTIVE IN PATIENTS WITH RELAPSED/REFRACTORY PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ ALL) HARBOURING T315I MUTATION: THE BOLOGNA EXPERIENCE

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Background. Ponatinib, a potent third generation pan BCR-ABL inhibitor, has recently showed a relevant activity against native and mutant forms of BCR-ABL, including the TKI resistant T315I mutant. The aim of this compassionate protocol was to confirm and evaluate the efficacy and the safety of the compound in patients with advanced Ph+ ALL and CML. Design and Methods Ponatinib was obtained through a compassionate personalized program, approved by Ariad and by Bologna Ethical Committee. After informed consent was signed, from May 2012 to March 2013, 14 patients (M/F: 6/8) have been treated with Ponatinib (45 mg orally, daily), including 12 Ph+ ALL (p190) and 2 BP (1 Myeloid and 1 Lymphoid, p210) of CML. All the patients were resistant or intolerant to previous TKIs administration. The median age of the patients was 55 years (range 18 -94). At the time of enrolment, median Hb, PLTs and WBC values were 11 g/dl (range 5.6-12.3), 70000/mmc (range 3000-255000) and 52620/mmc (range 5240-300000), respectively. At baseline, mutational analysis showed the presence of T315I mutation (8 pts), G250E (1 pt), T315I and Y253H (1 pt), T315I and Y253A (1 pt). No mutations were detected in 3 patients. Results. With a median follow up of 80 days (range 15-225+), a maHR was obtained in 12/14 patients (86%, with a median time to maHR of 24 days. After one month of treatment, a reduction of BCR-ABL fusion transcript was observed in 10/14 patients (71%). The level became undetectable in 4 patients (2 with T315I mutation). Median OS was 19 months (range 11-44+). A progression disease was observed in 4 patients. Mutational analysis after treatments, cytogenetic details, and further molecular data will be provided on site. At the time of this report, 7/14 patients are still on study (50%), whereas in the other 7 patients Ponatinib was discontinued due to lack of efficacy (5 pts) or to allow a stem cell transplantation (2 pts). Non-hematologic adverse events were described in 6/14 patients (grade >III skin rash in 3 patients; grade>II serum lypase increase in 2 patients; grade>II myalgia in 1 patient). Conclusion. In our experience, the activity of Ponatinib in advance Ph+ Leukemias, mainly in T315I mutated patients, was confirmed. No treatment-related deaths occurred. The understanding of molecular mechanisms responsible for resistance or lack of response to the drug will be necessary in order to early identify the patients who could take advantage from this treatment.

CO033

DEREGULATED EXPRESSION OF CHECKPOINT KINASE 1 (CHK1) IN BCR-ABL-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (BCR-ABL+ ALL) SUGGESTS A NEW THERAPEUTIC TARGET

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The activation of Chk1 has been demonstrated to influence response to chemotherapy in patients with BCR-ABL-positive chronic myeloid leukemia patients (Nieborowska-Skorska *et al*, Cell Cycle 2006). Chk1 and Chk2 are serine threonine kinase activated by different insults leading to DNA damage and involved in DNA repair and cell cycle control. Since, their role has not been established in BCR-ABL+ ALL, we investigated the expression of Chk1/Chk2 in blast cells from adult BCR-ABL-positive ALL patients (n=48) compared with normal bone marrow precursor cells (n=9) using the BioMark instrument (Fluidigm) and Fluidigm Dynamic Array 48 x 48. The expression of Chk1 but not Chk2 was higher (p=0,0005) in BCR-ABL+ leukemia patients (median value 0,69) compared with normal samples (median value 0,27). In order to better understand the biological role of Chk1 in ALL, we included in the analysis different B (BCR-ABL-positive: BV-173 and SUP-B15; BCR-ABL-negative: REH, NALM-6 and NALM-19) and T (MOLT-4, RPMI-8402 and CCRF-CEM) leukemia cell lines. The expression of Chk1 was very homogeneous among the cell lines, with no difference between BCR-ABL-positive or negative cells. Since Chk1/2 inhibitors are currently available, we investigated the efficacy of Chk1/2 inhibition by PF-0477736 (Sigma) both as single agent and in combination with tyrosine kinase inhibitors (TKIs), imatinib and nilotinib, in BCR-ABL+ leukemia cell lines. Results showed that the combination (TKIs + Chk1/2 inhibitor) is more effective than the single inhibitors in inducing cell death. In conclusion, adult ALL patients show an higher expression of Chk1 compared with normal bone marrow precursor cells. This abnormal expression could be found also in different B-/T-ALL cell lines, suggesting that the inhibition of Chk1 could be a target for new therapeutic strategies in ALL. Acknowledgments: Supported by European LeukemiaNet, AIL, AIRC, PRIN 2010-2011, Fondazione del Monte di Bologna e Ravenna.

CO034

CLINICAL SIGNIFICANCE OF LOW SENSITIVITY PROBES IN MRD ANALYSIS OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background. MRD analysis is the most powerful indicator of the risk of relapse and is adopted in Acute Lymphoblastic Leukemia (ALL) patients for the risk-stratification. In most clinical trials the required sensitivity of the probe is at least 10-4. MRD evaluation is usually not performed with low sensitivity probes and patients are treated according to clinical risk stratification (high risk, HR or standard risk, SR). Aims:To evaluate the ability of low sensitive probes in revealing high MRD levels and classify patients as high risk of relapse due to leukemia persistence. Methods.The prospective Northern Italy Leukemia Group trial 09/2000 enrolled a total of 280 consecutive unselected patients with Ph-ALL. In this study post-consolidation treatments were administered

according to the MRD status. MRD was assessed by RQ-PCR using one or two patient-specific molecular probes. MRD was evaluated at weeks 10, 16 and 22 and results were used to categorize patients as MRDneg (negative at w 22 and negative or positive <10-4 at w16) or MRDpos. SCT or intensified chemotherapy was prescribed to MRDpos patients whether maintenance to MRDneg patients. Only probes reaching the sensitivity of 10-4 were used for treatment allocation; patients without a sensitive probe were treated according to clinical risk. Results. Of 280 registered patients 142 completed consolidation phase and were eligible to MRD risk stratification. Fifty eight patients were classified as MRDneg and 54 MRDpos. For 30 patients MRD was not available for reliable risk assignment. Within these 30 cases, 9 had a low sensitive probe (10-3) with 8 having the MRD evaluation performed on available sample. A positive MRD signal at 10-3 level or greater in at least one sample was found in 5 cases. Among these, one patient experienced an early relapse, one refused any treatment and one relapsed after SCT. Interestingly, two of these patients had been previously assigned to the SR class (one SR B and one SR T lineage ALL) while the transplanted patients was a HR B ALL. Furthermore, the only two MRD positive surviving patients had been classified as clinical SR but received intensified chemotherapy courses, followed by SCT in one case. Conclusion: Our analysis suggests the usefulness of MRD evaluation in ALL even with low sensitive probes to identify, especially in clinical SR group, patients with high residual disease in whom intensified treatments are needed to avoid impending relapse.

CO035

DEEP MOLECULAR CHARACTERIZATION OF ADULT B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (BC-ALL) NEGATIVE FOR RECURRENT FUSION GENES REVEALS A HIGH COMPLEX GENETIC HETEROGENEITY INFLUENCING PROGNOSIS

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Introduction. Genome-wide profiling of B/T-ALL identified many novel somatic alterations, several of which have clear implications for risk stratification or future therapeutic targeting. However, most of the studies focused on children and therefore a deep molecular characterization of adults is still challenging, especially for those cases lacking recurrent fusion genes. Subjects-Methods. In order to shed light on this ALL subgroup, until now we retrospectively analyzed 28 newly diagnosed BC-ALL subjects (19 males/9 females; median age 41.5 years; negative for known fusion genes). Karyotype was normal in 10/28 (36%), showed abnormalities in 5/28 (18%) and failed or was not available in 13/28 (46%) cases. Overall survival rate was very poor with a median of 14 months (range, 1-75). We analyzed copy number alterations (CNA) of IKZF1, CDKN2A/B, PAX5, EBF1, ETV6, BTG1, RB1, and genes within PAR1: CRLF2, CSF2RA, IL3RA by the SALSA MLPA kit P335 IKZF1 (MRC Holland). In addition, mutation status was assessed for TP53, CRLF2, JAK2, LEF1, PAX5 and IL7R by next generation sequencing (NGS) with GS Junior (Roche Applied Science; IRON-II study oligonucleotide primer plates). Moreover, SNP arrays analysis was performed in 57% of cases to more fully assess genomic complexity. Results. Overall 76% of subjects showed an abnormality of at least one of the analyzed genes: 7 (25%) had one, 4 (14%) had two, 6 (21%) had three, 6 (21%) had four or more alterations. In subjects showing no abnormalities, SNP arrays analysis revealed amplification of chromosome 1q in 2/6 (33%). Deletions of CDKN2A/B were the most frequent (39%) and in 73%, they occurred together with other abnormalities, suggesting that multiple events are needed to induce the full leukemia phenotype. Other common CNA included: deletions of IKZF1 (25%), ETV6 (25%), PAX5 (14%), EBF1 (11%), PAR1 region (11%) and RB1 (7%). NGS showed mutations of TP53 in 18% of cases (W147*, V172L/G, G245C, Del244-246, D259Y) while JAK2 and CRLF2 were mutated in 7% (R683S/G) and 4% (F232C), respectively. Importantly, subjects with no abnormalities showed better survival rates compared to those with one or more molecular alterations ($p<0.01$). Conclusions: BC-ALL lacking recurring fusion genes is a highly heterogeneous and complex disease. Current diagnostic procedures are in need of revision to improve risk assessment and to guide therapeutic decisions. Supported: ELN, AIL, AIRC, Fondazione del Monte, PRIN 2011, NGS-PTL project.

CO036

EVALUATION OF THE EFFICACY AND ANTITUMOR ACTIVITY OF THE PAN-CLASS I PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) INHIBITOR NVP-BKM120 IN HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

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Introduction. T-ALL is an aggressive malignancy and despite improvements in survival rates prognosis is still poor. A common aberrantly expressed pathway related to T-ALL and converging on anti-apoptotic and pro-survival signals activation is PI3K/Akt and its inhibition is an attractive strategy to improve current treatments. NVP-BKM120 is a highly selective pan-class I PI3K inhibitor which has shown antitumor activity in preclinical and clinical studies in solid cancers. Here we investigated its efficacy in T-ALL. Methods. A panel of T-ALL cell lines with up-regulated PI3K/Akt signaling and primary T-ALL blasts were treated with increasing concentrations of NVP-BKM120 and analyzed at different time points. Results. MTT assays documented a strong reduction of viability in all cell lines tested with a median IC50 lower than 2μM after 48 hours treatment that correlate with apoptosis as documented by Annexin V/PI analysis and activatation of caspases. To asses selective inhibition of PI3K, levels of the pathway components p-Akt(ser473) and p-S6RP(Ser235/236) have been evaluated by western blotting showing a strong decrease in a dose and time dependent manner. Efficacy of NVP-BKM120 was also tested in an ex-vivo model of primary blasts from T-ALL patients with assessed constitutive activation of PI3K/Akt pathway and results were consistent with *in vitro* studies. Comparison between NVP-BKM120 and PI3K selective inhibitors p110 isoforms provided its stronger effect in terms of viability and pathway inhibition. The drug was also able to synergize with dexamethasone and vincristine both *in vitro* and ex-vivo. To note, NVP-BKM120 extents pro-apoptotic effects also in Jurkat cells co-cultured with MS-5 stromal cells which mimic bone marrow microenvironment suggesting a potential overcome of its protective effect toward leukemic cells *in vivo*. Finally cell cycle has been analyzed by flow cytometry revealing a strong accumulation in the G2/M phase while immunofluorescent analysis identified an increased number of mitotic cells with disorganized mitotic spindle compared to control, suggesting an impairment in G2/M regulation and in mechanisms involved in cell cycle progression. Conclusion: NVP-BKM120 showed viability reduction and apoptosis induction in T-ALL cells as well as in primary T-ALL blasts due to PI3K pathway inhibition supporting its clinical evaluation in T-ALL.

Hemostasis and Thrombosis

CO037

PREVALENCE OF CLONAL POPULATIONS OF HEMATOPOIETIC CELLS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PHENOTYPE IN PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS

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Background. Venous thromboembolism is a common complication in patients with paroxysmal nocturnal hemoglobinuria (PNH) and frequently occurs in the splanchnic veins. PNH screening is recommended in patients with splanchnic vein thrombosis (SVT) and signs of hemolysis or cytopenia, but the presence of mutant PNH clones may cause complement-mediated endothelial cell damage resulting in a prothrombotic state even in the absence of hemolysis. The prevalence of PNH clones in non-selected SVT patients is currently unknown. We carried out a multicentre cross-sectional study to determine the prevalence of PNH clones in a group of patients with SVT and without overt PNH. Methods. Patients with objective diagnosis of SVT within the previous 2 years were eligible for the study. Information was collected on demographic characteristics, risk factors, and site of SVT. All patients underwent blood sampling and the presence of PNH clone was centrally assessed using high sensitivity flow cytometric analysis to identify an abnormal population of 0.01% PNH cells or more. Results. 202 SVT patients were tested, 118 (58.4%) were males, mean age was 54.6 years (range 17-94). Site of thrombosis was portal in 103 patients, mesenteric in 74, splenic in 39, and supra-hepatic in 10. Major risk factors for SVT included cirrhosis in 31patients (15.3%), recent surgery in 22 patients (10.9%), and a myeloproliferative neoplasm in 21 patients (10.4%); in 70 (34.6%) patients SVT was unprovoked. Median time elapsed between SVT diagnosis and testing was 349 days. Cells with the PHN phenotype confirmed in two independent samples were detected in 2 (0.99%, 95% CI 0.17-3.91) patients, one with mesenteric vein thrombosis and inflammatory bowel disease, the second with unprovoked portal vein thrombosis. The clone size was 0.014% and 0.16%. The prevalence of PHN clones in patients with unprovoked SVT was 1.43% (95% CI 0.07-8.77) Conclusions Small PNH clones can be detected in patients with a history of SVT and no clinical manifestations of disease. Future studies are needed to explore the potential role of this finding in the pathogenesis of SVT and to better define in larger cohorts the cost- effectiveness of screening for PNH clones in this population.

CO038

THE JAK2 V617F MUTATION IN PATIENTS WITH CEREBRAL VENOUS THROMBOSIS IS ASSOCIATED WITH AN INCREASED RISK OF RECURRENCE

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Background. Cerebral venous thrombosis (CVT) is a rare and severe event. Recently a part of patients has been reported to carry the molecular marker of myeloproliferative neoplasm (MPN) JAK2 V617F. Little is known about the follow-up of CVT patients. Aim of the study: To investigate in a cohort of patients with CVT the rate of recurrent venous thromboembolism (VTE) and the risk for recurrence associated with either thrombophilia and JAK2V617F. Patients and methods: We investigated an ambispective cohort of 114 CVT patients; 25 (M/F 18/7) were aged <18 years (median 5, range 0-13). The 89 adults (M/F 16/73) had a median age of 35 (range 18-82). Patients with cancer were excluded. Inherited thrombophilia, antiphospholipids, and JAK2V617F were searched in all patients. A follow-up >6 months after CVT was recorded in 72 patients (60 adults), being prospective in 50 (31 adults). The probability of recurrence was estimated by the Kaplan–Meier method. Results. In pediatric patients the leading causes of CVT were loco-regional infections (n=13, 52%). Thrombophilia was diagnosed in 5 (20%); no child had JAK2V617F. In adults CVT was associated with oral contraceptives or pregnancy in 35 (48% of females) and 16 (22% of females), respectively, with other risk circumstances in 13 (14% of cases), and was unprovoked in 24 (27%). Thrombophilia was diagnosed in 27 (30%). Eight patients had JAK2V617F (9%); only 1 CVT was unprovoked. Four of them had diagnosis of MPN at the CVT event. In the 60 adult patients with an evaluable follow-up (median 2.3 years, range 0.5-32.6, total 336) the incidence of recurrent VTE was 3.1% pt-years (11 events in 10 patients: 3 CVT, 6 DVT- 2 in the same patient -, 2 splanchnic thromboses). Recurrences occurred in 1 of 31 reference patients without either thrombophilia and JAK2V617F (3.2%), 3 of 21 patients with thrombophilia (14.3%), and 6 of 7 patients with JAK2V617 (86%). No increase in risk was associated with thrombophilia (hazard ratio, HR, 1.11, 95%CI 0.18-6.76); in contrast, the overall HR ratio for recurrence associated with JAK2V617F was 4.75 (95%CI 1.81-35.77). The incidence of recurrence was 9.1% pt-years in the JAK2V617F-positive patients and 1.8% pt-years in those JAK2V617F-negative. Conclusions. In CVT patients the overall risk of recurrence is low and is not associated with thrombophilia. However, JAK2V617F is associated with an exceedingly high risk of recurrence, suggesting need for long-term anticoagulation.

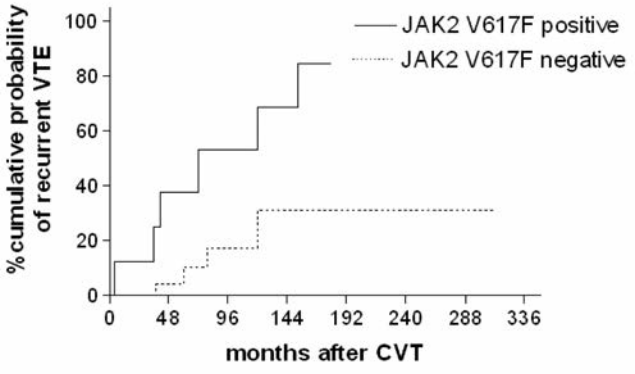

Figure 1.

CO039

CONDUCTING A TRIAL IN PREVIOUSLY TREATED PAEDIATRIC SUBJECTS WITH SEVERE HAEMOPHILIA A IN ACCORDANCE WITH EUROPEAN MEDICINE AGENCY (EMA) GUIDELINES: EXPERIENCES FROM THE TUROCTOCOG ALFA TRIAL (GUARDIAN TM 3, NN7008 3545)

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Introduction. The EMA Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products, requires clinical data on paediatric subjects to be provided with the initial application for marketing authorisation within the EU. According to EMA the clinical study in children 0-<12 years should be initiated following review of pharmacokinetic (PK) and efficacy/safety data from 20 previously treated patients (PTPs) ≥12 years for at least 50 exposure days (EDs). PK followed by investigation of efficacy and safety for at least 50 EDs each in 50 paediatric PTPs is required. Novo Nordisk conducted a paediatric trial (NN7008-3545; guardiaTM 3) with turoctocog alfa, a human rFVIII, for treatment and prevention of bleeds in PTPs with severe haemophilia A. Methods. This was a multi-centre, multi-national, non-controlled, open-label, safety, efficacy and PK trial. Paediatric PTPs <12 years of age with severe haemophilia A (FVIII≤1%) without inhibitors and with at least 50 EDs to FVIII were included in the trial. Results. A clinical trial application was submitted to Health Authorities and ethical committees/institutional review boards at 28 sites in 11 countries worldwide

with a mean approval time of 2-3 months. The NN7008-3545 trial was initiated June 2010 and 60/69 screened patients completed the trial. Total recruitment time was a little more than 12 months with an enrolment rate of 5-6 subjects/month during the last 9 months. Last patient's last visit was in Nov 2011. After completion, all patients chose to participate in the extension trial (guardian TM 2 trial). Conclusion: In children with haemophilia, growth and development influence FVIII PK that in turn influences replacement therapy. The revised EMA guideline introduces the requirement for pre-licensing trials in paediatric haemophilia subjects. The guardian TM 3 trial is the largest completed paediatric trial in severe haemophilia A without inhibitors and the first following the revised EMA guidelines. Fast completion was made possible through global trial allocation. Only 10 % of the patients were recruited at sites in EU countries.

CO040

CORRELATION OF BLEEDING PHENOTYPE/FXI ACTIVITY (FXI:ACT) IN CONGENITAL FXI DEFICIENCY

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Background. Bleeding phenotype in FXI deficiency is variable and generally related to surgery/trauma. Moreover, there is a poor correlation between bleeding and baseline FXI:Act. Aim. To describe the hemorrhagic phenotype of our FXI deficient population and to relate the phenotype with FXI:Act. Patients and Methods. We have been following 94 FXI deficient patients: 43F, 51M; diagnosis median age: 28.7 years (0.9-83.9); median follow-up: 0.9 years (0.1-36.2); median FXI:Act of all patients: 38% (range 0.5-69%; normal values: 70-140); FXI:Act ≤1% in 5 patients, >1≤5% in 12, >5≤10 in 3, >10≤20 in 6, >20≤70 in 68. Excessive bleeding is reported as described in medical records. Results. Fifty six patients experienced bleeding episodes not surgery-related. Prior to diagnosis, 64 patients underwent 133 surgeries. Prophylactic treatment was administered in 3/133 procedures: tranexamic acid (TA) in 1, fresh frozen plasma (FFP) in 2. Twenty eight/133 (21%) post-surgery hemorrhages were reported in 19 patients; in 12/28 cases, transfusional therapy (FFP and/or red blood cells units) was needed. Median FXI:Act of bleeder patients was 28% (0.5-53%). Twenty nine spontaneous deliveries (SD) and 8 caesarian sections (CS) were performed without prophylaxis: 4 post-partum hemorrhages occurred (patients FXI:Act: 2, 6, 27, 52.3% respectively). In 3 cases transfusional therapy was necessary. After diagnosis, 23 patients underwent 34 surgeries. Prophylactic treatment was administered in 23/34 procedures: TA in 7, FFP in 2, desmopressin in 4, FFP+TA in 8, desmopressin+TA in 2. The only bleeding reported (1/23, 4%) was after an emergency appendectomy performed under TA administration, in a patient whose FXI:Act was 2.8%. In 2/11 surgeries performed without prophylaxis, an excessive bleeding was reported but transfusional therapy was not necessary; FXI:Act was 29% for both bleeder patients. Four SD and 5 CS were performed with prophylaxis: FFP in 4, TA in 2, desmopressin in 3. No post-partum hemorrhages occurred. Conclusions. We confirm the wide variability in bleeding phenotype in FXI deficient patients, not related to the baseline FXI:Act levels. We highlight that a good management of prophylaxis treatment dramatically reduces the percentage of bleedings in case of surgery (21% *vs* 4%) and deliveries. Because of the low correlation between FXI:Act and the phenotype, we highlight the need of laboratory-based prognostic factors for a better management of these patients.

CO041

CHARACTERIZATION OF THROMBIN GENERATION POTENTIAL IN POLYCYTHEMIA VERA PATIENTS ENROLLED IN THE CYTO-PV ITALIAN CLINICAL TRIAL

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The pro-thrombotic effect of an elevated hematocrit (HCT) has been clearly demonstrated in polycythemia vera (PV) patients by the observation that at progressively higher HCT values there is an increase of thrombotic risk. Current guidelines for PV management recommend to maintain HCT below 45% in males and below 42% in females through phlebotomies and/or the use of cytoreductive drugs. Recently, the results of CYTO-PV clinical trial showed that PV patients with an HCT target <45% have a significantly lower rate of cardiovascular death and major thrombosis than those with an HCT in the range 45-50% (Marchioli *et al*, NEJM 2013). In the setting of the CYTO-PV trial was planned a biological sub-study to assess: 1) thrombin generation (TG) potential according to HCT levels, and 2) TG predictive value for bleeding and/or thrombosis. One hundred twenty-four PV patients (HCT<45%: n=66, HCT 45-50%: n=58; age 42-87 years) were enrolled in the biological sub-study. Blood samples were collected at randomization, and then after every 6 months for 5 years. TG potential was determined by the calibrated automated thrombogram assay (CAT assay, Stago), in platelet-poor plasma spiked with 5pM tissue factor (TF). TG was also performed in the presence of activated Protein C (APC) to evaluate APC resistance, and results expressed as normalized APC sensitivity ratio (nAPCsr). Fifty-one healthy subjects acted as the control group. At baseline, HCT levels were not different between patients randomized at HCT<45% *vs* HCT 45-50%. A significant correlation was observed between HCT values and TG parameters [ETP (R=0.501) and Peak (R=0.329)]. At baseline we also observed that APC markedly inhibited ETP and Peak of TG in both PV patients and controls; however, patients were more resistant to the anticoagulant action of APC compared to controls, resulting in significantly higher plasma nAPCsr. During follow-up, in patients randomized at HCT <45%, nAPCsr showed an overall decrease over time towards control values, while it remained higher than controls in the HCT 45-50%. The low rate of thromboses (n=9) and major hemorrhages (n=2) in the tested group did not allow us to assess the predictive values of TG for the thrombohemorrhagic events.In conclusion, our data show that in PV patients a more resistant to APC phenotype occurs compared to healthy controls. The more aggressive cytoreductive regimen, *i.e.* HCT <45% as target, appeared to be associated to a less APC resistant features.

CO042

ANTITHROMBOTIC PROPHYLAXIS IN PREGNANT WOMEN WITH ANTITHROMBIN CONGENITAL DEFICIENCY IS EFFECTIVE IN PREVENTING ADVERSE OUTCOMES

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Background. Type I (quantitative) or II (qualitative) antithrombin (AT) deficiency is caused by more than 200 mutations of AT gene. Type II deficiency is subclassified according to dysfunction of the reactive site (RS) or heparin binding site (HBS) or pleiotropic effects (PE). Type II HBS is associated with a low thrombotic risk. Aim of the study. To investigate the rate of thrombotic and obstetric complications in pregnant women with AT congenital deficiency and the effect of antithrombotic prophylaxis on the outcome of pregnancy. Patients and methods. We investigated in 35 women with congenital AT deficiency the outcome of their pregnancies with or without antithrombotic prophylaxis. Twenty-two women had type I deficiency and 13 type II (9 HBS, 2 RS,

2 PE); 26 were probands, and nine were relatives of nine probands. Nine women (none with HBS deficiency) had VTE prior to the first pregnancy. The outcomes were venous thromboembolism (VTE) or obstetric complications (OC) (early/late fetal loss, intrauterine growth restriction (IUGR), preeclampsia). Heparin prophylaxis was administered as therapeutic unfractionated or low molecular weight heparin (UFH or LMWH) in 21 women with type I or II (RS or PE) deficiency and in one with HBS deficiency, as a sequence therapeutic UFH-warfarin-UFH in five women with type I deficiency and as prophylactic LMWH in four women with HBS deficiency. Results. We analyzed ambispectively 75 pregnancies. Forty-seven non-HBS pregnancies (19 women) were not prophylaxed, and 53% were complicated: seven by VTE (four antepartum), 18 by OC, one was interrupted. Fifteen non-HBS pregnancies (11 women) received intensive antithrombotic prophylaxis, and IUGR occurred in one (relative risk 0.12, 95%CI 0.01-0.84, *vs* no prophylaxis). Thus, intensive prophylaxis reduced by 88% the risk of pregnancy complications. Four HBS pregnancies in four women were conventionally prophylaxed and uneventful; one HBS woman with other risk factors (FV Leiden, obesity, varicose veins) received therapeutic LMWH; finally, four HBS women had eight pregnancies not prophylaxed, with IUGR in one pregnancy. Conclusions: Use of therapeutic dosages of heparin in pregnant women with AT deficiency is effective in preventing adverse outcomes; either prophylactic dosages of LMWH or clinical surveillance seem enough in the low-risk HBS deficiency.

Hodgkin's Lymphoma

CO043

TUMOR BURDEN IN HODGKIN'S LYMPHOMA: MUCH MORE THAN THE BEST PROGNOSTIC FACTOR

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The clinical lesions of Hodgkin's lymphoma are the final result of a complex network of active autocrine and paracrine secretion of cytokines primarily triggered by the scarce neoplastic component and then amplified by the prevalent inflammatory and stromal cells. The intensity of this immunologic crosstalk is responsible for histopathological features, lymph node enlargement, and systemic symptoms of the disease. Under this point of view, the tumor burden (TB) can be considered the final expression of the whole cytological disorder, while other prognostic factors generally depend on the activity of a few, not prevailing, cytokines. This is likely the reason for the confirmed superior predictivity of (TB) – whatever the method of assessement – over every other prognostic factor. This report is the conclusive result of the investigation on the TB of 506 patients from 3 distinct trials (ABVD *vs* BEACOPP in advanced stages; ABVD + IF-RT in early-stage unfavorably presenting disease with two different schedules according to early response; and VBM + IF-RT in early, favorable-stage disease). TB was measured through the evaluation of the diagnostic whole body CT scan and revealed to be strongly related to the resistance to treatment as it is clinically expressed by failing to achieve complete remission at the end of treatment or to maintain it for at least 12 months. Interestingly, the relationships between TB and resistance are very different according to the type of treatment and the curves illustrating these relationships are distinctly separated (Figure 1). The relative risk of early treatment failure can be predicted on the basis of the TB at diagnosis and the therapy administered, so that, the treatment could be perspectively chosen on the basis of the measured TB and a fixed level of acceptable risk. Moreover, TB can offer an absolute measure of the strength and efficacy of each type of treatment, with a follow-up of only 12 months from the end of treatment. The relative complexity of the TB assessment has been an obstacle to its application. But, now, a simple and indirect estimate of the TB has been developed maintaining a prognostic advantage over every other determinant, IPI score included. Furthermore, promising results are coming from a semi-authomatic PET/CT scan measuring metabolically active volumes, instead of whole visible masses. TB in Hodgkin's lymphoma is a productive field of investigation.

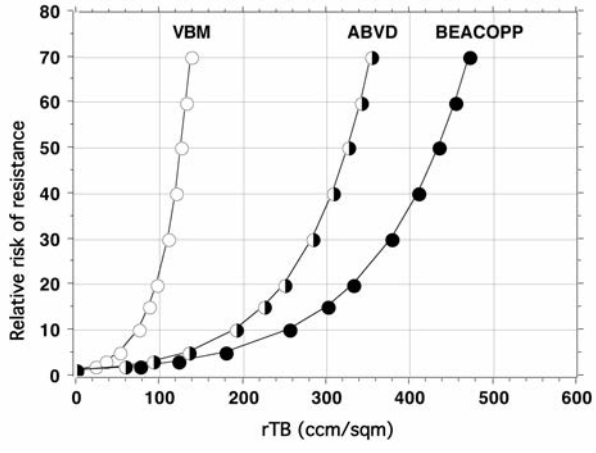

Figure 1.

CO044

PROGNOSTIC FACTORS IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY (R/R) HODGKIN'S LYMPHOMA (HL) TREATED WITH IGEV AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT): A NATIONWIDE RETROSPECTIVE SURVEY FROM FONDAZIONE ITALIANA LINFOMI (FIL)

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Introduction. The gold standard for relapsed/refractory HL patients is induction chemotherapy followed by ASCT. IGEV (Ifosfamide, Vinorelbine, Gemcitabine) attains both high complete remission (CR) rate and high mobilizing potential.Nowadays in Italy,it is the most widely used induction regimen in this setting. Thus, on behalf of FIL, we carried out a retrospective analysis in a homogeneous cohort of patients treated with IGEV in order to reassess the most common prognostic factors. Methods. We collected data of pts treated with IGEV plus ASCT from all FIL Centres between 1997 and 2007. Patients were required to be ≥18 year-old and they were scheduled to receive 2 to 4 pre-transplant IGEV courses. For each prognostic factor, the survival distribution was estimated through Kaplan–Meier method. Log-rank test was used to test differences between survival distributions in univariate analysis. Results. Data of 330 patients were available for the analysis. Main clinical characteristics: median age 32; M/F 57%/43%; previous regimens 1 64%, > 2 36%; refractory/relapsed: 51%/49%; B symptoms 15%, bulky disease 21%, extranodal disease 13%. Post-IGEV CR was obtained in 39% of cases evaluated with CT scan, and in 52% of 204 cases performed by PET. Overall, 269 pts proceeded to transplant after IGEV or further chemotherapy. With a median follow-up of 57.7 months for the whole population, median PFS was 50,6 months and median OS was not reached. In multivariate analysis, refractory disease had negative impact on both PFS and OS, whereas age, bulky disease and B symptoms affected OS. Table 1. Conclusions. factors influencing PFS and OS were identified as reported in Table 1, the further step ongoing is the creation of a prognostic score based on IGEV homogenously treated R/R HL patients.

Table 1.

Characteristics	PFS 4 yrs	P value	OS 4 yrs	P value
All	52.3		73.3	
Age		0.793		0.015
<40	53.3		78.0	
≥40	50.9		63.3	
Systemic symptoms		<0.001		<0.001
A	57.3		79.0	
B	33.6		52.9	
Bulky		0.191		0.008
No	53.8		75.9	
Yes	44.1		57.7	
Stage		0.028		0.227
I-II	59.5		77.2	
III- IV	44.4		68.8	
Disease Status		<.001		0.007
Relapse > 12	62.7		82.6	
Relapse <12	62.1		84.1	
Refractory	42.6		63.0	

CO045

FDG-PET4 NEGATIVITY OBTAINED WITH BEACOPP DOES NOT OVERCOME THE RELAPSE RISK OF FDG-PET2 POSITIVITY IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA PATIENTS TREATED WITH IGEV AND HIGH-DOSE CHEMOTHERAPY: A SINGLE CENTER EXPERIENCE

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Background. FDG-PET (PET) negativity (neg) before high-dose chemotherapy (HDC) predicts survival in relapsed/refractory (rel/ref) Hodgkin Lymphoma (HL) treated with HDC and autologous stem cell transplantation (SCT). Moreover, early PET positivity (pos) during salvage chemotherapy (CT) seems able to identify patients (pts) with a dismal prognosis (Castagna L,BJH 2009). Aims. To evaluate the impact of BEACOPP/escBEACOPP regimens in early PET pos HL pts receiving IGEV as salvage CT, in terms of PET neg before HDC and progression-free survival (PFS). Patients and Methods. Rel/ref HL pts receiving a PET-oriented salvage CT. PET evaluation was performed at baseline and after 2 courses of IGEV (PET2). PET2neg pts continued with IGEVx2 cycles and HDC; responsive pts with PET2 pos received BEACOPP/escBEACOPPx2 cycles. In both groups PET was evaluated after the 4th course of CT (PET4). Chemoresponsive pts received 1-2 cycles of HDC with SCT. Results. We analyzed 23 consecutive pts with rel/ref HL receiving IGEV as salvage CT between 2010-2012. All had received ABVD+/-RT as first line treatment. Sixteen pts (69%) were primary refractory (11 pts PET pos after ABVDx2) and 7 pts (31%) were in first relapse (early in 4 pts and late in 3). Median age 33 ys (17–53); stage III/IV 83%, B symptoms 35%, bulky disease 21%. After IGEVx2, 8 pts (35%) had PET2 neg, 13 pts (57%) PET2 pos (with partial response) while 2 pts had disease progression (PD) and are not evaluable. PET2neg pts remained negative except 1 who had PD after 4th IGEV; 8/13 PET2 pos pts (61%) converted to PET4 neg (6/9 pts who received BEACOPP and 2/4 escBEACOPP). Five/8 pts with PET2 pos and PET4 neg relapsed, as did 2/5 pts with PET2 pos and PET4 pos (who received tandem SCT auto-auto/auto-allo). After a f-up of 28 ms (14-39) the 2-ys overall survival and PFS of all 23 pts were 85% and 60%. The 2y-PFS of PET2 neg and PET2 pos pts were 87.5% and 49% (p=ns). The 2y-PFS of PET2 pos pts who converted to PET4 neg with BEACOPP/escBEACOPP was 47% compared to 60% of pts with persistent PET4 pos (p=ns)(see Fig1). Conclusions: This study confirms the prognostic significance of early PET in HL pts receiving IGEV as salvage chemotherapy. BEACOPP/escBEACOPP seems effective in inducing PET4 neg in early PET2 pos pts; however relapse rate even after HDC remains substantial and independent from the achievement of PET4 neg. Therefore new experimental approaches are needed in HL pts showing early PET2 pos during salvage.

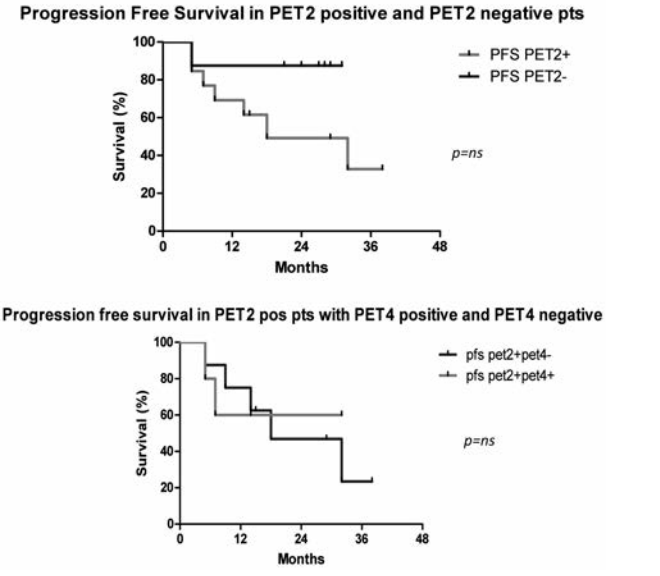

Figure 1.

CO046

ROUTINE BONE MARROW EVALUATION IN NEWLY DIAGNOSED HODGKIN LYMPHOMAS STAGED WITH PET/CT: IS IT REALLY NECESSARY? EXPERIENCE OF TWO ITALIAN HEMATOLOGICAL CENTERS

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In a recent retrospective study published by El-Galaly *et al* (JCO, 2012), 454 patients (pts) with a new diagnosis of classical Hodgkin Lymphoma (cHL) were staged with PET/CT. In this cohort, no positive bone marrow biopsies (BMB) were observed in pts in stage I to II according to PET/CT; moreover, neither in limited nor in advanced stages, a positive BMB caused a modification of treatment planned. In order to confirm these observations, we retrospectively analyzed data from pts with newly diagnosed cHL referring to Hematology Divisions of Florence and Udine University Hospitals since 2006 to 2012. All pts underwent to both unilateral BMB and PET/CT; stage and risk assessment were defined according to the Ann Arbor classification and the German Hodgkin Study Group (GHSG) criteria, respectively. Stage and risk group obtained with PET/CT alone were compared to those resulting from PET/CT combined to BMB. In this survey we included 212 pts, median age 33 (range, 14-71 years); 116/212 pts were male (55%); 36/212 pts (17%) presented one ore more focal skeletal lesions at PET/TC and 7/212 pts (3%) had a positive BMB; other patients characteristics are summarized in Table 1.

Table 1.

Staging, risk and skeletal lesions according to PET/CT and BMB

	negative BMB (n=204)		positive BMB (n=7)	
	N	%	N	%
Ann Arbor according to PET/CT				
I	10	5	0	0
II	107	52	0	0
III	51	25	2	29
IV	37	18	5	71
GSHG risk group				
Early	37	18		
Intermediate	72	35		
Advanced	96	47	7	100
Focal skeletal PET/CT lesions				
Unifolcal	10	5	2	29
Bifocal	6	3	1	13
Multifocal	15	7	2	29
No focal lesion	174	85	2	29
Homogeneous diffuse skeletal FDG uptake	8	4	0	0

BMB did not upstaged any patient who resulted in stage I-II according to PET/CT, and in none of the 212 pts BMB modified the therapeutic approach initially planned on the basis of PET/CT. In 2/212 pts with a PET/CT negative for skeletal lesions BMB was positive, causing an upstaging from stage III to stage IV. Focal skeletal lesions at PET/CT had a sensitivity and specificity of 85% each to detect a positive or a negative BMB. The positive (PPV) and negative predictive value (NPV) of focal skeletal PET/CT lesions to detect a positive BMB were 17% and 99%, respectively. Concluding, consistently to data previous reported: a) we did not registered any positive BMB in stages I-II according to PET/CT; b) in patients with a PET/CT staging we did not observed any influence of BMB on the planning of treatment; c) we remark the very high NPV (99%) of PET/CT for bone marrow involvement. On these grounds, the role of BMB in cHL patients staged with PET/CT seems questionable.If it is time to exclude BMB form staging of HD will be evaluated in further future studies.

CO047

INTERIM RESULTS OF IIL-HD0801 STUDY ON EARLY SALVAGE WITH HIGH-DOSE CHEMOTHERAPY AND STEM CELL TRANSPLANTATION IN ADVANCED STAGE HODGKIN'S LYMPHOMA PATIENTS WITH POSITIVE POSITRON EMISSION TOMOGRAPHY AFTER TWO COURSES OF CHEMOTHERAPY

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A prospective multicenter study on early salvage with high-dose chemotherapy and autologous stem cell transplantation (ASCT) in advanced stage Hodgkin's lymphoma (HL) patients with positive positron emission tomography (PET-2 positive) after two courses of doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD) and on comparison of radiotherapy versus no radiotherapy in PET-2 negative patients in complete remission after 4 additional chemotherapy courses is ongoing. At the time of interim analysis 417 patients were evaluable. In particular the focus was on PET-2 positive patients and on their outcome after salvage approach. PET-2 positive patients were scheduled for 4 courses of ifosfamide, gemcitabine, vinorelbine and prednisolone (IGEV) chemotherapy. After IGEV, a second PET evaluation was carried out: PET-IGEV negative patients received high-dose BEAM chemotherapy followed by ASCT, PET-IGEV positive patients received high-dose chemotherapy followed by two ASCT or one ASCT and one allogeneic stem cell transplant depending on donor availability. PET-2 positive (n=81) and PET-2 negative (n=336) patients didn't differ for baseline characteristics. Baseline characteristics of PET-2 positive patients were: 42 (52%) males, median age was 31 years, 73% (n=59) nodular sclerosis HL, 42 (52%) stage IV and 36% (n=29) bulky. 55 PET-2 positive patients were evaluable after 4-IGEV courses: 32 (58.2%) obtained a negative PET and underwent ASCT. 26 patients were restaged after ASCT, with 24 (92.3%) patients having a final negative PET, while only two having a positive PET. 23 (41.8%) patients were PET positive after IGEV: 6 went out of therapy and the others are ongoing. At the time of analysis median time of follow up was 19 months. Updated results will be presented at the meeting. These preliminary results showed that patients resistant to the initial treatment for residual PET-positive masses after the first two course of ABVD can be salvaged by early shift to high-dose chemotherapy supported by stem cell rescue.

C0048

IMMUNOSUPPRESSIVE PROPERTIES OF NEUTROPHILS IN HODGKIN`S LYMPHOMA

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Background. In Hodgkin Lymphoma (HL) elevated neutrophil count is a well recognized negative prognostic factor but its biological meaning is not elucidated. Material and Methods. In neutrophils (N) obtained from 15 HL patients we tested phagocytic activity, enzymatic activity of arginase (ARG-1), expression of ARG-1 and pro-angiogenic factor PROK-2, and suppression of healthy T-lymphocytes activation in co-culture experiments. Amount of ARG+ cells was also evaluated in HL lymphonodes. Results. We observed an increase of ARG-1 expression in N-HL up to 100 folds and of PROK-2 up to 36 folds compared to healthy subjects matched for age and sex (p=0.001), independently from tumor load and other well-known prognostic factors, including sex, anemia, stage, bulky disease and IPS. In the lymphonodes, ARG-1 evaluated in immunohistochemistry showed a granular pattern distribution in lack of overlapping with CD68+ staining. N-HL exhibited a reduced phagocytosis (93.2±1.9 % *vs* 73.1±3.7, p=0.0008) and an increased arginase activity up to 15 times compared to healthy subjects matched for age and sex. Finally, we co-cultured lymphocytes isolated from healthy subjects (h-Ly) with neutrophils isolated from fresh peripheral blood of HL patients (HL-N) or healthy subjects (h-Ne) and we evaluated markers of activation after stimulation with PHA-P at different time-points. After PHA-P stimulation, CD69 and CD25 were increased in h-Ly from 6 to 24 hours with peak at 24 hours and declining thereafter. CD71 increased slowly from 6 to 24, 48 and 72 hours, while HLA-DR maintained low expression with increase at 72 hours. Expression of these activation markers was down-regulated by co-culture of h-Ly with HL-N at ratio 1:4 and 1:8 at all tested time-points. Conclusion. Taken together our findings indicate that in HL patients, neutrophils are dysfunctional and may have a role in the immuno paresis that characterizes HL.

Autologous Transplantation

C0049

PROGNOSTIC VALUE OF FDG-PET IN RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA PATIENTS TREATED WITH HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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High-dose chemotherapy followed by autologous stem cell transplant (ASCT) is the standard treatment for patients with relapsed/refractory Hodgkin's lymphoma (HD). Aim of this study was to determine the prognostic value of FDG-PET scan in predicting the outcome of patients with poor risk HD undergoing ASCT. Data were retrospectively reviewed in 83 poor risk HD patients with a median age of 29 years (12-61) consecutively treated with ASCT at our Institution from 2002 to 2012. The M/F ratio was 45/38. Poor risk disease was defined as sensitive relapse (23 cases), response to 2nd line treatment (28 cases), more advanced disease (>2 lines of treatment, refractory relapse) (32 cases). The median number of prior chemotherapy lines was 3 (2-4). Bulky disease at diagnosis was present in 53% (44/83) of patients, extranodal disease at baseline or at relapse was recorded in 33% of cases (28/83). Pre-ASCT PET/TC was positive in 36 patients, negative in 47. Within the latter group, 19/47 patients had measurable disease ≥4cm. The conditioning regimens have varied during the years. BEAM was employed until 2011 (74 patients); thereafter, Carmustine was replaced by Bendamustine 400 mg/m^2 (3 patients) and by Fotemustine 300 mg/m^2 (6 patients).The pre-ASCT PET/TC was correlated with progression-free survival (PFS) and overall survival (OS) using the Kaplan-Meier method, focusing in particular on the prognostic significance of the presence of measurable disease pre-ASCT in PET-negative patients. The two-year projected OS of the whole population was 87% (95% CI: 78-97) and the pre-ASCT PET evaluation had no influence on OS (p:0.2). The two-year projected PFS was 73% overall (95% CI: 63-83). Pre-ASCT PET negativity was the only factor influencing PFS both in univariate (85% *vs* 58%; p:0.002) and in multivariate analysis including in the model disease status, lines of treatment, bulky disease and extranodal involvement as covariates. Within PET-negative patients, the presence of residual measurable disease was not associated with a worse outcome (p:0.71). In our series, a pre-ASCT negative FDG-PET identified patients with a better outcome probability and was a useful prognostic factor, independently of the disease status prior to ASCT. A positive pre-ASCT PET identifies a patient population with a more unfavorable prognosis. Future strategies will need to include for such patients more intensive approaches such as allogeneic stem cell transplantation and monoclonal antibodies.

C0050

AT HOME MANAGEMENT OF APLASTIC PHASE FOLLOWING HIGH-DOSE MELPHALAN (200 MG/M^2) WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS FOR MULTIPLE MYELOMA

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Aim of the Study is evaluate the appropriate use of health care resources to reduce costs and waiting lists associated with autologous (Auto) hematopoietic progenitor cell transplantation (HPCT). We report the results of an outpatient (OUT), home-treatment program for multiple myeloma patients (pts) receiving high-dose melphalan,HDM(200 mg/mq) and HPCT. Pts and Methods Eligible pts received treatment with a single or double course of HDM followed by Auto-HPCT. The home care (HC) cohort consisted of pts who were discharged the day after HPCT and then received all subsequent supportive care at home, without any check-up in hospital, with the assistance of specialized HC transplant professionals. In case of pts whose travel time to hospital exceeded 45 minutes, refusal of OUT treatment or lack of available caregivers, they were included in OUT or inpatient (IN) cohort, respective-

ly. OUT pts were discharged the day after HPCT, but supportive care was delivered daily in the outpatient clinic. OUT cohort was provided with free-of-charge suites equipped with emergency phone by the Associazione Italiana contro le Leucemie(CASAIL). In IN cohort, the supportive care designed for at home use was administered in hospital until neutrophil recovery to 0.5 x 10(9)/l. Results. 58 pts were treated with 84 cycles of HDM (33, 17 and 8 pts and 44, 25 and 15 courses in the IN, OUT and HC cohorts respectively. IN cases were older than OUT and HC ones but were comparable for disease status. Granulocyte recovery time was similar between the study groups, while a lower number of stem cells were infused in OUT cohort. There was no difference in platelet engraftment. The number of episodes and duration of grade III-IV mucositis were similar in all groups. Fever occurred in fewer OUT and HC cases and a significance statistical difference was observed in median days of fever >38C° and median days on broad spectrum antibiotics in these cohorts. In pts not hospitalized, readmissions during aplastic phase were uncommon (8% in OUT and 13 HC group, respectively). No deaths or unexpected emergencies occurred in the OUT or HC cohort. Conclusions:At home management in the aplastic phase after HDM and HPCT by community-based professionals is feasible without signs of increased toxicity . There is a striking reduction in the incidence of fever, days of fever and the use of antibiotics in pts not hospitalized. The study was conducted with the partial financial support of AIL Sezione A. Neri RC

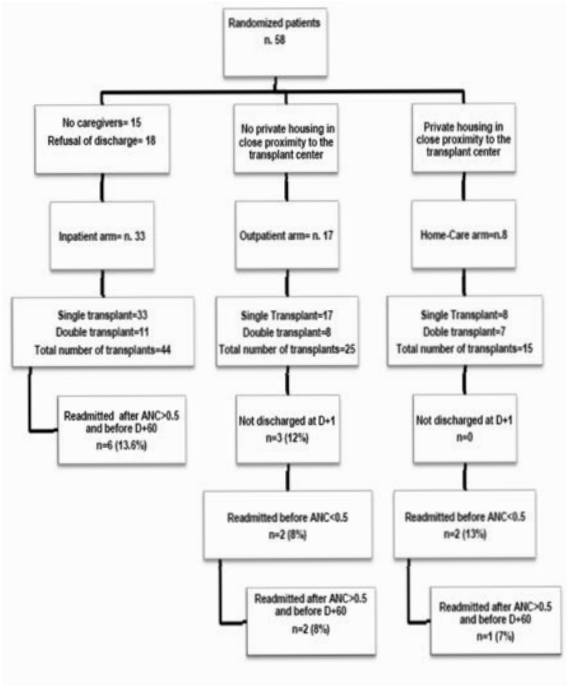

Figure 1. Patients randomization and results flow-chart

CO051

BENDAMUSTINE, ETOPOSIDE, CYTARABINE AND MELPHALAN (BEEAM) FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION INDUCE LONG-LASTING COMPLETE REMISSIONS IN A HIGH PROPORTION OF RESISTANT/RELAPSED LYMPHOMA PATIENTS: 32 MONTHS FOLLOW-UP UPDATE

Visani G,[1] Stefani PM,[2] Capria S,[3] Malerba L,[1] Galieni P,[4] Gaudio F,[5] Specchia G,[5] Meloni G,[3] Gherlinzoni F,[2] Giardini C,[1] Falcioni S,[4] Cuberli F,[6] Gobbi M,[6] Sarina B,[7] Santoro A,[7] Ferrara F,[8] Rocchi M,[9] Picardi P,[1] Guiducci B,[1] Ricciardi T,[1] Loscocco F,[1] Isidori A[1]

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We previously demonstrated the safety of a new conditioning regimen with bendamustine, etoposide, cytarabine, and melphalan (BeEAM) prior to autologous stem cell transplant (ASCT) in resistant/relapsed lymphoma patients (EUDRACTnumber2008-002736-15). However, median follow-up for surviving patients was short (18 months) at the time of publication. Therefore, it was not possible to draw final conclusion on the efficacy of the BeEAM regimen. For this reason, we evaluated the efficacy of the BeEAM regimen in terms of disease-free (DFS) and overall survival (OS) after a median follow-up of 32 months. Forty-three patients (median age 47 years, range 18-70) with resistant (21) or relapsed (22) NHL (28) or Hodgkin lymphoma (HL, 15) were consecutively enrolled in the study. At transplant, 14/43 patients (34%) were in CR, 22/43 (50%) were in partial response (PR) and 7/43 (16%) were either resistant or in progression. At the time of publication, after a median follow-up of 18 months, 35/43 patients (81%) were in CR. Disease type (NHL versus HL) and disease status at transplant (chemosensitive versus chemoresistant) were the only statistically significant variables influencing PFS (p=0.01;p=0.007). Disease status at transplant (chemosensitive versus chemoresistant) had a significant impact also on OS (p=0.004). We updated the follow-up at 32 months after transplant. Thirty-one out of 43 patients are still in CR (72%), as documented by both PET and CT scan. Two patients with HL were refractory and rapidly died, whereas 10/43 patients (23%) relapsed after a median time of 7.5 months (range:3-35) from transplant. Five patients died (3 NHL, 2 HL), whereas 5 patients are still alive after relapse. Median PFS and OS were still not reached. Interestingly, disease type at transplant is no longer influencing PFS (p=0.4), and still does not influence OS (p=0.3). On the other hand, disease status at transplant (chemosensitive *vs* chemoresistant) is still a strong predictor of both PFS and OS (p=0.03 and p=0.04, respectively). At present, one patient developed myelodisplasia after transplant. No other late effects were observed up to now. The new BeEAM regimen confirms its efficacy, even after 32 months of follow-up from transplant. Interestingly, the statistical difference between NHL and HL patients in terms of both PFS and OS was not confirmed in the long run. Acknowledgements: supported in part by AIL Pesaro Onlus.

CO052

SEQUENTIAL ALTERNATING R-CHOP AND R-FM CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION RESULTS IN HIGH RATES OF LONG TERM REMISSION IN ADVANCED FOLLICULAR LYMPHOMA

Gandolfi L, Derenzini E, Broccoli A, Casadei B, Pellegrini C, Stefoni V, Quirini F, Tschon M, Papadopulos F, Narducci R, Stefani G, Maglie R, Argnani L, Zinzani PL

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Autologous stem cell transplantation (ASCT) is a potentially curative treatment option for relapsed Follicular Lymphoma (FL) patients, but to date available data do not support the use of ASCT as first line consolidation, given the lack of overall survival (OS) advantage compared to standard therapy. R-CHOP (Rituximab-Cyclophosphamide, Vincristine, Doxorubicin, Prednisone) and R-FM (Rituximab, Fludarabine, Mitoxantrone), have comparable efficacy and are widely used as first and second line combinations. The best way to sequence the available therapies

in FL is still undefined. Here we show the long term results of a phase II trial of sequential chemotherapy alternating CHOP and FM plus Rituximab followed by ASCT in patients with stage III-IV and/or bulky FL either at disease onset or first relapse, conducted in our Institution from 2002 to 2008. Patients at diagnosis or first relapse were treated in sequence with R-CHOP for 4 cycles, Endoxan 7g/m^2 followed by hematopoietic stem cell harvest, R-FM for 4 cycles and ASCT. The ASCT conditioning schedule was BEAM (BCNU, ARA-C, Etoposide, Melphalan). 24 patients were enrolled, 12 pts were male. Median age was 44 years. One patient did not undergo ASCT for insufficient left ventricular ejection fraction and was excluded from the analysis. 13 patients were treated upfront whereas 10 patients at first relapse. After a median follow-up of 10 years, progression free survival (PFS) and OS in the whole study cohort were respectively 65% and 87%, with a complete response (CR) rate after the completion of sequential treatment of 100%. PFS and OS for patients treated at disease relapse were 60% and 70% (4 relapses, 3 deaths) respectively. Remarkably PFS and OS for the 13 patients treated upfront were 70% and 100% (4 relapses) respectively. To date, no secondary malignancies were observed. Sequential treatment alternating standard R-CHOP and R-FM followed by ASCT results in impressive long term PFS and OS, both in first line and at relapse. These data represent the proof of principle of a sequential therapy containing alternating alkilating agents and purine analogs followed by ASCT in FL.

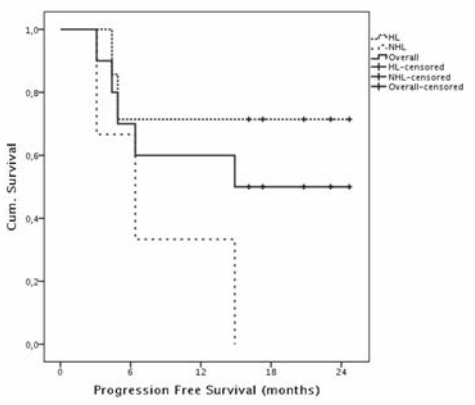

Figure 1. Progression Free Survival

CO053

TOTAL LYMPHOID IRRADIATION (TLI) AND HIGH-DOSE MELPHALAN WITH AUTOLOGOUS HEMATOPOIETIC STEM-CELL TRANSPLANTATION (AHSCT) FOR RELAPSED AND REFRACTORY LYMPHOMA

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Background. For patients with relapsed/refractory Hodgkin's disease (HD) and non-Hodgkin lymphoma (NHL), salvage chemotherapy followed by aHSCT is the standard of care. Excellent outcomes in HD using accelerated hyperfractionated TLI followed by aHSCT have been reported (ASH Annual Meeting Abs 2012 120: 2024). Helical tomotherapy is a novel rotational technique which delivers highly conformal radiation dose with great sparing of critical organs. We report on a phase I/II trial testing TLI in patients with advanced NHL and HD eligible to aHSCT. Patients and methods: From February 2011 to March 2012, 10 pts with relapsed/refractory HD (n=7), Diffuse Large (n=2) and follicular B-Cell NHL (n=1) were treated. Median age was 41 years (20-61), median number of prior therapies was 3 (2-4) and 4 pts had received prior ABMT. Salvage chemotherapy was chosen by treating physician. All pts had chemosensitive disease with complete remission (CR) in 6 patients (HD=5, NHL= 1), and partial remission (PR) in 4. Conditioning consisted of melphalan (140 mg/sqm) associated to daily hypofractionated TLI at 400 cGy for 3 consecutive days. Total dose of 1200 cGy, biologically equivalent to 1600 cGy in classical daily fractionation, was delivered to all nodal sites. In 3 pts, radiation dose was increased with a simultaneous integrated boost over the region of residual disease. Results. Median number of CD34+ cells infused was 5,5 x 106/kg (2,1 - 10,0). All pts engrafted with median time to ANC and PLT recovery of 13 (9-21) and 12 days (9-21), respectively . Median follow-up was of 19,2 months (7.3–26.1). The 2 HD pts in PR before transplant achieved CR. No treatment-related death occurred, 5 pts (50%) developed FUO, and 3 had grade 3/4 mucositis. None experienced grade 3/4 of extra-hematologic toxicity. Overall, the 2-year PFS and OS were 50% and 90%, respectively, whereas in HD the 2-year PFS and OS were 71% and 100% (fig.1). Relapse occurred in 5 pts (HD=2 and NHL=3) at a median time of 4.9 months and 1 NHL died of disease at 7,3 months after aHSCT. Conclusions: Our results show that hypofractionated TLI with tomotherapy in association to high-dose melphalan is safe and feasible in advanced lymphomas. In terms of clinical efficacy, the small number of patients limits the interpretation. However, survival curves seem to be quite encouraging and larger prospective study with TLI as part of the conditioning in patients with refractory/relapsed HD should be offered.

CO054

COLLECTION OF HEMATOPOIETIC STEM CELLS AFTER PREVIOUS RADIOIMMUNOTHERAPY IS FEASIBLE AND DOES NOT IMPAIR ENGRAFTMENT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN FOLLICULAR LYMPHOMA

Casadei B, Derenzini E, Broccoli A, Pellegrini C, Stefoni V, Gandolfi L, Quirini F, Tschon M, Papadopulos F, Narducci R, Stefani G, Maglie R, Argnani L, Zinzani PL

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Major concerns about radioimmunotherapy (RIT) administration early in the course of follicular lymphoma (FL) therapy are the long term toxicity and the theoretical impairment of hematopoietic stem cell (HSC) harvest, but few data are available about mobilization rates after RIT. On the other hand, autologous stem cell transplantation (ASCT) is a suitable treatment option for relapsed FL patients. The present study evaluates the impact of prior therapy with RIT (Yttrium-90 Ibritumomab Tiuxetan) and different chemotherapy regimens in all FL patients (n=103) attempting HSC mobilization at our Institution in the last 7-years period from January 2005 to March 2012. Sixty-nine patients had received R-CHOP (Rituximab-Cyclophosphamide-Doxorubicin-Vincristine-Prednisone) or CHOP-like regimens, 21 patients R-FM (Rituximab-Fludarabine-Mitoxantrone), 13 patients RIT with Yttrium 90-Ibritumomab Tiuxetan before HSC mobilization. All pts received chemotherapy plus granulocyte colony stimulating factor (G-CSF) 5 microgr/kg (n=95) or G-CSF alone (10 microgr/kg) (n=8) as mobilization regimen. Median CD34+ cells yield at first mobilization was 7.2x10^6/kg in the R-CHOP group, *vs* 4.3 in the R-FM group, *vs* 1.7 in the RIT group (p=0.02 R-CHOP *vs* R-FM; p<0.0001 R-CHOP *vs* RIT; p<0.02 R-FM *vs* RIT). 62 pts had only one prior treatment, 32 pts had 2, 9 pts had 3. The number of previous treatments did not significantly affect the amount of PBSC collected. Although 8/13 patients initially failed to collect enough HSC after RIT, a second and/or salvage harvest was succesfully performed in 7 patients, with 10/13 patients (77%) finally undergoing autologous stem cell transplantation (ASCT). The activity of the CXCR4 inhibitor Plerixafor, which was used with G-CSF in 3 cases, was particularly promising, allowing 2 patients to collect ≥ 2x10^6 CD 34+ cells/Kg. No differences in engraftment kinetics were observed between the three groups. Although mobilization was significantly impaired in patients previously treated with RIT, a salvage HSC harvest and ASCT after RIT were safe and feasible in the majority of patients.

Infections

CO055

USE OF 1,3-BETA-D-GLUCAN FOR THE DIAGNOSIS OF PNEUMOCYSTIS PNEUMONIA IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Introduction Pneumocystis pneumonia (PCP) remains an important cause of interstitial pneumonia in allogeneic hematopoietic stem cell transplant (HSCT) recipients, particularly in those who discontinued or could not tolerate the prophylaxis. Early diagnosis and treatment are important to order to reduce morbidity and mortality. 1,3-beta-D-glucan (BG) is a serum diagnostic assay, which is positive in most invasive fungal infections, including PCP, with the exception of Zygomycetes and Cryptococcus. The aim of this study was to assess the performance of BG, and to compare it with qualitative real time PCR for P. jirovecii in bronchoalveolar lavage (BAL) fluid, for the diagnosis PCP in HSCT recipients. Patients and methods Overall, 61 consecutive BAL samples performed from January 2009 to January 2012 in HSCT recipients from our center were analyzed. PCP was defined clinically as the presence of acute progressive dyspnea; bilateral, symmetric, fine reticular interstitial infiltrates on CT scan images; clinical response to targeted treatment (trimetoprim-sulphametoxazole) or death in case of non-treatment and the absence of other documented infection compatible with the clinical presentation. Colonization was defined as detection of Pneumocystis jirovecii by PCR but without clinic criteria of PCP. BG was performed with Fungitell assay, with the cut-off for positivity of 80 pg/ml. Patients who did not perform PCR for P. jirovecii or BG were excluded from the study. The comparisons were performed with Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables; two-tailed p was considered significant if <0.05). Results Seven of 61 patients were diagnosed with PCP (11%) and PCP related mortality was 40%. Four patients developed PCP after 1 year from HSCT, 1 patient after 2 months, while 2 had an early-onset PCP (within 3 weeks after HSCT). The results of BG and PCR testing are shown in Table 1. The sensitivity, specificity, positive and negative predictive value were, respectively, for PCR: 57%, 91%, 44%, 94%; for BG: 57%, 83%, 31%, 94%; for PCR and BG: 44%, 99%, 87%, 92%. Conclusions Combined BG and PCR in BAL fluid have an 87% positive and a 92% negative predictive value for a diagnosis of PCP, and should be used to confirm or exclude the diagnosis with a high level of probability.

Table 1. The results of serum 1,3-beta-D-glucan (BG) and PCR for *P. jirovecic* in bronchoalveolar lavage (BAL) in patients with and without Pneumocystis pneumonia (PCP)

	Patients with PCP, n=7	Patients with no PCP, n=54	p
BG value (pg/ml), median (range)	152 (<7 - 332)	<7 (<7 - 312)	0.023
Positive BG	4 (57%)	9 (17%)	0.043
Positive BG and PCR	3 (43%)	0	0.001
Positive PCR for Pneumocystis	4 (57%)	5 (9%)	0.007
Negative BG and PCR	2	40	0.008

CO056

AUTOPSY ANALYSIS ON EPIDEMIOLOGY AND SITE OF INVOLVMENT OF INVASIVE FUNGAL INFECTIONS (IFI) IN HEMATOLOGICAL MALIGNANCIES :A RETROSPECTIVE STUDY AT HEMATOLOGIC TERTIARY CARE DEPARTEMENT

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Background. Clinical diagnosis of IFI is difficult ,due to lack of sensitive and specific diagnostic tools. An assessment of trends concerning the prevalence of IFI is a challenge and postmortem data may be useful to monitor the local epidemiology ,the frequency and the disease patterns. AIM :The aim of this retrospective analysis is to determinate the local epidemiology and the prevalence at autopsy of IFI, occurring in hematological malignancies at a single center over a eleven years period. METHODS : We have retrospectively reviewed 161 patients – median age 62,5 yrs, range 22-83 - with hematological malignancies ,who underwent autopsy between 2002 -2012. Acute Myeloid Leukemia (AML) were 77, Acute Lymphoid Leukemia (ALL) 11, Lymphoproliferative disorders (LPD) 56 and other disorders 17. Acute leukemia pts received systemic antifungal prophilaxis, whereas the others not absorbable prophilaxis. None patients received transplant procedures. An experienced pathologist evaluated the organ involvement and the IFI pathologic pattern. Fisher's Exact test was used to recognize the IFI prevalence, the main occurring pathogens and the involved site; a p-value of <0.05 was considered statistically significant. Results. The analysis of 161 consecutive autopsies identified 40 pts.(25%)resulting to have IFI; of these, 22 were AML (55%) ,6 ALL (15%),11LPD (28%) and 1 other. Aspergillus spp. infection was detected in 20 cases (50%), Mucor spp in 8 (20%) and Candida spp. in 12 (30%). Moulds were prevalent in acute leukemia pts. and Aspergillus spp. is the leading pathogen with respect to Candida and Mucor spp. (p 0,0396),with a statistically significant prevalence in ALL (p 0,0186).The site more involved resulted lung (p 0.0002). Whereas the standardized EORTC/MSG criteria applied *in vivo* were conclusive for IFI in 6 pts (15%) only, the postmortem findings revealed fungal infections in 34 pts (85%).Conclusions. This analysis confirms that the IFI diagnosis is still an unresolved issue in hematological malignancies. Acute leukemias remain the subset with the higher prevalence of mould infections. As in other largest studies, in our experience Aspergillus spp and lung proved to be the most recurrent pathogen and site of involvement. At now, the diagnostic methods are not still completely able to identify the underlying IFI, thus the autopsy rate should be increased to achieve a better knowledge of epidemiology and to critically review previous misdiagnosis.

CO057

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

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Background/Aims. The risk of developing an invasive fungal disease (IFD) depends on multiple factors, including hematological malignancy, environment, genetic pattern, immune status. The role hospital independent exposure is not known. Aim of the study was to investigate the potential relationship between pre-hospital exposures to fungal sources and (IFDs) in adult acute myeloid leukemia patients (AMLs). Methods. From January 2010 to April 2012, all consecutive patients (pts) with newly diagnosed AMLs were registered in 31 Italian participating centers. Information about personal habits and possible environmental exposures were investigated. We collected data about comorbidities, job, hygienic habits, work and living environment, voluptuary habits (*i.e.*smoking, alcohol, illicit substances abuse), hobbies, pets. Other well-known post-therapy risk factors were analysed (*i.e.* neutropenia, mucosal damages). We focuses on pts treated with conventional chemotherapy only, in order to make our study population more homogeneous. All IFDs occurring until the 30th day after therapy were recorded. .Results. 1,192 pts were enrolled in the study; of them, 887 received intensive chemotherapy and were included in the present analysis. 214 (24%) developed an IFD; proven/probable cases were 73 (incid.8.2%): 53 molds (IMI), 20 yeasts. At univariate analysis we found a significant association between IMI and age (p 0.01), performance status (PS) (p<0.001), diabetes (0.005), COPD (0.005), smoking (0.02), cocaine (0.006), type of job (0.01) or hobby (0.01), body weight (0.04), home restructuring (<0.001). As for post-treatment variables, an association was found for urinary catheter (<0.0001), esophagitis (0.007), posaconazole prophylaxis (0.001). Multivariate analysis confirmed PS, COPD, job, body weight, home restructuring, esophagitis and posaconazole to be significant for IMI. No pre-hospital variables resulted to be correlated with yeasts, while CVC (0.03), esophagitis (<0.001), urinary catheter (<0.001), posaconazole (<0.001) emerged both at uni and multivariate analysis. Conclusions. Several hospital-independent variables emerged as potentially influencing IMI onset. Assessing the presence of these factors at time of admission may be helpful in defining patient' risk category and in better targeting prophylaxis, intensive monitoring and early treatment.

CO058

PERIPHERALLY INSERTED CENTRAL CATHETERS (PICCS) CAN BE SUCCESSFULLY UTILIZED IN HAEMATOLOGICAL PATIENTS RECEIVING INTENSIVE CHEMOTHERAPY OR ALLOGENEIC/AUTOLOGOUS STEM CELL TRANSPLANTATION

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Patients with haematological disorders frequently require the insertion of central venous catheters (CVCs); actually, little data exist on their use for intensive chemotherapy and blood progenitor cell transplant. Methods. Evidence-based interventions were implemented in our department from November 2009 to July 2012: 1.An high level nurse education program; 2) The use of ultrasound guide for the insertion of the tip of PICCs 3)Bedside placement and confirmed PICC tip placement by chest radiography; 4)Maintenance of maximum sterile barrier precautions; 5)chlorhexidine preparation, replace 10% povidone iodine for skin antisepsis; 6)adoption of PICC patient nurse archive (weekly PICC line review for each patient). Aim. Here, we carried out a clinical prospective investigation to determine the efficacy of these interventions in reducing the rate of PICC-related complications in hematology patients receiving intensive chemotherapy compared to allogeneic/autologous stem cell transplant recipients. Results. Three hundred sixty-four (364) PICCs were in place in 299 patients for a total of 41.111 PICC days (range, 1-482 days; mean 112,94 days); 292 were inserted in patients receiving conventional chemotherapies, and 72 in patients undergoing allogeneic or autologous hematopoietic stem cell transplantation (SCT). Sixty-six (60) PICCs were inserted during severe thrombocytopenia (platelets <50x10^9/L), seventy (70) during severe neutropenia (neutrophils <0.5x10^9/L). The rate of major complication was very low: 15 thrombotic complications PICC-related (4%; 0.36 per 1,000 CVC days), and 3 CRBSI (0,8%; 0.07 per 1,000 CVC days). Interesting, lymphoma and leukemia patients have, respectively, an increased risk of developing a CRBSI and a thrombotic PICCs-complication when submitted to hematopoietic stem cell transplantation (SCT). However, compared with allogeneic/autologous stem cell transplant group, the intensive chemotherapy group was associated with a marginally lower incidence of CRBSI complication rate (0.6 % *vs* 1.0 %, 0.10 *vs* 0.60 per 1,000 CVC days) [odds ratio (OR) 2,042]; no relevant differences in terms of thrombotic complications between the two cohorts (4.11 % *vs* 4.17%), 0.29 *vs* 0.39 per 1,000 CVC days) [odds ratio (OR) 1.014]. Conclusions. Our findings suggest that PICC devices are a viable and safe option for management of the haematology patients receiving intensive chemotherapy such as patients receiving blood stem cell transplantation.

Table 1. Outcomes according to underlying disease and relative adhibition

	Chemotherapy		ASCT		Statistics						Odds Ratio	
Diagnosis	% Definite CRBSI	% Thrombosis	% Definite CRBSI	% Thrombosis	Definite CRBSI (%)	Definite CRBSI per 1000 PICC days	Thrombosis (%)	Thrombosis per 1000 PICC days	Range (days)	Means (days)	OR CRBSI	OR Thrombosis
Lymphoma (n. 182))	0,69	4,16	2.63	2,63	1,10	0.10	3,85	0,34	4-336	146,3	3,86	0,62
Acute Leukemia (n 120))	0.95	4.76	0	13.33	0,83	0.07	5,83	0,46	99-432	213.8	0	3.07
Others (n. 62)	0	2.32	0	0	0,00	0	1,61	0,19	6-287	79.2	0	0
Overall	0.68	4,11	1.39	4,17	0,82	0.07	4,12	0,36	0-482	112.94	2,042	1,014

CO059

BLOODSTREAM INFECTION CAUSED BY CARBAPENEMASE–PRODUCING KLEBSIELLA PNEUMONIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background/Aims: Increasing rates of different phenotypes of drug resistance among Gram-negative bacteria [*e.g.* extended-spectrum-ß-lactamases (ESBLs) producing Enterobacteriaceae and multidrug resistant isolates of P. aeruginosa] have been widely reported in several settings during the last decade, including the hematological malignancy setting (HM). As a consequence, carbapenems were largely used for both empirical and target antibiotic treatments. However, very recently Klebsiella pneumoniae (Kp) strains producing K. pneumoniae carbapenemases (KPCs) have caused many infection outbreaks in different Countries. Aim of the study was to evaluate the incidence and outcome of bloodstream infections caused by KPCs in our institution. Methods. A retrospective, single center study in HMs over 2009-2012. We reviewed the hospital records of all adult inpatients with HM who were diagnosed with gram negative bacteremia. Data collected from the hospital charts and the laboratory database included patient demographics, disease and disease stage at time of bacteremia, type of HSCT (autologous or allogenic), medical history, clinical/laboratory findings, and treatment and outcome of infection. Results. We registered a progressive increase of BSIs caused by KPC-Kp isolates in patients with HMs, while the number of BSIs due to Gram-negative strains remained unchanged (Table). The KPC-Kp BSIs-attributable mortality rate was 57.6%, despite the use of a combination of ≥2 antibiotics with *in vitro* activity against the KPC-Kp isolate in more than 50% of patients.

Table 1. Prevalence and attributable mortality for bloodstream infections caused by Klebsiella pneumoniae carbapenemase producing Klebsiella pneumoniae during the years 2009-2012

	2009	2010	2011	2012	Total
All BSI by Gram negative bacteria	30	39	41	37	147
Death	5 (16.6)	7 (17.9)	9 (24.3)	11 (29.7)	32 (21.7)
Non KPC producing Kp BSI	1	2	5	4	12
Death	0	0	1 (20)	1 (25)	2 (16.6)
KPC producing Kp BSI	0	1	13	12	26
Death	0	1 (100)	7 (53.8)	7 (58.3)	15 (57.6)

Abbreviations: BSI, Bloodstream infection; Kp, Klebsiella pneumoniae; KPC, Klebsiella pneumoniae carbapenemase

This mortality rate was almost three times greater than that for BSI caused by all Gram-negative bacteria (32/147, 21.7%). Surprisingly, a high proportion of KPC-Kp BSI cases occurred in low-risk patients, in terms of both underlying malignancy (*e.g.* those with diseases other than acute leukemia), and phase of treatment (*e.g.* autologous-HSCT recipients). The rates of non-susceptibility to colistin, tigecyclin, and gentamicin were 19.2%, 30.8%, and 34.6%, respectively. Conclusions: In endemic areas for KPC-Kp, early identification of patients likely to be colonized and/or infected by KPC-Kp strains represents an important step in prevention and containment of their spread among hospitalized patients. Current policies on the empirical treatment might need to be revised, according to the possibility of serious infections caused by carbapenem-resistant Enterobacteriaceae.

CO060

HUMAN HERPES VIRUS-6 (HHV-6) REACTIVATION AND DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. HHV-6 is increasingly recognized as an opportunistic and potentially life-threatening pathogen in recipients of allogeneic Hematopoietic Stem Cell Transplantation (AlloSCT). Reported clinical manifestations of HHV-6 infection in transplanted patients are skin rash, interstitial pneumonia, bone marrow suppression and encephalitis. Moreover, some clinical reports suggest that HHV-6 can facilitate the occurrence of severe clinical complications of AlloSCT, increasing transplant-related mortality. Methods. From January 2009 to February 2013, we retrospectively evaluated 54 consecutive adult patients (median age 50 years) who developed positivity to HHV-6 after AlloSCT for high-risk hematological malignancies. Stem cell donors were family haploidentical (37), HLA identical sibling (8), unrelated volunteer (6), cord blood (3). The viral load was determined by quantitative PCR in cell-free body fluids such as plasma, bronchoalveolar lavage (BAL), cerebrospinal fluid (CSF), bone marrow (BM) aspirates or in gastrointestinal biopsies. Results. Median time from AlloSCT to HHV-6 reactivation was 34 days (range: 0-705). In plasma HHV-6 was detected in 31 pts, 9 in BM, 33 in gut biopsies or BAL, 7 in CSF. All pts were receiving acyclovir as viral prophylaxis except 5. Twenty-nine pts had acute GvHD (grade III-IV in 22) requiring high dose steroids in 26 cases; a concomitant CMV positivity was detected in 15 pts. The median absolute count of CD3+ lymphocytes was 262 cells/mcl. In 52 cases we reported HHV-6 clinical manifestations: fever (43), skin rash (22), hepatitis (19), diarrhoea (24), encephalitis (10), BM suppression (18), delayed engraftment (11). HHV-6 positivity led to antiviral pharmacological treatment in 37 cases, using as first choice therapy foscarnet. Amongst the total 54 patients with documented HHV-6 positivity 31 solved the clinical event. However the mortality rate was relatively high in this population (only 30% of pts were alive). A better overall survival is significantly associated with CD3+ cells higher than 200/mcl and time after AlloSCT more than 2 months. Conclusions. HHV-6 reactivation is associated with high morbidity and mortality in patients after AlloSCT. The regular monitoring of HHV-6 DNA, using a real-time PCR assay, may be useful for identifying active HHV-6 infection and for the introduction of a pre-emptive treatment, possibly reducing the incidence of the most severe clinical complications.

Myeloproliferative Disorders

CO061

ANALYSIS OF METHYLOME IN CD34+ CELLS IN MYELOFIBROSIS

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Background. Recent evidence indicate that MPNs are characterized by aberrant transcriptional profiles that are largely ascribed to the dysregulated JAK2V617F kinase activity; however, mutations in several genes intervening in epigenetic gene regulation through histone modification and/or DNA methylation have been discovered in MPN. Patients and Method: We conducted genome-wide analysis of DNA methylation to compare the methylation profile in matched CD34+ peripheral blood (PB) and spleen (SP) derived cells, as well as granulocytes (GN) from the same patient (pt). We used the Illumina Infinium Human Methylation 450 BeadChip that allows to analyze more than 450,000 methylation sites, within and outside of CpG islands, including microRNA promoter regions. We studied 5 pts with myelofibrosis (3 PMF,1 PET-MF,1 PPV-MF) and we also included bone marrow CD34+ purified from 5 health subjects. Differential methylation analysis was performed using the IMA R package. Results. Results showed widespread alterations in DNA methylation in MF and discovered numerous significantly and uniformly hypomethylated loci compared to normal subjects. In the comparison between control and MF PB cells we found 1524 differentially methylated genes (674 hyper- and 850 hypo-methylated); considering SP cells, we found 3487 differentially methylated genes (1135 hyper- and 2352 hypo-methylated), and by comparing with GN we found 2343 differentially methylated genes (579 hyper- and 1764 hypo-methylated). We failed to observe significant differences in the methylation pattern between PB and SP CD34+ cells, suggesting a common origin source of these cells. We also found that 538 genes presented a superimposable methylated pattern among MF different cellular types, suggesting that these genes are intrinsically associated with the neoplastic clone and do not vary according to the source or differentiation state (CD34+*vs*GN). Among differentially methylated genes/miRNAs we found some hypermethylated such as DNTT and LY86 that we previously showed to be down regulated, and some hypomethylated such as TM4SF1, GAS2, LEPR, STAT4 and miR-16-2 already found overexpressed in GEP analysis. Conclusion. these preliminary results show epigenetic differences in PMF CD34+ *vs* normal cells and reveal a substantial reproducibility of methylomic signatures among different cell subtypes of the clone. The role of these abnormally methylated genes in disease pathogenesis is the topic on current investigations.

CO062

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

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Philadelphia-negative Myeloproliferative Neoplasms (MPN) include Polycythemia Vera (PV), Essental Thrombocythemia (ET) and Myelofibrosis, both Primary (PMF) and secondary to PV or ET (PPV-MF and PET-MF); a MPN is the underlying cause of portal vein thrombosis in around 30% and Budd Chiari syndrome in 40% of cases. In patients with MPN and splanchnic vein thrombosis, splenomegaly can arise as the consequence of both the hematological disease and blood flow abnormalities. On turn, splenomegaly and the compensatory enlarged splancnic vessels are responsible for several complications in particular esophageal and gastric varices. Furthermore, splenomegaly per se may be massive and mostly symptomatic. Current treatment strategy for MPN patients with high risk disease because of previous thrombosis include cytoreductive therapy, usually hydroxyurea, that may have little influence in the control of splenomegaly. New drugs like Ruxolitinib, capable of decreasing the spleen volume, can consequently reduce the local pressure in splancnic vessels, and produce both symptomatic improvement of splenomegaly-related symptoms and contribute to improve the blood flow abnormalities. We design a phase 2 study of Ruxolitinib in patients with splenomegaly due to an underlying MPN associated with splanchnic vein thrombosis with the primary goal of evaluate the proportion of subjects achieving ≥50% reduction in spleen length measured by palpation or ≥ 35% reduction in spleen volume by MRI or CT at week 24. The secondary objectives include the assessment of changes in splanchnic circulation, hyperdynamic arterial circulation and stiffnes of epathic/splenic parenchyma through Doppler analysis and evaluation of oesophageal varices before and after 24 week of treatment, evaluation of safety of Ruxolitinib and Quality of Life assessment. Exploratory objectives include evaluations of JAK2V617F or MPLW515 allelic burden, screening for known mutations at baseline and their association with response, evaluations of cytokine and microRNAs profiles and quantification of circulating endothelial cells as putative markers of response to treatment. At the time of abstract submission 5 patients have been enrolled out of 21, and two completed the planned six-month treatment period; we will present updated results at the SIE meeting.

CO063

DEFERASIROX IN THE TREATMENT OF IRON OVERLOAD DURING MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background. Deferasirox is an oral iron chelator widely employed in the treatment of iron overload during thalassemic syndromes and myelodysplastic syndromes. Aim At present, very few data are available on the treatment with deferasirox in patients with Ph- Myeloproliferative Neoplasms(MPN) and transfusional requirement. Methods. To address this issue, we report here on 20 patients (M/F 14/6) with MPN and iron overload secondary to transfusional requirement enrolled in the database of our regional cooperative group who received a treatment with deferasirox. Of them, 17 had a primary Myelofibrosis, 2 a post essential thrombocythemia myelofibrotic phase and 1 a post Polycythemia Vera myelofibrotic phase. Results. The main features of the patients at diagnosis and at baseline of deferasirox treatment are reported in the Table. Treatment with deferasirox was started after a median interval from transfusion requirement of 8.7 months (IR 5.7 – 16.4). Starting deferasirox dose was 1,500 mg/day in 8 patients, 1,250 mg/day in 4 patients, 1,000 mg/day in 7 patients and <1,000 mg/day in 1 patient. All patients were evaluable for toxicity: extra-hematological toxicity was reported in 12/20 patients and consisted of gastro-intestinal symptoms in 6 patients, renal impairment in 4 patients and skin reactions in 2 patients. A dose reduction/temporary discontinuation was needed in 11 cases, but no patient went off treatment due to toxicity. Eighteen patients were considered evaluable for response (≥6 months of treatment) and 2 were too early. As to the iron overload decrement, after a median treatment period of 14.4 months (IR 7.1 – 21.3) 2 patients achieved ferritin levels <500 ng/ml, 7 patients ferritin levels <1,000 ng/ml and 9 patients did not have any ferritin reduction. As to hematological improvement, 5/18 patients showed an unexpected and persistent rise of Hb levels >1.5 g/dl, with disappearance of transfusional requirement in 3 cases. Conclusions. Treatment with deferasirox is feasible and effective in MPN with iron overload. Moreover, also in this setting an hematological improvement can occur in a sizeable rate of patients.

Table 1. Clinical features of patients at diagnosis and at baseline of deferasirox treatment

	DIAGNOSIS	BASELINE
Median age, years (IR)	70.3 (64.3 – 74.1)	71.1 (67.6 – 74.8)
Median Hb, g/dl (IR)	8.3 (7.6 – 9.3)	7.7 (6.9 – 8.3)
Median ferritin, ng/ml (IR)	405 (172 – 887)	1,415 (1,197 – 1,690)
Median creatinine level, mg/ml (IR)	NR	1.0 (0.8 – 1.1)

CO064

REAL-WORLD CYTOREDUCTIVE TREATMENT PATTERNS FOR ESSENTIAL THROMBOCYTHAEMIA IN EUROPE: ANALYSIS OF 3643 PATIENTS IN THE EXELS STUDY

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Introduction. The Evaluation of Xagrid Efficacy and Long-term Safety (EXELS) study is an observational cohort study of essential thrombocythaemia (ET) in high-risk patients. The study is being conducted in 13 European countries and is sponsored by Shire Pharmaceutical Development Ltd. The objective of this analysis is to observe the disease characteristics and cytoreductive treatment patterns of ET in routine clinical practice across Europe. Methods. High-risk patients (>60 years of age; history of thrombosis; platelet count >1000x10^9/L) with ET who were receiving cytoreductive therapy at the time of study registration were enrolled. Cytoreductive treatment selection occurred prior to enrolment and patients were managed according to local practice. Data were collected every 6 months for 5 years using an electronic data capture system. Here we describe findings from a data-cut taken in September 2011, 2.5 years since the last patient was enrolled. Results. Approximately 70% of the patients were continuing in the study at this data-cut. In total, 3643 patients (61.3% females and 38.7% males), across a wide range of ages (<40 years, 6.9%; 40–59 years, 25.5%; ≥60 years, 67.6%) were enrolled into the study. At enrolment the majority of patients (80.6%) had been previously treated with a cytoreductive therapy. The two main cytoreductive treatments prescribed as monotherapy were hydroxycarbamide (65.1%) and anagrelide (22.2%). Other treatments included interferon, busulphan, pipobroman, 32P and hydroxycarbamide/anagrelide in combination. At enrolment, a greater proportion of patients receiving anagrelide were <60 years (59.3%), compared with those receiving hydroxycarbamide (19.3%). There was considerable variation in rates of treatment selection between countries both at enrolment (hydroxycarbamide 38–80%; anagrelide 10–51%; other 4–50%) and at the time of data-cut (hydroxycarbamide 33–75%; anagrelide 9–50%; other 6–50%). Conclusions. EXELS provides real-world evidence of the patterns of cytoreductive treatment used for high- risk patients with ET across Europe. Hydroxycarbamide was the most frequent treatment of choice in nearly all of the participating countries across Europe; however, patient age strongly influenced the choice between hydroxycarbamide (older) and anagrelide (younger) therapy. In general, the treatment pattern for ET observed in EXELS is in accordance with expert recommendations in Europe.

CO065

CHARACTERISATION OF DIFFERENT REGIMENS FOR INTRODUCING SECOND-LINE ANAGRELIDE: RESULTS FROM A MULTICENTRE STUDY OF 177 PATIENTS IN FRANCE

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Anagrelide (ANA) is indicated in the EU for at-risk patients (pts) with essential thrombocythaemia (ET) in whom prior therapy (PT) is not sufficiently effective or well tolerated. This study aimed to identify the switch modalities used when introducing ANA treatment and determine their influence on 6-month (mo) outcomes (efficacy, tolerability and maintenance). This Phase IV observational study (NCT01192347) was conducted in 44 clinical centres across France. High-risk pts (aged >60 years; history of thrombosis; platelet count >1000x10^9/L) with ET were enrolled within 1 mo of switching to ANA; relevant data were collected and recorded from pt records after the 6-mo follow-up. Overall, 177 pts were enrolled: 62% female, 76% aged >60 years, median baseline platelet count 553x10^9/L. Intolerance to therapy (65%) and inefficacy (41%) were the most frequent reasons for treatment switch (factors not mutually exclusive). The Summary of Product Characteristics (SPC)-recommended ANA starting dose (1 mg/day) was used most frequently (53%); a notable proportion of pts (41%) started on 0.5 mg/day and starting doses ranged from 0.3 to 1.5 mg/day. Median ANA dose at study end was 1.5 mg/day. The method of ANA introduction was consistent with the SPC in 76% of pts. Almost all pts switched to ANA from hydroxycarbamide (93%). Most pts discontinued PT before ANA was introduced (66%; Group A). 22% discontinued PT after introduction of ANA (Group B; 17% within the 1st mo [Subgroup B1] and 5% in the subsequent 5 mos [Subgroup B2]). A further 9% had not discontinued PT by the end of the follow-up (Group C) and 5 pts (3%) were determined to have no PT. At the end of the follow-up, 85% of pts were still continuing on ANA, Groups: A (82%), B1 (93%), B2 (100%), C (81%). 71% of pts achieved platelet responses, Groups: A (67%), B1 (83%), B2 (100%), C (56%); 42% full response (<400x10^9/L) and 29% partial response (400–600x10^9/L or a reduction of ≥200x10^9/L). Median final platelet count was 412x10^9/L; absolute median change from baseline was -94.5x10^9/L. 75% of pts who received ANA in line with the SPC achieved platelet response *vs* 54% (pts not in line with the SPC). 85% of pts remained on ANA at the end of the 6-mo follow-up. The most frequent adverse drug reactions were palpitations (13%) and headache (11%). This real-world evidence shows that introducing ANA according to the SPC, and subsequently discontinuing PT, was associated with the highest platelet response rates.

CO066

GENE EXPRESSION DRIVES INTERFERON TAILORED THERAPY IN ESSENTIAL THROMBOCYTHEMIA

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Background. Interferon alpha (IFN) is able to induce hematological response in about 75% of ET patients but some of them could be defined as bad responders. IFN binding its receptor results in tyrosine phosphorylation of the JAKs proteins, Tyk2 and Jak1, that activate STAT family members such as STAT1 and STAT3. These proteins induce the transcription of SOCSs, whose role is to extinguish cytokine signaling by inhibition of JAK activity. JAK-STAT-SOCS cascade allows IFN-α and TPO pathway to cross-talk. Aims. In order to identify molecular markers that discriminate responders from non-responders to IFN, we analyzed transcript levels of specific genes involved in the IFN pathway. In particular we investigated the mRNA expression of JAK1, TYK2, STAT1, STAT3, SOCS1 and SOCS3. Methods. We analyzed 60 ET patients treated with 3MU of IFN-α-2b 5 times a week as induction (3 months), and 3 times a week as maintenance. Two groups of response were identified: Good-Responders(R) (n=44), who achieved complete response according to European Leukemia. Net criteria, and Bad-Responders(NR) (n=17) who failed. The mRNA expression of genes of interest was measured in bone marrow samples from ET patients by RTq-PCR and tested for their predictive value using receiver operating characteristics (ROC) curves. Data were normalized as following: [mRNA normalized copy number (NCN)=mRNA target gene/mRNA GUSB]. An IFN score was calculated as an average in $\log^2$ of mRNA levels of genes differently expressed between R and NR. Results. Main clinical characteristics were similar between the two groups of response. JAK2 V617F mutation was detected in 56,8% of R and 58,8% of NR (p=0,81) and no difference was found in JAK2V617F allele burden (p=0,17) and mRNA expression (p=0,2). R compared with NR showed higher mRNA expression of JAK1 (13,4 *vs* 4,4; p<0.00001), STAT3 (4,9 *vs* 2,4; p=0.0002) and SOCS3 (1,8 *vs* 1,03; p=0,015). The AUC, using the normalized gene expression values, was 0.88 for JAK1, 0.81 for STAT3 and 0.7 for SOCS3. Average expression in $\log^2$ of these three genes was calculated and used as IFN score. The analysis reveled an AUC of 0.9 for this IFN signature (p<0,00001). The optimal cut-off point for IFN score to discriminate between R and NR was 15,75 with a sensitivity of 94,1%, specificity of 88,6% and likelihood ratio of 9. Conclusion. We identified this set of three genes whose expression could be translated into IFN score that showed a significant correlation with response in ET.

Cytogenetics and Molecular Genetics - Laboratory Investigation in Hematology

CO067

ANALYSIS OF MOLECULAR ABERRATIONS IN CHRONIC MYELOMONOCYTIC LEUKEMIA BY NEXT GENERATION SEQUENCING

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Chronic myelomonocytic leukemia (CMML) remains a diagnostic and therapeutic challenge due to its highly heterogeneous features, which vary from mainly dysplastic (MD) to predominantly proliferative (MP). DNA mutations and epigenetic alterations of genes involved in different cellular pathways have been identified in recent years, but their contribution to disease onset and evolution remains unclear. The observations that MP-CMML patients have a significantly shorter survival compared to those with MD-CMML, and that some cases of MD-progress to MP-disease suggest that the two forms may be discriminated at the molecular level. We previously reported a higher frequency of proliferation-related mutations in MP-CMML patients, which associated with a shorter median survival, and acquisition of RAS mutations in concomitance with progression to MP-CMML, suggesting these lesions to act as second hits able to confer a proliferative advantage to the malignant clone. Here we further investigated the spectrum of aberrations involved in CMML onset and progression by comprehensive next generation sequencing (NGS) of 44 selected genes. NGS (Oxford Gene Technology, Oxford UK) was performed on genomic DNA from 21 peripheral blood mononuclear cells (PBMNCs) samples of 12 CMML patients. Seventeen samples were collected from 9 patients with MD-CMML at diagnosis and subsequently during the disease course (showing either long-lasting stable MD-disease, or progression to MP-CMML or AML), while 4 samples were obtained from patients with MP-CMML. Candidate mutations were validated by Sanger sequencing. Deep sequencing analysis confirmed TET2 mutations as the most frequent (10/12 patients, 83%) and, the earliest known event in CMML, being present since diagnosis in 100% of our cases with sequential samples. Among other investigated genes, we documented mutations of ASXL1, SRSF2, SF3B1, EZH2, CBL, DNMT3A, MPL, NOTCH1, NOTCH2, N- and K-RAS in variable proportions. Of note, in one case the sequencing of DNA from purified CD3+ cells unveiled the presence of TET2, ASLX1 and CBL mutations in a significant fraction of T-lymphocytes, suggesting the aberration to possibly arise in a multipotent progenitor. A combined analysis of serial samples and single-cell-derived colonies is currently ongoing to clarify the order of mutations acquisition and their role in clonal evolution, which could provide new insights on molecular mechanisms contributing to CMML development and progression.

CO068

TCR REARRANGEMENTS ARE NOT ENOUGH FOR DISTINGUISHING THE CHURG-STRAUSS SYNDROME FROM THE HYPEREOSINOPHILIC SYNDROME

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Background. A sustained and elevated blood eosinophilic count of unknown origin exceeding 1500 cells/μL and leading to organ infiltration characterizes both Churg-Strauss syndrome (CSS) and idiopathic hypereosinophilic syndrome (HES), leading to overlapping classifications, especially in ANCA negative patients. Important therapeutic implications may derive from the understanding of the pathogenetic pathways underlying these two conditions. Aim of the study. Monocentric, cross

sectional study aimed at assessing the prevalence of TCR gamma/delta chain gene rearrangements in consecutive patients with a diagnosis of CSS in order to: a) compare the prevalence of TCR rearrangements in CSS *vs* HES; b) explore any correlation between TCR rearrangements and clinical and laboratory features in patients with CSS and HES. Methods. Consecutive patients with a diagnosis of CSS (ACR criteria) or HES (Chusid criteria) were enrolled in the study from January 2010 to November 2012. Salient features prospectively collected in the study were 1) demographic data, 2) clinical, immunologic, and molecular features at the onset of the disease and during disease evolution. Eosinophil cationic protein (ECP), IL2, IL4 and IL5 were measured as biomarkers of eosinophilic activation. TCR gamma/delta rearrangements were evaluated by fluorescent PCR. Statistical analysis was performed by chi-square test, ANOVA and t-test. Results. Twenty-four patients with a diagnosis of CSS and 19 patients with a diagnosis of HES were enrolled in the study. Nine CSS patients out of 24 showed positive TCR rearrangements, with an overall prevalence of 37.5%. Out of them, 2 CSS patients had a confirmed positive serology for ANCA and 3 a histologically proven necrotizing vasculitis. No statistically significant difference was detected in the prevalence of TCR rearrangements in CSS in comparison to HES (37.5% CSS *vs* 57.8% HES, p=0.22). In patients with mild to severe eosinophilia, TCR rearrangements correlated only with the presence of constitutional symptoms. No correlation was found between TCR gene rearrangements and eosinophilic count, IL2, IL4 and IL5. Conclusion. This study demonstrated a comparable prevalence of TCR gene rearrangements in CSS and HES. A cross-talk between eosinophils and clonal T-cells may play a potential role in the pathogenesis of both HES and CSS.

CO069

HIGH-SENSITIVITY HEMATOPOIETIC CHIMERISM BY QPCR FOR RELAPSE PREDICTION AND SPECIFIC IDENTIFICATION OF HLA LOSS LEUKEMIC VARIANTS

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Leukemia relapse after allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is a major clinical issue, and therapeutic options remain to date unsatisfactory. Thus interest has risen in how to anticipate the detection of residual malignant cells by more sensitive techniques, such as those based on quantitative PCR (qPCR). Moreover, our group has recently described novel variants of leukemia post-transplantation relapse, characterized by genomic loss of the mismatched HLA (Vago *et al*, N Eng J Med, 2009), that are not detected by routine diagnostic assays and that should be treated differently from "classical" relapses, warranting the development of novel specific assays. Here we prospectively validated a commercial hematopoietic chimerism assay based on qPCR (AlleleSEQR® Chimerism Assay, Celera Genomics) in follow-up bone marrow samples harvested from 87 transplanted patients, comparing it with standard Short Tandem Repeat (STR) chimerism analysis. To discriminate between "classical" and "HLA loss" relapses, we developed chimerism assays targeted to specific HLA-A allele groups, based on the same qPCR technology of the commercial assay. qPCR chimerism displayed higher sensitivity in detection of residual host cells as compared to STR:108/339 samples (31.9%) exhibited a host chimerism above 0.5% and 75/339 (22.1%) above 1%, whereas 79/386 (20.5%) were positive in STR. qPCR chimerism could predict impending relapse with a sensitivity of 37.5% and a specificity of 73.1% for a 0.5% host threshold, and with a sensitivity of 29.2% and a specificity of 83.3% for a 1% host threshold, comparing favourably with STR (sensitivity 25%, specificity 85%). To provide early differential diagnosis of classical and HLA loss relapse, we designed 5 qPCR reactions targeting 9 of the most frequent HLA-A allele groups in Caucasians and providing an informative marker to more the 50% of our patients. The newly developed assays were validated on 70 samples harvested from transplanted patients, and, used in combination with the commercial qPCR assay for polymorphisms outside the HLA complex, allowed clear discrimination between HLA loss and classical relapses. In conclusion, hematopoietic chimerism detection by qPCR appears promising, displaying a higher sensitivity as compared to techniques currently in use, and allowing the simultaneous detection of disease variants characterized by genomic HLA loss.

CO070

DETECTION OF BCR-ABL FUSION PROTEINS AND SURROGATE MARKER PCRLK BY FLOW CYTOMETRY(FC) ASSAYS: UPDATED RESULTS OF A PROSPECTIVE MULTICENTER SCREEN STUDY IN CHRONIC MYELOID LEUKEMIA (CML)

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Background. Facilitating the detection and monitoring of BCR-ABL fusion protein (FP) activity may potentially complement the traditional genetic testing in CML follow-up (FU). We previously evaluated the utility of a FC-bead immunoassay [(BCR-ABL Protein Kit, BD Biosciences), FCBA] to detect FPs using a sample cohort of CML pts in a prospective multicentre study (SCREEN group, Hematology Units, Sicily and Calabria, Italy). We update our data using a larger pt cohort and introduce FC screening for phosphorylated CrkL (pCrkL), a BCR-ABL adaptor protein reported to serve as a surrogate marker of FP activity. Methods. PB or BM samples (n=235) from CML pts (n=72), ALL (n=17) and other disorders (n=15) were analyzed. PB from 88 healthy controls were used to calculate MFI- cut-off values. The anti-pCrkL antibody (BD Bioscience) detects the %pCrkL in CD34+ cells. Results. FCBA was positive in all newly diagnosed CML and in 4/7 accelerated phase CML cases (including one p230). MRD evaluation showed MFI+ signals in 55/125 FU samples; detectable FP corresponded to .07-100% BCR-ABLIS. For the remaining CML pts with undetectable FP (58), BCR-ABLIS was <4.5%. Pts with other disorders (n=15; ET, IMF, AML, LMMC, MM, LNH) tested negative both in FCBA and qPCR. Of the Ph1+-ALL cases, 3/10 pediatric pts were both FC and qPCR positive, 1/5 adult ALL were FCBA+. Correlation between FCBA *vs* qPCR (n=198) was highly significant (RHO=.802, p<.0001). Comparing FISH and FC-assays (n=54); all cases testing FCBA+ also tested FISH+ (18/18), while 4/36 (11.1%) of the FISH negativesamples were FCBA+ although BCR-ABLIS was>1%. ROC analysis showed that a FCBA+ assay could detect BCR-ABL transcripts up to BCR-ABLIS=1.37% (AUC=.951, P<.0001). Subdividing MFI into quartiles showed a strong proportional increase in relative %MFI corresponding to BCR-ABLIS transcripts (P<.0001), indicating MFI values could be used as a semi-quantitative scale. The degree of pCrkL did not correlate with either qPCR or FCBA. Conclusions. The FCBA is a rapid and easy technique able to detect BCR-ABL FPs with high specificity and sensitivity, potentially able to circumvent genetic-based FU of the disease and it appears to be more sensitive than FISH in detecting FP+ samples. The ongoing SCREEN study will further test the relationship with FISH and molecular analysis and evaluate the combination of FCBA and pCrkL as a feasible method to detect effective TKI inhibition/CML-reactivation by gating the residual leukemic population within the stem cell compartment in a larger cohort of pts.

CO071

FORTY MINUTES MOLECULAR DETECTION OF PML-RARA BCR1, BCR2 AND BCR3 TRANSCRIPTS BY RETRO TRANSCRIPTION LOOP MEDIATED AMPLIFICATION (Q-RT-LAMP) PERFORMED ON THE LIAISON IAM INSTRUMENT

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Background. The availability of ultra rapid screening tests, easy to be performed even in not specialized laboratories, can significantly enhance the management of patient affected by Acute Promyelocytic Leukemia, allowing rapid initiation of therapy. Methods. the PML-RAR Q-RT-LAMP method consists in two fluorescent multiplex assays for the molecular detection of the most frequent (bcr1 and bcr3) and the more rare bcr2 transcripts starting directly from total RNA. Both the assays also detect the endogenous GUS housekeeping RNA as internal reaction control. The reactions are performed into the Liaison IAM instrument (DiaSorin), which incubates at constant temperature for 40 minutes, monitoring the fluorescent signals in real-time and returning objective elaborated final results. Results. The analytical specificity has been established on 318 replicates of wild type RNA from 8 cell lines. In all replicates exclusively the GUS internal control RNA was amplified, demonstrating 100% specificity. The analytical sensitivity of the triplex (bcr1-bcr3-GUS) and duplex (bcr2-GUS) RT-LAMP assays has been determined on serial dilutions of RNA from the APL derived NB4 cell line (for bcr1 transcript) or from patients at diagnosis (for bcr2 and bcr3 transcripts) into wild type RNA (from HL-60 cell line). The triplex assay showed a level of sensitivity of 10-3 on both the bcr1 and bcr3 transcripts, while the duplex assay showed a detection limit of 10-2 within 40 minutes. The method, validated on 96 clinical samples previously analyzed by conventional RT-PCR, showed 100% concordance. Interestingly, the positive samples at diagnosis (n=34) were already amplified in less than 10 minutes Conclusions: The PML-RAR Q-RT-LAMP in combination with the Liaison IAM instrument is a novel system that ensures ultra-rapid, highly specific, sensitive and reliable detection of PML-RAR bcr1, bcr2 and bcr3 transcripts. The isothermal single-step format, monitorable in real-time, allows diagnosis of APL within 40 minutes starting directly from patient RNA. The simple and close-tube set up allow applicability in not highly specialized laboratories, representing an effective solution to improve management of APL patients.

CO072

DETECTION BY FLOW CYTOMETRY OF CIRCULATING MEGAKARYOCYTE-DERIVED CELLS IN THE DIFFERENTIAL DIAGNOSIS OF ACUTE MEGAKARYOBLASTIC LEUKEMIA AND MEGAKARYOCYTE NEOPLASMS

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Acute panmyelosis with myelofibrosis (APMF) and acute megakaryoblastic leukaemia (AMKL) are included among acute leukemias according to WHO 2008 classification; however a differential diagnosis with other myelofibrotic disorder is problematic, mostly because of insufficient cellularity. We report on a cohort of about 300 patients admitted to our marrow failure unit because of cytopenia, focusing on a series of on 8 patients who shared a distinct clinical evolution due to an aggressive megakaryocyte (MK) neoplasm. All patients were evaluated by standard peripheral blood (PB) and bone marrow (BM) cytology, karyotype analysis and BM trephine biopsy. Flow cytometry was performed at presentation and during the follow up. Two patients received diagnosis of AMKL (one case with complex karyotype). Blast cells were CD34+CD38+CD45+CD117+CD33+CD13+; in PB, we detected an aberrant cell population CD45-CD42b+ (CD34+ in one case and CD34- in the other), seen on the blood smear as MK fragments or giant platelets. Other three patients showed the same finding of abnormal giant platelets at the blood smear, resembling MK fragments, that flow cytometry identified as CD45-CD42b+CD61+ cells (CD34+ in one case). These patients had severe pancytopenia, dry tap and massive fibrosis on trephine biopsies, normal karyotype without any genetic lesion typical of primary myelofibrosis (PMF); nevertheless, they were initially classified as PMF, even if APMF could not be ruled out. Everyone progressed to AMKL and typical CD34+CD45+ blast cells were accompanied by a progressive increase of CD45+CD42b+CD61+ cells. This aberrant MK-derived cell population (which could not been detected in essential thrombocytopenia) was also identified in three additional patients with previous hematologic disorders: two pure red cell aplasia (successfully treated by immunosuppression), and one a 5q- melodysplastic syndrome (responding to lenalidomide). In all of them flow cytometry detected CD45-CD42b+ cells in the PB, which appeared as giant platelets/MK fragments, anticipating the rapid progression to AMKL (5q- was detected in 2 of 3 cases). We demonstrate that aberrant circulating MK-derived cells detectable by flow cytometry may be useful in the differential diagnosis of myelofibrotic disorders. These giant platelets or MK fragments, regardless the initial diagnosis, were associated with early evolution into AMKL, likely representing a surrogate marker for aggressive neoplasms of the MK lineage.

Myelodysplastic Syndromes

CO073

DETECTION OF COMMON BREAKPOINT REGION IN SEVEN MDS/AML PATIENTS WITH A DEL(11)(Q13-Q14)

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In MDS and AML patients a deletion of band q13 of the long arm of chromosome 11 is a recurrent, yet rare chromosomal defect. In fact, in large MDS patients series the incidence of this chromosomal lesion is about 1.0% (Solè *et al.*, 2005; Bernasconi *et al.*, 2007). The International Prognostic Scoring System (IPSS) includes single del(11q) within the intermediate-risk cytogenetic category, whereas the "New Comprehensive Cytogenetic Scoring System" includes it within the very good category. The present study was aimed to identify a commonly deleted region in 7 MDS/AML patients (2 RCMD, 1 RARS, 3 AML and 1 t-AML) with a 11q13-14 deletion on Conventional Cytogenetic (CC) studies and to correlate this finding with morphological and clinico-hematological data. Their median age was 62 years (range 42-84) and median follow-up was 4 months (range 1-51). In these cases, peripheral blood and bone marrow blasts showed similar morphological features, with large round nuclei, loose chromatin, small nucleoli, abundant, agranular, often vacuolated cytoplasm. Needle-like Auer bodies were observed in rare cells. Most blasts were peroxidase positive with strong reactivity of the cytoplasmic vacuoles and Auer bodies. CC and FISH analysis were carried out as already reported (Bernasconi *et al.*, 2007). FISH was applied to all the 7 patients with commercial probes from Vysis (Abbott Molecular/Vysis, North Chicago, IL, USA) and BAC probes provided by the Sanger Institute (Wellcome Trust Sanger Institute, Cambridge, UK). It revealed a deletion in five patients and an amplification in two who presented 11q material translocated onto other chromosomes. Furthermore, in order to establish the extent of chromosome 11q defect, twenty-one BAC probes spread over band 11q13.4-q22.3 were chosen. In 5 patients, 2 low-risk MDS and 3 AML, the breakpoint region was comprised between the BAC probes RP11-451K5 and RP11-23O14 (between bands 11q13.4 and 11q14.2). In 3 of these patients we succeeded in further reducing the breakpoint area to a region comprised between the BAC probes RP11-19P3 and RP11-23O14 (between bands 11q14.1 and 11q14.2). In conclusion i) del(11)(q13-q14) is a very rare clonal defect in MDS/AML, ii) AML patients with this terminal deletion present cytoplasmic vacuoles and Auer bodies as the unifying morphological feature, iii) despite the fact that the size of 11q deleted area is quite variable some patients my harbor a Commonly Breakpoint Region (CBR).

CO074

STUDY OF HEMATOLOGIC AND IMMUNOPHENOTYPIC FEATURES OF SHWACHMAN-DIAMOND SINDROME PATIENTS

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Introduction. Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive multisystem disorder characterized by bone marrow (BM) failure, pancreatic insufficiency and skeletal abnormalities. The SBDS gene, located on chromosome 7, is mutated in 90% of the cases SDS. Besides chromosome 7 abnormalities, del(20)(q11) is the most chromosomal abnormality encountered in SDS. SDS patients show frequently cytopenia (neutropenia, thrombocytopenia and anemia) and increased risk of MDS/AML evolution. Aim of the study was to evaluate the immunophenotypic features of BM cells of SDS patients for detecting abnormalities in the stem cells and hematopoietic precursors, and correlating these alterations with hematological characteristics to predict MDS/AML evolution. Materials. We studied 15 patients with SDS (M/F: 10/5; median age 7.61 years, range 0.5-36.67) and 9 controls (5 male and 4 female; median age 5.88 years, range 0.27-15.2). In all cases, cytological, cytogenetic and immunophenotypic evaluation of BM was performed. Moreover, peripheral blood counts were recorded. Results Neutropenia, anemia and thrombocytopenia were observed respectively in 80%, 13% and 20% of all SDS patients. No patients showed pancytopenia. BM smear evaluation showed mild hypoplasia in 33% of patients and reduction of megakaryocyte precursors in 53% of patients. None of the patients showed BM aplasia or hyperplasia. Cytogenetic abnormalities were found in 20% of BM samples (i(7)(q10) and intdel(20) in 13% and 7% respectively). Immunophenotypic analysis of BM from SDS patients showed a reduction in CD34+/CD38- hematopoietic stem cells, an increase in CD34+/CD38+ progenitor cells, and alteration in hematopoietic lineage maturation as compared to controls. On the basis of morphological and hematological characteristics, MDS was diagnosed in two patients (Refractory Cytopenia with Unilineage Dysplasia, according to WHO classification); they showed abnormalities of myeloid differentiation with an increase of immature cells (promyelocytes and myelocytes), as reported in the literature. Conclusions This is the first study correlating the clinical features of patients with BM immunophenotypic analysis. The future perspective is to increase the case number and the observation period to try to identify early abnormalities predictive of hematologic complications.

CO075

EFFICACY OF DECITABINE IN ADVANCED CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) PATIENTS

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Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic malignancy characterized by features of both a myeloproliferative neoplasm (MPN) and a myelodysplastic syndrome (MDS), and thus defined as an MDS/MPN overlap syndrome. The clinical presentation of this syndrome is quite heterogeneous and despite a smouldering onset, the prognosis is poor and progression to overt leukemia not rare. At present, therapy is limited to antiproliferative drugs, mainly for the 'proliferative ' subtype, but clear management indications are lacking. Recent biological and molecular characterization did not lead to identification of effective new therapies. We evaluated efficacy and safety of decitabine 20mg/m2/day for 5 days every 28 days for a minimum of 6 cycles in a group of CMML patients. Evaluation of response was performed after 4 and 6 cycles of therapy, according to IWG 2006 criteria. We enrolled 44 patients, 43/44 were evaluable; according to WHO 27/43 pts were classified CMML-I with organomegaly and 16/43 CMML-II. Median age was 71 (42-84) yrs, median number of cycles 8 (1-25). Eleven patients received <4 cycles, 17>4 to 6 cycles and 15>6 cycles. Eighty one percent of patients with CMML-I received >4 cycles. After 4 cycles, CR was obtained in 14% of cases, mCR in 18.6%, PR 2.3% and SD in 34.9% . After 2 more cycles, the CR rate increased to 16.2%, mCR 24.3%, SD decreased to 18.9%, while the patients failing to respond were 30%. Interruption of therapy with decitabine was mainly due to progressive disease (35% of cases), death (23%) and in 7% of the cases to toxicity of the drug. At present, 16.3% (7/43) of patients are still in treatment. Response duration after the sixth cycle is 9.7 months. Grade 3 / 4 toxicity was in the majority of cases hematological, and present all over treatment courses. In 7/43 patients severe infections occurred, and 1/43 had grade 3 / 4 cardiac and gastrointestinal toxicities respectively. In conclusions, our observations confirm activity of decitabine in CMML, with 40.5% CR plus mCR. Responses were achieved progressiviley prolonging decitabine cycles, consistently with DNA hypomethylating effect, which is the main mode of action of this drug. From our small cohort of patients it was not possible to extrapolate clinical or cytogenetic characteristics indicating responding patients. Parallel to this clinical study, we evaluated the pattern of DNA methylation of selected CMML patients.

CO076

POLYMORPHISMS IN GENES INVOLVED IN DNA REPAIR AND FOLATE PATHWAY SIGNIFICANTLY IMPACT ON SURVIVAL IN LOW-RISK, UNTREATED MYELODYSPLASIA

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The impact of the polymorphisms of DNA repair and folate pathway genes on the prognosis of patients with low or intermediate-1 IPSS myelodisplastic syndromes (MDS) has not been investigated up to now. We prospectively genotyped 54 MDS patients (median age 75 years) with IPSS low (n=23) or intermediate-1 (n=31) treated with best supportive care only. Genomic DNA was isolated from 1ml of peripheral blood by means of commercially available kits. Polymorphisms were determined by PCR-HRM (High Resolution Melting) assay and restriction digests of PCR products. All samples were analyzed for the following polymorphisms: XRCC1 194 (rs1799782 C/T, Arg/Trp) and 399 (rs25487 G/A, Arg/Gln), XRCC3 241 (rs861539 C/T, Thr/Met), TS 5'-UTR (2R/3R and rs183205964 G/C) and 3'-UTR Ins/Del (rs11280056 6bp+/6bp-), MTHFR 677 (rs1801133 C/T, Ala/Val) and 1298 (rs801131 A/C, Gln/Ala), APE1 148 (rs1130409 T/G, Asp/Glu). The characteristics and laboratory features of MDS patients with each polymorphisms were compared using 2-test and Mann-Whitney test. No significant association between polymorphisms and demographic, clinical or prognostic characteristics was observed. When comparing all the allele and genotype frequencies according to OS, the groups of patients with XRCC1 399 Arg/Arg, TS5'-UTR 2R/3G, 3C/3G, 3G/3G, TS3'-UTR Del/Del and MTHFR 677 Val/Val genotypes showed longer OS (XRCC1 399 Arg/Arg *vs* non-Arg/Arg P=0.015; TS5'-UTR 2R/3G, 3C/3G, 3G/3G *vs* 2R/2R, 2R/3C, 3C/3C P=0.03; TS3'-UTR Del/Del *vs* non-Del/Del P=0.04 and MTHFR 677 Val/Val *vs* non-Val/Val P<0.001). On the other hand, no statistically significant association between XRCC1 Arg194Trp, XRCC3 Thr241Met, MTHFR Gln1298Ala polymorphisms and OS was found. In multivariate analysis XRCC1 399 Arg/Arg (P=0.007), TS5'-UTR 2R/3G, 3C/3G, 3G/3G (P=0.038), TS3'-UTR Del/Del (P=0.041) and MTHFR 677 Val/Val (P=0.001) genotypes were independent prognostic factors, significantly associated with longer survival. BER gene and folate pathway gene polymorphisms may affect the prognosis and survival of patients with low-Int-1 myelodisplastic syndrome treated with best supportive care only. If confirmed in larger series, these polymorphisms could help to identify a subset of patients with short survival, who could benefit from an early treatment with hypometilating agents which are, at present, not indicated for the treatment of patients with MDS and low/Int-1 IPSS. Acknowledgements: The study was supported in part by AIL Pesaro Onlus.

CO077

CLONAL ACTIVATION OF AKT IN LOW-RISK MDS PATIENTS WITH DEL(5Q) TREATED WITH LENALIDOMIDE

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Lenalidomide is currently used in the treatment of del(5q) low-risk MDS patients, where it may suppress the del(5q) clone and restore a normal erythropoiesis. The exact molecular mechanisms underlying the effect of Lenalidomide in MDS cells are still unclear, although Akt phosphorylation is inhibited in Lenalidomide-sensitive del(5q) cell lines. The activation of Akt/mTOR or Akt/PI-PLCgamma1 pathways have been demonstrated in high-risk MDS, thus affecting stem cell proliferation, differentiation and apoptosis, *i.e.* critical processes for low-risk MDS, that usually show a marked apoptosis and a low proliferation rate, which can be rapidly reversed, thus leading to a worse clinical status. Here we studied the effect of Lenalidomide on inositide signalling pathways in 6 patients diagnosed with del(5q) MDS (IPSS: Low or Int-1) who were given Lenalidomide. Given the limited number of cells, we analyzed bone marrow total mononuclear cells. As for Akt phosphorylation, we analyzed its localization along with RPS14, in order to specifically detect the del(5q) clone. Moreover, by Real-Time PCR analyses, we assessed the expression of Beta-Globin, to evaluate the effect of the drug on erythropoiesis. In our case series, 4/6 del(5q) low-risk MDS patients responded to Lenalidomide and showed an activation of erythropoiesis, in that Beta-Globin levels increased, as compared with baseline. Moreover, these subjects also displayed an activation of PI-PLCgamma1 and Akt. Interestingly, Akt resulted to be specifically phosphorylated in cells not showing the 5q deletion, hinting at a clonal activation of this pathway. The 2 non responder patients early discontinued Lenalidomide for adverse events, and for these patients neither a clinical assessment of Lenalidomide effect, nor a molecular analysis, were possible. Our data show Akt/PI-PLCgamma1 activation during Lenalidomide treatment, and confirm the activation of erythropoiesis in responder patients. In addition, our results indicate that Akt is specifically phosphorylated in normal cells without del(5q). Therefore, it is conceivable that Lenalidomide may act not only by inducing apoptosis in clonal del(5q) cells, but also by enhancing the proliferation of normal non clonal cells, as described in non del(5q) MDS (Ebert, 2008), allowing the restoration of the normal erythropoiesis. This finding might be useful not only for understanding MDS pathogenesis, but also for the development of innovative targeted therapies.

CO078

ELTROMBOPAG FOR THE TREATMENT OF THROMBOCYTOPENIA OF LOW AND INTERMEDIATE-1 IPSS RISK MYELODYSPLASTIC SYNDROMES: RESULTS OF A MULTICENTER, RANDOMIZED, TRIAL

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Patients with myelodysplastic syndromes (MDS) may experience severe thrombocytopenia associated with an increased risk of hemorrhage. Eltrombopag is an oral agonist of the thrombopoetin-receptor (TPO-R) indicated for treating chronic immune thrombocytopenic purpura. Eltrombopag's potential in increasing PLT counts in lower risk MDS has not yet been evaluated. We present interim results of a Phase II, multicentre, placebo-controlled, single-blind study (EQoL-MDS) to evaluate safety and efficacy of eltrombopag in low and intermediate-1 IPSS risk MDS patients with thrombocytopenia. Secondary endpoints include changes in quality of life (QoL), PLT transfusion requirement, bleeding, and survival. Adult patients (N=171) are being included if: PLT<30 Gi/L; ECOG performance status <4; ineligible for, relapsed or refractory to other treatments; and naive to TPO-R agonists. Eltrombopag/placebo (2:1) will be administered at a 50 mg daily starting dose with 50 mg increases every 2 weeks to maximum 300 mg to target PLT 100 Gi/L. Dose interruptions or reductions are required for PLT >200 Gi/L or adverse events. PLT response is defined as Response (R) if: 1)

baseline PLT>20 Gi/L: absence of bleeding and PLT≥50Gi/L; 2) baseline PLT<20 Gi/L: PLT>20 Gi/L and increase by at least 100%, not due to PLT transfusions; and Complete Response (CR) if PLT≥100 Gi/L and absence of bleeding. QoL scores are evaluated by EORTC QLQ-C30 and QOL-E instruments. Thirty-one patients (21 on eltrombopag – Arm A), have been randomized and 5 are in screening at the time of this report. Mean age is 66 (SD 12) years, M/F 18/13. Baseline mean PLT count was 16 (SD 8) Gi/L. Three cases in Arm A and 1 in Arm B had significant bleeding requiring PLT transfusions. At a 12-week follow-up, 12 out of 15 cases in Arm A obtained PLT responses at median 100 mg dosing associated with disappearance of bleeding and PLT transfusion independence. There were no responses in Arm B. PLT count increased by mean 64 (SD 77) Gi/L (p=0.006) in Arm A versus no significant changes in Arm B by week 12. QoL improved significantly from baseline in Arm A. Grade III-IV unrelated adverse events occurred in 6 patients in Arm A. Bone marrow blasts decreased in 3 cases in Arm A, versus 2 progressions and 1 improvement in Arm B. Preliminary results suggest safety and efficacy of eltrombopag in low and Intermediate-1 risk IPSS MDS patients with thrombocytopenia.

Chronic Lymphocytic Leukemia

CO079

PROGNOSTIC SIGNIFICANCE OF LAIR-1, CD49D AND CD38 AS ASSESSED BY FLOW CYTOMETRIC ANALYSIS IN A PROSPECTIVE SERIES OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukaemia (CLL) is a haematological malignancy with marked clinical heterogeneity reflecting its biological diversity. In last years several markers with prognostic significance have been identified with significant and independent impact on patients' outcome. Among them, immunoglobulin heavy chain mutational status (IGHV), cytogenetic profile, and surface markers expression by flow cytometry have been the most widely used to identify patients with high risk CLL. The Leukocyte-Associated Ig like Receptor-1 (LAIR-1) is a transmembrane glycoprotein acting as inhibitory receptor controlling kinases pathway involved in cell proliferation. This protein expression varies during B-cell differentiation, and it has recently been demonstrated that LAIR-1 expression is higher among patients with low risk biological profile. However, its correlation with most biological variables and its prognostic significance remain unknown. We investigated 275 patients (median age 66, 39% females, 81% Binet A) as part of the "CLL Veneto" project, which is recruiting consecutive patients with CLL from Institutions of Veneto region since 2008. All patients had complete immunophenotype, fluorescence in situ hybridisation (FISH), and IGHV status. Overall, 38% of patients had unmutated IGHV, 15% had high risk FISH abnormalities, 23% were CD38+, 38% were CD49d+ and 60% were LAIR-1+. Expression of LAIR-1 was inversely related to CD38 expression (p=0.01), but was not associated with CD49d expression (p=0.12). Conversely, CD49d expression was strongly associated with CD38 expression (p<0.0001). LAIR-1 expression was expressed at lower levels in high risk FISH subcategories (p=0.01) and in patients with unmutated IGHV (p=0.001). Univariate analysis revealed that unmutated IGHV (p<0.0001), high risk FISH (p=0.0004), high expression of CD38 (p=0.0001) and CD49d (p<0.0001) and low expression of LAIR-1 (p<0.0001) were significantly associated with a shorter time to first treatment (TTFT). Of immunophenotypical variables, CD49d high expression (HR 0.35, p=0.001) and LAIR-1 low expression (HR 0.39, p=0.0006) maintained an independent significant association with shorter TTFT in multivariate analysis, while CD38 did not. Our results from a prospective series of widely characterized patients uniformly diagnosed and followed- up within the CLL Veneto project reveal that CD49d and LAIR-1 expression, but not CD38, can be used to independently predict TTFT in patients with CLL.

CO080

ENDOTHELIN-1/ETA RECEPTOR SIGNALING MEDIATES GROWTH AND PROLIFERATIVE SIGNALS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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The endothelin axis, comprising endothelins (ET-1, ET-2 and ET-3) and their receptors (ETAR and ETBR), has recently emerged as relevant player in tumor growth and metastasis. Here, we investigated whether ET-1/ETAR signaling pathway mediates growth and proliferative signals in chronic lymphocytic leukemia (CLL). Purified CLL cells treated with recombinant ET-1 experienced the activation of ERK/MAPK signaling pathway through ETA receptor triggering. Moreover, CLL cells were activated by ET-1 as measured in an MTT assay in which metabolically active cells convert MTT to formazan (n=10, FC=1.25 compared to unstimulated control, p=0.018). In contrast, the blockage of ETAR throughout BQ-123 selective antagonist was able to abrogate ET-1-mediated CLL activation. Furthermore, the direct contact between CLL cells, labeled or not with CFSE, and endothelial layer (HUVEC) induced CLL activation and increased the number of divided cells (p=0.003). Both effects were significantly reduced pretreating cells with BQ-123 (p=0.003). The levels of ET-1 precursor (big ET-1 peptide) in plasma samples collected from a multicenter cohort of CLL patients (n=101) ranged from 0.32 pg/mL to 28.9 pg/mL (median=3.7). Higher levels of big ET-1 were detected in patients with advanced Binet stage (p=0.004), high 2 microglobulin (p<0.0001), unmutated IGHV status (p=0.003), and intermediate/high FISH risk (p=0.002). Patients with higher levels of big ET-1 (≥5.4 pg/mL) showed shorter time to first treatment (TTFT), as compared to CLL with low big ET-1 levels (median TTFT, 88 *vs* 155 months, p=0.005). Moreover, the comparison of big ET-1 plasma levels between two sequential plasma samples collected from 8 CLL cases with median interval of 5 years, showed no differences over time in patients with stable disease (n=4). Conversely, variations of big ET-1 plasma levels between serial plasma samples were measured in patients experiencing disease progression (n=4, 4.3 pg/mL at diagnosis and 11.9 pg/mL pre-treatment). Collectively, our data describe for the first time a role of ET-1/ETAR signaling in CLL pathobiology. ET-1 mediates activation and proliferative signals in CLL cell that can be blocked by selective inhibition of ETAR. *in vivo*, higher plasma levels of big ET-1 characterize patients with progressive disease.

CO081

NEXT GENERATION SEQUENCING BY ION TORRENT TECHNOLOGY IN UNTREATED CLL PATIENTS: CLINICAL AND BIOLOGICAL CORRELATIONS

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Background. Chronic lymphocytic leukemia (CLL) is a hematological malignancy with clinicobiologic heterogeneity. Next-generation sequencing (NGS) techniques identified several mutated genes previously unrelated to CLL and have established correlations with clinical outcome. Ion Torrent is a Personal Genome Machine sequencer that uses semiconductor sequencing technology. It is the first commercial sequencing machine that does not require fluorescence and camera scanning, resulting in higher speed of analysis and lower cost. Objectives. To study the genetic complexity of untreated CLL patients and to correlate mutational status with clinicobiologic parameters. Methods. Agilent HaloPlex Target Enrichment kit was used to construct libraries of spot exonic regions of 20 genes (ATM, BIRC3, BRAF, CDKN2A, CTNNB1, DDX3X, FBXW7, KIT, KLHL6, KRAS, MAPK1, MYD88, NOTCH1, NRAS, PIK3CA, POT1, SF3B1, TP53, XPO1, ZMYM3) starting from genomic DNA of peripheral blood samples. Following emulsion PCR, enriched template-positive Ion Sphere Particles were loaded into a Ion chip 316 and sequenced using the Ion Torrent PGM. Sequencing data were aligned to the human reference genome (GRCh37) and analyzed using the Torrent Suite. Results. 30 untreated CLL patients were included in this study. Somatic mutations were identified in 15 cases. Mutated genes and corresponding number of cases were: TP53 (3 with >20% mutated cells, 5 with 5- 19% mutated cells), SF3B1 (3), POT1 (3), ATM (2), NOTCH1 (1), MYD88 (1), FBXW7 (1), MAPK1 (1), DDX3X (1), KLHL6 (1), KRAS (1). 8 cases presented one mutated gene, 5 cases 2 mutated genes, 1 case 3 mutated genes and 1 case 4 mutated genes. The size of the mutated population ranged from 6.4 to 76.5%. The presence of mutations correlated with high risk FISH (11q- and/or 17p-) (p=0.044) and unfavourable cytogenetic (11q-, 17p- or complex karyotype) (p=0.011) findings. No correlations were instead observed with sex, age, Binet stage, CD38 and IgVH. Mutated patients showed a significant (p<0.05) shorter median time to first treatment in comparison to those without mutations (35 months *vs* not reached at 76 months). Conclusions: The frequency of mutations in the 20 investigated genes is in line with data published in the literature using whole exome sequencing. This study suggests that in untreated CLL patients NGS by Ion Torrent is feasible and might represent an important tool for the characterization of CLL genetic heterogeneity and clinical-prognostic outcome.

CO082

IT IS REALLY POSSIBLE TO CURE HAIRY CELL LEUKEMIA PATIENTS ONLY WITH FRONT-LINE THERAPY?

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The last 2 decades have represented an exciting period for clinicians involved in the care of patients with hairy cell leukemia (HCL). Four new drugs, alpha-interferon (alpha-IFN), 2 -deoxycoformycin (DCF), 2-chlorodeoxyadenosine (2-CdA) and rituximab, have been identified as highly effective in the treatment of this rare disease. Although a proportion of patients are primary resistant to these agents and another significant proportion of patients who achieved remission will ultimately relapse, prolonged remissions are usually seen after only one treatment. Aim of the present study was to retrospectively review our institutional series of HCL patients searching for the presence of a subset of patients in continuous complete response (CR) after the front-line treatment. The whole sample consisted of 144 HCL patients: 32/144 (22.2%) cases in CR after first-line were found. We analyzed the outcome of the different lines of therapy in these patients followed in our institute from 1986 to 2008, with a median follow-up of 10.6 years. Twenty-eight were males and 4 were females; the median age at diagnosis was 57 years (range, 36-72 years). At presentation, splenomegaly was present in 16 (50%) patients and 4 patients had also hepatomegaly. According to the front-line therapy, 26 patients had 2-CdA, 3 patients DCF and the remaining 3 patients underwent alpha-IFN. All these patients obtained a CR and they are in continuous CR with a median duration of response of 9.8 years (range, 5.5-22.8 years). After therapy the median hairy cell index was 0.01 (range, 0.0001-0.08). There is a need for continuous study in this field to better define the optimal therapeutic regimen and, in particular, the biologic issues since at least 20-25% of HCL patients can be cure with only one treatment.

CO083

PRESENCE OF THE JAK2V617F MUTATION IN PATIENTS AFFECTED BY B-CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. JAK2 alterations appeared to be a prerogative of myeloid diseases in which they have a well-described pathogenic role. Recently, the constitutively activating JAK2V617F mutation has been identified in few B-CLL patients with a concomitant MPN. These double disease patients can represent a model to understand if the myeloid and lymphoid disease derived from the same ancestral clone. Moreover, the positivity for JAK2V617F mutation in these cases should be of great interest for the use of JAK inhibitors as a new potential therapeutic approach. We screened the JAK2V617F mutation in a large cohort of B-CLL patients using a new, highly sensitive and rapid molecular assay. Patients and Method: We analyzed DNA collected from 158 B-CLL patients at diagnosis or during follow-up. DNA samples were tested for JAK2V617F by an innovative non PCR technique using a Loop mediated isothermal AMPlification which specifically detects the JAK2 mutated allele (AS-LAMP) within 1 hour, reaching a 0.1% sensitivity and allowing a Real-Time monitoring thanks to the addition of an intercalating dye. Results and Discussion: We detected the JAK2V617F mutation in 4 cases (about 2.5%)(Table 1). In cases #1 and #3 no evidence of myeloid proliferation was detected at diagnosis and during clinical follow-up and the JAK2V617F allele burden was around 0.5% as established by accurate comparison with calibrators. Patient #2, bearing a low JAK2V617F allele burden, presented a thrombocytosis at diagnosis and during the follow up while patient #4 had a diagnosis of PV preceding of 20 years the B-CLL. In this latter case, with an allele burden higher than 50%, an ASO-PCR analysis was performed on the genomic DNA obtained from purified myeloid and lymphoid subpopulations. The CD3 and CD19 positive cells were proved negative for the JAK2V617F mutation while the CD15 positive cells were positive. Our data demonstrated that the JAK2V617F mutation can be present also in B-CLL, even if frequently in presence of a concomitant MPN. The analysis conducted on the DNA derived from different subpopulation of the patient #4 demonstrate that JAK2 was mutated only in the myeloid cells, suggesting that two coexisting diseases were not clonally related. Unfortunately, the analysis of chromosome X inactivation on the 2 different compartment to confirm that the two diseases derived from two different hemopoietic stem cells were not possible because of the male gender of the JAK2V617F positive patients.

Table 1. Chromosomal rearrangements involving JAK2 in Acute Leukemia and Lymphoma

Chromosomal Rearrangement	Type of AL or Lymphoma	Reference
PAX5-JAK2	B-ALL	Nebral et al, Leukemia 2009 Coyaud et al, Blood 2010
PCM1-JAK2	secondary AML and early pre-B-ALL T-cell lymphoma	Reiter et al, Cancer Res 2005 Adelaide et al, Leukemia 2006
SEC31A-JAK2	cHL	Van Roosbroeck et al, Blood 2011
SSBP2-JAK2	pre-B-ALL	Poitras et al, Genes Chromosomes Cancer 2008
TEL-JAK2	early pre-B-ALL	Peeters, Blood 1997
	pediatric T-ALL	Berger, Leukemia 1997
	pediatric T-ALL	Lacronique, Science 1997

CO084

THE MD ANDERSON CANCER CENTER SCORE PREDICTS TIME TO FIRST TREATMENT OF INDIVIDUALS WITH CLINICAL MONOCLONAL B-CELL LYMPHOCYTOSIS: RESULTS OF PROSPECTIVE ANALYSIS

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WHO and IWCLL 2008 criteria define monoclonal B-cell lymphocytosis (MBL) as a clonal B-cell expansion where the B-cell count is less than $5x10^9$/L and no symptoms or signs of lymphoproliferative are detected. Based on the number of clonal B cells, MBL is further divided into low-count and clinical (c)-MBL. cMBL is virtually indistinguishable from Rai stage 0 CLL regarding immunopheno- typic, genetic, and molecular features and carries a risk of progression to CLL requiring treatment of 1% to 2% per year. We sought for the applicability of score system originally proposed by MD Anderson Cancer Center (MDACC) group to predict time to first treatment (TTFT) in patients with early CLL in a prospective series accounting for 84 individuals diagnosed as having cMBL at different Italian hematologic institution between January 2006 and December 2010 and registered prospectively within 12 months from diagnosis in a national database (O-CLL1 protocol; clini- caltrial.gov identifier NCT00917540). Since variables included in the MDACC score were either clinical (*i.e.*, number of lymph node sites involved, lymph node size in neck, LDH) or biological (*i.e.*, mutational status of IGHV, presence of 11q or 17p deletion by FISH) for the purpose of this analysis only LDH, mutational status of IGVH and presence of 11q or 17 p deletion were considered. After calculating in each of 84 individuals with cMBL total point score according to MDACC formula, we utilized the cut-off value (*i.e.*, 25) previously validated in Rai stage 0 patients. After a median follow-up time of 33 months (range, 1-62 months) 7 (8.3%) out of 84 individuals developed active CLL requiring therapy. Interestingly, only one (1.5%) out of 66 individuals with total point score > 25 experienced a transformation to an active CLL phase requiring therapy, while such an event occurred in 6 (21.4%) out of 28 cMBL individuals with total point score ≥25. Finally, the likelihood of treatment of individuals with total point scores ≥25 was substantially greater than that of individuals with total point scores < 25 (P=0.02). This translates into a C-statistic value highly significant (c=0.80) that underscores concordance between observed and predicted TTFT. These data emphasize the relationship between cMBL and early CLL also regarding the use of clinico-biological parameters relevant for predicting TTFT and provide no evidence to separate these entities by a threshold of clonal B-cells.

Platelet Disorders

CO085

VENOUS THROMBOEMBOLIC EVENTS IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS AFTER SPLENECTOMY OR DURING THROMBOPOIETIN RECEPTOR AGONISTS (TPORA) THERAPY: SINGLE-CENTRE EXPERIENCE

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Recent evidences suggest that chronic ITP patients could have an higher risk of venous thromboembolism (VTE) compared with general population. Between second-line approaches, splenectomy and TPOra are regarded as the most suitable therapies in adults with chronic ITP. Although laparoscopic approach have reduced complications of splenectomy, VTE may occur. Equally, occurrence of VTE was demonstrate in extend trials with TPOra. Aim of this study was to evaluate the rate of VTE in ITP patients after splenectomy and during treatment with TPOra. We retrospectively evaluated 37 ITP patients who underwent splenectomy (20 patients) or TPOra (17 patients) between 2008 and 2012 at our Institution. Among splenectomized patients, 8(40%) were male and 12(60%) female, with a median age at diagnosis of 40 years(11-68) and a median age at splenctomy of 45 years (16-78). At splenectomy, 14(70%) patients had a chronic ITP, 5(25%) a persistent ITP and 1(5%) patient a newly diagnosed ITP. Laparoscopy approach was used in all patients. Among TPOra-treated patients, 6(35%) were male and 11 female (65%), with a median age at diagnosis of 58 years (11-86) and a median age at the start of TPOra treatment of 64 years(28-89). 5 patients received TPOra because of refractory, splenectomized ITP, while 12 patients were not suitable for splenectomy. The median observation time was 15 months in both groups (range 1-59 and 3-45 respectively). Two thromboembolic events were recorded in the splenectomized group (cumulative incidence 10%). A splanchnic vein thrombosis (SVT), occurred in a 38-years old male with persistent ITP, while a VTE (pulmonary embolism and deep vein thrombosis at leg) occurred in a 70-years-old female with chronic ITP. Both events occurred after few days from splenectomy, despite peri-surgery thromboprophylaxis with low molecular weight heparin. Among TPOra- treated patients, two SVT was recorded (cumulative incidence 11.7%). The events occurred in a 55-years-old female with refractory ITP and in 62-years-old female with chronic ITP, after one month and two years from the beginning of TPOra, respectively. In this preliminary study, a similar cumulative incidence of VTE was observed in two group of chronic ITP patients after splenectomy and during TPOra treatment; 3/4 VTE were SVT, an event quite rare in ITP. In one case a diagnosis of criptogenetic cirrhosis was made. A prospective clinical trial is desirable to better evaluate any difference in thrombophilic risk.

CO086

EFFICACY AND SAFETY OF ELTROMBOPAG FOR TREATMENT OF CHRONIC IMMUNE THROMBOCYTOPENIA. THE R.E.P. (A.H.N. APULIAN HEMATOLOGICAL NETWORK) EXPERIENCE

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Eltrombopag is a thrombopoetin-receptor agonist able to increase the platelet count in patients with immune thrombocytopenia (ITP). To date only a few data are available in the literature about its clinical efficacy and safety. In this report we propose a retrospective multicentric analysis of data about the clinical use of eltrombopag in patients with refractory ITP. We analyzed data coming from 23 patients with ITP, 58.1(45.5) years old [mean(SD)], 73.9% females, diagnosed with ITP 84.8(27.7) months before the treatment with eltrombopag, 73.9% of them without splenectomy. All patients were previously treated with steroids as first line therapy. The mean number of previous lines of therapies was 2.8(0.7) including rituximab (21.7% of cases) and romiplostim (17.4%). The patients were considered responsive to the eltrombopag when platelet count was >50,000/L, or at least doubled in comparison with the baseline. In our cohort the duration of the treatment with eltrombopag was 9.74(5.7) months with an average dosing of 53.3(17.4) mg. After three months 20 out of 23 patients were responders to eltrombopag (86.9%): one of them became non-responder afterward. Two patients out of 23 (8.7%) experienced significant adverse events (gastrointestinal disorders): they withdrew the therapy. Two out of 3 non-responders to eltrombopag had been treated previously with rituximab but 3 patients who relapsed after rituximab discontinuation were responders to eltrombopag. Two out of 4 patients previously treated with romiplostim (50%) were responders to eltrombopag. Eight patients withdrew eltrombopag: no-response in 3 cases, loss of response in 1 case, toxicity in 2 cases, no-treatment-related death in 1 case and stable response without need of further therapy in 1 case. The response to eltrombopag was more frequent in non-splenectomized patients (X2-test $p < 0.0001$). Our results demonstrate that after about 10 months of therapy eltrombopag is effective and safe in patients with ITP. The drug can be effective even in pre-treated patients with rituximab or romiplostim. Longer follow-up and wider cohorts of patients are mandatory to evaluate the long-term toxicity of the drug and the stability of the response of the patients after its withdrawal in order to assess the eventual safety of the discontinuation of the therapy.

CO087

DIFFERENTIAL DIAGNOSIS OF THROMBOTIC MICROANGIOPATHY: DATA FROM PADOVA TTP CASE SERIES

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Thrombotic thrombocytopenic purpura (TTP) is a rare disease. Differential diagnosis is often difficult, due to similarity in presentation with hemolytic uremic syndrome (HUS) and other thrombotic microangiopathies (TMA). Our aim was to investigate differences in clinical and laboratory features between TTP, HUS and other TMA in our case series. We retrospectively analyzed data from patients with a first clinical diagnosis of acute TMA. TTP and HUS diagnosis were clinically made as reported in the literature.

All patients not fitting these criteria were included in the other TMA group. ADAMTS13 activity and IgG anti-ADAMTS13 were assessed using commercially available FRETS-VWF73 and ELISA assays respectively. Severe protease activity deficiency was considered as ≤10%, as reported in the literature; however two subgroups were made (protease activity ≤5% and 6-10%) to compare different levels of the assay sensibility. IgG anti-ADAMTS13 was considered present for title ≥17 U/mL, according to the manufacturers instructions. Chi-square test and Anova analysis were used for groups comparison. Between June 2005 and November 2012, 88 TTP, 9 HUS and 22 other TMA patients were collected (M/F=44/85; mean age 44±17, range 3-82) (Table 1). When comparing the three groups together, TTP patients had a higher prevalence of neurological impairment (p 0.006) and lower mean platelet count ($p<0.001$); HUS patients showed higher prevalence of renal failure ($p<0.001$). Bleeding prevalence was significantly different too (p 0.014). ADAMTS13 activity and IgG anti-ADAMTS13 were tested in 106 patients: none of TTP ones had normal values of ADAMTS13 activity, while none of HUS or other TMA patients had severely reduced levels of the protease at diagnosis ($p<0.001$). When comparing HUS and other TMA groups, ADAMTS13 activity was not different. Clinical features at presentation can guide differential diagnosis when TMA is present: a low platelet count and predominant neurological impairment orient toward a diagnosis of TTP, while predominant acute renal failure with high levels of creatinine can be more specific for HUS diagnosis. Very low levels of ADAMTS13 activity are specific for TTP diagnosis, so that a protease activity >10% at onset can reasonably exclude TTP. There seems to be no difference when choosing a threshold of 5 or 10% activity to define a severe reduction with FRETS-VWF73 assay. ADAMTS13 activity, however, seems not useful to distinguish HUS and other TMA.

Table 1. Clinical and laboratory features of patients with acute TMA (Padova case series, June 2005-November 2012)

	TTP (88 pts)	HUS (9 pts)	Other TMA (22 pts)	P value
Fever	36 (41%)	5 (56%)	10 (45%)	n.s.
Neurological impairment	57 (65%)	3 (33%)	7 (32%)	0.006
Acute renal failure	25 (28%)	9 (100%)	11 (50%)	< 0.001
Bleeding	68 (77%)	3 (33%)	15 (68%)	0.014
Plts (G/L, mean ± SD)	18 ± 17	53 ± 36	48 ± 28	< 0.001
Hb (g/L, mean ± SD)	87 ± 21	94 ± 21	83 ± 16	n.s.
LDH (U/L, mean ± SD)	1721 ± 1151	2320 ± 1555	2141 ± 2684	n.s.
Creatinine (umol/L, mean ± SD)	101 ± 63	448 ± 363	157 ± 155	< 0.001
ADAMTS13 activity (%)				
- ≤ 5%	68 (77%)	0	0	< 0.001
- 6-10%	5 (6%)	0	0	
- 11-64%	3 (3%)	4 (44%)	14 (64%)	
- ≥ 65%	0	4 (44%)	8 (36%)	
- untested	12 (14%)	1 (12%)	0	
Anti-ADAMTS13 IgG (%)				
- negative	6 (7%)	6 (66%)	20 (91%)	< 0.001
- positive	70 (79%)	2 (22%)	2 (9%)	
- untested	12 (14%)	1 (12%)	0	

CO088

RESPONSE TO TPO-MIMETIC DRUGS ELTROMBOPAG AND ROMIPLOSTIM IN A SINGLE CENTER EXPERIENCE

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Background. Recently, thrombopoietin (TPO) mimetic drugs have been introduced in the treatment of immune thrombocytopenia (ITP). Two drugs are currently approved: eltrombopag (ETP) and romiplostim (RPL). There are no trials comparing directly these two drugs, neither information is available about the respective efficacy of each TPO-mimetic agent in patients who did not response to the alternative agent. Aim of the study: To assess the response to ETP and RPL in patients with ITP treated with a single agent or receiving both agents subsequently. Patients and methods: Fourteen patients with ITP (M/F 11/3, median age 53 years, range 13-71) received in our Center ETP or RPL or both agents from 2009 to 2013. Twelve patients had received >2 treatment lines before the first administration of TPO-mimetics; 4 patients had had splenectomy. Overall, patients received at different times of their clinical history 27 cycles of treatment with either agent (11 with ETP and 16 with RPL). We analysed only the weeks of therapy distant at least 21 days from treatments other than standard-dose steroids. Therefore, only 20 cycles of treatment were evaluable for analysis of response. Overall complete response (OCR) or overall response (OR) were defined as a platelet count >100,000/mmc or between 30,000 and 100,000/mmc in at least 4 consecutive weeks, respectively. Durable complete response (DCR) or durable response (DR) were defined as a platelet count >100,000/mmc or >30,000/mmc in at least 6 of the last 8 weeks of treatment, respectively. All definitions excluded responses within 4 weeks after rescue medications. Results. The number of weeks of treatment with either agent, the rates of response and the duration of response are shown in Table. The number of weeks on OCR was higher during treatment with RPL; however, no difference was found between ETP and RPL either in OR and DR. Because of lack of response, 2 patients were switched from RPL to ETP, and 2 patients were switched from ETP to RPL: in all cases no response was achieved after the switch to the other drug. Conclusions: In this cohort ETP and RPL showed no difference in the overall efficacy. Further studies on larger patient cohorts are needed to confirm these findings, which should allow to choose treatment with TPO-mimetics on the basis of parameters such as patient's compliance or preference, drug manageability, and drug cost.

Table 1.

	Eltrombopag	Romiplostim	P
Patients treated with a single TPO-mimetic	4	5	
Patients treated with both TPO-mimetics	5 *		
Responders, n patients	4 / 9 (44%)	5 / 10 (50%)	1.00
Weeks of treatment, n	182	247	
Platelet count > $100x10^9$/L (OCR), weeks	85 (47%)	142 (57%)	0.03
Platelet count 30 - $100x10^9$/L (OR), weeks	57 (31%)	65 (26%)	0.27
OCR + OR, weeks	142 (78%)	202 (82%)	0.39
OCR + OR, n cycles	4/8 (50%)	8/12 (67%)	0.64
Durable response (platelet count > $30x10^9$/L), n cycles	4/8 (50%)	7/12 (58%)	1.00

*1 patient received 2 evaluable cycles of romiplostim; 1 patient received additional 2 cycles (either romiplostim and eltrombopag) not evaluable because of concomitant rescue medication

CO089

THROMBOPOIETIN MIMETIC AGENTS (TPO MIMETICS) FOR PRIMARY IMMUNE-MEDIATED THROMBOCYTOPENIA (ITP): A LIFE-LONG THERAPY? RESULTS FROM A RETROSPECTIVE CASE SERIES

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Introduction. ITP is due to increased platelet (plt) destruction coupled with inappropriate plt production, resulting in peripheral thrombocytopenia. TPO mimetics offer new potentials for treating refractory ITP pts, enhancing production of plt. Because of their mechanism of action, TPO mimetics where thought to be a life-long therapy; however, this may not be the case. We report on a small series of pts treated with romiplostim (R) who were able to discontinue R after achieving response without relapsing at long-term follow-up. Patients. 18 pts (11F) refractory to steroid/IVIG received R through 2010-12: 4 early diagnosis (<3 mos from ITP onset); 9 splenectomized; 9 not eligible to splenectomy; 3 median lines of previous therapies, range 1-5. Median age at diagnosis of ITP 46.5 yrs, range 11-79; median age at R treatment 55.5 yrs, range 20-82. Median time from initiation of any treatment and R administration: 12 mos, range 1-264. Results. Response to R was achieved in 15/18 pts: 9 CR, 5 PR; 1 pt relapsed at 10 mos and antibodies against R were detected. At response, R doses ranged from 1 µg/kg every other week to 5 µg/kg/week. Stable plt levels were obtained in 12/14 responding pts; in these pts, R had been administered for a median of 7 mos, range 3-30, before attempting withdrawal. No specific schedule of withdrawal was selected: R was discontinued when long-term plt response was stable. 5 pts needed occasional re-exposure to R, because of re-occurrence of thrombocytopenia (TP), months after weekly administration was discontinued. Median follow up from R withdrawal is 11.5 mos, range 2-32. Discussion: In some ITP pts, thrombopoiesis is worsened by inappropriate plt production. TPO mimetics provide an additional stimulus to increase megakariocyte's (M) survival and plt production. However, this stimulation doesn't seem to be needed indefinitely: it appears that R is able to circumvent whatever inhibitory event is taking place within the bone marrow and restore M ability to respond to plt destruction. If confirmed, our finding has relevant implications: concern about TPO mimetics long-term sides effects (marrow fibrosis, thrombotic events) would be greatly reduced and treatment cost would be less of an issue thus making R use more feasible in newly diagnosed severe ITP not responding to first line therapy.

CO090

ROLE OF PLATELET KINETICS IN IMMUNE THROMBOCYTOPENIA (ITP) IN THE RITUXIMAB ERA: A SINGLE-CENTER STUDY OF 78 PATIENTS WITH A MEDIAN FOLLOW-UP OF 22 YEARS

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Platelet kinetic studies have been widely used in order to confirm immune thrombocytopenia (ITP) diagnosis and to provide predictive factors for response to splenectomy.The advent into clinical practice of other therapeutic options,including rituximab and TPO-receptor agonists,has increased the need to identify those patients more likely to fail surgery.Available data on platelet kinetics come from either small cohort of patients or with limited observation time. Autologous ^{111} In platelet-labelling was performed according to the recommendations of the International Committee for Standardization in Haematology Panel on Diagnostic Applications of Radionuclides (ICSH, 1988).Responses to treatment were defined according to current guidelines.Between 1972 and 1999, 78 ITP patients (male 27, female 51)underwent a platelet kinetic study before splenectomy and have now a median follow-up after surgery of 22 years (range, 1-37).All patients were primary resistant to or relapsed after front-line steroid therapy. Median interval from diagnosis to splenectomy was 14 months (range 1-187);median age at splenectomy was 31 yr (range, 9-65).Overall, 70 (89.7%) patients responded to splenectomy (CR, 80.7%; R, 9%).Nine out of 70 (12.8%) patients relapsed after initial response after a median time of 8 months (range, 2-65);a long-term stable response to splenectomy was therefore achieved in 61 (78%) patients.The pattern of platelet sequestration was predominantly splenic in 59 patients (76%), predominantly hepatic in 13 patients (17%) and diffuse in 6 (8%).No baseline characteristics could predict response or site of platelet sequestration. Patients with no-splenic (diffuse and hepatic) sequestration showed significantly lower response rates and lower incidence of stable responses to splenectomy compared to patients with splenic captation (Table 1).No significant correlation was found between platelet turnover/lifespan and outcome.Site of platelet sequestration could identify those 25% patients who had a lower benefit from surgery. The incidence of long-term stable responses ("cure") in those low-responders was at least comparable to that observed in patients treated with Rituximab,where the CR rate is around 20% at 5 yrs.Although confirming its negative predictive value, platelet sequestration study was not sensitive enough to isolate those patients who should avoid splenectomy,with a predicted response rate to surgery lower than the expected response to rituximab.

Table 1. Response to splenectomy after patients' stratification according to site of platelet sequestration.

Site of sequestration	No. of pts (%)	Response (CR+R)	p	Response (CR)	p	Stable response*	p
Diffuse and hepatic	19 (24%)	13 (68.4%)	0,002	11 (57.8%)	0,007	3 (23%)	0,018
Splenic	59 (76%)	57 (97%)		52 (88%)		51 (86%)	

* Patients in ongoing response, without any treatment after splenectomy, with a median follow-up of 22 yrs.

Chronic Myeloid Leukemia

CO091

CHRONIC MYELOID LEUKEMIA WITH VARIANT T(9;22) REVEALS A DIFFERENT SIGNATURE FROM CASES WITH CLASSIC TRANSLOCATION

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The t(9;22)(q34;q11) generating the Philadelphia chromosome and the BCR/ABL1 fusion gene represents the cytogenetic hallmark of chronic myeloid leukemia (CML). About 5–10% of CML cases show variant translocations with the involvement of other chromosomes in addition to chromosomes 9 and 22. The prognostic significance of variant t(9;22) was unclear and debated in the pre-imatinib era, whereas recent studies of large CML series showed that the presence of variant translocations has no impact on the cytogenetic and molecular response or on prognosis. However, the molecular bases of differences between CML patients with classic and variant t(9;22) have never been elucidated. We report a gene expression profile (GEP) analysis of 8 CML cases with variant t(9;22) and 12 patients with a classic t(9;22) identifying a set of 59 genes as differently expressed. Querying the Database for Annotation, Visualization and Integrated Discovery (DAVID) showed that the enhanced biological process in our gene set involved the intracellular protein kinases cascade. The kinases list included 5 genes: TRIB1 (tribbles homolog 1), STK17B (serine/threonine kinase 17b), PTK2B (PTK2B protein tyrosine kinase 2 beta), C5AR1 (complement component 5a receptor 1) and ZFP36 (zinc finger protein 36, C3H type, homolog). Further Ingenuity Pathways Analysis (IPA) yielded strong indications that 19 out of 59 dysregulated genes from our dataset are involved in the "Haematological System Development and Function, Cellular Development" network. A central role in this network is played by several proteins that are known to be activated in BCR/ABL1 cells, namely ERK1/2 (extracellular signal-regulated kinases), p38MAPK (p38 mitogen-activated protein kinase), JNK (c-Jun N-terminal kinase), and cell cycle regulator AKT (RAC-alpha serine/threonine-protein kinase). Noteworthy, the upregulated kinase genes revealed by DAVID analysis are also enclosed in the network identified by IPA. Moreover, TRIB1, PTK2B and C5AR1 kinases are involved in the regulation of the RAS/MAPK pathway. In conclusion, our GEP analysis performed on CML cases with variant t(9;22) improved the understanding of the biological mechanisms at the basis of the CML heterogeneity. Overall, our results reveal that in CML cases with variant t(9;22) there is an enhancement of the MAPK pathway deregulation already known to underlie the CML pathogenesis and point out the role of interesting candidate genes, such as TRIB1, PTK2B, and C5AR1.

CO092

RUXOLITINIB SYNERGIZES WITH TYROSINE KINASE INHIBITORS TO OVERCOME DRUG RESISTANCE RELATED TO BONE MARROW STROMA MICROENVIRONMENT IN CHRONIC MYELOID LEUKEMIA (CML)

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Most patients with CML in chronic-phase (CML-CP) treated with Imatinib has shown an overall survival rate of 85% in a 8 year result update of IRIS trial, but only a minority of Imatinib-treated patients achieve complete molecular response (CMR). Although second-generation tyrosin kinase inhibitors (TKIs) yield higher rates of CMR versus Imatinib there is no still evidence to support the eradication of CML

stem cells. Recent evidence suggests that upon TKI treatment, CML stem cell survival is BCR-ABL kinase independent. The BM is a dynamic microenvironment with high concentration of soluble factors that regulates haematopoiesis, enhances leukemia blast survival and modulates their resistance to pharmacological treatment. Ph+ K562 cells were cultured in RPMI medium, defined as regular media (RM). The human stroma cell line HS-5 serum-free supernatant was used as feeder (HS5/SCM). The apoptosis of Ph+ K562 cell line, treated with clinical doses of Imatinib, Nilotinib or Dasatinib on HS-5 monolayer is significantly reduced (18%±13%, 50%±6%, or 10%±10%, respectively), respect to RM (46%±12%, 84%±15%, or 53%±20%, respectively). Moreover, the TKI-resistance is also related to soluble factors produced by HS-5 cells. Indeed, apoptosis is greatly reduced when K562 cell line is treated with Imatinib, Nilotinib or Dasatinib in the presence of HS-5/SCM (20%±9%, 29%±18%, or 17%±6%, respectively), respect to RM. Furthermore, the IC50 of Imatinib, Nilotinib or Dasatinib is significantly increased when K562 cell are cultured on HS-5/SCM (7957nM, 889nM, 2.5nM, respectively) *vs* the IC50 calculated in RM (545nM, 13.93nM and 1.12nM, respectively). Upon SCM exposition, Ph+ cells, treated with TKIs, preserve long-term ability to re-start proliferation *in vitro* after TKI withdrawal. Indeed, the resistance to TKI treatment in this stromal co-culture experimental model is associated to BCR-ABL-independent STAT-3 activation. Furthermore, we demonstrated that JAK inhibitor Ruxolitinib synergizes with TKIs to induce apoptosis in progenitor CML cells and to reduce their clonogenic potential. Importantly, Imatinib, Nilotinib, and Ruxolitinib, alone or in combination did not significantly impair the formation of normal erythroid and myeloid colonies. Taken together, our data provide a rational for the therapeutic combination of TKIs and Ruxolitinib to the aim for eradication of primary BCR-ABL+ cells homed in BM niches.

CO093

BIN1 AND DNM1L GENES MODULATION IN CHRONIC MYELOID LEUKEMIA

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Introduction. The role of Bcr-Abl in the pathogenesis of Chronic Myeloid Leukemia (CML) is well established, however the mechanisms involved in progression remain poorly understood. By making use of our Drosophila Melanogaster transgenic model for human Bcr-Abl gene, we have identified the involvement in CML progression of different genes regulating the recycling and the degradation of tyrosine kinase (TK) receptors through the assembly of clatrin coated vesicles. Among them, we focused our attention on Bridging integrator 1 (BIN1) and Dynamin 1-like (DNM1L). Methods. Bin1 and Dnm1l expression was measured by quantitative Real Time PCR (qRT-PCR) in 60 samples from CML patients and in 10 healthy control samples. Among CML, 26 samples were collected at diagnosis and 34 during tyrosine kinase inhibitors (TKI) treatment, 18 of which during complete molecular remission (CMR). Bin1 e Dnm1l expression was also analysed by qRT-PCR in K562 cell line, after treatment with Imatinib (1nM, 100nM, 1 M) at different time (6, 24 and 48h). Results. We found that in CML patients both Bin1 and Dnm1l expression was significantly decreased at the time of diagnosis (p=0.0001), as compared to healthy subjects. Overall, during TKI therapy, the transcript levels of Bin1 were up-regulated if compared with diagnosis time, while Dnm1l expression still remained down-regulated. Interestingly, both Bin1 and Dnm1l transcript analysis showed an increase in CMR phase if compared with diagnosis (p=0.001 and p=0.0016, respectively) and during TKI treatment without CMR (p=0.0011 and p=0.0029, respectively). Bin1 expression was increased in K562 cell line at 24 and 48h after Imatinib treatment instead Dnm1l was decreased; these experiments confirm the qRT-PCR data obtained from patients during TKI treatment. Conclusions. Our experiments show a significant association between Bin1 and Dnm1l expression and clinical phases of CML, suggesting a direct correlation with Bcr-Abl levels. This study proposes a new deregulated mechanism in CML indicating Bin1 and Dnm1l as possible players in the maintenance of the abnormal signalling in this haematological disease.

CO094

COMBINATION OF EUTOS SCORE AND 3-MONTH BCR-ABL TRANSCRIPT LEVEL IDENTIFIES A GROUP OF GOOD-RISK CML PATIENTS WITH FAVOURABLE RESPONSE TO IMATINIB FRONT-LINE

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Background. The availability of three TKIs approved for the front-line treatment of Chronic Myeloid Leukemia (CML) raises questions about the optimal treatment strategy in individual patients. Two risk factors at diagnosis, spleen volume and basophils percentage (the EUTOS score), and one dynamic risk factor, reduction ≤10% of BCR-ABL transcript level after 3 months of imatinib, have been separately proved able to predict the long-term outcome of treatment. Aims and Methods. To identify patients with greater probability of good response to imatinib, we retrospectively tested the combination of EUTOS score and 3-month BCR-ABL transcript level in 169 consecutive CML patients treated front-line with standard dose (400 mg daily) imatinib. Patients were stratified into "good" (low EUTOS score and 3-months BCR-ABL ≤10%); "intermediate" (high EUTOS score or 3-monthsBCR-ABL >10%) and "poor" (high EUTOS score and 3-months BCR-ABL >10%) risk groups. Optimal responses were defined according to the European LeukemiaNet recommendations. Time to treatment failure (TTF) was measured from the start of imatinib to any of the followings: progression to accelerated or blastic phase (AP/BP), death for any cause at any time, hematologic or cytogenetic resistance leading to imatinib discontinuation. Progression free survival (PFS) was measured from the diagnosis to AP/BP or death for any cause at any time.

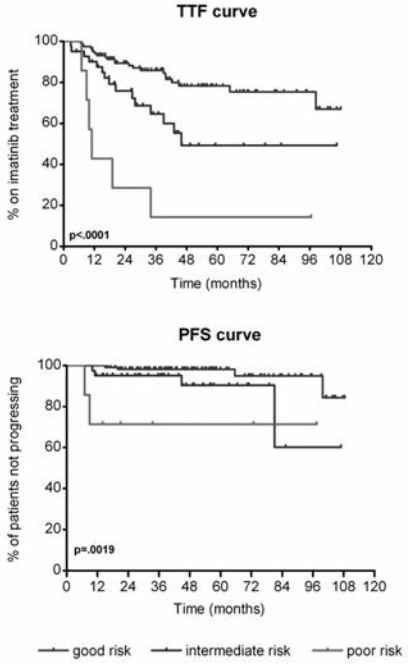

Figure 1.

Results. Median age of patients was 55 years (range 19–84). According to the EUTOS score there were 150 low risk (88.8%) and 19 high risk patients (11.2%); 3-month BCR-ABL transcript was ≤10% in 133 (78.7%) and >10% in 36 patients (21.3%). Patients with "good", "intermediate" and "poor risk" profiles were 71.6%, 24.3% and 4.1%, respectively. Patients in the "good risk" group had a significantly higher probability of achieving optimal response to imatinib: 6-month PCyR rates were 91.3%, 78.4% and 42.9% for good, intermediate and poor risk, respectively (p=0.0042), 12-month CCyR rates were 91.2%, 69.7% and 20% (p=0.0001), 18-month MMR rates were 77.9%, 26.1% and 0%, (p<0.0001). After a median follow-up of 43.2 months (range 11–108), the probabilities of imatinib failure were 18.2%, 36.6% and 85.7% respectively. Also TTF and PFS were significantly different (Figure). Conclusions. The combination of EUTOS score and 3-month BCR-ABL level identifies a group of about two thirds of CML patients with a very good outcome on imatinib front-line treatment.

CO095

VEGFR-2 SIGNALING MAY BE IMPLICATED IN IMATINIB (IMA) RESISTANCE THROUGH THE REGULATION OF SHP-2 PHOSPHATASE

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In the field of CML, although the vast majority of patients respond to TKI therapy, primary or acquired resistance may occur. We previously show that the tumor suppressor tyrosine phosphatase SHP-1 plays a key role in BCR-ABL-independent IMA resistance modulating the activation signals that SHP-2 receives from both Bcr/Abl and membrane receptor tyrosine kinases in both CML cell line and patients1. Several groups show that the signaling from VEGFR-2 is necessary for proliferation, chemotaxis and cell survival of several tumor systems. The elucidation of the BCR-ABL independent regulatory pathway in IMA resistant CML cells is important to develop new therapeutic strategy to overlap IMA resistance. In this study we investigated the role of SHP-1 and SHP-2 in VEGFR-2 signaling in KCL22 Ph+ cell lines (IMA sensitive/KCL22-S and IMA resistant/KCL22-R). To evaluate if VEGFR-2 interact with SHP-1/SHP-2, we perform specific co-immunoprecipitation assay. Moreover, we carried out WB experiments to evaluate the phosphorylation status of VEGFR-2 protein in our CML system. To further characterize the functional role of VEGFR-2 in IMA resistance, we knockdown VEGFR-2 in KCL22-R cell line by sh-RNA. RT-PCR and WB analysis showed that VEGFR-2 is up-regulated in KCL22-R respect to KCL22-S cell line. In addition, we demonstrated that VEGFR-2 forms a complex with SHP-1 and SHP-2 in sensitive KCL22-S cell line. In contrast, VEGFR-2 interacts only with SHP-2 in KCL22-R cell line, in which SHP-1 is down-regulated. Ectopic SHP-1 expression was able to establish VEGFR-2/SHP-1 interaction also in resistant cell line. Recently, Bhattacharya *et al*, demonstrated that SHP-1 regulates negatively VEGFR-2 signaling by dephosphorylation of specific tyrosine residues in HUVEC cell line. Thus, we hypothesized that SHP-1 might modulate negatively the VEGFR-2 signals also in our CML system. In particular, resistant cell line, lacking of SHP-1, but not KCL22-S cell line, show phosphorylation at Y996 and Y1059 of VEGFR2 also after IMA treatment. Moreover, ectopic SHP1 expression in KCL22-R was able to dephosphorylate VEGFR-2 at the same sites. To better clarify the direct role of VEGFR-2 activation in IMA resistance, we knockdown VEGFR-2 in KCL22-R cell line, restoring sensitivity to IMA. Our data indicate the involvement of VEGFR-2 in the Bcr-Abl independent pathway of IMA resistance and that the axis SHP-1/SHP-2 may modulate VEGFR2 signaling in CML cell lines.

Ref. 1. Esposito N, et al. Blood 2011.

CO096

IMATINIB MESYLATE FOR NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE: A LONG-TERM ANALYSIS BY THE GIMEMA CML WORKING PARTY

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Second generation TKIs have been approved for the treatment of chronic myeloid leukemia (CML) in early chronic phase (ECP), but imatinib (IM) still represents the golden therapeutic standard. The long term outcome is important to assess the treatment efficacy and to decide on the allocation of resources. In the IRIS trial, at 8 year, 55% of patients were still on IM and the overall survival (OS) was 85%. Other published reports have shorter follow-up. We analyzed 559 patients enrolled within 3 studies of the GIMEMA CML WP (NCT00514488, NCT00510926, observational trial CML023). Response monitoring: according to 2009 ELN recommendations. Definitions: progression, transformation to advanced phases; failure, according to 2009 ELN criteria; event, treatment discontinuation for any reason (information on survival and progression regularly collected) or lost to follow-up. All deaths, at any time and for any reason, were included. All the analysis were made according to the intention-to-treat principle. Baseline characteristics: median age 52 (18-84) years; high Sokal, Euro, EUTOS scores, 22%, 7%, 7%, respectively; CCA in Ph+ cells 4%; b2a2 BCR-ABL transcript 36%. Median follow-up: 78 (7-99) months. The cumulative incidence of CCgR, MMR and MR4 was 88%, 85% and 61%, respectively. Sokal, Euro and EUTOS scores were able to predict significantly lower CCgR and MMR; a high Sokal score also predicted significantly inferior MR4. Reasons for IM discontinuation: lack of efficacy (19%), toxicity or death (9%), withdrawal of informed consent (4%); 4% of patients were lost to follow-up. The 8-year event-free survival (EFS), failure-free survival (FFS), progression-free survival (PFS) and OS were 60%, 74%, 84% and 85%, respectively. Sokal and Euro scores were able to predict significantly lower EFS, FFS, PFS and OS; the outcome differences between low and intermediate risk patients were significant. Despite rarely, progressions were also observed among low risk patients. High EUTOS risk patients had poorer outcome, but PFS and OS differences were not significant. Age, performance status and b2a2 transcript resulted independent prognostic factors on PFS and OS. In a large nationwide multicentric experience, high response rates produced favourable outcomes in the majority of patients, particularly in low risk patients. Further therapeutic strategies are needed to overcome the adverse prognosis of high risk patients.

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Myeloma and Monoclonal Gammopathies II

CO097

BORTEZOMIB IMPROVES BONE INTEGRITY IN MULTIPLE MYELOMA PATIENTS BY BLOCKING OSTEOCYTE DEATH AND AUTOPHAGY

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Osteocytes are critical in the maintenance of bone integrity regulating bone remodeling through the cell death and authophagy. Recently we have demonstrated that an increased osteocyte death is involved in multiple myeloma (MM)-induced osteolysis. Bortezomib (BOR) currently used in the treatment of MM stimulates bone formation however its effects on osteocytes are not known and have been investigated in this study. We performed a histological evaluation on bone biopsies of a cohort of 37 newly diagnosis MM patients 31 of them with symptomatic MM and 6 with smoldering MM (SMM). The 55% of patients with MM have evidence of osteolytic lesions at the X-rays survey. Bone biopsies were obtained at the diagnosis and after an average time of 12 months of treatment or observation. Osteocyte viability was evaluated in a total of 500 lacunae per histological sections. A significant increase of the number of viable osteocytes was demonstrated in MM patients treated with Bortezomib (BOR)-based regimen as compared to those treated without BOR (% median increase: +6% *vs* +1.30%; p=0.017). Patients treated with BOR alone showed the highest increase of osteocyte viability, as compared to either patients treated without BOR (+11.6% *vs* +1.3%, p=0.0019) or those treated with BOR plus Dexamethasone (DEX) (+11.6% *vs* +4.4%, p=0.01). A reduction of osteocyte apoptosis was demonstrated by TUNEL assay. On the other hand, any significant difference was not observed in patients treated with Thalidomide (THAL) or Immunomodulatory drugs (IMiDs) than in those untreated with these drugs (p= 0.7). A multiple regression non-parametric analysis showed that BOR had a significant positive impact on osteocyte viability (p=0.042) whereas THAL/IMiDs as well as Zoledronic acid (ZOL) treatments have not (p=0.2). BOR also counterbalanced the negative effect of DEX treatment (p=0.035). The potential effect of BOR on osteocyte viability was confirmed *in vitro* in murine osteotcytic cell line MLO-Y4 and in the human pre-osteocytic one HOB-01. BOR and the proteasome inhibitors MG262 and MG132 significantly blunted MM-induced osteocytic cell death. Interestingly, BOR also blocked osteocytic authophagy in co-culture with MM cells as well as that induced by DEX treatment. Finally PTH short-term treatment potentiated the *in vitro* effects of BOR. Our data suggest that proteasome inhibitors block osteocyte death and autophagy supporting their use to improve bone integrity in MM patients.

CO098

ROLE OF 18F-FDG PET/CT IN THE ASSESSMENT OF PATIENTS WITH SMOLDERING MULTIPLE MYELOMA

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Background. Smoldering multiple myeloma (SMM) is characterized by absence of organ damage (hypercalcemia and/or renal insufficiency and/or anemia, and/or lytic bone lesions, BL) and is left untreated. Whole body X-ray (WBXR) and magnetic resonance imaging (MRI) are the cornerstone imaging procedures of staging. Aim of the study: To investigate the role of 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT) for the assessment of patients with SMM without BL in standard imaging. Patients and methods: Forty-six patients (M/F 23/23, median age 67.5 years, range 39-8) with SMM were recruited from 2008 to 2012. MC components were IgG (26 patients), IgG (7), IgAk (7), IgA (4), light chain (1), and biclonal (1). All patients performed at diagnosis WBXR, MRI of the spine and pelvis, and PET/CT; the latter was repeated in 13 during follow-up (median 11.5 months, range 5-31). Results. In 12 cases with negative first WBXR at diagnosis, PET/CT detected focal increased FDG uptake and upstaged the disease (26%). All the abnormal areas corresponded to BL identified by MRI (n=10) or CT (n=2) (100% sensitivity); in 1 patient bone lesion was confirmed by a a novel detailed X-ray. Notably, the BL were outside the spine and pelvis and were confirmed by a second MRI in 3 cases (6.5% of the overall cohort and 25% of the PET/CT-positive patients). In 2 additional cases PET/TC detected synchronous solid neoplasms (renal and prostatic). Overall, PET/CT was advantageous in 14 patients (30.4%) and prevented underdiagnosis in 5 (3 with BL outside the spine and pelvis and 2 with another neoplasm) (10.8%). In 4 of the remaining 32 cases (25%) without FDG uptake at diagnosis, PET/CT allowed to classify as osteoporosis some BL doubtful at standard imaging. In 3 cases PET/CT was falsely negative (91% specificity): in 1 patient cuneiform vertebra bone biopsy showed plasmocytoma, and 2 patients developed during the follow-up BL detectable at MRI in X-rays areas which were suspected at diagnosis but were PET/CT-negative. During the follow-up, PET/CT revealed evolution of disease in 4/13 patients (30.7%). Conclusions: PET/CT can help to upstage SMM at diagnosis or during the follow-up in at least one-quarter of cases and should be included in the imaging work-up of SMM, preceding MRI to allow targeted imaging in cases of increased FDG uptake outside the spine and pelvis.

CO099

META-ANALYSIS OF 6383 INDIVIDUAL PATIENT DATA: SECOND PRIMARY MALIGNANCIES (SPM) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS ACCORDING TO LENALIDOMIDE EXPOSURE

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Background. Three randomized trials recently reported an increased risk of second primary malignancies (SPMs) in newly diagnosed myeloma (MM) patients treated with lenalidomide (Len). The excess risk with lenalidomide compared with non-lenalidomide has not been described. We performed an individual patient data meta-analysis to estimate the incidence of SPM according to Len exposure. Methods. Relevant studies, from PubMed and ASCO/IMW/ASH abstracts (after 2000), that met the following criteria were included: (1) randomized trials of newly diagnosed MM patients; (2) randomization to treatment with Len in at least one arm (Lenalidomide-trials); (3) randomization to treatment to at least one new drug but not Len (no-Lenalidomide-trials); (4) available data of SPMs. The primary end point was the cumulative incidence of hematologic and solid SPMs estimated accounting for competing events. Results. 6383 patients were included in the analysis (median follow-up: 30 mos). Total cases of SPMs were 420 (6.6%), including 188 (2.9%) hematologic and 232 (3.6%) solid cancers. 3218 patients were enrolled in the lenalidomide-trials and were available for a direct comparisons between lenalidomide (Len, N=2620) *vs* non-lenalidomide (No-Len, N=598) treatments. The cumulative incidence rates of solid SPMs were similar in the two groups (Table) with no difference in different lenalidomide combinations. The cumulative incidence of hematologic SPMs were 1.4% at 3 years and 3.1% at 5 years in the Len group and 0.4% at 3 years and 1.4% at 5 years in the No-Len group. The observed difference was mainly attributed to the increased incidence in patients receiving Len+melphalan (1.8%) as compared with len+cyclophosphamide, (0.3%), len alone (0.3%) and melphalan only (0.4%). In multivariate age-, gender- and cluster-adjusted analysis, the association of len+melphalan increased the SPM risk of about 4-fold (HR 3.8, p<0.001) with no excess in patients receiving len+cyclophosphamide (HR 1.27, p=0.72) or len alone (HR 0.84, p=0.72) as compared with melphalan alone. The risk of death for adverse events and progression disease was higher than the risk of SPM. Conclusions: An increase of SPMs was observed in patients receiving lenalidomide compared with controls, mainly attributed to the increased occurrence of hematologic SPMs, especially with the combination

len+melphalan. In the context of the observed survival benefit, the benefit/risk profile of lenalidomide treatment.

Table 1. Cumulative incidence at 3 and 5 years of SPMs and death

	3-year cumulative incidence, % (95% CI)		5-year cumulative incidence, % (95% CI)	
	Lenalidomide	No lenalidomide	Lenalidomide	No lenalidomide
SPMs				
Overall	3.9 (3.0-4.9)	3.3 (1.7-4.9)	6.9 (5.3-8.5)	4.8 (2.0-7.6)
Solid	2.6 (1.8-3.3)	2.9 (1.4-4.4)	3.8 (2.7-4.9)	3.4 (1.6-5.2)
Hematologic	1.4 (0.8-2.0)	0.4 (0.0-0.9)	3.1 (1.9-4.3)	1.4 (0.0-3.6)
Death				
All Causes	23.5 (21.4-25.7)	24.8 (20.6-29.6)	47 (43.1-51.1)	68.2 (56.9-78.9)
Myeloma	13.3 (11.6-15.1)	14.6 (11.0-18.3)	25.6 (22.4-28.8)	36.3 (25.9-46.6)
Adverse events	6.7 (5.5-7.9)	6.4 (3.8-8.9)	9.8 (8-11.6)	19.2(12.1-26.3)
SPMs	1.0 (0.5-1.5)	0.7 (0.0-1.5)	2.4 (1.3-3.5)	0.7 (0.0-1.5)

CI, confidence interval; SPM, second primary malignancies

C100

LENALIDOMIDE MAINTENANCE IMPROVES SURVIVAL IN NEWLY DIAGNOSED YOUNG MULTIPLE MYELOMA (MM) PATIENTS

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Background. High-dose chemotherapy with haemopoietic stem-cell improves quality of response and survival in multiple myeloma (MM). The incorporation of new drugs into induction, consolidation and maintenance therapy is changing the treatment paradigm in newly diagnosed MM pts. Aims: To compare in a prospective randomized trial conventional chemotherapy plus novel agents [melphalan-prednisone plus lenalidomide (MPR)] with tandem high-dose melphalan (melphalan 200 mg/m2 with stem-cell support; MEL200), both followed by maintenance with lenalidomide or no maintenance. Methods. A 2x2 factorial randomized trial was designed. We randomly assigned 402 patients with newly diagnosed multiple myeloma (<65 years) to one of four treatment groups: six cycles of MPR followed by maintenance (N=98), six cycles of MPR without maintenance (N=104), tandem MEL200 followed by maintenance (N=100) or tandem MEL200 without maintenance (N=100). The primary end point was progression free survival (PFS); secondary end points included safety and overall survival (OS). Results. Patients characteristics were well balanced. Lenalidomide maintenance did not significantly increase response rate: CR rate was 18% after MPR and 23% after maintenance, while it was 24% after MEL200 and 32% after maintenance. After a median follow-up of 45 months, the median PFS was 25 months with MPR and 39 months with MEL200 (corresponding to a PFS of 50% *vs* 68% at 2 years, HR=1.66; 95%CI 1.27- 2.18, p=.0002). Median PFS was 37.45 months for maintenance and 25.5 months for no maintenance (p<.0001). The 4-year OS was 62% with MPR and 72% with MEL200; (p=0.27), 75% for maintenance and 58% for no maintenance (p=.02). No meaningful interaction was detected between MPR/MEL200 and maintenance/observation effects. Conclusions: MPR at diagnosis was clearly inferior to MEL200 when PFS is used as the main endpoint. At present, OS is similar between MPR and MEL200. Lenalidomide maintenance significantly reduced the risk of progression and of death independently from the previous treatment. therapy.

C101

LENALIDOMIDE PLUS DEXAMETHSONE (RD) VS MELPHALAN-LENALIDOMIDE-PREDNISONE (MPR) OR CYCLOPHOSPHAMIDE-PREDNISONE-LENALIDOMIDE (CPR) IN ELDERLY COMUNITY-BASED NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: EFFICACY AND SAFETY RESULTS FROM A PHASE 3 TRIAL

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Rd and MPR showed to be effective and safe in newly diagnosed multiple myeloma (MM) patients (pts). Cyclophosphamide represents a valid less toxic alkylating alternative. A formal comparison between 2-drug and 3-drug lenalidomide-containing combinations has not been performed. The aim of this phase III trial was to compare a not-alkylating regimen (Rd) *vs* alkylating regimens (MPR/CPR) in a community-based setting of MM pts ≥65 years old or not eligible to autologous stem cell transplantation. Patients were randomized (1:2) to receive 9 28-day cycles of Rd or MPR/CPR. Upfront dose reductions of dexamethasone, melphalan and cyclophosphamide were performed, according to pt age (Rd: lenalidomide 25 mg/day for 21 days; dexamethasone 40 mg on days 1,8,15 and 22 in pts 65-75 years old and 20 mg in those >75 years; MPR: lenalidomide 10 mg/day for 21 days; melphalan orally 0.18 mg/Kg for 4 days in pts 65-75 years old and 0.13 mg/Kg in >75 years pts; prednisone 1.5 mg/Kg for 4 days; CPR: lenalidomide 25 mg/day for 21 days; cyclophosphamide orally 50 mg/day for 21 days in pts 65-75 years old and 50 mg every other day (eod) in >75 years pts; prednisone 25 mg eod). After induction, patients were randomized to receive maintenance with lenalidomide alone or with prednisone, until disease progression. The primary endpoint was progression-free survival (PFS). Between October 2009 and October 2012, 663 pts were enrolled (Rd:222, MPR/CPR:441). Patient characteristics were well balanced in the 2 groups. Median age was 73 years in each arm. Response rate (≥PR) was identical: 74% in both groups. After a median follow-up of 19 months, the 2-year PFS was similar in the 2 groups (47% in Rd and 55% in MPR/CPR, HR 0.81, 95% CI 0.57-1.16, p=0.25). In patients younger than 75 years old, the 2-year PFS was slightly inferior with Rd than MPR/CPR (HR 0.81, 95% CI 0.57-1.16, p=0.25). In patients older than 75 years the 2-year PFS was identical in both Rd and MPR/CPR (HR 1.07, 95% CI 0.68-1.68, p=0.76). At least one grade ≥3 hematological adverse event was reported in 28% with Rd and in 45% with MPR/CPR (p<0.001), with a significant difference between the two alkylating agents (62% MPR and 29% CPR, p<0.001). At least one grade ≥3 extra-hematologic toxicities were 25% in both Rd and MPR/CPR. In a community-based population triple*t al*kylating combinations does not offer PFS advantage over doublet therapy without alkylating agents. Grade 3-4 adverse events were higher in the MPR group.

C102

NOVEL AGENTS INCORPORATED INTO AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR NEWLY DIAGNOSED MM PATIENTS OVER 65 YEARS OF AGE: ANALYSIS OF OUTCOMES

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Whether incorporation of novel agents into ASCT can improve the outcome in comparison to novel agent-based treatments not followed by ASCT in newly diagnosed MM patients (pts) over 65 years of age still remains an open issue. In this retrospective analysis we evaluated the outcome of 63 pts with a median age of 68 years (range 66-72) treated up-front with novel agents and ASCT. At diagnosis, 50% of the pts had ISS 2-3 and 24% had renal impairment. Induction therapies were bortezomib-based (37 pts) or thalidomide-based (26 pts) and yielded at least VGPR in 57% and 36% of the pts, respectively. A median of 7.2x106 CD34+ cells/kg (IQR 5.8-10.9) was collected either following CTX (58 pts) or G-CSF alone (5 pts). Melphalan dose prior to ASCT was 200 mg/m2 in 39/63 pts, while it was reduced (140 mg/m^2) in the remaining pts due to impaired renal function. Sixteen pts received a tandem ASCT. Two cycles of consolidation therapy were given to 20 pts. Both platelets and neutrophils recovery occurred at day 12 (range 7-16 and 10-15, respectively). No transplant-related mortality was observed at 100 days. Two pts experienced grade 4 mucositis, while 13 pts (20%) presented grade 3 toxicities, mainly mucositis and infections. Overall, 97% of the pts achieved at least PR, including 71% at least VGPR and 30% CR. With a median follow up of 48 months, 4-years OS was 77%, median TTP and PFS were 42 and 43.4 months, respectively. On univariate analysis, ISS at diagnosis was the only variable related with a significantly prolonged survival: PFS 83.9 for ISS 1 *vs* 34 months for ISS 2-3 (p= 0.04), OS not reached for ISS 1 *vs* 75.2 months for ISS 2-3 (p=0.01). In conclusion, this analysis may suggest that ASCT is feasible and well tolerated in selected MM pts aged > 65, the outcome being at least comparable to that offered by novel agent-based regimens, including MPT and VMP. However, this comparison has several limitations, including possible differences between studies with respect to pts characteristics, treatments used and nature of the analysis. To more carefully evaluate the role of ASCT in this subset of older, but still fit, pts, results of a pair-matched analysis comparing ASCT *vs* the best novel agent-based treatment strategy will be presented at the meeting.

Non-Hodgkin's Lymphoma II

C103

LENALIDOMIDE PLUS RITUXIMAB-CHOP21 (LRCHOP21) IS SAFE AND EFFECTIVE IN ELDERLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL), REGARDLESS CELL OF ORIGIN (COO) SUBTYPING: LONG TERM RESULTS OF THE PHASE II REAL07 STUDY OF THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Standard treatment for elderly DLBCL is RCHOP21, however up to 40% of patients fail. In the phase I trial REAL07 FIL demonstrated that the association of LRCHOP21 was feasible in elderly untreated DLBCL and identified 15 mg lenalidomide from day 1 to day 14 as the maximum tolerated dose in combination with RCHOP21. The phase II trial REAL07 was designed based on Simon's two stage design to demonstrate an improvement of overall response rate (ORR) of 15% in LRCHOP21 compared to 70% of standard RCHOP21, according to 2007 Cheson criteria. Secondary endpoint was to correlate outcome with COO profile. Inclusion criteria were: age 60-80 FIT at the comprehensive geriatric assessment; 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: RCHOP21 plus 15 mg lenalidomide from day 1 to 14 for 6 courses. All cases were centrally reviewed by expert pathologist and COO profile was evaluated (analysis ongoing). From April 2010 to May 2011, 49 patients were enrolled. Clinical characteristics were: median age 69 years (range 61-80); stage III/IV 43 (88%), IPI IH/H 30 (61%).

Table 1.

Adverse Events (AEs)	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic AEs recorded in 277 cycles of treatment				
Leukocytopenia	27 (10%)	46 (17%)	42 (15%)	35 (13%)
Neutropenia	14 (5%)	32 (12%)	25 (9%)	62 (22%)
Febrile neutropenia	0	0	8 (3%)	2 (1%)
Thrombocytopenia	43 (16%)	21 (8%)	16 (6%)	19 (7%)
Anemia	92 (33%)	39 (14%)	12 (4%)	1(<0.5%)
Non-hematologic AEs in the 49-patient population				
Cardiac	1 (2%)	3 (6%)	1 (2%)	0
Gastrointestinal	11 (22%)	10 (20%)	1 (2%)	0
Neurological	10 (20%)	10 (20%)	2 (4%)	0
Renal	2 (4%)	1 (2%)	1 (2%)	0
Infection	1 (2%)	6 (12%)	1 (2%)	0
Deep venous thrombosis	0	0	1 (2%)	0

At the end of 6 LRCHOP21, ORR was 92%. Complete remissions (CR) were 42 (86%) and partial remission 3 (6%); 3 patients (6%) did not respond and one (2%) died for homicide. Preliminary data of CR according to COO showed: 15 (83%) CR on 18 germinal center (GC); 11 (79%) CR on 14 non-GC. At a median follow-up of 22 months, 2-year overall

survival (OS) was 92% (95% CI: 79-97) and 2-year PFS was 73% (95% CI: 57-84); 2-year PFS for IPI LI was 84% (95% CI: 59-95) and for IPI IH/H 65% (95% CI: 41-81). PFS according to COO is ongoing. Hematological and extra-hematological toxicities were mild, with no grade IV extra-hematological events and no toxic deaths during treatment (Table 1). Of the 294 planned courses of LRCHOP21, 277 (94%) were administered; median dose of lenalidomide delivered was 1185 mg (94% of the planned dose); at least 90% of the planned dose of each drug was administered in 91% of the RCHOP21 courses. Median interval time between RCHOP21 courses was 21 days (range 19-48). In conclusion, LRCHOP21 is effective, also in poor risk patients. The addition of lenalidomide to RCHOP21 is safe without unexpected toxicities and does not impair doses and timing of RCHOP21. These encouraging data warrant a future phase III randomized trial comparing LRCHOP21 *vs* RCHOP21 in elderly untreated DLBCL.

C104

EFFICACY OF NON-PEGYLATED LIPOSOMAL DOXORUBICIN IN ELDERLY PATIENTS WITH AGGRESSIVE B-CELL NON HODGKIN LYMPHOMA: A MULTICENTRIC STUDY

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We conducted a multicenter, phase II, double-arm trial comparing the efficacy and the safety of 2-weekly and 3-weekly R-COMP regimen. Patients with confirmed histological diagnosis of aggressive B-cell non-Hodgkin lymphoma, aged ≥65 years and with an IPI≥1 were eligible. Thirty-nine patients with an Activities of Daily Living (ADL)=6 were assigned to receive 6 cycles of dose-dense R-COMP14 regimen, whereas 90 patients with an ADL<6 were assigned to receive six cycles of R-COMP21 regimen. The clinical features were comparable in both study arms, without statistical differences. The median age was 74 years (65-89) at diagnosis and the study included 21 (16%) very elderly patients (≥80 years). The Performance status was WHO<2 in 70% of all patients, the median number of comorbidities was 2 (1-7) and the median LVEF was 60% with 16% of patients having LVEF ≤ 50%. The number of cycles administered was 234 in R-COMP14 and 481 in R-COMP21 with the relative dose-intensity for the regimens of 93% and 90% respectively. Toxicity was mainly hematological in both groups. Only 8/129 (6%) presented a grade II-IV cardiotoxicity. All patients were evaluable for response. With a median follow-up of 24 months the overall response rate (ORR) in R-COMP14 arm was 85%, with complete response (CR) rate of 72%, whereas the ORR in R-COMP21 was 90%, with CR rate of 73%. The event-free survival was respectively of 67% and 69 % in the two arms. Univariate analysis revealed that event-free survival was negatively impacted by high risk IPI (1-3 *vs* 4-5; P=0.02). The prognostic factors for shorter overall survival (OS) were age>70 years (P=0.009), high IPI score (IPI=1-3 *vs* 4-5; P=0.04), advanced-disease stage III-IV (P=0.01) and impaired WHO performance status 2-3 (P=0.003). Multivariate analysis showed that WHO performance status 2-3 (P=0.02) and age> 70 years (P=0.03) negatively impact the overall survival in both arms. The functional assessment of frailty, Activities of Daily Living (ADL), has allowed to identify really elderly, frail patients. The overlapping of ORR and EFS between the dose-dense R-COMP 14 regimen and the standard R-COMP 21 regimen suggest that the use of NPLD outruns the impact of a dose-dense immunochemotherapy, representing a therapeutic opportunity for frail elderly patients, not suitable for a dose-dense treatment. Acknowledgements: The study was supported in part by AIL Pesaro Onlus.

C105

THE ROLE OF RITUXIMAB AND PET IN THE TREATMENT OF PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

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Third-generation MACOP-B (adriamycin, cyclophosphamide, vincristine, bleomycin, methotrexate and prednisone) regimen in combination with mediastinal radiotherapy (RT) seems to improve lymphoma-free survival of primary mediastinal large B-cell lymphoma (PMLBCL). In addition, the role of consolidative mediastinal RT remains unclear also with the improvement of PET use. Until 2002 MACOP-B plus RT was recommended at our institution in all PMLBCL patients. Between 2002 and 2011, 74 previously untreated PMLBCL patients were diagnosed and treated with MACOP-B plus rituximab and consolidative mediastinal RT (30-36 Gy). Fifty patients had stage II and 24 stage IIE-IV, bulky disease was documented in 93% of patients. Median age was 34 years (range, 17-62) and 59.5% were females. All patients were evaluated by CT and PET scan. After the final PET evaluation, PET-negative patients were observed while PET-positive patients underwent mediastinal RT. Finally, 61 (82.4%) patients achieved a complete response (CR); 51 (68.9%) presented a positive final PET and were treated with local RT, while the other 23 (31.1%) had a negative PET. Five patients relapsed within 12 months. At 10 years, overall survival was 82%, progression-free survival was 87.6% and disease-free survival (DFS) of the 61 patients who achieved CR was 90.5% (median follow-up 4 years). Regarding the DFS curve, no statistically significant differences were observed between the patient subset treated also with RT (PET-positive) and patients only observed (PET-negative): 90.7% (4/51 relapses) *vs* 90% (1/23 relapse) (p=0.85), respectively. Considering our institutional historical records when the front-line for PMLBCL patients included MACOP-B plus RT without any decision related to PET results (before 2002), the 10-year DFS was 82.8%. This study indicates that adding rituximab does not change the final results in terms of CRs and DFS utilizing third-generation regimens. In addition, the introduction of the PET-guided RT approach after MACOP-B plus rituximab leads to a patient tailored treatment which preserves the outcome and, at the same time, allows to reduce the use of RT.

C106

FLUDARABINE-MITOXANTRONE-RITUXIMAB (FMR) REGIMEN IN UNTREATED INDOLENT NON-FOLLICULAR NON-HODGKIN'S LYMPHOMA: EXPERIENCE ON 143 PATIENTS

Quirini F, Casadei B, Derenzini E, Broccoli A, Pellegrini C, Stefoni V, Gandolfi L, Tschon M, Papadopulos F, Narducci R, Stefani G, Maglie R, Argnani L, Zinzani PL

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Indolent non-follicular lymphomas (INFL) are generally regarded as incurable, apart from extranodal MALT lymphomas, which can be partially cured by surgery, local radiotherapy, or antibiotic treatment. An observational retrospective study was conducted on 143 INFL patients with biopsy-proven and bidimensionally measurable disease providing that their first chemoimmunotherapy performed was fludarabine, mitoxantrone and rituximab (FMR) regimen and diagnosis from September 2000 to March 2010. In particular, there were 32 small lymphocytic lymphoma (SLL), and 111 marginal zone (MZL) lymphomas [specifically, 49 extranodal MALT, 17 splenic marginal zone lymphoma (SMZL), and 45 nodal MZL (NMZL)]. All patients were characterized by symptomatic disease needing immediate conventional antilymphoma therapy (including gastric Hp+ MALT lymphoma with persistent disease after Hp eradication). At the time of assessment after six cycles of FMR regimen, overall response rate was 96.5% with 88% of complete responses (CR) and 8.5% of partial responses (PR). With a median follow-up time of 48 months, 10 of 125 (8%) CR patients had disease relapse, yielding an estimated 9-year disease free survival (DFS) of 74.9% and an estimated 10-year overall survival of 92.8%. The estimated 9-year progression free survival was 70.5%. In particular, all the 10 relapsed patients showed lymphoma recurrence within 52 months; after this time the DFS curve presented a plateau configuration. Regarding the

histology, among the global 10 relapses, 2 were SLL and 8 were MZL. In particular, evaluating the DFS curves of the 3 different subsets (extranodal MZL, SMZL, and NMZL) there is a statistically significant difference (P=0.03) where the NMZL predicts the worst DFS. Only 2 (1.4%) patients developed a secondary haematological neoplasia. This study has shown promising findings for the use of a fludarabine-based regimen in combination with rituximab in the front-line treatment of symptomatic INFL with a real high percentage of CR associated to interesting long-term DFS and favourable acute and long-term safety profile.

C107

ROLE OF FRONT-LINE HIGH DOSE THERAPY WITH STEM CELL TRANSPLANT IN PERIPHERAL T-CELL LYMPHOMAS. A SINGLE CENTER EXPERIENCE

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Introduction. Consolidation of first response with high dose therapy (HDT) and autologous stem cell transplant (SCT) may improve outcome of peripheral T-cell lymphomas (PTCL). However, no comparative trials are currently available. Material and Methods. We retrospectively evaluated the outcome of previously untreated patients with PTCL, excluding primary cutaneous PTCL and ALK positive anaplastic large cell lymphoma (ALCL). Treatment plan was stated after diagnoses and patients were stratified accordingly. Conventional therapy (CT) consisted of CHOP or CHOP-like therapy. Patients assigned to first-line consolidation with autologous or allogeneic SCT were assigned to HDT group. Results. At diagnosis, 43 patients were planned to receive HDT-SCT but only 26 (60%) eventually received it (22 autologous, 4 allogeneic) while the remaining experienced early death (8 patients), progression (8 patients) or mobilization failure (1 patient). The median age of this first cohort was 47.9 years (range 23-63), that also showed an advanced stage (III-IV) or an int-high/high IPI in 77% and 58% of the cases, respectively. The rate of complete remission (CR) was 57% with 21% of patients dying during treatment (5 patients not evaluable for response, 3 responders and 1 with progressive disease). Eighty-one patients were treated according to a CT strategy. Median age was 66.7 years (25-85), an advanced stage (III-IV) or an int-high/high IPI was present in 73% and 61%, respectively. The CR rate was 57% with 19% of the patient dying during treatment (11 patients not evaluable for response, 2 responders and 1 with progressive disease). With a median follow up of 1.63 years (0-25) and 82 deaths, by intention to treat analysis the 5-years overall survival (OS) was 43% and 32% for HDT and CT (p=.90, Figure 1), respectively, while the 5-years OS of those patients eventually receiving SCT was 64%. Irrespectively from the adopted treatment strategy, patients who achieved a CR showed a similar 5-years OS and disease free survival that were 57% and 52% in the CT group and 80% and 64% in the HDT cohort (p=.43 and p=.44), respectively. Conclusions: The overall clinical outcome of most PTCL patients remains unsatisfactory, with a large fraction of patients not responding to front-line treatment. The advantage of a post-remission consolidation with SCT has to be confirmed by appropriate ad hoc designed clinical studies.

C108

MODIFIED HYPER-CVAD AND RITUXIMAB FOR THE TREATMENT OF B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND BURKITT LYMPHOMA

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Background. The category of B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BL-INT) was introduced into the 2008 WHO classification to define an infrequent subtype of aggressive mature B-cell NHL. It has been suggested that the deregulated expression of MYC and BCL-2 or BCL-6 oncoproteins, along with the genetic complexity of these tumors, contributes to the poor outcome of BL-INT when treated with standard R-CHOP. Aims and Methods. From 1995 we have been treating patients with advanced, aggressive B-NHL using a modified HyperCVAD regimen alternating sequential cyclophosphamide, vincristine and adryamicine plus intrathecal prophylaxis (cycle A) with high-dose methotrexate and high-dose cytarabine (cycle B). From 2002 standard-dose Rituximab has been added to each cycle. The diagnostic specimens of all patients treated into this protocol were revised according to the WHO 2008 criteria, and 20 patients were classified as BL-INT. FISH analysis for MYC translocation using a Break Apart probe and additional IHC analyses were performed in more recent cases or when adequate frozen material was available. Results. Median age of 20 BL-INT patients was 40 years (range 19-72), males were 13 (65%). The large majority of patients had high-risk characteristics: Ann Arbor IV stage (n=17, 85%), ECOG Performance Status ≥2 (n=12, 60%), Ki-67 >90% (n=15, 75%), bulky disease (n=13, 65%). A leukemic presentation was present in 7 cases (35%) and 4 patients (20%) had CNS disease. MYC translocation was found in 7/11 cases (63.6%), BCL2 and BCL6 were hyper-expressed in 11/16 (68.7%) and 13/14 (92.8%) cases, respectively. Remarkably, a "mutated" p53+/p21- phenotype was found in 9/9 tested cases. Eighteen patients were evaluable for response to treatment, 4 were treated with chemotherapy alone and 14 received Rituximab plus chemotherapy (median 3 cycles A plus 3 cycles B). Complete remission (CR) was achieved in 17 patients and partial remission in one. Three patients suffered from relapse and another one died of fungal pneumonia while in CR. After a median folllow-up of 69 months the EFS is 72.2%, not different from what seen in BL treated with the same protocol (Todeschini *et al*, Am J Hematol 2011). Conclusions. Although in a small series, we observed that an intensive, short-term chemotherapy regimen determines a good outcome in BL-INT characterised by a very unfavourable clinical and molecular profile.

Acute Myeloid Leukemia II

C109

CD200 OVER-EXPRESSION CORRELATES WITH IMMATURE PHENOTYPE IN ADULT ACUTE MYELOID LEUKEMIA

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The over-expression of CD200 has been implicated in the pathogenesis of haematological malignancies and in acute myeloid leukemia (AML) CD200 up-regulation seems to be an indicator of poor prognosis. The aim of our study was to correlate the expression of CD200 with the main clinical and biological characteristics of a series of AML. We analysed bone marrow samples collected at diagnosis of AML from 92 patients (pts), median age 44 years (range 20-60), median white blood cells count (WBCc) 43.79x10^9/L (range 0,36-360x10^9/L). The cytogenetic/genetic information was available in 88/92 (96%) pts, 23 (26%) belonged to the category of "favorable-risk", 35 (40%) and 30 (34%) to the one of "intermediate-risk" and "adverse-risk", respectively. Forty-five pts (49%) had myelomonocytic or monoblastic/monocytic AML. CD200 was determined by multicolor flow cytometry and the results were expressed as a percentage. The threshold of positivity was set at >20%. CD200 positivity was observed in 51/92 (55%) cases. No correlation was found between CD200 positivity and WBCc or cytogenetic/genetic risk group. Six of 9 (66%) AMLs bearing RUNX1-RUNX1T1 rearrangement were CD200 positive. We found a significant correlation between CD200 positivity and CD34 (41/51, 80%, P=0.000001) or CD117 expression (51/51, 100%, P=0.0009). At the opposite, the myelomonocytic/monoblastic phenotype was associated with CD200 negativity (28/41, 68%, p=0.005). There was also a significant inverse correlation between CD200 positivity and the absence of CD19 and CD56 (43/51, 84% and 45/50, 90%, P=0.03 for both). In order to explore further the prognostic role of CD200, we analyzed its correlation with MRD persistence after induction and consolidation courses. While no sufficient cases were available for this analysis after consolidation, we observed that of 45 pts with a MRD measurement after induction, 27 (60%) were MRD positive and 19 of these 27 (70%) were CD200 positive at presentation. Further studies are in progress to evaluate CD200 expression on normal progenitors and to investigate its role as an aberrant marker. Our preliminary data suggest that CD200 is significantly associated with a stem cell-like feature in AML, evaluation on a larger series of AML is warranted to confirm the unfavorable prognostic significance of this molecule. Finally, CD200 might have a role as a novel marker contributing to the selection of aberrant phenotypes for purpose of MRD detection.

C110

EFFECT OF QUIZARTINIB (AC220) ON RESPONSE RATES AND LONG-TERM SURVIVAL IN ELDERLY PATIENTS WITH FLT3-ITD POSITIVE OR NEGATIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

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Background. Advanced age and FMS-like tyrosine kinase 3 internal tandem duplications (FLT3-ITD) in acute myeloid leukemia (AML) are associated with early relapse after standard chemotherapy and poor survival. Quizartinib (AC220), an oral FLT3 inhibitor active against ITD mutant and wild type FLT3, has shown promising activity in Ph 1 and 2 studies. Methods. Patients (pts) in a Ph 2 open label study (N = 333) of quizartinib monotherapy included 154 aged ≥60 y with known FLT3-ITD status and AML relapsed in <1 y or refractory to 1st line chemotherapy. Median duration of treatment was 14.2 wks (range 0.1 70.6 wks) for FLT3-ITD(+) pts and 9.5 wks (range 1.1 77.0 wks) for FLT3-ITD(-) pts. The composite complete remission (CRc) rate included complete remission (CR), complete remission with incomplete platelet recovery (CRp), and complete remission with incomplete hematologic recovery (CRi). Results. Of 110 FLT3-ITD(+) pts, 63 (57%) had a CRc (3 CR, 4 CRp, 56 CRi). Of 44 FLT3-ITD(-) pts, 16 (36%) had a CRc (2 CR, 1 CRp, 13 CRi). Median overall survival (OS) in FLT3-ITD(+) pts was 25.3 wks and 16/110 (15%) survived >52 wks. The median age of these pts surviving >52 wks was 69.5 y (range 66 80 y) and median OS was 76.3 wks (range 56.9 96.0 wks). All of these pts responded to quizartinib (2 CR, 2 CRp, 8 CRi, 4 partial remission [PR]). 2 pts were still alive >1 y (OS 93.0 and 96.0 wks). Median OS in FLT3-ITD(-) pts was 19.1 wks and 6/44 FLT3-ITD(-) pts (14%) survived >52 wks. The median age of these pts was 70.0 y (range 65 77 y) and their median survival was 76.6 wks (range 54.9 98.4 wks). 5 of these pts responded to quizartinib (1 CR, 3 CRi, 1 PR). Conclusions: These data for an FLT3-targeted agent show encouraging survival in a subset of elderly pts with relapsed/refractory FLT3-ITD(+) AML. Supported by: European LeukemiaNet, AIRC, AIL, PRIN 2010-2011.

Table 1. Efficacy in Elderly Relapsed/Refrctory AML pts

	FLT3ITD(+) (N=110)	FLT3-ITD(-) (N=44)
Cumulative CRc, n (%)	63 (57)	16 (36)
CRc+PR, n (%)	86 (78)	20 (56)
Median CRc duration, wk (95% CI)	12.1 (6.3, 15.7)	10.8 (8.1, 26.1)
Median overall survival, wk (95% CI)	25.3 (21.3, 30.0)	19.1 (12.0, 29.4)

CRc = composite complete remission; PR = partial remission

C111

PRETRANSPLANT MRD STATUS HAS A DIFFERENT IMPACT ON SURVIVAL IN ADULT AML PATIENTS SUBMITTED TO AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANT

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Allogeneic stem cell transplant (ASCT) is the treatment of choice for intermediate and high-risk karyotype acute myeloid leukemia (AML) that represents almost 60% of the entire population. Nonetheless, the extensive use of this option is hampered by the paucity of candidates (25-30%) with a full matched family donor. For the other patients, autologous stem cell transplant (AuSCT) or further chemotherapy are the only chances of treatment. We have demonstrated that an adjusted risk-stratification, based on the evaluation of pretreatment genetics/cytogenetics and minimal residual disease (MRD) status at the end of consolidation, refines risk-assessment. We distinguished two categories of patients: 1) Low-risk (LR): good/intermediate-risk karyotype (K) MRD negative; and 2) High-risk (HR): adverse-risk K, FLT3-ITD mutated and good/intermediate-risk K MRD positive. The aim of our study was to evaluate the impact of ASCT on prognosis of HR patients and the role of AuSCT as an alternative approach for HR patients without a matched sibling. We analyzed 111 HR and 43 LR patients of whom 64 were sub-

mitted to AuSCT and 46 to ASCT. Seventeen patients received no transplant because of poor performance status or insufficient stem cell harvest, whereas 26, all in the HR group, relapsed before transplant. In the AuSCT group 31/64 (49%) were LR and 33/64 (51%) HR, with LR group showing a superior overall survival (OS) (LR 54% *vs* HR 18%, p=0.004). In the ASCT group 41/46 (89%) were HR and 5/46 (11%) LR. Seventeen out of 46 ASCT (37%) were performed using matched unrelated donors (7) and haploidentical donors (10). HR and LR patients shared a comparable long-term OS (LR 60% *vs* HR 55%, p=NS) with a 37% survival gain for the HR population as compared to HR patients who received AuSCT. However, no survival advantage was demonstrated for LR patients whose OS was similar whatever the form of transplant delivered (OS 60% *vs* 54%, p=NS). Such a Figure may be due to the higher treatment related mortality observed in the ASCT group as compared to AuSCT one (2/5, 40% *vs* 2/31, 6%, p=0.026). In conclusion, ASCT confers a significant survival advantage to HR patients but expose LR patients to an excess of toxicity. Therefore, in LR patients the ASCT option might be postponed after a second remission. On the other hand, for HR patients AuSCT does not represent a valid substitute for ASCT which should be timely performed in first remission, also considering alternative sources of stem cells.

C112

VALIDATION OF A NEW PROPOSED RELAPSE RISK SCORE (CBC-SCORE) FOR NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA

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PETHEMA group stratified acute promyelocytic leukemia patients (APL) into 3 different subgroups (low, intermediate, high risk), according to WBC count (if > 10 x 10^9/L) and platelet count (40 x 10^9/L). Risk-adapted strategy based on this classification improved clinical outcome of APL patients. The so-called CBC-score has been recently proposed, which included haemoglobin level and improved prognosis in terms of complete remission rate (CR), early death rate and overall survival (OS). We validate this new score in 142 newly diagnosed APL observed at a single institution from March 1993 to December 2010. Diagnosis was initially established morphologically and subsequently confirmed in all cases by RT-PCR identification of PML/RARA fusion gene. Seventy-three patients diagnosed between January 1993 and May 2000 received uniform post-remission treatment based on the AIDA 0493 regimen, while 69 patients diagnosed after May 2000 were treated according to the risk-adapted regimen AIDA-2000. Molecular tests were performed after third consolidation and then every 3 months for two years and every six months after the end of maintenance. Molecular relapse was defined as a positive PML/RARA test detected in two successive marrow samples collected at any time after consolidation in the absence of morphologically detectable blasts in both marrow or peripheral blood. According to PETHEMA risk score 39 patients were classified as low risk, 66 as intermediate and 37 as high risk. Application of CBC score identified 35 patients with a score 0 (25%), 60 patients as score 1 (42%), 47 patients as score 2-3 (33%). Four patients identified as low risk with PETHEMA score were included in the CBC- intermediate category and the level of hemoglobin transformed other 10 patients in CBC-high risk. With this latter score, we identified a difference in the occurrence of differentiation syndrome (DS) during induction therapy: 8.5% in score 0, 15% in score 1 and 20% in score 2-3 (p=0.02). Early death occurred only in patients with score 2-3 (p=0.001) and relapse rate was significantly different: 17% in patients with score 0, 20% in score 1 and 27% in score 2-3 (p=0.02). After a median follow-up of 9 years, we identified also a different OS rate according to CBC-score: 91% for patients with score 0, 82% for score 1 and 79% for score 2-3 (p=0.001). CBC-score is able to stratify APL patients in terms of OS, incidence of DS and relapse rate with the same prognostic weight of PETHEMA score.

C113

HOW MANY AML PATIENTS DO RECEIVE EARLY ALLOGENEIC BONE MARROW TRANSPLANT? REAL LIFE DATA FROM GENOVA ACUTE LEUKEMIA REGISTRY (REGAL)

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We report a retrospective review of AML treatment in 132 patients enrolled in a regional acute leukemia registry with the purpose of evaluating transplant rate in first CR. From October 2010 to January 2013, 132 AML patients have been reported in the Genova Acute Leukemia Registry (REGAL). Median age was 66 years (range 16-87). Ninety-two patients had denovo disease (69%); in 40 patients (31%) AML was secondary to MDS (n. 24), chemo radiotherapy (n.9), and chronic myeloproliferative disorder (n.7). Fifteen patients (11%) had acute promyelocytic leukaemia. Relevant comorbidities were present in 75 patients (57%). ECOG performance status was 0 in 41 patients (31%), 1 in 51 (38%), 2 in 29 (22%), 3 in 10 (7%) and 4 in 1 (2%). Karyotype could be evaluated in 115 patients (87%) and was favourable in 17 patients (15%), intermediate in 80 (69%), unfavourable in 18 (16%). A molecular profile including study of FLT3 ITD, NPM1 gene mutations and expression of WT1 and BAALC genes was performed in 106 patients (80%).Five patients received supportive care only (4%), 33 low intensity chemotherapy (25%), 94 were eligible for intensive conventional chemotherapy (71%). In this last group 6 patients had an induction related death (6%), 58 achieved CR (62%). Induction related deaths were 2 (4%) and 4 (9%) in patients younger and older than 60 years, respectively. Complete remission rates were 78% and 43% in patients younger and older than 60 years, respectively. Fourty patients were considered eligible for early BMT (age < 65 years and unfavourable cytogenetic or intermediate cytogenetic plus high risk molecular profile or secondary AML), 12 were actually transplanted in first CR (30%) and 9 are disease free and waiting for BMT (22%). Three more patients were transplanted in second CR. Reasons for not receiving early BMT were infections (n. 6), refractory or relapsed disease (n. 12) and unavailable donor (n.1). Stem cell donors have been HLA identical siblings (n.2, 17%) and haploidentical siblings (n. 10, 83%). No patients transplanted in first CR died for transplant related reasons. ReGAL data show that 71% of newly diagnosed AML were treated with intensive chemotherapy and 13% of these were transplanted in first CR. BMT in first CR was performed in 30% of eligible patients. With the increased utilization of haploidentical donors, transplant related mortality has not changed and lack of donor is not anymore a reason for not receiving an early BMT.

C114

ARSENIC TRIOXIDE INDUCES APOPTOSIS ASSOCIATED WITH DOWNREGULATION OF THE LEUKEMIC NUCLEOPHOSMIN (NPM1) MUTANT PROTEIN IN NPM1-MUTATED AML

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AML carrying NPM1 mutations accounts for about one-third of adult AML, shows distinctive biological and clinical features and has been included as a provisional entity in the 2008-WHO classification of myeloid neoplasms. In spite of its relatively good prognosis, there are still patients with poor outcome, especially those associated with FLT3-ITD mutation and elderly. Therefore new, potentially less toxic, therapeutic strategies need to be explored. For this purpose, we have recently investigated the effect of Arsenic Trioxide (ATO) in NPM1-mutated AML *in vitro*. We tested ATO in different human AML cell lines: OCI/AML3 and IMS-M2 cell lines (harbouring NPM1 gene mutation) and U937, HL60 and OCI/AML2 cell lines (not harboring NPM1 gene mutation) used as counter part; and primary AML samples collected from AML patients at diagnosis upon informed consent. Interestingly, in NPM1-mutated AML cell lines, and not in the others, growth arrest and pro-apoptotic effects were evident after 24 hrs and marked after 48 hrs of treatment even with low doses of drug (0.1 - 0.3 μM), well below the doses within the therapeutic range (1-3 μM). Induction of apoptosis was associated with activation of caspase-8, suggesting involvement of the death cell recep-

tors pathway. Indeed, flow cytometric analysis showed 1.5-2 fold increased expression of TRAIL-receptor DR5 upon 24-48 hrs of drug treatment. However, concomitant treatment with a specific caspase-8 inhibitor did not prevent cell growth arrest and apoptosis indicating the activation of the death cell receptor pathway is not the only underlying mechanism. Upon treatment, also levels of p53 and p21 were upregulated. Moreover, ATO induced signs of differentiation in OCI/AML3 cell line with levels of CD11b markedly upregulated. Strikingly, we showed that levels of the leukemic NPM1 mutant protein (and not, or less, the NPM1 wild-type) were significantly reduced by ATO treatment. Importantly, results observed in cell line were confirmed in a series of primary AML cells from 15 patients at diagnosis (7 NPM1-mutated AML, 8 NPM1-wild type AML) (Fig 1). In primary AML cells, the higher sensitivity in terms of apoptosis of NPM1-mutated AML was statistically significant (P=0.04). Our preliminary data suggest the potential involvement of NPM1 mutant protein in mediating the higher sensitivity of AML cells to ATO and require further studies to better characterize the underlying molecular mechanisms.

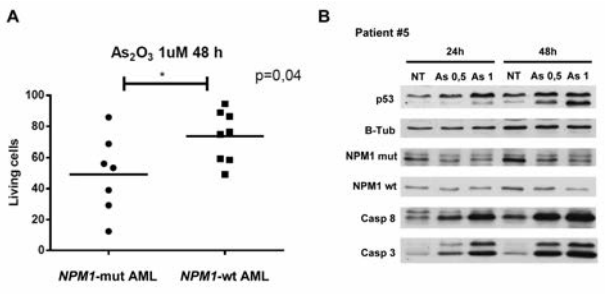

Figure 1.

Quality of Life and Support Therapy

C115

PERIPHERAL INSERTED CENTRAL CATHETERS (P.I.C.C.) INSERTION AND MANAGEMENT IN HEMATOLOGY PATIENTS: RESULTS FROM 5 YEARS PROSPECTIVE STUDY OF THE CAGLIARI'S DEPARTMENT OF HAEMATOLOGY

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Background. Central Venous Catheter (CVC) is crucial for a haematology patients appropriate clinical management. The purpose of our study was to evaluate if introduction of Peripheral Insertion Central Venous Catheter (PICC) in clinical practice is feasible and could simplify patients management. Methods. Since the start of the experience a prospective study was started to evaluate complication rate and usefulness of PICC device in hematology practice. All implantation procedures has been done under ultrasound guide. Probability of catheter life was calculated with K-M method. Univariable analysis was performed with the log-rank test. Results. From March 2007 to July 2012 321 consecutive PICC have been implanted in 305 hematology patients. There were 165 male and 140 females. Median age was 46 years, range 16-94. There were 108 (35,4%) Hodgkin Lymphoma, 61 (20%) non Hodgkin lymphoma, 48 (15.7%) ALL, 44 (14.2%) MM, 36 (11.8%) AML and 8 (2.6 %) patients with other hematological disease. Forty-two PICC (13.8%) have been used for autologous HSCT and 1 (0.3%) for allo HSCT. Catheter insertion was successful in 305 instances (95%), in 16 instances (5%) PICC insertion was not possible. At the time of this analysis 23 out of 305 PICC (7,5%) are still "in situ" and in use and 282 (61%) have been removed. Reason for removal was end of therapy in 186 instances (61%), accidental withdrawal in 27 (8.8%), patient death in 36 (11,8%) and catheter related complication in 33 (10,8%). Catheter related complications were the following: 2 (0.65%) catheter ruptures , 6 (1.96%) malfunctioning, 15 (4.9%) occlusions, 2 (0.65%) delayed abnormal dislocations, 6 (1.96%) suspected PICC-related sepsis, 1 (0.3%) local infection and 1 (0.3%) patient poor compliance. Of the six cases of suspected infection only 1 episode (0.3% - 0.02/1000 days/PICC) was confirmed (E.Coli septicemia). There were only 3 cases (0.98%) of symptomatic PICC-related thrombotic complications without the need for removal. PICC median life was 131 days (1-722) for a total of 45,674 days of implanted PICC. K-M probability of catheter life, censored for removal by the end of therapy and the patient's death, was 80% at day 180 and 51% at day 455. Neither disease nor white cells or platelets count had influenced on catheter life. Conclusions. The insertion easiness, duration of life and low rate complication of PICC encourage the use in the hematological patient.

C116

FARMAREL: AN ITALIAN PHARMACOVIGILANCE PROJECT TO MONITOR AND EVALUATE ADVERSE DRUG REACTIONS IN HAEMATOLOGICAL PATIENTS

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Background Adverse drug reactions (ADRs) reduce patient's quality of life, increase mortality and morbidity, and have a negative economic impact on health care systems. Nevertheless, the importance of ADR reporting is often underestimated. Aims The project "FarmaRel" has been set up with the aim of monitoring and evaluating ADRs in haematological patients and increasing pharmacovigilance culture among haematology specialists. Methods In 13 haematology units, an "ADR monitor" with the task of encouraging ADRs reporting and sensitizing healthcare professionals to pharmacovigilance, has been assigned. The ADRs occurring in haematological patients (both in hospital and at home) were collected electronically with the aid of a software specifically created. ADRs have been then retrospectively analyzed. Results. Between January 2009 and December 2011, 887 reports were collected. Number of ADRs was higher in elderly adults (528; 59%), in male (490; 55%), and in non Hodgkin lymphoma patients (343; 39%). Most frequently, ADRs were caused by antineoplastic agents, corticosteroids for systemic use and immunosuppressants; the 3 most suspected single drugs were rituximab (252 reports), cyclophosphamide (138 reports) and doxorubicin (118 reports). Majority of reactions were severe: 45% required or prolonged hospitalization, 6% was defined as life-threatening event, 2% produced permanent or severe disability and in 3% of cases patient died. In 51% of cases, ADRs were fully resolved at the time of reporting, in 23% were improved, in 7% were partially resolved, in 6% were unchanged or deteriorated, in 3% the patient died, in 10% outcome was not available. According to Schumock and Thornton criteria, a percentage of ADRs as high as 7% was found to be preventable *vs* 2% according to reporter opinion. Considering nature of ADRs, blood and lymphatic disorders were the commonest, followed by infections and infestations. Patients' haematological diagnosis, not age or gender, resulted the variable most influencing ADRs characteristics, among which severity and outcome. Conclusions. The employment of personnel specifically dedicated to pharmacovigilance is a successful strategy to improve number and quality of ADR reports. The project significantly contributed to the overcoming of the "Gold Standard" for pharmacovigilance in Italy and showed that an important percentage of ADRs in haematological patients are preventable. During the congress, an update of data will be presented.

C117

BONE METABOLISM AND RISK OF VERTEBRAL LOW-TRAUMA FRACTURES IN PATIENTS WITH LYMPHOMA

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The development of impaired mineral metabolism together with osteoporosis and related low-trauma fractures is higher in elderly patients and may be further promoted by treatment with steroids and/or cytotoxic agents. Despite this predisposing situation, the evaluation of mineral metabolism is generally not considered in the baseline work-up of patients with lymphomas and this medical practice may contribute to the development of bone fractures and alter patients' quality of life. We evaluated in 38 consecutive patients with a new diagnosis of lymphoma (20 DLBCL, 5 FOL grade 1-3A, 3 MZL, 5 Hodgkin lymphoma, 5 other histotypes), pre-treatment serum (PTH, Ca, P, albumin, 25-OH vitamin D, CTX, fT3, fT4, TSH, LH, alkaline phosphatase) and radiological (lumbar spine and hip dual x-Ray absorptiometry and vertebral x-Ray) parameters related with mineral metabolism, presence of osteoporosis and vertebral fractures (according to Genant's classification). These factors were then related with patients and lymphomas baseline characteristics and clinical outcome. WHO-FRAX® algorithm (a predictive score of fracture) was performed in all the patients. Patients' median age was 61 years with female predominance (55%); 79 % had a stage III-IV. None had a previously established diagnosis of osteopenia, osteoporosis or related fractures. The results of this study indicated an increase of PTH in 19/38 patients (50%), with a median level of 57 pg/ml (range 2.5-164), evidence of osteopenia, osteoporosis and vertebral fractures in 20 (53%), 7 (18%) and 16 (42%) patients, respectively. All fractures were asymptomatic and in most cases of the mild type. A Stepwise logistic regression showed that higher levels of both PTH and FRAX®-score had a positive predictive value for the occurrence of vertebral fracture. With the limitation of this small survey, no correlation was found between age, sex, histotypes, stage, LDH, clinical outcome and the presence of serum or radiological parameters related with mineral metabolism impairment. Our results indicate that a significant proportion of patients with lymphoma have baseline signs consistent with an alteration of bone metabolism. Moreover it seems that FRAX® has a similar performance in patients with lymphoma as well as in general population. Despite the current absence of a pathological explanation bridging these conditions, major attention is warranted to this issue in order to recognize and treat patients at risk for vertebral fractures.

C118

NERER - NETWORK EMATOLOGICO REGIONE EMILIA ROMAGNA

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The NERER, that has been founded in 2008 through a grant sponsored by University of Bologna and the Emilia Romagna Region, has the purpose of reviewing and monitoring the hematological malignancies. The comparison between National and International rate of incidence and prevalence about hematologic malignancies in Emilia Romagna region was the primary objective. The project also included the monitoring of intra regional health mobility and of diagnostic and therapeutic protocols related to haematological malignancies. The project was extended from 2008 to 2010, and led to an overall registration of 3345 patients (> 17 years old) with the following haematological disease: chronic myeloid leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, acute lymphoid leukemia, polycythemia vera, essential thrombocythemia, idiopathic myelofibrosis, chronic myeloproliferative disorders, multiple myeloma, myelodysplastic syndrome. The rate of incidence and prevalence has been collected through this data collection. Subsequently, the data obtained were compared with data from the regional cancer registry, national data registry and data provided by the World Health Organization. In the study were recruited: 754 patients with multiple myeloma, 544 patients with chronic lymphocytic leukemia, 542 patients with acute myeloid leukemia, 493 patients with essential thrombocythemia , 398 patients with myelodysplastic syndrome, 232 patients with polycythemia vera, 162 patients with chronic myeloid leukemia, 118 patients with idiopathic myelofibrosis, 101 patients with acute lymphoid leukemia, 55 patients with myeloproliferative disorders. The rates of incidence and prevalence are similar to those reported in the current literature and World Health Organization. The intra-regional health mobility afflicts only 10% of patients with haematological disease in Emilia Romagna. In contrast with the higher incidence of the extra regional health mobility, confirming the excellence of the regional health system.

C119

DISABILITY EFFECT ON SURVIVAL OF HOME CARE ONCO-HEMATOLOGICAL PATIENTS

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Introduction. Reduction of capacity to attend to basic activities of daily living (ADL) is a frequent complaint in home care managed patients (pts) affected by hematological malignancies. Disability may arise both as an effect of comorbidities, hematological disease or its complication, and, lastly, treatment toxicity. Disability itself is a risk factor for complications (thrombosis, accidental falls), so that it could be expected to

affect overall survival (OS). Data about this issue are scarce. Aim. The aim of this analysis is to ascertain the presence of an effect of disability on OS in home care (HC) pts affected by hematological malignancies. Methods. At the moment of pts admission in our home care service for hematological pts, disability was assessed with Katz Index, as a part of data registration at pt entry. Pts with diagnosis other than hematological malignancies were excluded from analysis. OS from HC admission was calculated with Kaplan-Meier survival curves analysis; effect on OS of six variables (age, gender, diagnosis, chemotherapy lines before HC admission, comorbidities and disability at HC admission) was evaluated. Results. From September 2011 and March 2013, 93 pts were admitted in our HC service; 8 (8.6%) pts, having incomplete data recording, were excluded; among the remaining 85 pts, 56 (65,9%) pts were affected by hematological malignancies and were included to further analysis. Anagraphic, disease, comorbities, and disability data are reported in Table with statistical analysis result. Among the 6 examined variables, AML diagnosis and none/mild disability showed statistically significant negative effect on OS. Conclusions. Beyond diagnosis, OS is strongly influenced by disability. Disability is a useful and simple tool to stratify pts in order to identify prognosis. Intervention aimed to reduce or prevent disability could have effect on survival, although only clinical trials could address this issue.

Table 1. Patients data and statistical analysis.

		Kaplan-Meier survival analysis					
	n	Median	Min	Max	Category	Median OS	p
Gender							
f	22					72	NS
m	34					117	
Age		79	35	94			
					<79	47	NS
					>79	136	
Diagnosis							
AML	20				AML	67	0.016
MDS	11				No-AML	156	
Lym/CLL	10						
MPN	8						
MM	5						
ALL	2						
Treatment lines		1	0	6			
0	19				0	142	NS
1	16				>0	53	
2	7						
3	7						
>3	5						
Comorbidities							
No	21					60	NS
Yes	35					120	
Disability							
Very severe	6				Mild to very severe	53	0.002
Severe	7						
Intermediate 2	8						
Intermediate 1	1						
Mild	8						
Very mild	7				None – Very mild	185	
None	19						

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

C120

DEFERASIROX TREATMENT IN PATIENTS FREE OF TRANSFUSION AFTER BONE MARROW TRANSPLANTATION WITH IRON OVERLOAD

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Recipients of hematopoietic stem cell transplantation (HSCT) frequently have iron overload (IO) resulting from chronic transfusion therapy for haematological disorders. The commonest cause is an improper or complete absence of iron chelation therapy before and during the treatments which proceed transplantation. There is recent evidence that IO may affect outcome of allogeneic HSCT in term of higher transplant-related mortality and late complications, but the exact role of preventive iron chelation therapy with deferasirox (DFX) in transplant patients (pts) is not yet known. In order to evaluate the role of DFX to prevent organ damage in long term survival patients, we evaluated IO after HSCT in a particular subset of pts. We considered a group of pts who underwent HSCT with an high iron uptake (more than 20 blood transfusions). After a minimum period of 100 days after HSCT, we evaluated ferritin level and renal function. We selected a cohort of pts with a minimum ferritin level of 1500 ng/mL, with normal renal function, absolutely free of transfusion dependence, in complete remission and without severe HSCT complications, included severe active graft versus host disease. With the same criteria, we included also pts with a lower ferritin level, but with a histologic demonstration of liver IO. We treated them with low-dose of DFX (7-10 mg/Kg/day); suspension or reduction of treatment has been considered in case of appearance of side effects related to DFX. We overall treated 9 pts (2 of them for liver IO). Median initial ferritin level was 2476 ng/mL (1400-7482 ng/mL). Median duration of treatment was 6 months (1-16 months); 2 pts are still on treatment, 2 stopped for toxicity (renal impairment and diarrhea), 2 for personal refusal, 2 for late relapse and 1 for normalization of ferritin level. 5/9 pts experienced an high reduction in serum ferritin level, with an initial median value of 3139 ng/mL (1000-7482 ng/mL) and a final median value of 1266 (379-1996 ng/mL). Median initial haemoglobin level was 11.6 g/dL (9-14.4 g/dL), 2 pts presented an haematological response with an increase in haemoglobin level of 2 g/dL at the end of treatment. These preliminary data tell us that low dosage of DFX can be a safe and efficient option in transplanted pts who present IO in absence of transfusion uptake to prevent organ damage. Largest studies need to confirm this suggestion and to evaluate the haematological response in terms of haemoglobin improvement.

POSTERS

Acute Leukemia I

PO01

RAPID DIAGNOSIS OF PHILADELPHIA POSITIVE CELLS IN CEREBROSPINAL FLUID (CSF) WITH Q-PCR AUTOMATIC EQUIPMENT

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Background. The acute leukemia are malignant clonal pathologies characterized from genetic alterations of the hematopoietic stem cells. Some forms of acute leukemia Philadelphia + can show central nervous system implications. Aim: methodology applications in real time PCR, which forensic uses , and quantification in real time of the Ph+ cells in the liquor, can be clinically useful in order to evaluate in short and fast time the SNC implications for study of MRD. Equipments and Methods. We have studied one case of myeloid s acute leukemia already positive at onset at the t(9; 22) translocation(b3a2). Onset quantification performed by bone marrow samples by automatic equipment of Q-PCR for quantification, the BCR/ABL copy is resulted to be of 45 %. For molecular evaluation and evaluation of residual positive Philadelphia cells has been utilized for Q-PCR and qualitative PCR for isoforms study. The quantitative evaluation has been made after induction and consolidation treatment. After neurological signs, a Q-PCR evaluation of the liquor has been requested to evaluate Ph+ cells presence in the sample. Results. Molecular results (bone marrow) by Q-PCR(after 37 days from onset), show a reduction of initial couple of BCR/ABL (0.43 %); after showed clinical signs of neurological trouble has been made a real time determination of the liquor, result is positive but not quantifiable for an over range value of the ABL housekeeping gene. The CSF cells number detected in Nageotte room (direct count) were of 0.4/µl while, the BCR/ABL quantity in real time was not detected, but the instrument highlighted very clear ABL and BCR trends (Cycle threshold: 21,31), detecting not quantifiable positivity. In order to raise the determination sensitivity has been concentrated the liquor 5:1, but in the way, has not been possible, although this technical improvement, to quantify of BCR/ABL copies and the ABL gene was always over range. Conclusions: The Q-PCR instrumentations can be results to be a rapid method to detect quality and quantity level the Ph+ cells presence. In order to have optimal trends is better to concentrate the CSF , 5 times as much ,and to use 200 µl of concentrated sample. Being, Q-PCR instrumentation, for set on fixed values of minimum and maximum of the validity range of the ABL gene, it is not possible the quantification, because are present few Ph+ cell/ ul, but PH+ cells detection is performed in a safety, accurate and repentine way.

PO02

WILMS TUMOR 1 (WT1): PROGNOSTIC MARKER IN ACUTE MYELOID LEUKEMIA (AML) AS RELAPSE MOLECULAR INDEX IN PATIENTS WITH TRANSPLANT

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Background. Leukaemias are a group of hematopoietic diseases resulting from the clonal proliferation of stem cell. Acute myeloid leukemia is a heterogeneous disease characterized by several recurrent cytogenetic aberrations that provide the most important prognostic information at diagnosis. Molecular markers are specific and recognized tools with a predictive role when correctly applied. They can be used in the study of minimal residual disease. WT1 is over expressed in 90% of AML and is recognized as a prognostic marker in AML. Aim of the study. Is to identify molecular markers relevant in disease evolution and MRD control in order to early highlight molecular relapse. Materials and Methods. RNA from samples extracted from 35 LAM patients were subjected to the present study. WT1 gene over-expression level (regulation) was the most important marker studied in the LAM patients Follow-up. The average age of onset was 59 years. Among all, 11 patients have been previously subjected to transplant: allogenic (4 cases) and autologous (7 cases). The experimental analysis method used in the present study was the Real-Time Q-PCR platform with standardized commercial kits, chimerism study by polymorphism VNTR, and STR in qualitative PCR. Results. The range values of WT1 copies/10000 ABL (control) tested in a pool of normal samples was 0-20 for peripheral blood and 0-130 for bone marrow samples. Among our cases, 89% had WT1 gene over-expression with a WT1 average value in onset of 19578 copies /10000 ABL. Correlating WT1 results (gene over- expression) with other well known leukaemia markers, we have demonstrated that 4 patients were also FLT3 positive, one was positive for inv16 and one for the t(8; 21).For cases undergoing allogeneic transplantation (4 cases) was evaluated the chimerism qualitative study. Conclusions: In allogeneic transplantation, WT1 expression was early and predictive for the molecular relapse without an early chimerism indication. A follow-up case in remission, with negative results for both t(8;21) and WT1 expression, WT1 was early as 5 months before the morphological relapse (blasts over range in bone marrow) and molecular marker relapse (8.21), showed in our study with WT1over-expression monitored monthly. In conclusion, WT1 has demonstrated to be an early sensitive and practical marker to highlight alteration in clinical status with early molecular relapse, even before chimerism study or other specific markers such as t(8; 21).

PO03

NPM1 ISOFORMS GENE MUTATION AND CORRELATION IN PATIENTS WITH FLT3 ITD IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Acute myeloid leukemia is a heterogeneous disease characterized by different cytogenetic aberrations, the 45% of patients are cytogenetically normal. The study of nucleophosmin (NPM) provides us diagnostic and prognostic information about the disease. The mutations in NPM1's have been identified as: "Mutation A" with a frequency around 75-80%, "Mutation B" with a frequency of 10% and the rarest " Mutation D", with a frequency of 5%. Aims of the study. To study patients with acute leukemia alterations or gene mutations for selective prognostic and therapeutic decisions. Evaluate the diagnostic prognostic significance in all those cases where the presence of the mutation is related to the absence of FLT3-ITD. Highlight the advantages of Q- PCR diagnostics in detecting the presence of mutations in NMP1. Materials and methods: The analysis were performed on 41 patients, suffering from acute myeloid leukemia, 23 males and 18 females. Gene study was performed using Real-Time methodology, while for the FLT3 gene has been used qualitative PCR. All samples used (peripheral blood and bone marrow) come from the onset of AML. Results. We have studied, 15 NPM1(+) cases at onset (36.58%), including 10 females and 5 males; 4 were FLT3(+) (9.75%). Among NMP1 positive cases was possible to determine the frequency of NPM1 mutations: NMP1-A(86.67%), NMP1-B (13.34%), none for NMP1-D. It was made a comparison of the results of NMP1A and FLT3-ITD and evaluated how many patients have been found with positive NMP1A and negative FLT3-ITD, and those with negative NMP1A and positive FLT3-ITD. On 41 patients analyzed, ten good prognosis were obtained (25%), which will sent to chemotherapy protocol. Conclusions. The good prognosis group with cytogenetic(-)/FLT3 (-)/NPM1(+) is directed to therapy (3+7 cycles) based on (ARAC+Daunobicina), followed by consolidation (high-dose ARAC) and auto-transplantation. For all patients who have positive cytogenetic, but FLT3(+) and NPM1 (+), the therapeutic protocol is indicated by the type of cytogenetic alteration. Patients with cytogenetic(+), FLT3 (+), NPM1(-) are sent to specific chemotherapy cycles and are subsequently sent to allogeneic transplantation. The study and monitoring of NPM1 gene mutations are a sensitive target for study of minimum residual disease (MRD).

P004

TRANSFUSION IRON INTAKE IN TRANSPLANT ELIGIBLE PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA: RETROSPECTIVE ANALYSIS OF IRON LOAD AT THE END OF CHEMOTHERAPY PROGRAM AND BEFORE TRANSPLANTATION

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Iron overload is an important adverse prognostic factor for patients (pts) undergoing hematopoietic stem cell transplantation (HSCT): it increases the risk of infections, veno-occlusive disease and hepatic dysfunction. The majority of data derives from studies regarding pts with thalassemia and myelodysplastic syndromes, with established iron chelation strategies. On the contrary, the extent of transfusion iron overload in transplant eligible de novo acute myeloid leukemia (AML) and the need of iron chelation therapy (ICT) remains debated. We want to evaluate transfusional iron intake in pts with de novo AML who underwent HSCT or completed consolidation chemotherapy courses. We retrospectively analysed 44 potentially transplant eligible AML pts (20 males, 22 females) from March 2009 to February 2013. Standard induction treatment (cytarabine 100 mg/sqm twice daily for 7 days and idarubicin 12 mg/sqm daily for 3 days) was administred in 40 pts, while 4 pts received high dose induction chemotherapy with cytarabine 2g/sqm twice daily (day 1, 2, 8, 9) and idarubicin 18mg/sqm daily (day 3, 10). 11 of the 23 pts underwent transplantation in second complete remission after reinduction chemotherapy. Iron intake until HSCT or until the end of consolidation chemotherapy, was calculated as total amount of red blood cells (RBCs) transfused X 1.08. Mean transfusional iron intake was 0.65 mg/kg per day, among a mean treatment period of 6.6 months. Mean ferritin level at the end of the treatment or before transplant conditioning regimen was 2951 µg/L (median 2123 µg/L). This retrospective evaluation confirms a relevant transfusion iron load in de novo AML in a short time interval; of note, patients with -thalassemia major or other refractory anemias have a transfusion iron intake of 0.3-0.6 mg/kg per day. Serum ferritin is not specific for iron overload and is a poor predictor of body iron burden, and maybe it is even less specific in AML. Moreover, the optimal ICT in this setting of pts is still to be defined: subcutaneous deferoxamine infusion is inconvenient due to thrombocytopenia and neutropenia, while oral deferasirox may not be sufficiently rapid in his action if we consider the short time available before transplantation. Considering our data, it could be interesting to investigate the role of early low dose deferasirox administration at the beginning of chemotherapy program, in order to prevent transfusion overload and his prognostic impact at transplantation.

P005

INFLUENCE OF FLT3/ITD MUTATION ON OUTCOME OF PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Survival of de novo acute myeloid leukemia (AML), particularly in younger patients (pts), has improved in recent years. Relapse continues to remain an important obstacle to successful outcome. This is particularly true in AML patients with FLT3/ITD molecular mutations. The FLT3/ITD mutations occur in about 30% of AML pts; when compared with their FLT3 wild type counterparts, FLT3/ITD+ pts are at particularly high risk of relapse when treated with chemotherapy alone. These pts are often referred for hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1). However, there is no clear evidence that HSCT in CR1 decreases the relapse rate or improves overall survival (OS). We retrospectively considered 41 adults pts (median age 54 years) with de novo AML between January 2009 and January 2013. 39 pts who had FLT3/ITD mutation status available were included in the final analysis. The FLT3/ITD and NPM1 mutation assay was performed using fluorescent PCR with primers and amplification conditions. All pts received standard AML induction chemotherapy with cytarabine 100 mg/sqm twice daily (7 days) and idarubicin 12 mg/sqm (3 days) followed by consolidation chemotherapy with high dose cytarabine for 1–3 courses (18 g/sqm each course); among these pts, 11 underwent allogeneic HSCT. 8 pts were FLT3/ITD + (5 pts NPM1 mutated, 3 pts NPM1 wild type), 31 were FLT3/ITD- (12 pts NPM1 mutated, 19 pts NPM1 wild type). Log-rank (Mantel-Cox) test applied to Kaplan-Meyer method was employed to estimate PFS and OS. At 2 years we observed a relapse after consolidation chemotherapy in 4 of 8 pts FLT3/ITD+ pts and in 5 of 31 FLT3/ITD- pts. Among FLT3/ITD+ and FLT/ITD- pts, 5 pts and 6 pts were transplanted in CR1 or CR2, respectively. 2 years PFS was 16.7% for FLT3/ITD+ and 68.7% FLT3/ITD- pts (p 0.041); 2 years OS was 18.7% for FLT3/ITD+ pts and 72.2% for FLT3/ITD- pts (p 0.048). We observed a significant difference in 2 years OS and PFS among FLT3/ITD+ and FLT3/ITD- pts, indipendently by NPM1 mutational status. In our experience FLT3/ITD mutation has a poor impact on AML patients, although most of FLT3/ITD+ pts underwent HSCT. It is debated from literature the role of HSCT in overcoming the poor impact of FLT3/ITD mutation; in different studies FLT3/ITD mutation seems to have an adverse effect on outcome of HSCT in the same direction it does after chemotherapy. Our data seem to confirm this hypothesis.

P006

CONSOLIDATION CHEMOTERAPY WITH HIGH DOSE CYTARABINE FOLLOWED BY ALLOGENEIC HSCT IN DE NOVO ACUTE MYELOID LEUKEMIA PATIENTS: RESULTS FROM A SINGLE CENTRE EXPERIENCE ACCORDING TO DIFFERENCE RISK CLASSES

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High-dose cytarabine applied as consolidation therapy after attainment of a complete remission (CR) has become an established element in the treatment of acute myeloid leukemia (AML). There is no direct evidence either to suggest that any particular genetically defined subset of AML would benefit from high-dose levels of the drug. We report the outcome of 41 patients (pts,mean age 54 years) diagnosed as having AML between January 2009 and January 2013. They were divided at diagnosis into low (LR,15 pts), intermediate (IR, 17 pts) and high risk classes (HR,9 pts) according to European Leukemia Net classification. Among LR pts, 4 were diagnosed as CBF leukemias (2 inv(16) positive, 2 t(8,21) positive, all of them c-KIT wild type) and 11 were NPM1 mutated/FLT3-ITD-. Patients allocated in HR class had complex karyotype (4 pts) or carried del(7q) (1 pt); we included in HR class also FLT3-ITD+/NPM1 wild type pts (4 pts). Other pts were defined as IR patients. All pts underwent induction therapy (IT) with cytarabine 100 mg/sqm twice daily (7 days) and idarubicin 12 m/sqm (3 days). 36 patients obtained complete remission (CR) after IT and they underwent 2 or 3 cycles with high dose cytarabine (18 g/sqm per cycle, cumulative dose 36 g/sqm or 54 g/sqm). 12 pts underwent allogeneic HSCT in first or second CR (5 HR pts,6 IR pts). Log-rank (Mantel-Cox) test applied to Kaplan-Meyer method was employed to estimate progression free survival (PFS) and overall survival (OS). At 2 years 11 pts relapsed, 1 after HSCT and 10 without receiving HSCT: of them, 3 relapsed pts were not candidate to HSCT because they had a LR disease; they were all NPM1 mutated/FLT3-ITD-. Cumulative incidence of relapse at 2 years was 35.8%, 43.2% and 58.3% in LR, IR and HR pts respectively, with no statistical difference among risk classes. No difference was found also for PFS and OS: 2 year PFS was 64%, 42%, 57% for LR, HR and IR pts respectively (p 0.77) and 2 year OS was 65%, 50% and 53% for LR, HR and IR pts (p 0.83). In our experience high dose cytarabine based consolidation regimen (36 g/sqm or 54 g/sqm) followed by HSCT in selected pts (IR pts in CR2 or in CR1 with a sibling donor and HR pts) seems to give an equal OS e PFS among different risk classes. Thus, in our experience this kind of approach seems to reduce the poor impact of adverse cytogenetic abnormalities and molecular markers such as FLT3 mutation in AML, with a similar outcome among risk classes.

P007

IMMUNOPHENOTYPIC ANALYSIS OF HEMATOPOIETIC STEM CELL COMPARTMENT IN ACUTE MYELOID LEUKEMIA PATIENTS

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Introduction. Acute leukemias are the result of neoplastic transformation of hematopoietic stem cells (HSCs). Recent studies showed that immature cells with stem cell biological features (self-renewal, drug resistance) can be identified in leukemia and are responsible for disease development, maintenance and relapse. The presence of leukemia stem cells (LSCs) has been shown mainly in the bone marrow (BM) CD34+/CD38- cell fraction of patients with AML, where normally HSCs can be detected in healthy subjects. This study was aimed at identifying phenotypic abnormalities of LSCs to improve the minimal residual disease (MRD) monitoring in AML patients. Methods. Immunophenotype of immature cell compartment (CD34+/CD38- cells) was carried out according to a 6-color antibody panel (Table 1). First, we studied 11 AML patients at diagnosis and 6 healthy donors to identify the LSC-associated phenotype (LAP). Five/11 patients were further analyzed at disease relapse to evaluate the phenotypic stability. Subsequently, we combined the most significant LAP-markers to set up an antibody combination for the perspective study (Table 1). Therefore, we analyzed 9 patients with AML at diagnosis and during follow-up (3/9 patients). Results. In the comparative study AML patients showed a down-modulation of CD133, CD90 and CD117 in CD34+/CD38- cells, as compared to healthy donors. On the other hand, CD123 and CD45RA were up-regulated. These phenotypic abnormalities were stable at relapse. In the perspective study, putative LSC were found in 7/9 patients at the diagnosis. During the follow-up, the abnormal population was still present in all the studied patients. Conclusions. LAP identification in the CD34+/CD38- cell population can discriminate LSCs from normal HSCs. Consequently, further studies are in progress to demonstrate the clinical relevance of LSC quantification in AML patients.

Table 1.

RETROSPECTIVE STUDY						
	FITC	PE	PerCP	PE-Cy7	APC	Apc-h7
# 01	HLA-DR	CD133	CD38	CD34	CD117	CD45
# 02	CD25	CD90	CD38	CD34	CD33	CD45
# 03	CD56	CD123	CD38	CD34	CD7	CD45
# 04	CD45RA	CD45RO	CD38	CD34	/	CD45
PERSPECTIVE STUDY						
# 01	HLA-DR	CD90	CD38	CD34	CD123	CD45
# 02	CD45RA	CD133	CD38	CD34	CD117	CD45

P008

PURE ERYTHROID LEUKEMIA (PEL) EVOLVING FROM A THERAPY-RELATED MYELODYSPLASTIC SYNDROME (MDS) SECONDARY TO TREATMENT FOR LONG-LASTING AND REPEATEDLY RELAPSED BREAST CANCER

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PEL is a rare malignancy often related to a preceding MDS, as recently observed by us in a patient with a MDS, thought to be secondary to antineoplastic treatments for her breast cancer (BC). A 64-year-old woman was seen in hematological counseling for a macrocytic anemia and thrombocytopenia; the patient was on palliative treatment after a 16-years history of repeatedly relapsed BC. For several months she was receiving epoetin and folate. Given the loss of response to this agent, the increased need for transfusions and the worsening thrombocytopenia, she was referred to hematologic consultation. The morphological examination of peripheral blood showed prominent erythrocyte and platelet anisopoikilocytosis along abnormal hypogranulated neutrophils, but not circulating blasts. A MDS was suspected. Initial BM aspirate failed; the examination of the BM trephine biopsy showed hypocellularity with prevalence of erythroid progenitors along a severe of megakaryocytic dysplasia. However, a near-normal erythroid gradient maturation was recorded. The CD34 positive myeloblasts were less than 1%; cytokeratin staining with MNF116/AE1AE3 was negative. This framework was interpreted as MDS. However, the transfusion requirements soon after increased further and the thrombocythemia became even more severe; again, she began to present gastrointestinal bleeding. A disease re-evaluation, also to evaluate the possible use of hypomethylating agents, was performed. Gastrointestinal endoscopies were negative so the patient underwent capsule videoendoscopy that revealed a hemorrhagic duodenitis. The BM, although markedly hypocellular, showed a preponderance of primitive erythroblasts, which were characterized by an aberrant morphology, including binucleate and bizarre forms. On immunophenotype, blast cells were positive for Glycophorin–A: 80%, CD36 (a marker of immature erythroid cells): 80%, CD45: 10% and were negative both for myeloid (CD13 and CD33) and immaturity markers, such as CD34 and CD117 (Figure 1). Cytogenetic revealed complex abnormalities, such as:44,XX,der[(12)(p?-pter),der(14)t(14;21) (q11;q11),der(16) t(16;21)(q11;q11),der(17)t(17;21)(p12;q11), der (19)t (19;21)(p13:p13-pter;q11] in 100% of metaphases. Overall, these pathologic features were consistent with a PEL. The patient was treated with 5-azacitidine of which she receive two cycles before dying of hemorrhagic and infectious complications.

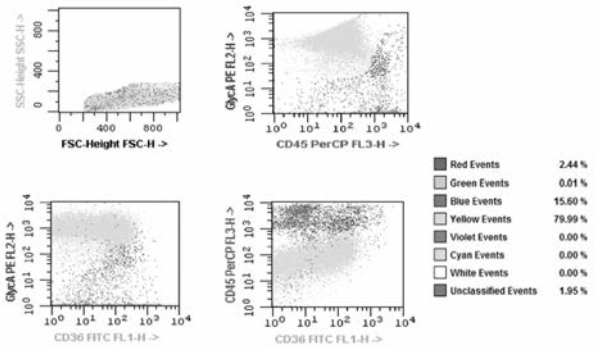

Figure 1.

P009

MYELOID SARCOMAS TREATED WITH AZACITIDINA AFTER INDUCTION WITH ETOPOSIDE, MITOXANTRONE AND CYTOSINE ARABINOSIDE COMBINATION

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Introduction. Myeloid sarcoma (MS) is a rare hematologic disease characterized by the occurrence of myeloid masses occurring in extramedullary sites concomitantly with or at relapse of acute myeloid leukemia (AML). The exact identification of MS is important for the therapy because it is often postponed for high misdiagnosis rate. Case report: A 75 year old man was admitted to our hospital for acute disease of gallbladder that need of surgery. After surgery the histology report

showed a diffuse infiltration of the gallbladder from myelo-monocytic blasts, picture compatible with myeloid sarcoma. TC scan and PET total body showed the enlargement of nodes in almost all sites of body with splenomegaly, epatomegaly and the involvement of different organs and the left orbit. The peripheral blood film was normal, without immature cells. The bone marrow aspirate showed a picture of classic acute myeloid leukemia. During the hospitalization he had obstructive renal failure and was placed the uretheral stents; when the normal renal function was restore he started chemotherapy with mitoxantrone, arabinoside C and etoposide combination obtaining a partial remission. After the 2th cycle the extramedullary mass reduced still and the blasts decreased to below 30 % and he started therapy with azacitidina at 75 mg/m2 daily for seven days every four weeks. After three cycles of azacitidina the hematological picture has improved and the blasts are reduced further. Discussion. MS is a tumor of immature granulocytes, monocytes or both, involving any extramedullary site; rarely precede peripheral blood or bone marrow involvement; it can also appear as an initial manifestation of relapse in a previously treated acute AML patient in remission; it is reported in 2 – 8% of patients with AML either as a single or as a multifocal tumor. MS is frequently mistaken for other neoplastic disease. The most common genetic abnormalities are an extra chromosome 8 and inv(16). The treatment with conventional AML-type chemotherapeutic protocols, is associated with favorable survival outcomes. Conclusion. MS is a rare disease. The patients with isolated MS may have a better prognosis compared with AML patients without MS. The therapy for isolated MS and MS occurring in AML patients is AML-type chemotherapy. The treatment with azacitidina could be a feasible treatment after induction chemotherapy for older patients.

P010

SIGNALING NETWORK IN T-CELL LYMPHOBLASTIC LEUKEMIA: RESULTS FROM A SINGLE-CELL MULTI-PARAMETRIC PHOSPHO-FLOW CYTOMETRY STUDY

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The molecular events occurring during T-cell acute lymphoblastic leukemia (T-ALL) pathogenesis determine intrinsic defects of leukemic cells as well as altered interpretation of external cues deriving from the microenvironment. Both cell-intrinsic defects and microenvironmental stimuli converge on the activation of regulatory signaling pathways involved in processes enhancing the capacity of self-renewal, overturning the control of cell proliferation, blocking differentiation, and promoting resistance to apoptosis. The identification of specific oncogenic signaling network profiles involved in the pathogenesis of T-ALL is challenging to gain an integrated, overall features of malignant cells. We used multi-parametric phospho-flow cytometry to simultaneously determine protein expression and protein post-translational modifications (*i.e.* phosphorylation) at a single cell level in T-ALL cell lines at different differentiation stages. We analyzed signaling pathways that are crucial for the survival and proliferation of T-ALL cells, *i.e.* Notch1, PI3K/Akt, MAPKs and Jak/STAT. Protein expression and signaling properties were measured in baseline and modulated conditions, using biologically relevant modulators for T-ALL, *i.e.* JAG1, CXCL12, IL-7. In unmodulated condition, signaling/expression profiles varied among the cell lines, ranging from a clear positive fluorescence in at least one of the protein measured to no change with respect to isotype. Modulation with physiologic stimuli evoked signaling profiles different from those measured in basal condition. Moreover, profiles are heterogeneous across treatments and cell lines. Pearson's correlation between nodes (a node is defined as a 3-dimensional vector where each dimension represents a single response to treatment) showed an overall high correlation between signaling/expression statuses following different treatments for each cell lines. Graph diagrams obtained using highly correlated nodes showed topologically different network across cell lines. This study showed that multi-parametric phospho-flow cytometry enables to distinguish signaling network maps that are associated with the T-ALL ontogeny stages. Combinations of different pathway information may identify biologically relevant signaling hubs, thus forming the basis for future studies testing the clinical validity of multi-parametric phospho-flow cytometry assay in primary T-ALL cells.

P011

GENOMIC ANALYSIS OF NOTCH MUTATIONS IN A CASE OF ALAGILLE SYNDROME WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Alagille syndrome (ALGS), or arteriohepatic dysplasia, is a congenital multisystem disease due to Notch signalling pathway mutations, most commonly affecting JAG1 (ALGS type 1), and more rarely NOTCH2 (ALGS type 2), leading to hepatic, lung, renal and ocular dysfunction (chronic cholestasis, peripheral pulmonary artery stenosis, dysplastic kidneys pigmentary retinopathy), mental retardation, and skeletal abnormalies (minor vertebral segmentation, characteristic facies, posterior embryotoxon/anterior segment defects). ALGS is an autosomal dominant disease, but it is characterized also by variable penetrance and clinical expression and somatic/germline mosaicism. A 20-year-old man with ALGS was admitted to the University Hospital of Verona because of pancytopenia. Following analyses led to the diagnosis of Philadelphia chromosome/bcr-abl-negative, CD10-positive, B-lineage acute lymphoblastic leukemia (common B-ALL). In order to identify the genetic components involved in this complex phenotype, we sequenced the exome of a bone marrow sample collected from the patient. By genome interpretation with Knome pipeline applied to the reference genome UCSC hg19, we found missense variants both in NOTCH2 (E38K) and JAG1 (P871R) genes that are mainly involved in the syndrome, although their effect on protein function was predicted not to be deleterious. However, we detected putative damaging mutations in genes such as PAX5 (R38H) and NOTCH1 (K1821N) which might be strongly related to the observed disease. In fact, PAX5 is a member of PAX protein family of transcription factors implicated into regulation of early development, that binds NOTCH2 and likely altering its functionality. On the other hand, NOTCH1 is involved in cell growth and proliferation and thus the predicted alteration of function of the corresponding protein may have an important role in neoplastic transformation.

P012

TP53 MUTATION SCREENING IN ADULT ACUTE MYELOID LEUKEMIA (AML) PATIENTS SHOWS ASSOCIATION WITH COMPLEX KARYOTYPE

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Introduction. AML is a heterogeneous disease with various chromosomal aberrations. The karyotype at diagnosis provides important prognostic information that influences therapy and outcome. The TP53 gene is the most frequently mutated gene in human tumors. The reported TP53 mutation rate in AML is low (2.1%). In contrast, the incidence of TP53 mutations in AML with a complex aberrant karyotype (CK-AML) is higher (69-78%). Aims. to investigate TP53 mutations in adult AML patients (pts) focusing on subgroups of pts with chromosome abnormalities. Patients and Methods. 65 AML pts with FAB-M0, M1, M2, M3, M4, M5, miscellaneous cytogenetic abnormalities and normal karyotype (nK-AML, 6/65 pts) were examined. Nineteen pts (29.3%) showed 3 or more chromosome abnormalities (CK-AML), 35 (53.8%) presented one or two cytogenetic abnormalities (other-AML) and in 5 cases the karyotype was not available. Genomic DNA and/or cDNA was isolated from

mononuclear AML blast cells. TP53 mutation screening was performed on all 65 AML pts, in particular in 42 from exon (ex) 2 to 11, in 7 from ex 4 to 11 and in 16 pts from ex 2 to 8. We focus our analysis on coding sequences (RefSeq GRCh37/hg19 NG_017013.2; http://www.ncbi.nlm.nih.gov/nuccore/383209646). Results. By PCR and subsequent Sanger sequencing, mutations of TP53were detected in 8 pts (12.3%). Two pts revealed 2 mutations. Eight of ten mutations (80%) were located in the DNA binding and in the carboxyl-terminal tetramerization and regulatory domains and they included L130P and K132R (ex 5); I195T (ex 6); R248W (ex 7); L264ins, R267G, R273H and the deletion 264-271 (ex 8). Outside these domains, we identified the E11K (ex2) and the 3' untraslated region C/A 7572841 (ex11). All the mutations in coding regions were classified in the IARC database (http://p53.iarc.fr/TP53GeneVariations.aspx) as deleterious. Six out eight mutations were found in CK-AML pts (6/19, 31.6%), one in nK-AML (1/6, 16.7%) and one in other-AML (1/35, 2.9%). Conclusion. Although this analysis is still ongoing, our data demonstrated that mutations of TP53 occur in 12.3% of AML with a higher frequency in the subgroup of CK-AML (p = 0.0095). Since TP53 mutations have predicted to be deleterious and correlated with prognosis, TP53 mutation screening should be recommended in these patients. Supported by: European LeukemiaNet, AIL, AIRC, PRIN 2010-2011, Fondazione del Monte di Bologna e Ravenna.

P013

PBX/E2A TRANSCRIPT POSITIVE B-LYMPHOBLASTIC LEUKAEMIA (B-ALL) PRESENTING WITH BILATERAL RENAL AND MYOCARDIAL INVOLVEMENT: A CASE REPORT

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In February 2013 a 56-year-old man was admitted to another hospital complaining abdominal pain. The patient's medical history was negative. Clinical examination revealed only a mild abdominal distension. No systemic symptoms were reported and laboratory data were within limits. An abdomen-ultrasound scan (US) showed multiple bilateral renal lesions with no other abnormalities. In the hypothesis of a secondary localization of neoplasm, a total body-CT scan was done, which confirmed the presence of multiple bilateral renal lesions. A testicular-US was negative. EGDS was normal, while a colonoscopy showed only diverticulosis. A subsequent US-guided biopsy of the left kidney was diagnostic for B-cell lymphoblastic lymphoma (B-LBL) and the patient was transferred to our unit. A diagnostic work-up for B-LBL/ALL was done: a peripheral blood smear showed few leukemic blasts (2%), whereas bone marrow aspirate displayed a large leukemic involvement (80%). Flow-cytometry analysis characterized blasts as CD10+/CD58+/CD38+/TdT+/CyCD79a+/CD22+. Molecular analysis was negative for BCR-ABL transcript but positive for PBX1/E2A. A further total body CT showed a bilateral renal enlargement (16 cm right-15 cm left) with multiple hypodense confluent lesions, while a total body FDG-PET indicated an intense uptake by both kidneys (SUV max 6.5) and by the anterior myocardial wall (SUV max 5.8). Laboratory data remained within range except for a mild creatinine raise. The myocardial involvement was analyzed through an echocardiogram that demonstrated a diffuse and severe dilatative cardiomyopathy and the absence of any hypokinetic segment. The ECG was normal. A thorax X-ray confirmed the enlargement of the heart profile while a revision of the thorax-CT scan showed a thickening of the left ventricular wall. The patient underwent HyperCVAD regimen with a rapid disease's response. Renal involvement is not an uncommon feature in B-ALL/LBL, but it is usually observed during the latter phases of disease. In fact, there are only few cases reporting a large bilateral renal involvement at disease presentation. Cardiac involvement is a very rare feature in ALL too. It can present as intracardial mass or as myocardial infiltration, with or without symptoms. PBX/E2A occurs in a small subset of ALL and has an uncertain prognostic value. This is the first report of an association of this transcript with such an unusual extranodal presentation.

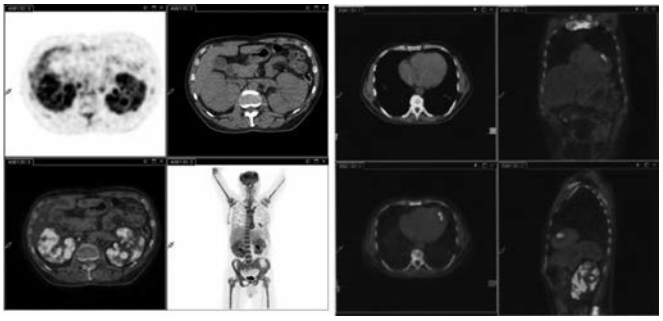

Figure 1.

P014

ACUTE MYELOID LEUKEMIA RISK NEAR STEEL PLANT MANUFACTORY IN TARANTO; AN EPIDEMIOLOGICAL AND PROGNOSTIC VALUATION

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Due to the established, recently reported, high risk of tumor's incidence in the area of Taranto as the consequence of concentration of carcinogens in the environment connected principally to a big steel plant manufactory, we investigated the incidence of AML and high risk MDS together the prognostic aspects of these patients. The incidence was extrapolated from the Taranto's registry of tumor related to 2006-08 period which has been recently validated; the prognostic analysis was performed on a retrospective cohort of patients observed from 2000 up to December 2012 and evaluated according to karyotypic alterations, response to therapy and survival. The standardized incidence of AML in Taranto is 4.7/100.000 /year over, 4.3 for females and 5.2 for men, respectively. The standardized incidence of high risk MDS is 2.7/10 0.000/year, 3.7 for men and 1.7 for females. A difference was recorded between the sites of Taranto town and Taranto country with 15% less incidence of AML and MDS out of the town. The karyotype was available in 163 pts of a cohort of 220 pts (74%); 19 pts had good karyotype alterations (12%), 92 pts had intermediate alterations or normal karyotype (56%) and 52 pts (32%) had bad karyotype alterations (especially complex karyotype). Response to therapy related to 220 pts who all did a well defined scheme according to GIMEMA's protocols consisted of 124 pts achieving CR (57%), 91 pts not achieving remission (41%), and 5 pts dead in induction (2%). CR rate was superior in pts with good karyotype (76%) than intermediate karyotype (51%) or bad karyotype (32%). The actuarial status show 31% patients alive with a follow up ranging from 4 months to 11 years (mean 2.5 years), 8% alive of those with bad karyotype. In synthesis this retrospective study on the AML incidence in Taranto's area shows an increase of number of leukemia in the metropolitan area and the comparison with literature is demonstrating a variety of leukemia at worst prognosis than expected. We consider that environmental factors in an area at risks, such as pollutions including several carcinogens, are the potential responsible key factors for prognostic outcome of patients. Epidemiological studies are ongoing for such evaluation.

P015

ALLOGRAFTING IN HIGH RISK ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is the most frequent acute leukemia in adults. The aim of the study was to evaluate outcomes in newly diagnosed patients, younger than 66, who achieved complete remission (CR)

after induction/consolidation therapy at the Divisions of Hematology at Città della Salute e della Scienza, Università di Torino, Torino, Italy, between 2000-2011. Three-hundred and two AML patients (except FAB-M3) were consecutively diagnosed and stratified by risk as follows: low risk included presence of t(8;21), inv(16)/t(16;16); high risk features included WBC>50.000/μL at diagnosis, secondary leukemia, presence of extramedullary AML, complex karyotype, chromosomal monosomy, no remission after induction, and FLT3/ MLL mutations (since 2004). Intermediate risk included patients who did not meet either low or high risk criteria. Moreover, the standard risk group included low+intermediate risk patients. Patients were treated according to Center guidelines or on clinical trials active at the time of diagnosis. All high risk patients were considered for an allograft since diagnosis. After induction/consolidation, 229/302 patients (76%) achieved CR: 16/229 (7%) were at low, 54/229 (24%) at intermediate, and 159/229 (69%) at high risk respectively. Eighty/159 (50%) high risk patients received an allograft as 1st line treatment; 56% from a HLA-matched sibling, 42% from an unrelated donor and 2% received a haplo-identical transplant. Seventy-nine/159 (50%) did not receive an allograft primarily because of failure to find a suitable donor either sibling or unrelated. At median follow-up of 53 months from induction therapy and 49 months from achieving CR, 5-year overall survival (OS) and 5-year event free survival (EFS) of the entire patient cohort were 45% and 35% respectively. Standard risk patients showed a 5-year OS of 56% and high risk patients of 40% (p=0.008). Five-year EFS was 37 and 34% in standard and high risk patients respectively (p=0.194). High risk patients who underwent an allograft up- front showed a 5-year OS of 53% and showed a statistically significant advantage as compared with those who did not receive a transplant (p=0.018). In conclusion, allografting plays a pivotal role in OS and EFS for high risk acute myeloid leukemia. The lack of a donor is associated with bad clinical outcomes. Clinical trials designed to evaluate the use of more readily available donors such as haploidentical siblings or parents are needed.

P016

MULTI-LINEAGE DYSPLASIA AS ASSESSED BY IMMUNO-PHENOTYPE HAS NO IMPACT ON CLINICAL-BIOLOGIC FEATURES AND OUTCOME OF ACUTE MYELOID LEUKEMIA WITH MUTATED NUCLEOPHOSMIN (NPM1)

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Background. Acute myeloid leukemia (AML) with multi-lineage dysplasia (MLD) is a separate subset in WHO classification. Morphologic study of residual hemopoiesis at diagnosis is the standard criteria for defining MLD. Its prognostic value is still under debate due to technical (residual non-blast cells are few at diagnosis and morphology is operator-dependant) and biological reasons: MLD-related poor prognosis is supposed to rely on progression from a myelodysplastic syndrome (MDS) but MLD might merely result from differentiation/maturation by AML clone. A major controversy concerns MLD value in NPM1-mutated (NPM1+) AML, since NPM1+ status correlates with good prognosis, especially when FLT3-wt. Falini *et al.* (Blood 2010) showed morphologic MLD has no prognostic weight. Aims. To study MLD in NPM1+ AML by an alternative technique: flow cytometry (FC) is emerging as a useful method to study dysplasia. Its application to AML allows: i) to study large cells' amount; ii) to quantify, refer to controls and thus standardize FC parameters. Methods. Patients: 70 NPM1+ pts were studied. Flow cytometry: FACSCanto II (BD) and Infinicyt (Cytognos) were used for data acquisition and analysis. We adapted to AML an approach previously described for MDS (Matarraz *et al.* 2008): MLD was appraised by an immuno- phenotypic score (IPS) including 18 parameters (14 for granulocytic and 4 for erythroid lines). Results. Median age was 57 (24-70). Median WBC count was 48.0 x 10^9/L. Karyotype was normal in 62 (88.6%) pts. FLT3-ITD occurred in 27 pts (38.6%). MLD was assessable by morphology in 66 pts; 24 (36.4%) showed MLD. IPS was calculated in all 70 pts; median IPS was 6.25 (0.5-18.5). Pts were grouped according to IPS higher (IPS+) or lower-equal (IPS-) than the median: age, WBC and platelet counts, incidence of morphologic MLD were not different. IPS+ group had lower FLT3-ITD incidence (25.7% *vs* 51.4%; p=0.048); interestingly, Falini *et al.* (Blood 2010) reported analogue results with morphology. CR rate was not different in IPS- (82.9%) and IPS+ (74.3%; p=0.56) pts. IPS did not affect disease-free and overall survival (Fig. 1A-B). FLT3 status confirmed its prognostic value in our cohort (Fig. 1C-D). Conclusions. This study provides evidence that MLD, as assessed by FC, does not influence clinical characteristics and outcome of NPM1+ AML. These findings further support NPM1+ AML to be considered as a separate entity and its prognostic assessment should not be based on MLD.

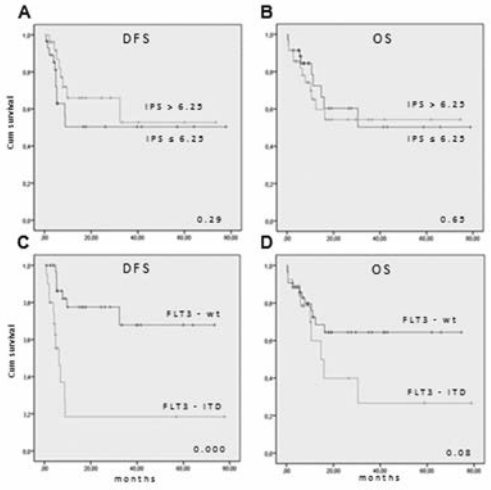

Figure 1.

P017

INTEGRATED CYTOGENETIC AND MOLECULAR DATA IDENTIFY THREE ACUTE MYELOID LEUKEMIA RISK GROUPS, WITH "INTERMEDIATE-RISK" PATIENTS EXPERIENCING AS POOR AN OUTCOME AS "HIGH-RISK" PATIENTS AND MOST PROBABLY BENEFITING FROM FIRST-LINE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Aims. We examined the prognostic role of cytogenetic and molecular data on a retrospective series of 102 consecutive patients, treated with curative intent in Treviso in 2003-12. Methods. we evaluated 102 AML patients (no APL) aged 18-74 yrs (median: 58). Median follow-up was 25 months (6-100). 60 patients (58.8%) were <61 yrs old. 25 patients (24.5%) had secondary AML. All patients were treated with intensive chemiotherapic regimens, *i.e.* FLAI5 (n=67), ICE (n=17), DA37 (n=14), MEC4 (n=2) and FLAN (n=2). In case of complete remission (CR) after induction, patients have been consolidated by high-dose Ara-C (3 gr/m^2 BID, dd 1-4) followed by either intermediate-dose Ara-C (1.5 g/m2) or transplantation (ASCT n=7; first-line allogeneic HSCT n=21). At diagnosis, all patients had karyotype analysis, while FLT3 and NPM1 assessment was available only for 67 (65.7%) and 66 (64.7%) cases, respectively. KIT mutation analysis was available for all patients with Core Binding Factor (CBF) AML (n=11). We combined cytogenetic and molecular data to obtain three risk groups: 1) low (n=20): CBF AML KITwt, normal karyotype (NK) FLT3wt NPM1mut AML; 2) intermediate (n=52): NK FLT3wt NPM1wt, NK FLT3-TKD, CBF AML KITmut; 3) high (n=30): NK AML FLT3-ITD, AML with cytogenetic abnormalities of any kind, complex + monosomal karyotype AML. 13 patients with normal karyotype and no molecular data were considered in the intermediate risk group. Results. Overall, 5-yr OS, DFS and EFS were 23.9%, 28.0% and 18.1%, respectively. The combined cytogenetic-molecular risk assessment identified three risk groups with 5-yr OS 56.5%, 19.3% and 11.8%, DFS 27.9%, 29.8% and 24.0% and EFS 26.5%, 19.0%, 10.9%, respectively. CR was higher in low-risk patients (95% *vs* 58.8% *vs* 53.3%, P=0.002), while there was no significant difference in terms of relapse (36.8% *vs* 56.7% *vs* 37.5%, P=NS). Age >61 yrs identified inferior prognosis in all risk groups, but only as a trend. First-line allogeneic HSCT was beneficial in terms of OS (P=0.012). The use of first-line Fludarabine-containing regimens did not yield advantages. At Cox mod-

eling secondary AML and absence of CR achievement after induction predicted poorer outcome, while first-line allogeneic HSCT was beneficial for OS (HR 0.44; 95%CI: 0.23-0.85, p=0.014). Conclusions: intermediate risk AML patients, assessed by combining cytogenetic and molecular data, experience as poor an outcome as high-risk patients and might benefit from first-line allogeneic HSCT.

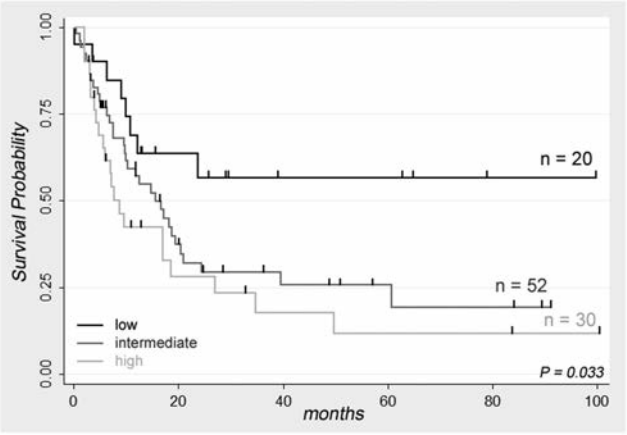

Figure 1.

P018

SIMILAR BUT NOT THE SAME: CHROMOSOMAL ABNORMALITIES T(8;21)(Q22;Q22) AND INV(16)(P13;Q22)/T(16;16)(P13;Q22) IDENTIFY SUBTYPES OF CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA CHARACTERIZED BY DIFFERENT SURVIVAL AND PROGNOSTIC FACTORS

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Aim. To address the heterogeneity of CBF AML patients-t(8;21)(q22;q22)/RUNX1-RUNX1T1 and inv(16)(p13q22)/t(16;16)(p13q22)/CBFB-MYH11- by assessing their characteristics at diagnosis and their response to therapy. Methods. we retrospectively studied 132 patients (t(8;21) n=58; inv(16)/t(16;16) n=74) treated with curative intent in 9 Hematology Centers between 1987 and 2012. Median follow-up was 66 months(10-294). Cytogenetic data at diagnosis were available for all patients, while only 39 had KIT(29.5%), 63 FLT3(47.7%) and 54 NPM1 assessment(40.9%). Patients were treated with regimens either based on DA37 and similar (n=102) or first-line Fludarabine-containing regimens (n=30). All patients achieving complete remission (CR) after induction were consolidated with 1 (n=18), 2 (n=49) or >3 (n=50) cycles with HiDAC. 29 patients underwent ASCT, and 15 first-line allogeneic HSCT. Results. median age was 44 yrs (15-79). 5-year OS, DFS and EFS of the whole series was 62%, 58.9% and 53.5%, respectively. While achieving high CR rates after induction (86.2% *vs* 90.5%, P=NS), patients with t(8;21) experienced worse 5-yr OS (57.3% *vs* 67.7%, P=0.09), DFS (47.0 *vs* 67.5, P=0.015) and EFS (43.6% *vs* 60.8%, P=0.045) than patients with inv16/t(16;16), due to a trend to higher relapse (48% *vs* 32.8%, P=NS) and poorer response to salvage treament. Considering all patients, age >61 yrs, grade-4 thrombocytopenia, elevated LDH and D816 KIT mutation were predictive for poorer OS, while the presence of extramedullary disease/granulocytic sarchoma, and D816 KIT mutation were prognostically relevant only for t(8;21) patients; conversely, age>61, leukocytosis and grade-4 thrombocytopenia were predictive only for inv16/t(16:16) patients. Noteworthy, D816 KIT mutation did not predict prognosis in inv(16)/t(16;16) patients. Additional trisomy 22 (n=7), trisomy 8 (n=5) and chromosome 7 alterations (n=6) were associated with a trend to better survival. Neither the presence of FLT3 mutations (ITD/TKD) nor the presence of NPM1 mutations affected prognosis. The use of first-line Fludarabine did not yield clear advantages, while the achievement of complete remission after induction had maximal impact on OS (HR 4.82; 95%CI: 2.29-10.16, p<0.001). We observed a clear survival benefit in a small selected series consolidated by first-line autologous (n=29) or allogenic HSCT (n=15, P=0.022) after HiDAC. Conclusions:t(8;21) and inv(16)/t(16;16) CBF AML should be considered as two distinct entities.

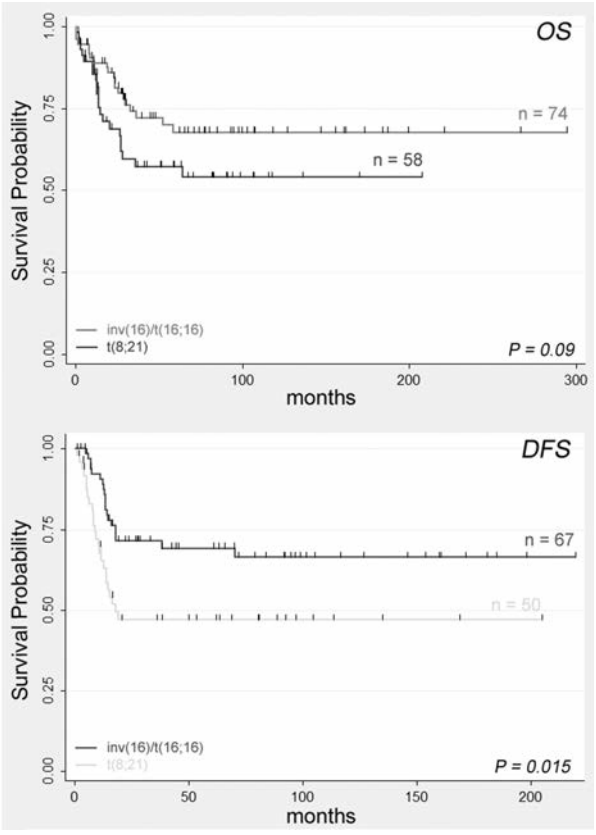

Figure 1.

P019

MAST CELL LEUKAEMIA (ALEUKAEMIC VARIANT) IN STABLE DISEASE WITH IMATINIB

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Mastocytosis is a heterogeneous disease characterized by an accumulation of mast cells (MC) in one or more organs. The WHO-classification defines 7 disease-variants: cutaneous mastocytosis (CM), indolent systemic mastocytosis (ISM), SM with an associated clonal haematological non-MC-lineage disease (SM-AHNMD), aggressive SM (ASM), MC leukaemia (MCL), MC sarcoma (MCS), and extracutaneous mastocytoma. The new 2008 WHO classification has included in the category of myeloproliferative neoplasms (MPNs), all forms of SM, that have been grouped with the term of mast cell disease (MCD). Mast cell leukaemia (MCL) is defined by increased numbers of MC in bone marrow smears (20%) and peripheral blood. MCL is relatively rare (about 1% of SM)

with median survival of 2 months only. In typical cases, circulating MC amount to 10% of leukocytes (classical form of MCL). The aleukaemic subvariant of MCL shows <10% MC on peripheral blood smears. We describe a case of a 65-year-old Caucasian woman presented to emergency department with flushing and hypotensive shock. Serum biochemistry and hematological parameters with morphological examination of peripheral blood smears was normal, except for serum total tryptase levels 2255 ng/mL; bone marrow aspirate and bone marrow biopsy showed a 70-80% infiltrate of atypical mast cells infiltrate (CD117+); immunophenotypic examinations of neoplastic mast cells by flow cytometry showed (CD117+, CD2+, CD25-); the KITD816V mutation not being observed. Computed tomography scan of chest and abdomen likes so vertebral magnetic resonance imaging appeared normal.The patient was been given antihistamine alone. Five months later we observed a worsening anemia, as far as 8g/dL., and serum tryptase level remained at about 2000 ng/ml. Because of the rarity of these forms, an effective standard of care is lacking and treatment has to be tailored to the needs of the individual patient. Imatinib is the only SM treatment currently approved by the Food and Drug Administration (specific indication is treatment of adult patients with ASM without the KITD816V mutation or with unknown KIT mutational status); so we started therapy with imatinib 400 mg/die, quickly lowered at 300 mg/die due to diffuse idric ritention. After five months from start of theraphy with imatinib the patient shows a stable disease (anemia transfusion-independent, tryptase levels 2.000-3.000 ng/mL, with episodes of flushing, diarrhea, abdominal pain, hypotension).

P020

COEXPRESSION OF THE NPM1 AND FLT3-ITD MUTATIONS ALTER HEMATOPOIESIS AND LEAD TO LEUKEMIA DEVELOPMENT IN THE MOUSE

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NPM1 and FLT3-ITD mutations have been frequently found to coexist in normal karyotype AML. The presence of FLT3-ITD determines a poor prognosis. NPM1 mutations are associated with improved outcome, but only in the absence of concomitant FLT3-ITD. These observations suggest that NPM1 and FLT3-ITD mutations functionally cooperate for leukemic transformation. Here, we investigated the cooperative effect of the NPM1 mutant (NPMc+) and FLT3-ITD in hematopoiesis and leukemia susceptibility *in vivo* by crossing our previously published NPMc+ (Sportoletti 2013) with FLT3-ITD mice (Lee 2007). Strikingly, concomitant expression of the NPMc+ and FLT3-ITD mutants in hematopoietic cells lead to rapid leukemia development. Indeed, 6 out of 6 mice that were homozygous for the NPMc+ mutant and heterozygous for FLT3-ITD become moribund with a median survival of 3.5 months. In contrast, none of single heterozygous mutant and wild type genotypes die after 1-year follow-up. Leukemic mice presented with massive splenomegaly, leucocytosis, anemia and thrombocytopenia. Blood smears and marrow cytospins were characterized by elevated blasts. Pathological analysis confirmed blasts in BM, spleen and liver showing the expression of cytoplasmic NPM1 at immunohistochemistry. We next investigated whether concomitant NPMc+ and FLT3-ITD expression is sufficient to induce a detectable phenotype, prior to the overt leukemia onset. NPMc+FLT3-ITD mice exhibited significant leukocytosis (WBC 20.8±18 x 109/L; N=20) due to increased neutrophils (29.34%±6.5) and monocytes percentages. NPMc+FLT3-ITD mice also developed mild anemia and macrocytosis (MCV 58.74±5.6 fl). Morphologic and flow cytometric analysis confirmed Gr1+Mac1+ myeloid cells expansion in PB, BM and spleen of double heterozygous mice. NPMc+FLT3-ITD mice display splenomegaly due to expansion of mature myeloid elements that were also infiltrating livers. The presence of the NPMc+ and FLT3-ITD alleles determined an expansion of granulocyte-monocyte progenitors and a decrease in megakaryocyte-erythroid progenitors in BM. We demonstrated that NPMc+ mutation and FLT3-ITD cooperate in leukemia development *in vivo*. Furthermore, NPMc+ and FLT3-ITD induce a "preleukemic state" characterized by leucocytosis and macrocytosis in 100% of the mice. This model will allow for further study of novel pathways involved in leukemogenesis and the exploration for potential therapeutic targets in AML.

Myeloma and Monoclonal Gammopathies I

P021

FREE LIGHT CHAIN ASSAY AND SEMI-AUTOMATIC INSTRUMENTATION: REPORT AFTER A YEAR OF ACTIVITY

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Monoclonal gammopathies are a group of disorders characterized by clonal expansion of B cells that usually secrete intact monoclonal immunoglobulins, monoclonal free light chains (FLC) or both. Until recently the combination of protein electrophoresis (PE) and immunofixation electrophoresis (IFE), in both serum and urine specimens, were the only lab assays to detect monoclonal gammopathies. They however are less effective in detecting FLC alone (light chain disease, amyloidosis) or minimal secretion of intact immunoglobulins (non secretor multiple myeloma). Recently a sensitive nephelometric assay has been developed to identify, characterize and quantitative serum kappa and lambda FLC; latex conjugated polyclonal antibodies to epitopes that are usually hidden in complete immunoglobulin were used. The FLC assay (FREELITE, The Binding Site Birmingham UK) was performed on a semi-automated nephelometer (MININEPHplus) using stored thawed serum. This assay consist of kappa and lambda quantification, moreover we calculated the FLC K/ ratio. The reference intervals recommended by manufacturer for K, and ratio K// are 3,3-19 mg/L, 5,7-26,3 mg/L and 0.26-1.65 respectively In this preliminary study we analyzed 87 cases: 79 affected by multiple myeloma (MM) and 8 with amyloidosis. The mean serum K, and K/ ratio values in patients with MM were (334.29 mg/L, 1362.02mg/L and 58.87) respectively. Twenty-seven patients with MM (32%) had normal serum K, and ratio K/ with mean values of 10.15 mg/L, 10.97 mg/L and 0.99 respectively, 52 patients had increased serum FLC and/or abnormal K/L ratio. Of the 26 patients that showed FLC normal values, 5 underwent peripheral blood antilogous stem cells transplantation. In patients affected by amyloidosis mean serum K, and K/ ratio values were 23,91 mg/L, 216,35 mg/L and 0,61 respectively. The semi-automated nephelometer is easy to use and enables laboratories to offer a cost efficient service for lower volume of serum free light chain assays. The automatic antigen excess is check for Free lite assay. The systems, assays and results are developed, optimized and validated together, ensuring quality assays and results. But in the laboratory practice, the time of experimental phase is too long for a low number of samples analyzed. Rates of dilution, for antigen excess affects the determinations number daily performed amplify the errors possibility for too samples handling.

P022

EARLY APPLICATION OF PERCUTANEOUS VERTEBROPLASTY DOES NOT AFFECT PERIPHERAL BLOOD STEM CELL (PBSC) COLLECTION AND TRANSPLANT IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

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Vertebral fractures occur in over 50% of MM patients and can cause pain, disability and poor quality of life. Appropriate antimyeloma therapy can lead to symptoms improvement in the majority of the patients, but these positive effects can take time to be perceived. Furthermore, application of radiotherapy prior to PBSC mobilization can severely impair stem cell collection. Percutaneous vertebroplasty has been proposed as a suitable option to rapidly relieve bone pain from vertebral fractures in patients with neoplastic diseases such as MM, but so far, little is known about the effects of this procedure on subsequent PBSC mobilization, collection and transplant. Eighteen consecutive patients (10M, 8F, median age 64.5 years) with untreated, symptomatic MM and painful vertebral lesions underwent percutaneous vertebroplasty prior to proceed to the planned transplant program at our Institution. Forty-one procedures were performed at C2-L5 levels, 10 patients were treat-

ed at a single level, a maximum of seven levels were processed in 1 patient. Complete or optimal pain control was achieved in 95% of the patients in an average of 2.3 days after the procedure. PBSC mobilization regimen consisted of cyclophosphamide + G-CSF in 15 patients and G-CSF + plerixafor in 3 patients; all the patients successfully mobilized PBSC; in a median of 1.8 apheresis, the median number of collected CD34+ cells was $10.8x10^6$/kg (range 3.4-16.5). All the patients underwent autologous stem cell transplant; hematological recovery averaged 11 days both for PMN (>500/mmc) and for platelets (>20000/mmc). In conclusion, percutaneous vertebroplasty is useful in MM patients with painful vertebral fractures as it allows rapid and durable achievement of pain control, without interfering with PBSC collection and transplant.

P023

EFFICACY OF LENALIDOMIDE/DEXAMETHASONE IN REFRACTORY/RELAPSED MULTIPLE MYELOMA: A RETROSPECTIVE MULTI-CENTER STUDY FROM "RETE EMATOLOGICA PUGLIESE" (REP)

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Introduction In this multi-center retrospective study we analyzed 290 patients with refractory/relapsed MM treated with lenalidomide/dexamethasone as salvage therapy. Patients and methods Two hundred ninety patients, 141 females and 155 males, median age 70 years (range 42-88), were treated in 12 haematogical centers (REP). Median Hb value was 11 gr/dL (range 7-17,2), absolute neutrophil count 3.200/µl (range 700-14.360), PLT count 182.500/µl (range 3000-542.000). Median clearance creatinina value was 80 ml/min (range 1-225). In 18/45 analyzed patients karyotype had cytogenetic abnormalities; namely, del 13q was observed in 12 patients, t(4;14) in 2 patients, t(11;14) in 2 patients, del 17p in 1 patient; complex karyotype was observed in 1 patient. Fifty-three % of patients presented with at least one severe comorbidity (diabetes mellitus, hypertension, atrial fibrillation, coronary heart disease, peptic ulcer, renal and hepatic dysfunction).The median number of previous lines of therapy was 3 (1-4). Ninety-nine patients had undergone autologous stem cell transplantation. The initial dose of lenalidomide was 25 mg/day in 170 patients, 15 mg/day in 56 patients, 10 mg/day in 54 patients and finally 5 mg/day in10 patients. In 14 cases lenalidomide/dexamethasone was associated to other drugs. Results Two hundred forty-six patients were evaluable for response. Median number of administered cycles was 7 (range 1-37). Overall Response Rate was 68%; namely, according to IMWG uniform response criteria, 27 patients (16%) achieved a CR (negative immunofixation), 60 patients (36%) a VGPR (reduction of M-protein >90%), 81 patients (48%) a PR (reduction of M-protein >50%). Median time to best response was 4 months (1-11). Median duration of response to treatment received was 12,5 months (1-40). Median time to progression was 7 months (1-40). Grade 3/4 haematological toxicities occurred in 29 patients (17%), non-haematological toxicities in 59 patients (35%), causing interruptions of the treatment or reduction of daily dose. Fifteen patients (9%) interrupted prematurely the treatment for progression disease after a haematological response (2 CR, 5 VGPR, 8 PR), 10 patients (6%) for haematological response (6 CR, 3 VGPR, 1 PR). Quality of response correlated with the number of previous treatments. Conclusions. Our study confirms that lenalidomide/dexamethasone is an effective combination in inducing significant responses in refractory/relapsed MM.

P024

CONTINUOUS ALTERNATE-DAY LOW DOSE LENALIDOMIDE IN COMBINATION WITH LOW DOSE PREDNISONE AS FRONTLINE TREATMENT FOR OCTOGENARIAN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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About 30% of patients with newly diagnosed multiple myeloma (NDMM) are older than 75 years. Immunomodulatory drugs (IMIDs) have improved response rates and outcomes of NDMM, except for patients older than 75 years more vulnerable to side effects of IMIDs because of their frailty and comorbidities. We evaluated efficacy, toxicity and health-related quality of life (HRQOL) associated with continuous alternate-day low dose lenalidomide (LD-R, 10 mg on alternate days) and low dose prednisone (15 mg/day) (LD-RP) in 7 octogenarian NDMM patients (5 males and 2 females) with a median age of 82 years (range 80-87). All octogenarian patients had IgG MM, except 1 oligosecretory lambda chain MM; all were in Durie-Salmon stage III, except 1 in stage II, and had poor WHO performance status (median: 2, range 1-3). Patients were evaluated at baseline and every 6 months for HRQOL according to MM-specific questionnaire QLQ-MY20 of European Organisation for Research and Treatment of Cancer (EORTC). All patients received aspirin thromboprophylaxis, 57% of them requiring from diagnosis erythropoietin and zoledronic acid treatment. In these 7 octogenarian NDMM patients completing at least three months of therapy, the overall response rate (ORR) was 86%, including 1 complete remission (CR), 2 very good partial remission (VgPR) and 3 PR. After a median follow-up of 12 months (range 3-24), the quality of response improved with continuous LD-RP treatment with a cumulative median reduction in monoclonal protein levels of 85% (range 20-100%); none of the patients required discontinuation of treatment secondary to specific hematologic and/or extra-hematologic toxicity. In addition, QLQ MY-20 questionnaires revealed that 70% of patients treated with continuous LD-RP reported improvements of QOL scores. Two out of 7 octogenarian patients died (1 for progression after 12 months and 1 for sepsis no treatment-related), and 2-year overall survival and progression-free survival estimates were 41% and 75%, respectively. Noteworthy, all patients treated with continuous alternate-day LD-RP showed progressive increase of circulating CD56+, CD3- natural killer cells regardless of treatment response. Our data provide evidence that continuous alternate-day low dose lenalidomide is a manageable and effective frontline treatment for octogenarian NDMM patients. These preliminary results require further validation in prospective larger studies.

P025

ASSOCIATION BETWEEN ABNORMAL SERUM FLC RATIO AND IMMUNOPARESIS IN PATIENTS WITH MGUS. A POSSIBLE PROGNOSTIC FACTOR OF EVOLUTION IN MULTIPLE MYELOMA?

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Monoclonal gammopathy of undetermined significance (MGUS) occurs in 3% of people older than 50 years and up to 10% in those older than 70; it is associated with a 1%/y risk of progression to Multiple Myeloma (MM). Free Lights Chains ratio, plasma cells immunophenotype and DNA aneuplody are now considered important parameters of progression, in addition to the already known prognostic factors (immunoparesis, type and amount of the monoclonal component (MC). Recent data report immunoparesis and abnormal Kappa/Lambda (K/L) ratio in 25% and 30% respectively of patients (pts) at diagnosis. Aim of this study was to assess the incidence of these two parameters in a cohort of 95 pts with MGUS, to verify if they are associated and if their incidence is influenced by other parameters (time from diagnosis, type of Immunoglobulin (Ig) and/or light chains). The patients evaluated were 44 males and 51 females with a median age of 67 years (45-91). Median time from diagnosis to the time of observation was 3 years (0-21). The

MC was IgA in 11 pts, IgG in 72, IgM in 12; 63 had a K and 32 a clonal lambda light chain. K/L ratio was abnormal in 47 pts (49.4%) and normal in 48 (50.5%).Immunoparesis was present in 47 pts (49.4%):17 with a normal (36.1%) and 30 with an abnormal K/L ratio (62.5%)(p-0.004). In 14 pts two classes of Ig were involved. An association between the two parameters occurred in 31.5% of the pts; it seems more frequent in IgA MGUS (55.5%) than in IgG (30.1%) and IgM (25%); we did not observe any differences between K MGUS (30.6%) and L MGUS (34.3%). The association between an abnormal K/L ratio and immunoparesis was present in 21.4% of pts with time from diagnosis of less than 3 years and in 47.2% of pts with a longer time from diagnosis (p-0.04). Our data show that immunoparesis is more frequent in pts with an abnormal K/L ratio. The association seems to be more frequent in case of IgA gammopathy; there are no differences between the two types of light chain. Our data suggest that the longer is the time elapsed from diagnosis, the higher are the frequency of an abnormal K/L ratio and the incidence of immunoparesis, with a greater probability of association. We need a larger number of pts with an adequate follow up to evaluate if the association between immunoparesis and abnormal K/L ratio has a prognostic value, although the higher frequency of association in the subset of pts with a longer time from diagnosis seems to contradict this hypothesis.

P026

PLASMA CELL IMMUNOPHENOTYPE AND CLINICAL OUTCOME IN PATIENTS WITH MULTIPLE MYELOMA (MM)

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Background. 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. 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. Moreover, both the normal plasma cells and those of patients with MGUS are usually positive for the CD43. Aim. We investigated the expression of CD117 and CD43 on bone marrow plasma cells of patients with MM, evaluating the correlation with the clinical course of disease. Methods. In the last 5 years we have analyzed, at diagnosis, the bone marrow blood of 71 patients affected by MM. 49 out of 71 presented a IgG component and the remaining 22 patients were IgA. On the basis of the staging criteria (ISS), 38/71 pts. were in stage II and 33/71 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. Results. The immunophenotype of bone marrow plasma cells demonstrated the expression of CD38 (very bright) and of CD138 while CD19 was absent; 49/71 were CD43+(dim) and 20/71 CD117+(dim). 15 out of 20 CD117-positive patients showed a specific immunophenotypic pattern (CD117+/CD43-). These patients were in stage II and showed a favorable clinical outcome, as demonstrated by a higher DFS and OS than the remaining patients, regardless of the treatment administered. Conclusions. The possible prognostic role of CD117 and CD43 in MM warrants further clinical investigation on a larger series of patients even on the basis of new therapeutic strategies.

P027

TRANSFERRIN-IMMUNE COMPLEX DISEASE – AN UNDERDIAGNOSED CONDITION?

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The combination of marked hypersideremia, hypertransferrinemia and monoclonal gammopathy of underdetermined significance should alert clinicians to the possible presence of an antitransferrin immunoglobin, this is an uncommon acquired disorder described as transferrin-immune complex disease. We have previously described a case of transferrin-immune complex disease with 100% transferrin saturation and liver iron overload, however the findings reported in the few cases described are heterogenous and the presence of high transferrin saturation and liver iron overload is not universal. Here, we report the identification of two additional patients with anti-transferrin monoclonal gammopathy, hypersideremia and hypertransferrinemia but with incomplete transferrin saturation and no hepatic iron overload. The antibodies from both the patients were purified by using transferrin as affinity bait and characterized. One subject showed a high titer monoclonal anti-transferrin IgM with a light chain, the first time that this has been reported. The other patient developed the disease after pregnancy. Her monoclonal antibodies were IgG with a light chain of an altered molecular weight. Our results evidenced that transferrin might induce the development of a monoclonal immune response of different classes and specificity . Moreover, the identification, in a single hematologic center, of three different subjects with antitransferrin monoclonal gammopathy suggests that the disease might represent a still underdiagnosed condition. By clinical point of view these patients must be followed both as MGUS that as hemochromatosis.

Table 1: Laboratory analyses for Patients 1, 2, and 3

	Patient 1*	Patient 2	Patient 3
Serum iron	700 µg/dL	400 µg/dL	385 µg/dL
Serum transferrin	570 mg/dL	500 mg/dL	600 mg/dL
Serum ferritin	800 µg/dL	Normal	Normal
Transferrin saturation	100%	61%	Normal
Hepcidin**	Low	Mild Low	3.37 nmol/L
Hb gr/dl	Normal	Normal	Normal
MCV	Normal	Normal	Normal
Liver iron overload***	Yes	No	No
Monoclonal	IgG	IgGk	IgM
Immunoglobulin, type& level g/dl	1.5	0.5	1.45

*See reference 3; **Normal levels: 1,45-5.39 nmol/L; *** by MRI

P028

CYTOGENETIC AND IMMUNOPHENOTYPIC PROFILE OF MONOCLONAL GAMMOPHATIES: RELEVANCE OF CHROMOSOME 5 IN MGUS PROGRESSION TO MULTIPLE MYELOMA

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Introduction Plasma cell (PC) disorders are characterized by the presence of recurrent genetic aberrations. We evaluated, by interphase Fluorescence In Situ Hybridization (FISH) and Flow Cytometry, chromosomal abnormalities and PC immunophenotype in patients with Monoclonal Gammopathy of Undetermined Significance (MGUS), Multiple Myeloma (MM) and Plasma Cell Leukemia (PCL). Methods. 277 patients (41 with MGUS, 36 with PCL and 500 with newly diagnosed MM)

entered the study. FISH was performed on bone marrow and peripheral blood purified PC. Slides were prepared for interphase FISH and DNA probes were used to detect ploidy status, 13q14, 12p13, 1p36 and 17p13.1 deletions; t(4;14)(p16;q32), t(14;16)(q32;q23), t(11;14)(q13;q32); 11q23 (MLL), 1qter and 5q gain. PC immunophenotype was assessed using quadruple combinations of MoAbs for the detection of the following antigens: CD38, CD138, CD56, CD45, CD40, CD19, CD20, CD52, CD117, cytoplasmic kappa/lambda. Results Results are shown in Table 1. A significant higher frequency of 13q14, 17p13.1, 12p13, 1p36 deletions and 1q21 gain was observed in PCL *vs* MM *vs* MGUS patients. The presence of both 1p36 deletions and 1q21 gain was more recurrent in PCL respect to MM and MGUS samples (24.1% *vs* 4.7% and 17.6%, p<0.01). No significant differences were found among these three groups regarding the IgH translocations. However comparing MGUS/MM with PCL patients, a significantly higher incidence of t(14;16) was found in PCL (16.7% *vs* 5.3%, p=0.04). PC immunophenotype showed a significantly higher expression of CD56 (66.7% *vs* 69.3% *vs* 48.5%; p<0.04) and CD19 (19.4% *vs* 10.7% *vs* 3%; p<0.001) in MGUS and MM compared to PCL patients. A lower CD117 expression was also observed in PCL group even if not statistically significant. In the MGUS group, 16 patients showed disease progression to MM. By comparing "evolving" with "non-evolving" MGUS, we found that the evolving variant was characterized by a higher frequency of 13q14 (62.5% *vs* 42.8%; p=ns), 12p13 (14.3% *vs* 0%; p=ns), 1p36 (35.7% *vs* 15.8%; p=ns) deletions, 1qter gain (64.3% *vs* 42.1%; p=0.04), t(11;14) (25% *vs* 15.8%; p=ns), t(4;14) (16.7% *vs* 10.5%; p=ns), t(14;16) (16.7% *vs* 0%; p=ns). 5q gain was found with a significantly lower frequency in the evolving group (18.2% *vs* 58.8%; p=0.03) suggesting its protective role in disease evolution. Conclusions. Our results highlight the role of 5q gain as protective marker in disease progression from MGUS to MM.

Table 1.

	MGUS	MM	PCL	
Genetic Pattern	**Frequency (%)**	**Frequency (%)**	**Frequency (%)**	**P value**
13q14-	48.7	53.9	80.7	0.011
17p13.1-	9.7	16.4	38	0.013
12p13-	5.9	14.3	31	0.014
1p36-	23.5	17	40	0.005
1qter+	50	43.8	70	0.026
5q+	44.8	38	26.7	ns
11q23+	52.9	49.5	39.3	ns
t(11;14)	18.7	16.2	10	ns
t(4;14)	12.5	19	24	ns
t (14;16)	6.2	5.2	16.7	ns
non-hyperdiploid	53.8	50.3	71.4	ns
Almost 1 IgH translocation	34.4	33.5	38.9	ns
Immunophenotype				
CD138	100	96	96.9	ns
CD45	35.1	24.3	35.3	ns
CD19	19.4	10.7	3	<0.001
CD56	66.7	69.3	48.5	0.044
CD40	100	99.7	88.9	ns
CD20	14.7	13.3	9.4	ns
CD52	22.2	12.1	16.7	ns
CD117	35.7	20.6	11.5	ns

P029

IDENTIFICATION OF EPHA3 AS A NEW POTENTIAL MOLECULAR TARGET IN MULTIPLE MYELOMA

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The past decade has witnessed a dramatic improvement in the therapeutic options in multiple myeloma (MM). However, the disease remains incurable, underscoring the need to identify new therapeutic approaches. In this setting, monoclonal antibodies against MM specific cell surface antigens represent a promising strategy, which is however hampered by a lack of appropriate target structures on all pathogenic MM cells. Increasing evidences implicate Ephrin receptor (Eph), a tyrosine kinase family, in human tumors. Basing on the role of EphA3 in tumors, an engineered IgG1 antibody targeting the EphA3 (KB004) was developed and it is now under phase I clinical trials in USA for the treatment of hematological malignancies refractory to conventional treatment and over-expressing EphA3. Thus, we investigated the EphA3 role and its preferential membrane–bound, GPI-linked ligand EFNA5, in MM patients in order to define EphA3 as a potential molecular target for a novel therapeutic approach with an anti EphA3 monoclonal antibody. The EphA3 expression was studied through a comparative proteomic analysis of bone marrow (BM) endothelial cells (ECs) in patients with MM (MMECs), MGUS, (MGECs), control subjects (ECs), and MM cells. Our data showed that EphA3 mRNA levels progressively increases from ECs to MGECs, reaching the highest values in MMECs. Subsequent analysis by western blot and immunofluorescence confirmed EphA3 protein up-regulation among the different EC types. The MMECs in which EphA3 was silenced revealed a protein level reduction of approximately 80% when compared to the controls. We could not detect major viability defects. Furthermore, *in vitro* angiogenesis inhibition was evident when compared to the not silenced counterpart by matrigel assay. To know whether EphA3 may impact not only MM angiogenesis but also neoplastic plasma cells (PCs), three MM cell lines were studied for the EphA3 expression. We found the PC lines gave constant over expression of EphA3. Finally, the preliminary data regarding EFNA5 mRNA expression level showed it is expressed in either MMECs and MM plasma cell lines. The evaluation of KB004 effect on MMECs in term of apoptosis induction and *in vitro* tube formation inhibition, as well as the analysis of EphA3 levels in primary MM PCs, are in progress. From this study we expect to characterize the role of the EphA3in MM and to provide *in vitro* experimental evidences supporting the possibility of using EphA3 as a new MM molecular target.

P030

ENDOTHELIN B RECEPTOR (ETBR) EXPRESSION BY HUMAN MALIGNANT PLASMA CELLS: PRELIMINARY EVIDENCE FOR A ROLE OF THE ENDOTHELIN AXIS IN MULTIPLE MYELOMA

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The endothelin (ET) axis, which consists of three small peptides (ET-1, ET-2, ET-3) and two ET receptors (ETAR and ETBR), plays an important role both in normal and tumor conditions. In solid tumors, ET-1 has been shown to act as an important paracrine or autocrine growth factor for neoplastic cells, thus participating to tumor development and progression. The initial dependence of malignant plasma cells on signals from

the bone marrow microenvironment together with our preliminary analyses showing the selective expression of the ETBR transcript by multiple myeloma (MM) cell lines, prompted us to test a possible role of the endothelin axis in supporting MM cells. Aim of our study was therefore: i) to confirm the expression of ETBR by malignant plasma cells from MM cell lines and patients' samples at both mRNA and protein level; ii) to analyze the expression of ETBR ligands by bone-marrow stromal cells; iii) to establish the effects of ET-1 on MM cell lines proliferation and/or inhibition of apoptosis under starvation or exposure to cytotoxic agents; and iv) to evaluate the capability of ETBR antagonists to reverse such effects. Our results demonstrated that MM cell lines (U-266, OPM-2, LP-1) expressed ETBR transcripts (RT-PCR) and proteins (Western blot and flow-cytometry). ETBR expression was also detected on primary malignant plasma cells in 50% of patients' bone marrow samples analyzed by flow-cytometry (Figure 1A) and immunohistochemistry. Interestingly, bone marrow-derived mesenchymal stromal cells (MSC) either undifferentiated or differentiated to adipocytes, chondrocytes and osteocytes as well as bone marrow fibroblasts and endothelial cells, revealed the expression of the ET transcripts at various levels. Moreover, *in vitro* experiments revealed that the addition of ET-1 rescued MM cell lines from starvation-induced apoptosis (Figure 1B). Taken together our results indicate that in MM bone marrow stromal cells may promote the survival of malignant plasma cells through the ET-1/ETBR axis. Ongoing analysis will confirm the capability of ET-1 to rescue MM cells from drug-induced apoptosis and the reversibility of such effects by ETBR antagonists, thus indicating their possible therapeutic application. A combined treatment in which the efficacy of established chemotherapeutic agents would be enhanced by targeting specific components of the ET axis could be a promising approach to be explored in clinical settings.

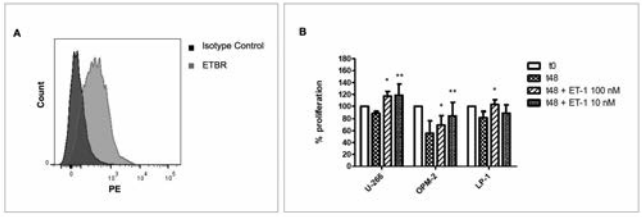

Figure 1. A) ETBR expression by primary malignant plasma cells. Representative case of 15 ETBR-positive cases out of 30 analyzed; B) ET-1 rescues MM cell lines from starvation. ET-1 (100 nM or 10 nM) was added to U-266, OPM-2 and LP-1 serum-deprived cells. Proliferation was evaluated after 48h-incubation (MTT assay). Mean ± SEM of 4 experiments. **P<0,005 and *P<0,05

P031

XBP1 EXPRESSION AS PROGNOSTIC MARKER IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB

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Plasmacytic differentiation is regulated by several genes such as the transcription factor X box binding protein 1 (XBP1), the interferon regulatory factor 4 (IRF4) and the transcriptional repressor B lymphocite-induced maturation protein 1 (BLIMP1). High mRNA levels of these genes have been detected in malignant plasma cells (PC) and are considered negative prognostic factors in patients treated with standard chemotherapy or thalidomide. Lenalidomide seems to overcome IRF4 negative prognosis while bortezomib seems to induced better responses in patients with high levels of XBP1. In this study, we assessed the prognostic role of these genes in a large cohort of multiple myeloma (MM) patients treated with a bortezomib-based regimen. Gene expression was assessed in purified PC (CD138+ bone marrow fraction) of well-characterized patients with newly diagnosed MM. 151 patients enrolled in two multicenter clinical trials (the phase II PAD-MEL100-LP-L and the phase III VMP-VMPT) were included in the study. Quantitative PCR to test gene expression of XBP1, IRF4 and BLIMP-1 was performed with Abi Prism 7900 using a relative quantification approach and GUSB as housekeeping gene. Data were analyzed using SPSS 21.0.0. No association has been observed between XBP1 expression and response to therapy: patients achieving a complete response (CR) had median XBP1 expression of 8.14 (IQR 4.68 – 13.76), those obtaining a very good partial response (VGPR) had a median value of 8.73 (IQR 3.56 – 12.72), those with partial response (PR) had a median of 8.26 (IQR 3.82 – 9.93) and patients with stable disease (SD) had a median of 7.68 (IQR 4.11 – 11.12). No differences in FISH risk profile were observed between patients with high and low XBP1 expression. XBP1 and response to therapy demonstrated to be two independent prognostic factors of progression-free survival (PFS) and overall survival (OS). The three-year PFS was 59% in patients with high XBP1 expression compared with 28% in patients with low XBP1 value (p=0.001). The respective three-year OS was 86% and 74% (p=0.067). High IRF4 expression was associated with better PFS (p=0.008) but similar OS (p=0.484). No PFS (p=0.444) and OS (p=0.529) differences have been observed according to BLIMP1 mRNA expression. Our results suggest that high expression of XBP1 is a marker of improved outcome in MM patients treated with bortezomib. Further analyses are required to confirm these data on independent cohort of patients.

P032

COMBINATION OF LENALIDOMIDE, LIPOSOMAL DOXORUBICIN AND LOW DOSE DEXAMETHASONE (RDD) IS FEASIBLE AND EFFECTIVE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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We asses safety and efficacy of Lenalidomide, Liposomal Doxorubicin and low dose dexamethasone (RDd) in relapsed/refractory patients. From June2008, 38patients were enrolled. Lenalidomide (25 mg/die, day 1-21), Liposomal Doxorubicin (30 mg/mq,day1) and dexamethasone (20 mg/die,day 1,8,15,22 of 28days cycle) was administered for 6 cycles. Patients in >PR received 3other RDd as consolidation. Responder patients allowed 10 mg/die Lenalidomide(day 1-21,every 35 days)until progression or intolerance. The characteristics of patients were summarized in Table 1. Seventeen (45%)patients had been heavly pre-treated: 50% and 74% with thalidomide or bortezomib. Nineteen patients (50%) had previously undergone autotransplant. The most frequent grade 2-3 adverse events were anemia (21%), thrombocytopenia (21%), neutropenia (50%), peripheral neuropathy (13%), fever (21%) with lenalidomide dose reduction or discontinuation in 8 (21%) and 21 (45%) patients. No grade4 toxicity was observed. Patients aged>65 years showed a higher incidence of anemia (87% *vs* 31%,p=0.001), thrombocytopenia (82% *vs* 37%, p=0.05), neutropenia (100% *vs* 75%, p=0.013), delaying administration of RDd more frequently (73% *vs* 31%, p=0.01). After 6RDd, the overall response rate (ORR) was documented in 72%of evaluable patients (36): 19VGPR (53%), 7PR (19%), 3SD (9%) and 7PD (19%), Figure 1. Fifteen patients completed the planned treatment.The ORR after consolidation was 93%(7%CR, 79%VGPR, 7%PR) and 1 patient relapsed. During the maintenance treatment,63% were in VGPR, 31% in CR,6% in PR. After 22 months (3-51) of median follow-up,the median TTP was12 months with IC95%(10-21). Kaplan Meyer estimated 1.5yTTP was 52%. At univariate analysis,prior thalidomide therapy (67% *vs* 32%, p=0.003) and <RP post 6RDd (73% *vs* 36%, p=0.005) were correlated to a lower TTP, Figure 2. Median OS is 4., 6 months with IC95%(38-66). Ten of 12refractory patients aged <65y were chemosensitive to RDd and were successfully transplanted. The+90daysORR

were: 3CR (30%), 3VGPR (30%), 2PR (20%), 1 (10%) NR, 1TRM. Five (50%) patients are still in >RP. Our experience suggests that RDd is tolerable and effective for refractory/relapsed patients. We obtained 72% of ORR, although really adverse prognostic factors of our series: 45% of patients were heavly pretreated,50%relapsed after autotransplant and 11% were older 75 years. The ORR and quality of responses improve after consolidation and during the maintenance,with a 1,5y TTP comparable with that reported in literature. RDd seems also an optimal option for young refractory patients as bridge to treatment.

Table 1. Patient's characteristics at enrollment

Patient Characteristics	N = 38
Median age, y (range)	69 (46-81)
> 75 years	4 (11%)
Refractory Myeloma	12 (32%)
Resistant Myeloma	26 (68%)
Type of Myeloma, no. (%)	
IgGL / IgGK	8 (21%) / 13 (34%)
IgAL / IgAK	4 (11%) / 8 (21%)
Lambda / Non secer	4 (11%) / 1 (2%)
Durie Salmon stage, no. (%)	
IIIA / IIIB / IIA	29 (77%)/ 2 (5%) / 7 (19%)
ISS, no. (%)	
I / II / III	13 (36%) / 15 (42%) / 8 (22%)
Median Time since initial diagnosis, months	31 (5-185)
Median number of prior chemotherapy, no.(range)	2 (1-6)
cycles >=2	17 (45%)
Prior transplant, no. (%)	19(50%)
Prior thalidomide-containing regimens, n (%)	19 (50%)
Prior Bortezomib-containing regimens, n (%)	28 (74%)

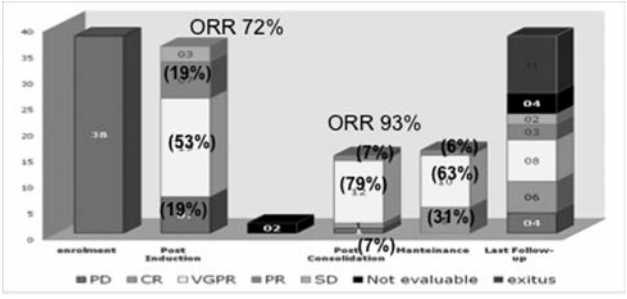

Figure 1. Response Rates during the different phases of treatment.

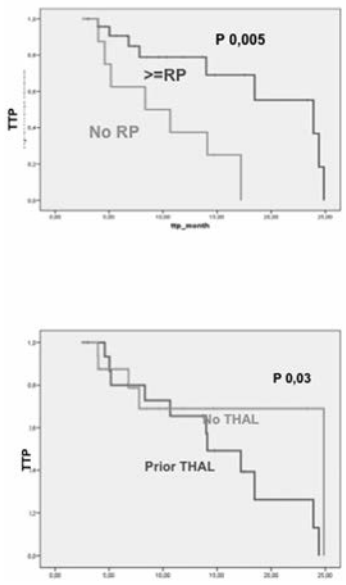

Figure 2. TTP and prognostic factors.

P033

HOMOZYGOSITY FOR KILLER IMMUNOGLOBIN-LIKE RECEPTOR (KIR) HAPLOTYPE A PREDICTS FAVORABLE OUTCOME OF AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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The large variability in clinical course and response to therapy observed in patients with multiple myeloma (MM) makes it plausible to hypothesize that genetic factors may be involved. Natural Killer (NK) cells have a prominent role in the innate immune response that is strongly implicated in tumor surveillance processes. The activity of NK cells is finely modulated by the inhibitory and/or activating function of killer immunoglobulin-like receptors (KIRs). The aim of the present study was to evaluate the possible influence of KIR genes, KIR haplotypes or KIRs and their respective HLA class I ligands on the onset of MM as well as response to autologous hematopoietic stem cell transplantation (AHSCT). One hundred and thirty MM patients were transplanted in three Hematology Centers of Cagliari, Reggio Calabria and Catania. A group of 121 healthy individuals from the Italian Voluntary Bone Marrow Donor Registry were used as controls. Ninety-four patients (71%) achieved complete remission (CR) or a very good partial response (VGPR) after AHSCT. The remaining 38 patients (29%) achieved a partial response (PR) and/or relapsed (R). KIR haplotype AA was significantly reduced in MM patients compared to the healthy controls [15% *vs* 33%, HR = 0.4, 95% CI: 0.2 – 0.66; p = 0.001]. Homozygosity for KIR haplotype A was also significantly associated with achievement of CR/VGPR after AHSCT [21.3% *vs* 0%, hazard risk (HR) = 0.05, 95% CI: 0.003 – 0.8; p = 0.0009]. These data suggest that KIR gene profiles may possibly influence susceptibility to the occurrence of MM and point to homozygosity for KIR haplotype A as a prognostic marker of CR/VGPR in MM patients after AHSCT. This parameter may serve as a useful tool for the identification of patients at high risk of relapse who require careful monitoring during AHSCT.

P0034

WHOLE-BODY DIFFUSION-WEIGHTED MAGNETIC RESONANCE IS SUPERIOR TO SKELETAL X-RAY AND MAGNETIC RESONANCE OF THE SPINE FOR THE ASSESSMENT OF BONE DISEASE IN MULTIPLE MYELOMA

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Bone disease in multiple myeloma (MM) is routinely assessed by skeletal X-ray (XR) and magnetic resonance of the spine (S-MRI). Diffusion-weighted MRI (DW-MRI) is a functional MRI that detects the changes of water diffusion through cells. This prospective, non-randomized, monocentric phase II study aimed at comparing whole-body DW-MRI with XR and S-MRI for the assessment of MM bone lesions. Thirty-six consecutive symptomatic patients at diagnosis or at relapse performed XR, S-MRI, whole-body MRI, and whole-body DW-MRI before treatment, after treatment, and 6 months after the end of the treatment.

MRI exams were performed in a single session, and clinical, laboratory and bone marrow evaluations were done at each time point. A substudy evaluated 12 asymptomatic patients at diagnosis, after 6 and 12 months. Radiology exams were independently read by 3 radiologists experienced in MM, and the techniques were compared by the count of segments with focal lesions (FL) (>=5 mm). Diffusion-weighted MRI significantly detected more segments with FL than XR (p=0.01) and S-MRI (p=0.02) through all timepoints. After treatment, the DW-MRI and S-MRI detected a significant change of FL consistent with response (p=0.04 for both techniques), whereas XR did not (0.55). Having >4 segments with FL by DW-MRI before treatment predicted a worse progression free survival (PFS, p=0.02) and relapse incidence (p<0.01). In Cox multivariate analysis adjusted for ISS stage, DW-MRI before treatment significantly predicted PFS (p<0.01). After treatment, a positive DW-MRI exam predicted a 3-year 83% relapse risk, and was associated with a worse PFS (p=0.01) and relapse incidence (p=0.01). Diffusion-weighted MRI was superior to whole-body MRI through all time points (p<0.01). The substudy of asymptomatic patients showed that DW-MRI is superior to XR (p<0.01) and similar to S-MRI (p=0.47). In conclusion, diffusion-weighted MRI is superior to XR and S-MRI in detecting FL in MM and before treatments predicts PFS and relapse. The findings of DW-MRI after treatment correlate with response and are predictive of PFS and relapse.

P035

THE THERAPEUTIC EFFECT OF LENALIDOMIDE TOWARDS MULTIPLE MYELOMA IS ENHANCED AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A CASE-MATCHED STUDY

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Introduction. Lenalidomide (Len) is a highly effective drug against multiple myeloma (MM). It 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 stem cell transplantation (AlloSCT), since Len may interact favourably with the graft-versus-myeloma (GVM) effect. Methods. In order to verify if Len is more effective when given after AlloSCT, we conducted a case-matched analysis comparing Len after autologous SCT (AutoSCT) *vs* Len after AlloSCT. In this retrospective study, the main matching criterion was represented by the number of treatment lines received before Len. Results. We collected data from 40 patients in each group. Baseline characteristics between Auto and Allo patients (pts) were similar, except for age at diagnosis (55 years, range 39-70, in Auto pts; 47 years, range 29 - 62, in Allo pts). The median number of previous lines of treatment was 3 (range 1-6) for both groups. Thirty-one (77%) Auto and 36 (90%) Allo pts received bortezomib. Similarly, 35 (87%) Auto and 21 (52%) Allo pts were previously treated with thalidomide. Before Len treatment, 14 (35%) Allo pts had acute graft-versus-host disease, and 15 (37%) pts had chronic GVHD. Median time between diagnosis and Len start was 65 months (range 14-162) in Auto, and 72 months (range 19-246) in Allo pts. Median time from transplant to Len start was 50 months (range 7-159) in Auto, and 21 months (range 6-134) in Allo pts. In all cases Len dosage was 25 mg, and it was combined with dexamethasone. Best responses for Auto and Allo patients were as follows: 5 *vs* 3 CR, 6 *vs* 8 VGPR, 12 *vs* 13 PR, 9 *vs* 8 SD, 8 *vs* 8 PD. Time from Len start to the best response was 4 months for both groups. With a median follow-up of 22 months, the median progression-free survival was 9 months in Auto, and 13 months in Allo patients (p=0.03). Overall survival was 22 months in Auto, and 51 months in Allo patients (p=0.04). No unexpected toxicities were observed. In the Allo group 2 (5%) patients experienced GVHD flare. Conclusions Late post-AlloSCT Len administration with dexamethasone seems to be more active, both in terms of PFS and OS, than its use in the AutoSCT setting, without an excess of toxicity. This finding supports the hypothesis that Len positively interact with the donor immune system enhancing the disease control.

P036

AUTOIMMUNE DISEASES IN COURSE OF IMIDS: SELECTIVE OCCURRENCE AFTER LENALIDOMIDE

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Introduction. Immunomodulatory drugs (IMiDs) are active drug for the treatment of multiple myeloma (MM), and have important immunomodulatory properties. This activity may contribute to autoimmune diseases (ADs) occurrence. Despite some case reports, a systematic evaluation on ADs after IMiDs is still lacking. Methods We did a retrospective study to evaluate the occurrence of ADs among patients affected by MM treated with immunomodulatory drugs (IMIDs). Patients were grouped into three classes depending on the type of IMID used. The first group was composed of patients treated only with thalidomide (Thal) (n=104), the second group with lenalidomide (Len) (n=79) and the patients in the third group were first treated with Thal and then with Len (n= 44). Results. A previous AD before MM diagnosis was documented in 7/104 patients (6%) in the Thal group, 5/79 patients (6%) in the Len group. None of the patients in the Thal-Len group had any prior ADs. The median line of therapy in which IMID was used was 1 (range 1-4) in the Thal group, 1 (range 1-4) in the Len group, while in the third group the median line of therapy for Thal was 1 (range 1-3), and 3 for Len (range 1-6). Median therapy duration of IMIDs in the Thal group was 7 months (range 1-143), and 10 months (range 1-89) in the Len group. In the third group Thal treatment had a median duration of 7 months (range 1-36), and of 7 months (range 1-62) for Len. No ADs cases have been observed in the Thal, nor in the Thal-Len group. However, 7 (9%) patients in the Len group developed an AD, in particular: one autoimmune anemia (AHA), one idiopatic thrombocytopenic purpura (ITP), one Evans syndrome (combination of AHA and ITP), one optic neuritis, one polymorphous erythema, one Grave's disease, one polymiositis. All ADs were managed with Len discontinuation and steroid treatment, except for Grave's disease, which required thyroidectomy, and the polymiositis, which was fatal. Odds ratio for ADs of Len respect to Thal was 21. The timing of occurrence revealed a double pattern, with a clustering in the first 3 months in 5 cases, and the occurrence after the second year in the remaining patients. Conclusions. ADs were exclusively observed in Len-treated patients. Interestingly, ADs occurred mainly when patients receive Len in the first line of treatment. No patient who received Len after the second line developed ADs. ADs where preferentially seen in the first 3 months, or after 2 years of Len treatment.

P037

BORTEZOMIB, NON-PEGYLATED LIPOSOMAL DOXORUBICIN, DEXAMETHASONE (PAD REGIMEN) AND AUTOLOGOUS STEM CELL TRANSPLANT IN NEWLY DIAGNOSED MULTIPLE MYELOMA: RESULTS OF A PILOT STUDY

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In Multiple Myeloma (MM) patients (pts) the combination of Bortezomib, Doxorubicin and Dexamethason is based on the rationale of dual apoptotic signaling leading to *in vitro* synergy between Bortezomib and Doxorubicin and additive activity of Dexamethason. A phase II pilot study in newly diagnosed MM pts was planned as Bortezomib, Non-pegylated Liposomal Doxorubicin and Dexamethason induction therapy followed by ASCT and maintenance with Bortezomib. Aims of our study were safety and efficacy in terms of ORR, OS and PFS. From January 2009 to March 2013, 32 pts (M/F: 17/15) with a median age of 63 years (range: 44–73) were enrolled; twelve pts (37,5%) were more than 65 yrs. At diagnosis 53% and 47% of pts had Durie and Salmon staging II and III, respectively. ISS 1, 2 and 3 were 6%, 35% and 59%, respectively. Five pts had a renal impairment. Extensive bone disease and extramedullary disease were documented in 19 and 4 pts, respectively. Fifteen pts had IgG, 6 IgA, 8 light chain and 3 non secretory MM. Unfavorable cytogenetic was recorded in 12 (37,5%) cases. Planned treatment: Bortezomib 1,3 mg/mq iv or sc d 1,4,8,11; Dexamethasone orally at the dose of 40 (20 in pts more than 65 yrs) mg/d on days 1,4,8,11; Non- Pegylated Liposomal Doxorubicin 30 mg/mq on d 1 of a 28-day cycle up to 4 cycles. After PAD regimen pts underwent to high-dose cyclophosphamide (4 g/m^2) with G-CSF support, peripheral stem cell harvest and ASCT (MEL 100 and 200 in pts over or above 65 years, respectively). After ASCT, all pts received maintenance with Bortezomib alone twice a month. Out of thirty-two enrolled patients, 28 concluded the courses PAD and 27 of them underwent to ASCT. After 4 PAD 27 pts (96,4%) achieved more than a PR including 54% (15/28) of CR, 36% of VGPR (10/28) and 7% of PR (2/28). After ASCT all pts achieved at least VGPR including 85% (23/27) of CR. At a median follow-up of 24 months (range 2-51), PFS and OS are 90,6% and 96,9%, respectively. PAD regimen resulted well tolerated and WHO grade 1-3 AEs included neuropathy (22%), hematologic toxicities (44%), infections (9%), gastrointestinal toxicities (18%). No case of cardiac toxicity was observed. In conclusion, sequential PAD, ASCT and Bortezomib as maintenance is an attractive regimen to maximize the efficacy of ASCT. PAD in front-line setting is a highly effective and well tolerated regimen.

P038

PROSPECTIVE STUDY WITH BORTEZOMIB, NON-PEGYLATED LIPOSOMAL DOXORUBICIN AND DEXAMETHASON (PAD REGIMEN) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Relapsed/Refractory Multiple Myeloma (R/R MM) patients (pts) have a poor outcome because of multi-drug resistance, low-performance status and toxicity to previous chemotherapy. Bortezomib enhances chemosensitivity to doxorubicin and overcomes drug-resistance. Indeed, a prospective study was planned by adding bortezomib to non-pegylated liposomal doxorubicin and dexamethason (PAD regimen) with the aims to improve outcome in terms of ORR, TTP and OS and minimize therapy-related toxicity. From November 2005 to March 2012 51 pts with R/R MM (relapsed n= 37, refractory n= 14) received PAD regimen: M/F ratio was 27/24; median age was 61 years (range 34-79); median time from diagnosis was 32 months (range 4-121); three therapy lines (range 1-5) were previously administered; three pts previously underwent to autologous and allogeneic hemopoietic stem cell transplantation (auto- and allo-HSCT) were 23 and 5, respectively. Planned treatment: bortezomib 1,3 mg/mq iv days 1,4,8,11; non-pegylated liposomal doxorubicin 30 mg/mq on day 1 and dexamethason 40 mg days 1-4 of a 28-day cycle up to 6 cycles. Forty-one pts (80,4%) received the planned treatment whereas 10 pts did not complete it because of toxicity (1 patient) and resistant or progressive disease (9 pts). Median time to best response was 3 months (range 2-6). The overall response rate was 75% with 10 CR (20%), 16 vGPR (31%) and 12 PR (24%). Fifteen of the responder pts underwent HSCT (auto-HSCT: 10; allo-HSCT: 5). The previous administration of anthracyclines (35 patients) and bortezomib (3 patients) did not seem influence the response. Median duration of response was 29 months (range 6-64 months). After a median follow-up of 66 months, 13 (25%) pts were alive and 7 (14%) of them, follow in CR. The safety profile was manageable: Grade 3-4 hematologic adverse events (AEs) was 28%; grade 3-4 non-hematologic AEs (sensory or motor neuropathy, infections, fever, fatigue, diarrhea) was 36%. Despite heavy previous treatments, including anthracycline-based, no significant cardiac toxicity was observed. In conclusion, according to our study, PAD regimen appears effective in both elderly and heavily pre-treated R/R MM pts. In fact a significant improved clinical outcome in our cohort of pts was observed.

P039

BENDAMUSTINE IS EFFECTIVE IN HEAVILY PRE-TREATED MULTIPLE MYELOMA PATIENTS

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Bendamustine has been proved to be effective in the treatment of relapsed and refractory (R/R) multiple myeloma (MM), both as a single agent and in combination. Aim. To retrospectively assess efficacy and toxicity of Bendamustine in R/R MM patients treated in our centre. Methods. A total of 25 MM patients were treated with Bendamustine in combination with steroids (dexamethasone, prednisone) between October 2011 and April 2013. 4 patients were excluded from response assessment. Thus, we retrospectively analyzed data from 21 subjects. The dose range of Bendamustine was 60 to 120 mg/m^2 on days 1 and 2 of a 21/28-day treatment cycle. The median number of cycles administered was 2 (range 1-10). All patients (median age 67; M/F 9/12) had received prior therapy with a median of 2.5 previous treatments (range 1–5). 9/21 (43%) had previously undergone single or tandem ASCT. All patients had been previously treated with novel agents and 11 of them (52%) had received both bortezomib and lenalidomide. 12 on a total of 17 patients (71%) assessed for standard karyotype and FISH, either at diagnosis or at relapse, were found to had high-risk cytogenetics. 8/21 (38%) had baseline renal insufficiency. Results. High quality responses were observed in 2 of 21 patients (1 CR, 1 VGPR) and partial and minimal responses in 5 and 4/21 respectively, for an overall response rate (ORR) of 52%. Although ORRs were comparable in patients with or without prior ASCT (66% *vs* 41%), the rate of high quality responses was superior in non-ASCT patients (0% *vs* 16%). The median time to progression from treatment initiation was 5 months for the overall population (median follow-up 6.5 months), with no significant difference between patients with or without prior ASCT. 4/12 (33%) patients with high-risk cytogenetics responded (1 VGPR, 1 PR 2 MR). None of the 2 patients carrying del(17p) was responsive. Complete and partial reversals of renal failure were observed respectively in 1 and 2 of 5 responding patients with baseline high creatinine levels. Compared to patients without prior ASCT, subjects previously treated with ASCT had a significant risk of grade 3-4 granulocytopenia (44% *vs* 25%) and thrombocytopenia (55% *vs* 8%) and serious infectious complications (44% *vs* 25%). Conclusions. Bendamustine is effective in heavily pre-treated MM patients, including patients with high-risk cytogenetics and renal failure. Toxicity profile is more favorable in patients without prior ASCT.

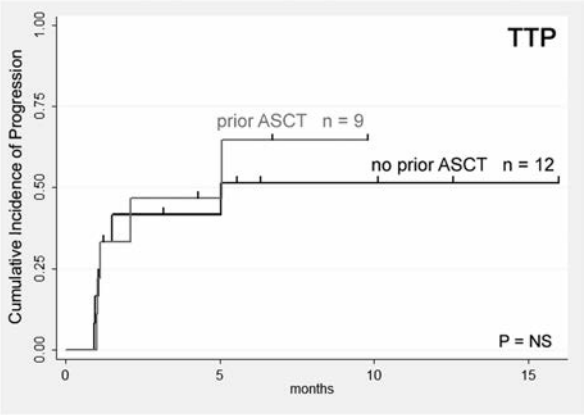

Figure 1.

P040

MANAGEMENT OF MULTIPLE MYELOMA WITH BENDAMUSTINE: A FURTHER OPTION FOR RELAPSED AND REFRACTORY PATIENTS

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Bendamustine has been proved to be effective either in relapsed/refractory and in new diagnosed Multiple Myeloma(MM) as single agent or combined with steroid and has also additive/synergistic activity with bortezomib. Here we evaluate retrospectively the efficacy and safety of bendamustine in patients with relapsed/refractory MM and focus on individual factors associated with outcome. 16 patients (9 M,7 F) with advanced MM, received a chemotherapy schedule containing Bendamustine. Median age at diagnosis was 62.6 y (range 39-82) while age at start of treatment was 67 y (r.48-83); median number of prior lines of treatment was 5.7(r.4-8). Cytogenetic characteristics were avaiable in 6 patients: 2 of them had abnormalities (del13q and t (11;14),respectively). The last treatment before bendamustine was a bortezomib-based regimen in 30%, an IMIDs-based regimen in 53%, a bortezomib/IMIDs-based regimen in 23%, while 15% of patients had received other chemotherapies. All the patients were relapsed and refractory to the last therapy received. We considered only patients who had completed at least two courses of Bendamustine. A total of 47 cycles was administered (median 3, r.2-6). In 91% of patients bendamustine was associated to bortezomib (66%), or IMIDs (25%) and only in 8% it was coupled to dexametasone. In our schedule, Bendamustine was given, at a median dose of 142 mg/sqm (r.100-200) on day+1 and +8 every 28 d. After a median follow-up of 3 months, median OS from diagnosis was 60.2 months, while OS from start of Bendamustine was 3.6 months (r.2-6 months).2/16 patients died for other causes (one for cardiovascular disease and the other one for gastric cancer). Grade 3 transfusion-dependent anemia occurred in 42% while in 57% grade 3 neutropenia occurred. However, only 1 patients interrupted the schedule due to hematologic toxicity. We observed no serious extra-hematologic toxicity, only grade 1 gastrointestinal side effect, treated by common antiemetic drugs. According to IMWG uniform response criteria, 11 out of 16 evaluable patients achieved a partial response after a median time of 3 months with an overall response rate of 68%. In particular, for 3 patients, Bendamustine was a bridge to second AutoBMT, after having achieved a PR. In our hands, Bendamustine can be considered an effective option also for advanced patients, relapsed and refractory to almost all available therapeutic resources, and moreover it could be considered as a bridge to second AutoBMT.

P041

PROLONGED MOLECULAR REMISSIONS AFTER TANDEM AUTOLOGOUS-NONMYELOABLATIVE ALLOGRAFTING IN NEWLY DIAGNOSED MYELOMA

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Allografting induces persistent molecular remissions (MR) in multiple myeloma (MM). We here present the results of minimal residual disease (MRD) analyses by nested qualitative PCR (Nested-PCR) and real time quantitative (RQ)–PCR in 26 patients (pts) with stage II-III MM treated with a tandem auto-allo approach. Transplants consisted of an autograft followed by non-myeloablative 200 cGy TBI and an allograft. BM samples were collected at diagnosis, after the autograft, at month 1, 3, 6 after the allograft and then every 6 months. Nested-PCR and RQ-PCR analyses were carried out using patient-specific primers as previously described (Voena, Leuk 1997; Ladetto, BBMT 2000). For outcome analysis pts were grouped according to reported criteria (Ladetto, ASH 2011): FullMR and StandardMR indicated MRD negativity on two consecutive samples by nested-PCR or by RQ-PCR respectively. Nineteen/26 pts had a molecular marker. At a median follow-up of 10,5 years (5,2-13,9) from diagnosis and 9,9 years (4,2-12,9) from the allograft, overall survival (OS) was 61% and median progression-free survival was 5,2 years. Transplant-related mortality occurred in 3/19 pts (16%), while 5/19 pts (26%) died of disease progression. Overall, cumulative incidence of non-relapse mortality (NRM) was 16%. MRD analysis showed that after the autograft 3/19 pts (16%) were negative by nested-PCR. After the allograft, the rate of PCR negativity remained low at month 1 (3/19, 16%) and 3 (5/19, 26%). However, PCR negativity went up to 44% (8/18) at 6 months and 47% (7/15) at one year post-transplant. Overall, 8 pts achieved FullMR at a median time from allograft of 6 months (1-12) and for a median duration of 33 months (6-102). Overall, 8 relapses occurred, 6 among 11 pts who never achieved FullMR and 2 in 8 pts who reached FullMR. Of these one has incomplete follow up and in the other one clinical relapse was heralded by a molecular relapse. Pts in FullMR had lower relapse incidence (27% *vs* 55% p=0,189) and better median OS (not reached *vs* p=0,027)) than pts who did not achieve FullMR. StandardMR occurred in 12/19 pts (63%) during the first 24 months post-transplant, at a median time of 2 months (1-18) and for a median duration of 27 months (3-102). Pts in StandardMR showed lower relapse incidence (RI) (27% *vs* 71% p=0,016) and better median OS (not reached *vs* p=0,05) as compared to pts with positive PCR.

P042

SIRT REGULATES THE MOLECULAR INTERACTION BETWEEN C-MYC AND HIF-1 IN MULTIPLE MYELOMA

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Multiple Myeloma (MM) is an incurable hematologic malignancy characterized by the accumulation of malignant plasma cells. Dysregulation of MYC by rearrangement or translocation are common somatic events described either in early or late stage of the disease, and transcriptional profiling of MYC pathway activation is observed in more than 60% of MM cell lines. Hypoxia Inducible Factor-1 (HIF-1) overexpression has been described in several MM cell lines and in about 30% of MM patients samples. In solid tumours, deregulation of c-MYC has been associated with HIF-1 upregulation. Here we explored the interaction between c-MYC and HIF-1 in a panel of MM cell lines. We had previously shown that treatment with EZN-2968, an antisense oligonucleotide against HIF-1, resulted in a significantly reduction of HIF-1 protein level after 48h of incubation. To confirm these results, MM1S cells

were treated with EZN-2968 for 48h, lysed, co-precipitated with p300, and incubated with anti-HIF- 1 antibody. We showed that HIF-1 was no longer associated with p300 in EZN-treated compared to untreated samples. We next observed that treatment with EZN-2968 induced a progressive accumulation of cells in S-phase with concomitant reduction of G2/M phase. We further verified the effect of HIF-1 inhibition on c-MYC protein level, and we showed that c-MYC protein expression was reduced in a time dependent manner and was almost undetectable after 72h of incubation. Recently, it has been shown that SIRT1, a transcription factor involved in a development, cellular stress responses, and metabolism, can modulate HIF-1 and c-MYC activity. By Immunoblotting assay, we observed that SIRT1 physically interacts with c-MYC and this interaction is up-regulated in the presence of EZN- 2968. These results were also confirmed at the transcriptional level, by Chromatin Immunoprecipitation (ChIP) assays using an anti-SIRT1 antibody. After 24h of treatment with EZN-2968, we found a significant increase of MYC promoter amplification signals in treated compared to untreated samples, suggesting that SIRT1 recruitment at MYC promoter is dependent on HIF inhibition. We showed that in MM cell lines the expression of HIF-1 and c-MYC are linked and mediated by SIRT1 deacetylase protein. The data suggests a new regulatory mechanism for controlling c-MYC and HIF-1 in MM cells.

P043

PROLONGED PROGRESSION-FREE SURVIVAL WITH AUTOLOGOUS TRANSPLANTATION COMPARED WITH CYCLOPHOSPHAMIDE-LENALIDOMIDE-DEXAMETHASONE IN NEWLY DIAGNOSED MYELOMA: A PHASE 3 TRIAL

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Novel agents improve the outcome of Multiple Myeloma (MM) patients, questioning the role of autologous stem cell transplantation (ASCT). This trial compared the efficacy and safety of Cyclophosphamide- lenalidomide-dexamethasone (CRD) versus Melphalan 200 mg/mq (MEL200) followed by ASCT in newly diagnosed young MM patients. 389 patients (<65 years) with newly diagnosed MM were enrolled. All patients received Rd induction (four 28-day cycles of lenalidomide 25 mg day 1–21 and low-dose dexamethasone 40 mg day 1,8,15,22). After induction all patients received stem cell mobilization and then were randomized to receive consolidation with CRD [six 28-day cycles of Cyclophosphamide (300 mg/mq day 1,8,15), dexamethasone (40 mg days 1,8,15,22) and lenalidomide (25 mg days 1–21)] or MEL200-ASCT [tandem melphalan 200 mg/m^2 with stem-cell support]. Primary study endpoint was progression-free survival (PFS). After induction 78% of patients achieved at least partial response, including 18% very good partial response (VGPR). After consolidation VGPR rate was 47% (including 16% CR) in the CRD arm *vs* 51% (including 17% CR) in the MEL200 arm. After a median follow-up of 28 months, the 2-year PFS was 60% for CRD arm and 72% for MEL200 arm (HR 0.68, P=.02) and OS was 92% and 88% respectively (HR 1.1, P=.28). Rate of grade 3-4 hematologic (86% *vs* 26%, P<0.001) and non hematologic (35% *vs* 18%, P=0.003) adverse events (AEs) were higher in the MEL200 arm compared with the CRD arm. The main non-hematologic AEs were infections (18% *vs* 5% respectively in the MEL200 and CRD arms, P=0.001) and gastrointestinal AEs (19% *vs* 4%, respectively in the MEL200 and CRD arms, P<0.001). MEL200 significantly improved PFS as compared to CRD. As expected AEs were higher with ASCT, but manageable with standard supportive care. Longer follow- up is needed to assess OS.

P044

A PHASE I/II, MULTI-CENTER, OPEN LABEL STUDY OF POMALIDOMIDE, CYCLOPHOSPHAMIDE AND PREDNISONE (PCP) IN PATIENTS WITH MULTIPLE MYELOMA RELAPSED AND/OR REFRACTORY TO LENALIDOMIDE

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Despite the progress made with the introduction of autologous stem-cell transplantation and the availability of novel agents, all multiple myeloma (MM) patients eventually relapse and become refractory to current therapies. Patients who are no longer responding to the available novel agents have a median event-free survival of 5 months and overall survival (OS) of 9 months. In this setting pomalidomide, in monotherapy or combined with low-dose dexamethasone, has shown significant activity. We designed a phase I/II study to assess the safety and the efficacy of the combination pomalidomide- cyclophosphamide-prednisone (PCP) in MM patients refractory or relapsed after lenalidomide therapy. In the phase I of the study, 24 patients were consecutively enrolled and 4 dose levels of pomalidomide (1, 1.5, 2, 2.5 mg/day on days 1-28) were tested in combination with cyclophosphamide (50 mg every other day) and prednisone (50 mg every other day, on days 1-28 of each 28-day cycle) for six 28-day cycles, followed by maintenance therapy with pomalidomide and prednisone. In the phase II of the study further 45 patients were enrolled and treated at the MTD of pomalidomide. In the phase II of the study, 55 patients received the MTD of pomalidomide (2.5 mg), and were evaluable after completing at least 1 cycle of PCP. The median age was 69 years (range 41-84). The median number of prior regimens was 3 (range 1-3). Patients were previously exposed to lenalidomide (100%), bortezomib (84%) and thalidomide (20%); 37 patients were refractory and 18 patients were relapsed after lenalidomide therapy, 22 patients were refractory to both lenalidomide and bortezomib. After a median of 6 PCP cycles (range 1-6), complete response rate was 5%,at least very good partial response rate was 24% and at least partial response (PR) rate 51%. At least PR was observed in 61% and 46% of relapsed and refractory patients, respectively. After a median follow-up of 14.8 months, 1-year progression-free survival was 48% (median 10.4 months) and 1-year OS was 69% (median not reached). Grade 3-4 adverse events were primarily neutropenia (42%),thrombocytopenia (11%), infections (9%), rash (7%) and neurologic (7%). Treatment discontinuation due to toxicity occurred in 5 patients (9%). The combination PCP induced high response rates and prolonged PFS in patients previously exposed to lenalidomide and bortezomib, with a manageable toxicity profile.

P045

IMMUNOPHENOTYPIC RESPONSE AFTER ALLOGRAFTING IN MULTIPLE MYELOMA

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In myeloma, data on immunophenotypic remission (IR) after an allograft are lacking. Our study compared the impact of IR to that of complete clinical remission (CR) in 66 consecutive patients, median age 54 years (35-66), transplanted between 2000 and 2011, and with at least a follow-up of 3 months. Disease response was evaluated by serum/urine electrophoresis and bone marrow aspirate at scheduled time-points. Skeletal survey or MRI were performed yearly or as clinically indicated (overt relapse or complaints of bone pain). IR was defined as absence of monoclonal marrow plasma-cells detected by 4 or 6-colour staining flow cytometry using CD38, CD138, CD56, CD19, CD45, cyKappa, cyLambda. CCR was defined according to standard criteria. Conditioning regimen was non-myeloablative in 55 patients, reduced-intensity in 10 and myeloablative in 1. Donors were HLA identical siblings in 58 patients and unrelated in 8. Thirty- five/66 (53%) received an allograft up-front. In patients surviving at least 3 months, treatment related mortality was

10.6% at 3 years. After a median follow-up of 69 months (19-147), incidence of acute and chronic graft-versus-host disease was 45% and 49%, with no significant difference between responsive and refractory patients. At follow-up, 24/66 (36%) patients achieved CR and IR (CR/IR), 22/66 (33%) achieved IR but not CR because of persistence of the M-component (noCR/IR), and 20/66 (31%) did not achieve either CR or IR (noCR/noIR). Median overall (OS) and event-free survivals (EFS) were not reached and 59 months in the CR/IR group, 77 and 15 months in the noCR/IR, and 30 and 5 months in the noCR/noIR respectively ($p<0.001$ for both OS and EFS). Belonging to the CR/IR group was the only statistically significant predictor for prolonged OS and EFS ($p<0.001$). Of note, cumulative incidence of extramedullary disease at first relapse post-trasnsplant was 4.4% in the CR/IR, 31.8% in the noCR/IR and 15.0% in the noCR/noIR groups respectively ($p<0.001$). In conclusion, the achievement of IR showed a significant impact on clinical outcomes including patients who did not clear the M-component. Discrepancies between IR and CR, and a higher incidence of extramedullary relapse in the noCR/IR group suggest that myeloma cells may escape immune control outside the bone marrow. In this group, positron emission tomography may be indicated to detect early relapse.

Lymphomas I

P046

THE BERLIN-FRANKFURT-MÜNSTER PROTOCOL FOR THE UPFRONT TREATMENT OF BURKITT'S LYMPHOMA: THE BOLOGNA EXPERIENCE

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Burkitt's lymphoma (BL) is a highly aggressive B-cell lymphoma; because of its fast growing rate it often represents a clinical emergency. Intensive treatment approaches are required for adult BL, although a univocal standard of care still does not exist. From 2003 to 2011, 18 HIV-negative patients affected by BL received an intensive treatment according to the Berlin-Frankfurt-Münster (BFM) protocol at our institution. Treatment plan consisted of initial cytoreduction followed by 3 blocks, A (ifosfamide, vincristine, methotrexate, etoposide, cytarabine), B (vincristine, cyclophosphamide, methotrexate, doxorubicin), C (vindesine, methotrexate, etoposide, cytarabine), each repeated twice, every 28 days, with rituximab at day 1 each block. All patients received central nervous system (CNS) prophylaxis with intrathecal methotrexate. Autologous stem cells harvest was done after 4 cycles, with reinfusion (ASCT) at the end of the 6-blocks after BEAM (carmustine, etoposide, cytarabine, melphalan) conditioning. Four patients were female, 14 were male; median age at onset was 34 years. Twelve patients had stage III-IV disease; bulky disease occurred in 9 patients and extranodal involvement in 12, mainly at the gastrointestinal tract (44.4%). CNS involvement was rare (5.5%). Five patients required a preliminary surgical approach, mainly because of bowel occlusion. All but one patients received rituximab during treatment; 14 patients completed all the 6 blocks. Stem cell harvest was performed in 15 patients (83.3%) who all received a subsequent ASCT. Treatment withdrawal occurred due to renal toxicity in 2 cases (patients now alive after alternative salvage treatment) and early patient death in 1 case. Severe cytopenias, all transient and easily manageable, were documented in those who received ASCT; 7 patients developed cytopenia-related oral mucositis. After ASCT, 13 patients (72.2%) achieved a complete response (CR), whereas 2 (11.1%) presented with progressive disease, and then died. At a median follow up of 3.5 years, all the patient in CR are alive and disease free; one developed myelodysplasia 1 year after ASCT. Overall survival of the entire population is 79.3% at 7.2 years (83.3% for those who received ASCT), with disease-free survival of 100%. Intensive treatment according to BFM protocol, with rituximab and ASCT, appears feasible, safe and highly effective in adult patients with BL, as demonstrated by long-term survival rates of patients in continuous CR.

P047

LINFOMI A CELLULE B PRIMITIVI DELLA CUTE: UN'ENTITÀ RARA CON MOLTE SFACCETTATURE

Maglie R, Pellegrini C, Broccoli A, Gandolfi L, Casadei B, Stefoni V, Derenzini E, Quirini F, Tschon M, Papadopulos F, Narducci R, Stefani G, Argnani L, Zinzani PL

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Primary cutaneous B-cell lymphomas (PCBCLs) are a distinct but heterogeneous group of primary cutaneous lymphomas that are generally classified into three distinct subgroups: primary cutaneous follicle-center lymphoma (PCFCL), primary cutaneous marginal zone lymphoma (PCMZL), and primary cutaneous diffuse large B-cell lymphoma, leg-type (PCDLBCL, LT). Aim of the present study was to review effectiveness of the vary treatment strategy and patients' survival after first line approach. Study group included 46 patients with PCBCLs who were treated and observed in our Institute during a 23-year period (1989 to 2012). Median age at diagnosis was 53.7 year (range 21.0-88.8 years). 32% are females and 68% are males. All of patients are in stage IE without systemic symptoms. At the diagnosis 41.3% of patients had multiple lesions and 58.7% one single lesion. PCFCL accounted for 63% of occurrences (n=29), followed by PCDLBCL (n=10, 21.7%) and PCMZL (n=7, 15.2%). Patients were treated in first line according to guidelines

for each histology: rituximab single agent was administered in 32% of patients, radiotherapy in 30%, chemotherapy in 32% and the remaining 6% underwent surgery. Overall response rate for the whole samples was 95.6% (complete response rate). At 12.3 years, the progression free survival was 45.6% and the disease free survival 65.7%. According to histology, at 15.2 years survival were as follows: PCFCL presented a PFS of 40%, a DFS of 79.5% and an OS of 100%; PCMZL reported a PFS of 20.8%, a DFS of 26.7% and an OS of 75%; for PCDLBCL PFS was 67.5%, DFS 83.3% and OS 90%. Our data showed that disease histology remains the most important prognostic factor for patients' survival. PCFCL and PCMZL are indolent lymphomas that infrequently disseminate to extracutaneous sites and are associated with an excellent long-term survival but frequent relapse. PCDLBCL, an aggressive lymphoma, has a better prognosis. Furthermore, one third of patients (except PCDLBCL) were treated with rituximab monotherapy with a good result and without toxicity suggesting that is a valid alternative to classic treatment (radiotherapy and surgery).

P048

HIGH DENSITY OF CD68+/CD163+ TUMOR-ASSOCIATED MACROPHAGES (M2-TAM) AT DIAGNOSIS IS A POOR PROGNOSTIC FACTOR IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Tumor-Associated Macrophages (TAM) can both have an anti-tumoral function and play a key role in tumor promotion, depending on the specific acquired immunophenotype (M1 and M2 respectively). Recent studies have shown that M2-TAM density is related to poor prognosis in most hematologic malignancies, including Hodgkin lymphoma and angioimmunoblastic T-cell lymphoma, while contrasting results were obtained for Diffuse Large B-Cell Lymphoma (DLBCL). The aim of this study was to identify and to quantify the infiltration of TAM in DLBCL at diagnosis and to evaluate the presence of a significant correlation between the prevalent subtype of macrophages and clinical and biological features of disease. From January 2002 to December 2012, a total of 102 patients with a diagnosis of DLBCL were treated in our Institution. Of these 102 patients, 61 were included in our study, whereas the remaining 41 were excluded (because diagnosis was made in other Institutions or because residual unstained sections were not available). The lymph-node histological sections of these 61 DLBCL patients were analyzed at diagnosis. Different subtypes of TAM were marked as a double immunofluorescence, CD68+/HLA-DR+ (M1) and CD68+/CD163+ (M2) and identified with confocal microscopy. The cut-off used for definition of high TAM density was established according to published studies. Our results show that high M2-TAM density has a statistically significant correlation with clinical unfavorable prognostic factors (age ≥65 years: P=0,02; PS ≥2: P=0,034; high IPI score: P<0,001; albumin <3,5 gr/dl: P=0,001; advanced Ann-Arbor stage: P=0.017) and with biological features of disease aggressiveness (high Ki-67: P=0,004; activated B-cells immunophenotype: P=0,01). In addition, high M2-TAM density significantly correlates with poor response to treatment (P<0,001).

P049

ORAL CHEMOTHERAPY: AN INNOVATIVE CHOICE

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Despite improvement of lymphoma treatments, many patients still relapse, the majority of these patients are elderly and reluctant or unable to receive intravenous chemotherapy. Patients at 3°or 4° treatment line received an oral chemotherapy in an outpatient setting expressely designed to offer a well tolerating and easy to administer therapeutic option.It's known that treatment with all-oral protocols has been lowering the management costs, without a reduction of efficacy. This program was planned by clinicians, psycho-oncologists and hospital pharmacists, the latter providing detailed technical information about drugs management. Molecules of widespread use and moderate cost have been employed according to the following scheme: NIET: Idarubucin 30 mg/sqm(d1), Procarbazine 100mg/sqm (d1-4), Etoposide100mg/sqm (d1-4), Dex 20 mg(d1-4) for high grade lymphoma, FC Fludarabine 25 mg/sqm, Cyclophosphamide 150 mg/sqm(d1-4),L Chlorambucil 10mg (d1-10) for low grade lymphoma. A total of 71 patients were evaluated: 66 were able to start the treatment. Median age of the patients was 77,9y (63 – 95), the majority were unfit 25 (38%) or frail 33 (50%). Only 8 (12%) heavily treated patients were fit ;36% had DLBCL/T lymphomas/HL and were treated with NIET; 11% were FL/WM/MCL and received NIET or FC; 52% had CLL/SLL and were treated with FC or Chlorambucil. Objective benefit from the therapy after 3 cycles was evaluated for 56 patients: in 71% of the patients there was an objective benefit, 16% acheived a stable disease, 13% had a progressive disease. Haematologic toxicity was evaluated over 257 cycles: we detected neutropenia G3(6%) and G4(6%), thrombocytopenia G3 (4%), G4 (4%), anaemia G3 (2%) and G4 (3%). Non haematologic toxicity was infective G1-2 (3%), asthenia G1-2 (11%), nausea G1-2(7%), neurological G1-2 (2%). 13 patients (20%) needed G-CSF support, 16 patients (24%) were supported with EPO. Psycho-oncological evaluation was made on a subgroup of 20 patients before (T0) and after (T1) chemotherapy. Results showed a reduction of depressed patients (45% at T0 *vs* 30% at T1) and patients judged positively the oral treatment (85%) and the presence of the pharmacist (90%). The all-oral approach has demonstrated to be an efficient alternative to traditional intravenous chemotherapy for ederly and frail patients with no cure expectation. The therapy shows some efficacy, improves the comfort from symptoms, is well tolerated and has restrained costs.

P050

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

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Background. WM is an incurable disease, with an overall medial survival of only 5-6 years. Age, hemoglobin level, platelet count, (2) microglobulin, and monoclonal IgM concentrations are characteristics required for prognosis. First-line therapy of WM has been based on single-agent or combination therapy with alkylator agents (*e.g.* chlorambucil or cyclophasphamide), nucleoside analogues (cladribine or fludarabine), and the monoclonal antibody rituximab. Novel therapeutic agents that have demonstrated efficacy in WM include thalidomide, lenalidomide, bortezomib, everolimus and bendamustine. Methods. We report the treatment outcome for 16 (9 male, 7 female; median age: 70y, range: 67-78) relapsed/refractory Waldenström's macroglobulinemia (WM) patients. Treatment consisted of bendamustine (90 mg/m^2 I.V. on days 2, 3) and rituximab (375 mg/m(2) I.V. on day 1) for all patients. One rituximab- intolerant patient received bendamustine alone. Each cycle was 4 weeks, and median number of treatment cycles was 4. Results. The clinical stage (remission, progression or stable disease) was defined with clinical re- evaluation after chemotherapy and re-staging 6 months after end of therapy. At best response, median serum IgM declined from 3500 to 500 mg/dL, and hematocrit rose from 29.9% to 37.8%. Overall response rate (CR + PR) was 81.2%. Overall therapy was well tolerated. Prolonged myelosuppression was more common in patients who received prior nucleoside analogues. Conclusions. Bendamustine in combination with Rituximab demonstrates an excellent effectiveness in previously treated WM patients, with an acceptable toxicity profile. These agents, when compared to traditional chemotherapeutic agents, may lead in the future to higher responses, longer remissions and better quality of life for patients with WM.

P051

THE LYMPHOCYTE TO MONOCYTE RATIO IMPROVES THE IPI-RISK DEFINITION OF DIFFUSE LARGE B-CELL LYMPHOMA WHEN RITUXIMAB IS ADDED TO CHEMOTHERAPY

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Background. The peripheral blood lymphocyte to monocyte ratio (LMR) at diagnosis can be clinically relevant in patients with diffuse large B-cell lymphoma (DLBCL). Patients and methods: We reviewed the outcome of 1,057 DLBCL patients followed from 1984 to 2012 at four centers. LMR was analyzed as a clinical biomarker by ROC analysis and Harrell's C-statistics. The relationship between IPI and the LMR was analyzed by the Fisher exact test. Univariate and multivariate analysis were performed by the log rank and Cox proportional-hazards models. Results. Patients were characterized by a median age of 61 years, IPI >2 in 39% and they were treated with a rituximab-containing chemotherapy in 66%. LMR proved strongly predictive for survival in patients treated with rituximab-based programs, but not in those receiving chemotherapy alone. Additionally, a LMR value ≤2.6 (as determined by ROC analysis) was associated with a worst performance status, a higher LDH, an advanced clinical stage and a higher IPI score (p= 0.000). In patients treated with rituximab containing programs, a LMR value < 2.6 was found in most of the primary refractory patients (75%) and proved as the best cut off to predict both response and survival (p=0.018). Finally, multivariate analysis and Harrell's C-statistic confirmed the IPI-independent role of LMR on survival (p= 0.0000). Conclusion. LMR is a simple and potent predictor of clinical response and survival in DLBCL treated with rituximab-containing chemotherapy. In this group LMR improves the IPI risk definition.

P052

BENDAMUSTINE IN HAIRY CELL LEUCEMIA: A SINGLE CENTER EXPERIENCE

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Hairy Cell Leukemia (HCL) is a low grade Non Hodgkin lymphoma that presents with trilinear cytopenia and, usually, splenomegaly. Diagnosis is confirmed by the presence of villous lymphocytes CD103, CD25, CD11c positive on bone marrow histopathology. Recent treatment is based of purine analogs with which patients often reach an hematological response. However relapse still occurs suggesting the presence of residual disease. Recently it has been discovered the presence of B-RAF V600 mutation in virtually all patients with HCL, and experience with B-RAF inhibitor had shown major responses. Bendamustine experience has grown in various hematologic neoplasms with clinical and long lasting response, especially in low grade lymphomas. The drugs acts primarily as an alkylating agent but it shares some characteristic with purine analogs. We treated a total of 6 patients with Bendamustine (60-70 mg/ m^2 days 1,2) in association with rituximab (375 mg/m^2 day 3). Cycles were repeated every 28 days. Patients characteristics are summarized in Table 1. All patients were considered for data analysis. Treatment was well tolerated in all patients, with mild neutropenia without severe infective complications. One patient had to stop treatment due to gastrointestinal bleeding for congenital angiodysplasia with subsequent surgery, however bone marrow histology showed hematological complete remission. Among the whole group overall response rate (ORR) was 100 % with 5/6 complete response a 1/6 partial response (patient in 2nd relapse). Patients in response had absence of marrow involvement and improvement of splenomegaly. Unfortunately we didn't have minimal residual disease evaluation in all patients. So far all the patients hold their response with a median follow up of six months. In two patients a molecular complete response was obtained. Bendamustine data in HCL as first line therapy are not yet described in literature. Our experience showed a very good safety and tolerance profile also in elderly patients with very satisfactory responses in the whole group also with the observation of complete molecular response. Newer drugs would be available in the future for HCL (B-RAF inhibitors) but bendamustine deserve to be studied in these patients' subset in the meanwhile. These early data should be confirmed with longer follow up and more patients, with the monitoring of minimal residual disease in order to understand the depth of possible response.

Table 1.

Paziente	Eta'	Sesso	Tp predecenti	Status pre tp	N di cicli	Tossicita'	Risposta
Paziente 1	46	M	Cladribrina-rituximab	Prima recidiva	4	Nessuna	RC
Paziente 2	58	M	-	Diagnosi	4	Nessuna	RC
Paziente 3	63	M	Cladribrina	Prima Recidiva	4	Nessuna	RC
Paziente 4	73	M	IFN-a, cladribina	Seconda recidiva	4	Nessuna	RP
Paziente 5	67	M	-	Diagnosi	5	ematologica	RC
Paziente 6	78	M	-	Diagnosi	6	ematologica	RC

P053

LENALIDOMIDE FOR RELAPSED OR REFRACTORY AGGRESSIVE B-CELL NON HODGKIN'S LYMPHOMA

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Lenalidomide is an immunomodulatory agent with proven antitumor activity in B-cell malignancies. We retrospectively analyzed efficacy and toxicity of single-agent lenalidomide in the unfavourable setting of relapsed-refractory aggressive B-cell non Hodgkin's lymphomas (NHL). Fifteen consecutive patients (pts) with relapsed or refractory aggressive NHL received oral lenalidomide 25 mg once daily on days 1–21 every 28 days. A disease reassessment was planned every three cycles; in responding pts lenalidomide was administered until progression. Median age was 62 years (range: 31-80), 10 pts (67%) were male. Five pts (33%) had de novo diffuse large B-cell lymphoma (DLBCL), 3 pts (20%) had transformed lymphoma (TL) with previous follicular histology in two cases and marginal zone histology in one pt, and 7 pts (47%) had mantle cell lymphoma (MCL). Median time from diagnosis to lenalidomide was 97 months (range: 19.1-194.3) and median number of prior therapies was 3 (range 1-8); nine pts (60%) failed a previous autologous stem cell transplantation. Median number of administered cycles of Lenalidomide was 2 (range: 1-12). Lenalidomide was discontinued during the first cycle in 7 pts (47%): reason for discontinuation was grade ≥3 cutaneous rash in 2 pts and disease progression in 5 pts. The overall response rate (ORR) for the entire population was 27%. We registered no response for DLBCL and TL pts, while 4 MCL pts achieved a partial response. Median duration of response was 18.1 months (range: 0-44.8). Therapy is on-going in 2 MCL pts. Grade ≥3 toxicity was mainly haematological: 40% pts experienced thrombocytopenia, 33% neutropenia, 6% anemia. Grade ≥3 cutaneous rash was observed in two cases and completely resolved after lenalidomide discontinuation. In conclusion, lenalidomide had a manageable toxicity profile with severe cutaneous reaction in a small proportion of pts. Lenalidomide proved to be effective as expected in relapsed or refractory MCL, with an ORR of 57% and a median duration of response of 18 months. We had no response in DLBCL and TL pts. Median time between diagnosis and lenalidomide treatment is significantly longer in our series than the median interval reported in prior studies, despite a comparable median numbers of previous treatments. This difference can possibly explain the disappointing result in DLCL and TL. These data suggest that Lenalidomide outcome could be improved if the drug is employed in earlier phases of disease.

P054

MINIMAL RESIDUAL DISEASE (MRD) DETECTION BY NEXT-GENERATION SEQUENCING AND REAL-TIME QUANTITATIVE PCR: A METHODICAL COMPARISON IN ALL, MCL AND MM

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Real-Time-Quantitative(RQ) PCR based MRD detection using primers derived from the immunoglobulin heavy chain variable region(IGH)is a disease monitoring tool in ALL,MCL and MM. It is highly sensitive and has been standardized in the context of European Scientific Foundation for Laboratory Hemato-oncology (ESHLO).It has some limitations, including marker identification failure and false negatives due to clonal evolution.To verify if IGH-based next-generation sequencing (NGS) might overcome RQ-PCR,we have performed comparison of the two methods on diagnostic (DG) and post-treatment follow-up (FU) samples. 378 samples were collected from 55 pts (15ALL,30MCL,10MM).IGH RQ-PCR was carried out as previously described [Ladetto,BBMT 2000; Brüggemann,Blood 2006], according to the ESHLO criteria [van der Velden, Leukemia2007]. NGS was performed at the Sequenta facilities. Using universal primer sets, we amplified IGH-V-D-Jgene segments from DNA.Amplified products were sequenced to obtain a high degree of coverage and analyzed using standardized algorithms for clonotype determination.Tumor-specific clonotypes were identified for each patient based on their high-frequency in DG sample and then quantitated in FU samples. NGS analysis was performed independently under blinded conditions. Comparability of results by RQ-PCR and NGS was assessed by bivariate correlations between methods. Discordances were classified as:a positive/negative discordance was defined as major when the positive result was >1E-05 and minor when ≤1E-05; a quantitative discordance was defined as the presence of two positive results with a quantitative discrepancy >1 log.51 pts (93%) were evaluable with at least one tool, 43 (78%) with both and 4 (7%) with none. Disease-specific success rates are shown in Table 1. Sequences identified with both tools were identical in 41 cases and unrelated in 2.Overall, 330 samples (87,3%) were evaluated with at least one tool and 265 (70%) with both. In terms of MRD output, concordance was significant ($p<0.001$) and 214 (80,8%) samples had an optimal concordance.Of these major discordances were 16(6%); minor discordances were 24 (9,1%);quantitative discordances were 11(4,1%). In 2ALL clonal evolution hampered straightforward MRD assessment.In 1 case IGH RQ-PCR underestimated MRD while a second RQ-PCR marker(TCRD)overlapped NGS. In a second case NGS did not detect the tumor diagnostic clone due to loss of the complete IGHV at relapse whereas the preceding IGHDJ was preserved and detected by RQ-PCR.

Table 1. Rates of success of RQ-PCR and NGS among ALL, MCL and MM by patient

Disease	Patients	Patients evaluable PCR	Patients evaluable NGS	Patients evaluable with both tools	Patients evaluable with at least one tool	Patients not evaluable
ALL	15	15	15	15	15	0
MCL	30	22	26	22	26	4
MM	10	8	8	6	10	0
TOT	55	45	49	43	51	4

Abbreviation. RQ-PCR: real-time quantitative polymerase chain reaction, NGS: Next Generation Sequencing, ALL: Acute Lymphocytic Leukemia, MCL: Mantle Cell Lymphoma, MM: Multiple Myeloma

P055

HUMAN NPM-ALK POSITIVE CELL LINES GROWN AT HIGH AP26113 DOSES DEVELOP DIFFERENT MECHANISMS OF RESISTANCE

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ALK is a tyrosine kinase receptor involved in a broad range of solid and haematological tumors, such as 5% of cases of Non Small Cell Lung cancer, 50% of cases of Inflammatory Myofibroblastic Tumor and in rare cases of other widespread malignancies. It has been shown that 70-80% of ALK+ Anaplastic Large Cell Lymphoma (ALCL), an aggressive Non Hodgkin T-cell lymphoma, are caused by the aberrant oncogenic fusion protein NPM-ALK. Crizotinib was the first clinically relevant ALK inhibitor, now approved for the treatment of late stage and metastatic cases of lung cancer and still in clinical trial for other ALK related diseases. As expected from experience with other tyrosine kinase inhibitors, several patients developed Crizotinib resistance, mainly due to the appearance of new point mutations located in ALK kinase domain. Currently, other ALK inhibitors are available and already in clinical trial, hopefully representing the second line therapy able to overcome Crizotinib resistance. In our work we focused our attention on the phase I/II dual ALK/EGFR inhibitor AP26113 (Ariad Pharm.). Two NPM-ALK+ human cell lines, KARPAS-299 and SUP-M2, were grown in the presence of increasing doses of AP26113; 8 cell lines able to grow at high AP26113 doses were selected. All cell lines show an AP26113 50% inhibitory concentration (IC50) value much higher than the one observed in parental cells, respectively 130 – 998.4 nM versus 1 nM. KARPAS-299 cells resistant to AP26113 show NPM-ALK overexpression as the main cause of resistance, detected both at transcriptional and at protein level (increase was quantified at 16-25.5 fold and 3.69-6.08 fold respectively), while SUP-M2 cell lines living and proliferating in the presence of the drug harbour several point mutations spanning the entire ALK kinase domain. In particular, we identified the following aminoacid substitutions: L1122V, F1174V, L1196M, L1198F, and S1206C. Interestingly, all cell lines carrying NPM-ALK overexpression developed drug addiction, meaning that AP26113 withdrawal caused cell growth arrest and apoptosis. This knowledge about the possible appearance of new clinically relevant mechanisms of drug resistance is a useful tool for the management of new TKI resistant cases.

P056

FDG-PET FOR INITIAL AND END-OF-THERAPY STAGING OF T-CELL LYMPHOMA

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Few data are available on the role of fluoro-deoxy-glucose positron emission tomography (FDG-PET) in T-cell non Hodgkin Lymphoma (NHL). We retrospectively reviewed 92 FDG-PET scans performed in 36 T-cell NHL pts and we focused on 54 scans for which a simultaneous standard contrast-enhanced computerized tomography (CECT) was available for comparison. Patients' median age was 56 years (range: 25-77) and 20/36 pts (51%) were females. As regards histology, 17 pts (47%) had peripheral T-cell lymphoma not otherwise specified, 5 pts (14%) had angioimmunoblastic T-cell lymphoma, 13 pts (36%) had anaplastic T-cell lymphoma and 1 pt (3%) had nasal-type T-cell lymphoma. Baseline FDG-PET scans were 27, while FDG-PET performed for end-of-therapy disease reassessment were 27. At baseline staging, PET and CECT pointed out the same disease sites of involvement in 7.4% (2/27) of cases. PET identified a higher number of disease sites compared to CECT in 66.6% (18/27) of the pts; on the contrary, in 26% (7/27) of cases PET showed a lower number of disease sites than CECT. Additional sites identified by PET were both nodal and extra-nodal: nodal sites in 12 pts, spleen in 2 pts, nasopharinynx in 2 pts, bone and testicle in 1 pt each. FDG-PET

results led to change the an Ann Arbor stage in 51.8% (14/27) of cases, with an up-staging in 44.4% (12/27) of pts. At end-of-therapy restaging, 74% (20/27) pts had a negative FDG-PET scan; CECT was concordant and consisting with complete response in 75% (15/20) of cases, while in the remaining 25% (5/20) of cases CECT showed a partial response with residual lesions. In the 7/27 final PET positive pts, CECT was concordant and pointed out a partial response in 5/7 pts, while was negative in 2/7 cases. A survival analysis according to final PET result was not performed due to the low number of final PET positive pts. In conclusion, in our hands FDG-PET allowed a more accurate baseline disease extension evaluation: it could identify additional sites, both nodal and extra-nodal, in two-thirds of pts. A change in clinical stage according to FDG-PET was observed in 51.8% of cases, with an upstaging in the majority of pts. A high level of concordance was observed between PET and CECT at restaging. Nevertheless, PET pointed out minimal residual disease in 2/7 final CECT negative pts and complete remission in 5/20 final CECT positive pts, allowing to optimize response evaluation in small but critical subgroups of pts.

P057

IDENTIFICATION AND CHARACTERIZATION OF CANDIDATE MYELOID DERIVED SUPPRESSOR CELLS IN THE MONONUCLEAR FRACTION OF PERIPHERAL BLOOD SAMPLES FROM PATIENTS AFFECTED BY NON-HODGKIN LYMPHOMA (NHL), HODGKIN LYMPHOMA (HL), AND MULTIPLE MYELOMA (MM)

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Myeloid derived suppressor cells (MDSCs) constitute a heterogeneous population of myeloid cells characterized by the ability to suppress T lymphocytes' immune responses. MDSC include two major subsets (*i.e.* monocytic and granulocytic), defined by the expression of distinctive antigens (CD11b+CD14+HLA-DRlow/ and CD11b+CD33+CD15+), and by the content of specific immune suppressive molecules. Currently, a large number of MDSC phenotypes have been described in different human diseases, including solid tumors, infections and inflammatory diseases. However, the existence of MDSCs in human hematological malignancies remains debated and to date myeloid CD11b+CD14+HLA-DRlow/ or CD11b+CD33+CD15+ cell populations with putative immunosuppressive functions have been described by two studies only conducted respectively in a NHL and in a MM series. Aim of our study was to identify candidate MDSCs in the peripheral blood mononuclear fraction (PBMC) of peripheral blood samples obtained from patients affected by NHL, HL and MM.

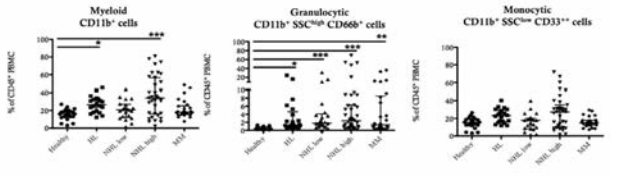

Figure 1. Frequencies of total myeloid, granulocytic and monocytic cells within the total CD45+ PBML are reported. Data are presented as mediamvalues with interquartile ranges

Upon informed consent, 107 newly diagnosed patients affected by NHL (60), HL (21), MM (26), and 39 age- sex-matched healthy donors were enrolled in the study. Based on the analysis of CD11b, CD66b, CD33, CD16, CD14 and CD45 by flow-cytometry, we evaluated within the PBMC fraction of each sample the frequency and the absolute number of total myeloid (CD11b+), monocytic (CD11b+ SSClow CD33++), and granulocytic (CD11b+ SSChigh CD66b+) cells. Noteworthy, the latter are supposed to sediment in the mononuclear layer upon activation-induced degranulation. Our data revealed that the frequency of total myeloid cells (mostly composed by CD11b+ SSChigh CD66b+) was significantly increased in PBMCs of patients as compared to healthy donors (Figure 1). Of interest the frequency of total CD11b+ and SSChigh CD66b+ cells resulted significantly reduced in PBMCs of patients in complete remission as compared to the same patients at the diagnosis.Moreover, preliminary *in vitro* experiments have shown that the depletion of CD11b+ elements from patients' PBMCs restored the proliferation of autologous T lymphocytes, thus indicating a suppressive activity of myeloid cells. Our data suggest the presence of candidate CD11b+ MDSCs in the PBMC fraction of patients affected by NHL, HL and MM. Further *in vitro* studies are ongoing in order to specifically assess the immunosuppressive activity of SSChigh CD66b+ cells within the CD11b+ component of PBMCs.

P058

PROGNOSTIC ROLE OF F-18 FDG PET/CT QUANTISATION PARAMETERS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AT DIAGNOSIS: STANDARDIZED UPTAKE VOLUME (SUV) VS METABOLIC TUMOUR VOLUME (MTV)

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Diffuse large B-cell lymphomas (DLBCL), the most frequently occurring lymphoma entities in Europe and North America, exhibit an initial aggressive behaviour which, however, ultimately contributes to their elevated cure rate, generally being highly responsive to combined immuno-chemotherapy. F-18 FDG PET/TC is rapidly becoming a routine measure for staging and follow-up of patients with aggressive lymphoma. We compared the prognostic significance of the F-18 FDG PET/CT quantitative assessment by standardized uptake value (SUVmax) with that of the metabolic tumour volume (MTV) in patients complying with DLBCL. SUVmax is currently considered the gold standard measure of neoplastic activity, whereas MTV is a novel index which represents the extent of FDG uptake by tumour tissues and is used for the estimation of gross tumour volume. Thirty-two patients (16 males, 16 females; mean age, 67.6±19 years) with DLBCL underwent F-18 FDG PET/CT at diagnosis for staging 2±1 months before the initiation of the treatment. The mean SUVmax and the summed MTV (cm3; 42% threshold) were registered for all the detected lesions. Global mean SUVmax was 15.2±7 and global mean MTV was 50.6±44 cm3. The median SUVmax value was 14 and the median MTV value was 24 (cm3). The patients were categorized into two groups, according to SUVmax and MTV median values, and homogeneously treated with R-CHOP or R-CHOP-like regimens. The follow-up was 22±9 months. Six patients (19%) relapsed during the period of observation and 5 of them (16%) died. The Kaplan-Meier survival analysis according to SUVmax evidenced a significant difference in clinical outcome, showing a better event-free survival (EFS) (p=0.007; HR, 8.1, log-rank test) in patients presenting higher values as compared to those having less than 14. Conversely, the tumour burden estimated by the summed MTV was not suitable for predicting EFS (p=0.07; HR, 0.2, log-rank test). Our data, although still preliminary, suggest that the magnitude of the glycolytic activity, rather than the amount of the metabolically active burden, could hold a predominant value for predicting the possibility of relapse in patients with DLBCL. This study is still on-going and updated results, including a multivariate analysis comprising IPI score and neoplastic proliferative activity measured by Ki67 evaluation on histological samples, will be presented.

P059

R-CHOP21 VS R-CHOP14 IN 1024 DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: RESULTS OF A MULTICENTRIC RETROSPECTIVE STUDY FROM FONDAZIONE ITALIANA LINFOMI (FIL)

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Diffuse large B cell lymphoma (DLBCL) is the most common histotype of non-Hodgkin's lymphoma. R-CHOP21 (C21) is considered the standard therapy but a large number of studies tested the dose dense regimen R-CHOP14 (C14). The aim of our multicenter retrospective study was to evaluate the efficacy in terms of Overall Survival (OS) and Progression free survival (PFS) of the two regimens C21 and C14 in a large cohort of patients (pts) treated with curative intent. Patients diagnosed consecutively between January 2002 to December 2011 were considered for the study: 1024 pts were collected in thirteen Italian Haematoloy Departments. 702 were treated with C21 and 322 with C14. The two cohorts of pts were balanced for all clinical characteristics except for age higher than 60 years (66% in C21 *vs* 37% in C14 (p 0.000), high-intermediate and high risk IPI (33% C21 *vs* 28% C14; p 0.01). All pts in C14 used primary prophylaxis with G-CSF, and patients treated with C21 used G-CSF as secondary prophylaxis. After induction therapy 817 pts (80%) obtained a complete remission: 553/702 (79%) after C21 and 264/322 (82%) after C14. After a median period of observation of 36 months 101 pts out of 817 CR pts relapsed, 69/553 (12,4%) in the C21 arm and 32/264 (12.1%) in the C14 arm. OS at 3 years was 81% in C21 and 85% in C14 (p:0.1); PFS was 70% in C21 and 72% in C14 (p:0.4). Univariate statistical analysis showed that OS was significantly superior in younger pts (<60 year), Ann Arbor stage I-II, absence of B-symptoms, no bulky disease, negative bone marrow biopsy, low and low-intermediate risk IPI; PFS was significantly superior for the same characteristics. Multivariate analysis showed that OS was affected by age (p .002) and IPI (p .0000) and PFS by stage (p .002) and IPI (p .0000). The results of univariate analysis performed stratifying for therapy shown that C14 is able to overcome some negative prognostic factors (symptoms and bulky disease). No differences in haematological or extra-haematological toxicities were observed in the two arms; four deaths for sepsis were observed (1 in C14 and 3 in C21). Our results confirm that C14 does not improve either OS or PFS in comparison with standard C21 in the whole lymphoma population analysed. However, in univariate analysis the intensified therapy reduced the prognostic impact on OS of some important factors such as symptoms and bulky disease in comparison with standard C21, suggesting that C14 could be useful in a subset of pts.

P060

A COMBINED MONITORING APPROACH WITH TELEMEDICINE AND BIOMARKERS REVEALS FREQUENT SUBCLINICAL CARDIOTOXICITY IN LYMPHOMA PATIENTS TREATED WITH CLASSICAL OR LIPOSOMAL ANTHRACYCLINES

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Anthracyclines (AC) are the mainstay first line drug in many lymphoma patients:their use,however,is limited by the occurrence of Cardiac Toxicity(CT).The exact prevalence of AC CT occurring after widely used regimens such as R-CHOP or ABVD is unknown and there is uncertainty about the best monitoring method and possible prophylactic or therapeutic interventions. Methods.We started a prospective observational trial in lymphoma patients undergoing treatment with classical or liposomal AC.We used a comprehensive approach to monitor for AC CT,using a telemedicine(TM)system integrating echocardiography,ECG and biomarkers(Troponin I-TnI). Results.In this interim analysis,41 patients completed the planned treatment(23 males and 18 females).Median age was 51.3 years(range 19.1 to 78.5 years),and 10 patients were >65 years.11 were HL and 30 NHL(20 were DLBCL).Liposomal AC was used in 11 patients and classical AC in 30,with mean cumulative doses of 276 and 291 mg/m2,respectively. Overall 12 patients(29%)showed at least 1 relevant alteration among the baseline parameters to be monitored.The primary endpoint(reduction in LVEF greater than 10% to a final value <50%,evaluated 3 months after the end of treatment)occurred in 1 patient(out of 18 evaluable)who,however,at the time of the planned evaluation,had already undergone stem cell transplantation. 6/41 patients(15%)developed a TnI rise above 0.08 ng/mL and 20(49%)above 0.03 ng/mL. With both cut- offs,the rises occurred more frequently at cumulative doses >200 mg/mq (Figure 1).No difference was noted between those treated with liposomal or classical AC.In two patients the TnI rise reflected asymptomatic acute coronary syndromes detected with TM system,requiring myocardial revascularization.These patients completed the planned AC treatment and they are well.5 other patients developed minor reductions in the LVEF:none of them had a TnI rise >0.08 ng/ml. Regarding feasibility,the average time to perform an echocardiogram was 7 minutes,while it took about 4 minutes on average by the cardiologist to produce a report. Echocardiograms were not evaluable only in 2.6% of cases. Conclusions. Even with low cumulative doses,subclinical signs of AC CT were found in at least 29% of patients.In a low-risk setting for AC CT,a monitoring strategy combining clinical,imaging,instrumental and biomarker data seems to enhance the sensitivity of separate Methods. This strategy is feasible and resource-saving thanks to the integration in a TM system.

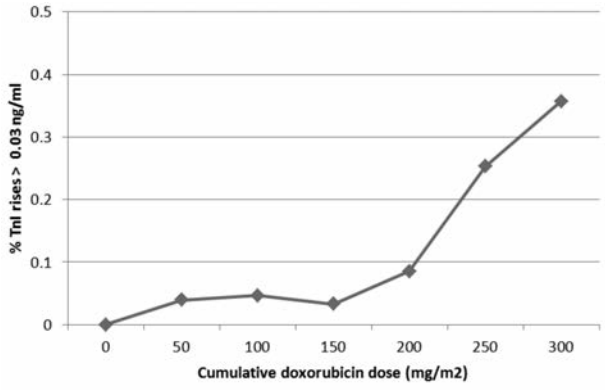

Figure 1. Proportion of TnI controls above 0,03 ng/ml

P061

CYCLOPHOSPHAMIDE, VINCRISTINE, MYOCET, AND PREDNISONE WITH OR WITHOUT RITUXIMAB (COMP+/-R) AS FRONT-LINE THERAPY IN ELDERLY PATIENTS WITH AGGRESSIVE NON HODGKIN'S LYMPHOMA (A-NHL). PRELIMINARY RESULTS OF A PROSPECTIVE, MULTICENTER PHASE II STUDY OF THE RETE EMATOLOGICA PUGLIESE (REP)

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Background. CHOP-21 regimen is standard of care for young and elderly patients (pts) with newly diagnosed A-NHL. However,elderly pts often receive lower dose of effective drugs or lower number of planned

cycles than younger pts resulting in poor CR and 5y OS rates.In the attempt to reduce toxicity, increase the dose intensity (DI) and CR rates of elderly pts,the REP started a phase II study (EudraCT-Number 2008-004156-76) with COMP+/-R regimen given to elderly pts with A-NHL. The primary end points of study were to evaluate the CR rate, and incidence of pts completing the 6-8 planned cycles. The secondary end points were to evaluate the 3-y PFS and OS. Methods. From Jan-2009 to Oct-2011, 69 pts, median age 73 yrs (range 65-86), and diagnosis of DLBCL (n=68),MCL (n=3) and ALCL,(n=1) were enrolled into study. At entry 61% of pts had stage III-IV, 19% BM+ve, and 71% aa-IPI score more than 1. Serum LDH level and B2M above UNL was recorded in 47% and 65% of cases respectively. The COMP+/-R regimen consisted of standard CHOP-21 regimen in which doxorubicin was replaced by liposomal doxorubicin (Myocet) at dose of 50 mg/m^2 IV on day1; rituximab was given at 375 mg/m^2 IV on day 1 of each cycle; pts were planned to receive 6-8 cycles of chemotherapy at investigator discretion. IF-RT was allowed to residual or primitively bulky disease. All pts but one received R-COMP chemotherapy and 10 pts (15%) IF-RT. Results. Pts received a median of 5.8 cycles (1-8) with 81% of them (n=53) receiving six (n=41) or more cycles. On ITT analysis CR was achieved in 40 pts (61%; IC95% 48-73%) and PR in 11 (17%) with an ORR of 78% (IC95% 67-87%).Twelve pts (18%) had SD/PD. When only pts receiving 6 or more cycles were evaluated the CR rate was 73% (n=35) and PR 17% (n=9) with an ORR of 90%. The median DI of cyclophosphamide and Myocet was 0.98 and 0.96 respectively. Hematological toxicity was acceptable with few pts experienced WHO grade 3-4 anemia (8%), neutropenia (32%), or thrombocytopenia (3%). Grade 3-4 infection were observed in 3 pts (5%).Seventeen pts died, including 10 for disease progression and 5 for treatment-related toxicity. There was 1 pts died for cardiac toxicity after the 4th cycle. After a median follow-up of 17 mos (1-48) the 3-yr PFS was 58.9% (IC95% 39.6-73.9) and 3-yr OS 68.2% (IC95% 52.7-79.5). Conclusion. These preliminary results show that R-COMP is safe and effective regimen when given as front-line therapy in very elderly A-NHL.

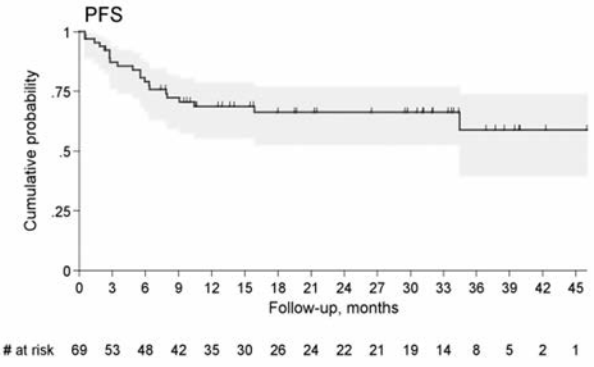

Figure 1.

P062

OUTCOME OF ELDERLY PATIENTS WITH AGGRESSIVE LYMPHOMA DIAGNOSED IN A HOMOGENEOUS GEOGRAPHICAL AREA (AREA VASTA ROMAGNA): ANALYSIS OF AN UNSELECTED PATIENTS POPULATION

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Elderly patients with aggressive lymphoma (HG-NHL) represent a therapeutic challenge as the pursuit of disease eradication should be weighted against patient's co-morbidities and preservation of a good quality of life. The aim of the present study was to evaluate the outcome of all the patients aged >60 years that were consecutively diagnosed with HG-NHL in all Hematological Centres of Romagna/ area vasta romagna (AVR) in the period january 2010-december 2012. We retrospectively evaluated 155 patients, their median age at diagnosis was 73 (range 61-93 years). The CIRS-G score was evalueted in 68.9% and 72% of them were classified in the category of fit (less than 3 grade 3 and no grade 4 co-morbidities). The majority of the patients (89.3%) had B cell NHL, the most common histotype was diffuse large B cell lymphoma (75.7%). Rates of other disease-related factors were as follows: stage III-IV, 66.9%; performance status (ECOG) <2, 52.2%; international prognostic index 1, 2, 3 and >4, 20.1%, 21.6%, 32.8% and15.7% respectively; B symptoms, 48%. Chemotherapy with curative intent, mostly R-CHOP or CHOP like regimens with or without Rituximab, was administered to 87% of the patients , and 37% of them received a dose adjustment. All treated patients were evaluable for response: 60.2% achieved a complete remission and 14.1% a partial response with an overall response rate of 74.3%. After 9 months median follow-up, 52.9% of patients are alive and disease-free. A wider use of comprehensive geriatric assessment scores would probably improve the management of elderly patients with HG-NHL, helping the clinician to talilor the treatment basing on individual patients' needs.

P063

RITUXIMAB AND CHLORAMBUCIL AS FIRST LINE THERAPY OF LOW-GRADE OCULAR ADNEXAL LYMPHOMAS (OALS): LONG TERM FOLLOW-UP RESULTS

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Ocular adnexal lymphomas represents 8% of primary extranodal lymphomas, 80% of OALs constitutes extranodal marginal zone lymphomas (EMZL). Radiotherapy is associated to high rates of local disease control, but also to the risk of relapse and immediate and delayed complications. Single-agent chemotherapy with alkylating agents and rituximab are used for the treatment of low-grade lymphomas. We investigated the efficacy and the safety of a combination of chlorambucil and rituximab as 1st line therapy in patients (pts) with OALs. Staging included Chlamydia psittaci (Cp) detected by PCR; therapy consisted of chlorambucil (0,1 mg/Kg/die for 45 days, then on days 1 to 15 monthly for 4 mths) and rituximab (375 mg/sqm weekly for 4 doses, then monthly for 4 infusions). Since November 2003 to November 2012, 22 consecutive OALs (20 EMZL, 2 FL) have been treated according to protocol.The median interval between onset of the 1st symptoms and diagnosis was 13 mths (range, 4 mths -3 yrs). Eight pts were male (36%) and 14 pts were female (64%). Median age at diagnosis was 68 yrs (range,35-86 yrs). Disease was localized in the conjunctiva in 16 pts (72%), in the lacrimal glands in 3 pts (14%) and in other orbital sites in the last 3 pts (14%). Twenty-one pts presented a stage I disease, 1 stage IV, no pts showed B- symptoms and LDH was within normal range in 19 of 22 pts (86%).We evaluated PCR for Cp in the 1st 10 consecutive pts and it was negative. All pts completed the treatment without delay; there was no grade III-IV toxicities neither hospitalizations and haematological toxicity was mild. At the end of treatment 21 pts (95%) resulted in CR, and 1 obtained a PR (5%). After a median follow-up of 62 mths (range,10-106 mths) all pts are alive, 17 maintained CR and 4 relapsed after 3,4,5 and 7 yrs; 3 pts were retreated with the same protocol because they relapsed with the same histology, the other relapsed systemically as FL. The median PFS was 50 mths (range, 1-98 mths). All pts performed ophthalmologic follow-up: we didn't report ocular toxicities, and all pts conserved a normal visual function, including acuity. No secondary myelodisplatic syndrome or neoplasms are reported. After a long follow-up the combination of rituximab and chlorambucil proved to be low toxic, feasible and effective therapy for primary OALs. No delayed ocular or haematological complications are reported. For this reason our regimen should be considered for the 1st line of this indolent lymphoma.

Anemias and Hemoglobinapathies - Cytogenetics and Molecular Genetics - Laboratory Investigation in Hematology

P064

EXCESSIVE MENSTRUAL BLOOD LOSSES CAN CAUSE SEVERE IRON DEFICIENT ANEMIA IN WOMEN WITH INHERITED OR ACQUIRED COAGULATION DISORDER FOR ORAL ANTI-COAGULANT TREATMENT

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Background. Excessive menstrual blood losses (EMBL) are a frequent symptom of a inherited bleeding disorder (IBD) but they can also complicate treatment with oral anticoagulants (OAT). EMBL can lead to severe iron deficient anemia, sometimes requiring red blood cells transfusion. The quantitative determination of menstrual blood losses can effectively support the management of this complication through an early detection that can help to avoid excessive iron losses and maintain a positive balance of iron homeostasis. Objective. To quantitatively determine in a prospective evaluation, menstrual blood and iron losses in women with a diagnosis of inherited or acquired coagulation disorder secondary to OAT referring to our Center for iron deficient anemia. Methods. Alkaline Hematin Method (AHM) was applied for the quantitative determination of menstrual blood losses (MBL) in each enrolled woman. A personal and gynecological history was recorded and all other potential known causes of iron deficiency were excluded in each enrolled woman . Sanitary protection wears used during menses were carefully collected and analyzed in a central laboratory. Menses were studied from 1 cycle to 3 consecutive cycles. A group of healthy women with normal hemoglobin and ferritin levels was enrolled as control. Blood cells count and ferritin serum levels were determined before enrollment and at least two weeks after the last menses. EMBL were defined as MBL >80 mL. Iron losses were determined from the hematin values. Results. Sventeen women with a known diagnosis of inherited bleeding disorder (IBDs)or under OAT, with a therapeutic INR (range:2-3), were enrolled for iron deficient anemia: von Willebrand's Disease (vWD, n=3), Factor VII deficiency(n= 6), OAT for Deep Vein Thrombosis or Pulmonary Embolism (n=8). Mean age at enrollment was 30.2 years (SD:5,19). Mean Hemoglobin levels were: 9,7 g/dL(SD:2,4); mean serum ferritin levels: 5 ng/dL (SD:3,37); mean menses duration was 6 days,(SD:2); mean MBL was 95 ml (SD:12,7). Median amount of iron lost was 5.2 mg/cycle. In the control group (n=25), mean age at enrollment was 29,9 years (SD:6,4), mean Hemoglobin levels were: 13,5 g/dL (SD:0,9); mean serum ferritin levels: 36,2 ng/dL (SD:20); mean menses duration was 4,5 days,(SD:1); mean MBL was 45 ml (SD:17,2). Median amount of iron lost was 0,8 mg/cycle Conclusion: EMBL can be the leading cause of moderate to severe iron deficient anemia in women with IBDs or under OAT.

P065

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA TREATED WITH ECULIZUMAB IN 5 PATIENTS: HETEROGENEITY AND PECULIARITIES

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Paroxysmal nocturnal hemoglobinuria (PNH) is caused by nonmalignant clonal expansion of hematopoietic stem cells harboring somatic mutations of the X-linked PIG-A gene. It leads to the loss of GPI membrane- anchored proteins from cell surface. The resulting intravascular hemolysis is caused by uncontrolled activation of the complement cascade and determines hemoglobinuria, anemia, fatigue, renal impairment, pulmonary hypertension, smooth muscle dystonias and thromboembolism, with a bad quality of life. We describe 5 PNH patients: 3 males and 2 females; median age was 36 year (18-54). All developed PNH after other haematologic disorders. One was a rare case occurring after autologous stem cell transplantation(ASCT) for non-Hodgkin's lymphoma; another had experienced an aplastic anemia, successfully treated with immunosuppressive therapy; two were affected by anemia and thrombocytopenia of unspecified nature; in the fifth young patient (18 ys) hereditary spherocytosis had been diagnosed 2 years before, based on erythrocyte membrane protein analysis and splenectomy had been performed. All patients had strong intravascular hemolysis and flow cytometry immunophenotyping study suggesting PNH. After vaccination against Neisseria meningitides the terminal complement inhibitor eculizumab (EC) was started. The intravascular hemolysis was completely controlled in all patients. Only one patient continues transfusions; she shows significant extravascular hemolysis, building up GPI-C3d coated erythrocytes (DAT ++), and is candidate for splenectomy. Despite the different haematological response all 5 patients experienced a dramatic clinical improvement in the quality of life. The transplant patient showed a reduction and ultimately extinction of the pathological clone; this provided the unique possibility to discontinue treatment after 12 months, then maintaining remission for years. Probably in this case, EC did not play any direct role in the kinetics of the PNH clone, while the conditions which led to expansion of the clone were somehow unique and possibly related to the ASCT and resulted transient. Our small series, including even 2 rare cases (one pediatric and one after ASCT) shows how variable is the clinical and biological spectrum of manifestations in this strange and fascinating disease; moreover its clinical evolution in each single patient remains frequently unpredictable.

P066

SPLENOMEGALY: UNCOMMON PRESENTATION OF COBALAMIN DEFICIENCY. A CASE REPORT AND REVIEW OF THE LITERATURE

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A 47-y old woman was admitted in our hospital for splenomegaly, pancytopenia, referring progressive fatigue and dyspnea. She had mild scleral icterus and palpable spleen extending 8 cm below the left costal margin, petechiae of the face and limbs. Neurologic system was normal. The TB CT Scan confirmed splenomegaly. CBC showed Hb 6.2 gr/dL, (the patient received a RBC transfusion as emergency) MCV 92 fl, WBC 2500/mmc, N 1500/mmc, PLT 27.000/mmc, reticulocytes 42.000/mmc, hyperbilubinemia of 1.8 mg/dL, and LDH was 1000 UI/L (n.v. 100-290). Marrow was hypercellular. Erythroid precursors were incresead and showed megaloblastosis; granulocites were decresead with many giant metamyelocites. Megacaryocites were decreased and dysplastic. The bone marrow findings was considered for severe megaloblastic anemia and a parenteral B12 vitamin therapy was begun. In g+6 we have seen reticulocyte crisis with ret 397.000/mmc. Gastroscopy showed atrophic gastritis. Iron binding capacity and ferritin were normal. JAK2 and circulating CD34 cells were normal. Serum Vit B12 level was 18 mcgr/l. In about 10 days leucocytes and platelets normalized and hemoglobin was 9.6 gr/dl at the time of discharge. After 2mo echotomoghraphy showed the spleen had decreased in size of 13 cm. At our time, megaloblastic anemias are uncommon, with some cases in the eldery. They are most seen in patients who assume drugs interfering with DNA synthesis. The most important causes, include inadequate secretion of intrinsic factor as in autoimmune atrophic gastritis, and inadequate release of cobalamin with foods. Deficiency can also be present in malabsorbiment for celiac disease, ileal resection and bowel chronic inflammatory disease. Dosage cannot be reliable because of the levels of B12 binding proteins. Serum homocysteina and methylmalonic acid are incresed in Vit B12 deficiency, even if MAA can be elevated in renal insufficiency. Deficiency of vit B12 may result in severe pancytopenia and it can be mistaken for acute leukemia. In fact, leukoerytroblastosis of peripheral smear can be seen. In these cases, bone marrow smear observation can indicate right diagnosis. Modern textbook fail to mention splenomegaly but old published experiences reported a prevalence between 3-8%. Today the exact prevalence of splenomegaly is not known.This case reminds that severe megaloblastic anemia, may present characteristics such as pancytopenia, erytroblastos, splenomegaly suspicious for malignant disorders.

P067

LIPOSOMIAL IRON HAS AN ANTI-INFLAMMATORY EFFECT AND IS BETTER THAN IRON SULFATE IN CORRECTION OF ANEMIA OF CHRONIC INFLAMMATORY DISEASE OF YOUNG WOMEN

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Background. Liposome has a described anti-inflammatory effect and transports its content directly in blood, beyond gastric and enteric wall. Aim. Aim of this study is to verify if liposomial iron is most effective than iron sulfate in correction of anemia of chronic inflammatory disease of young women. Patients and Methods. In group A 9 patients (4 with systemic erythematous lupus, 3 with mixed connectivitis, 2 with rheumatic fibromyalgia), median age 32 years (R27-42), Hb 8.5 g/dL (R8-10), saturation of iron binding capacity <20%, with a median ferritin level of 100 ng/mL (R90-250), ESR 35 mm/1st hour (R22-95), CRP 18 mg/I (R12-24), normal B12 and folate, received liposomal iron 60 mg/day orally for 3 months. In group B 12 patients (6 with systemic erythematous lupus, 3 with mixed connectivitis, 3 with rheumatic fibromyalgia), median age 38 years (R29-45), Hb 9 g/dL (R8-9.5), saturation of iron binding capacity <20%, with a median ferritin level of 120 ng/mL (R80-190), ESR 33 mm/1st hour (R20-87), CRP 15 mg/I (R13-27), normal B12 and folate, received iron sulfate 210 mg/day orally for 3 months. Results. After treatment, group A showed a median hemoglobin level of 11.5 g/dl (R10.5-12), a median ferritin level of 260 ng/ml (R 190-280), a ESR decrease to a median value of 8 mm/1st hour (R 3-10) and a median CRP 3 mg/I (R2-4). After treatment, group B showed a median hemoglobin level of 9.5 g/dl (R8-9.5), a median ferritin level of 100 ng/ml (R 90-180), and ESR and CRP don't showed any improvement. 4 patients showed hepygastralgia, 2 stipsis, 5 diarrohea. Conclusion. Liposomial iron is most safe, effective, well tolerated, effective than iron sulfate in increase hemoglobin level and reduce inflammatory markers in correction of anemia of chronic inflammatory disease of young women.

P068

INTRAVENOUS FERRIC GLUCONATE (IFG) THERAPY SIGNIFICANTLY REDUCES PLATELETS COUNT (PC) AND NORMALISES BOTH THROMBOCYTOSIS AND THROMBOCYTOPENIA IN PATIENTS WITH IRON DEFICIENCY ANEMIA (IDA)

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Background. Iron is a powerful regulator of megakariocytopoiesis *in vivo* and *in vitro*.Iron deficiency (ID) is typically associated with IDA.In addition to anemia,abnormal PC also have been reported in both adults and children with IDA.Severe and/or long lasting IDA has been associated with both thrombocytosis and thrombocytopenia,which have been reported in up to 50% and 2-3%, respectively of patients (pt). Most studies so far pubblished on PC in pt with IDA are retrospective in nature and are often lacking in detailed information about therapy (route of iron administration,doses,PC pre-and post-treatment ecc.).We here report that IFG significantly reduce PC in outpatients with IDA of different etiology. Patients and methods. Starting on 1st October,2012,53 outpatiens were treated for IDA diagnosed by standard criteria.The formula recommended by the package insert was used to calculate the IFG pt's dose. Pt with active cancer or hemorrages were excluded. Peripheral blood counts were performend before and after 4-5 weeks completing IFG. After a test dose with 62,5 mg of Fe diluted in 100 cc of saline,125 mg/day in saline 250 cc were amnistered in subsequent days, possibly 5 days a week,until total dose, if tolerated. Results. A total of 797 doses were administered in 53 pt (40F,13M) mean age 55,83 (range 15-89).Menhorragia and vegetariansism were the most frequent causes of IDA.Three women intrerrupted IFG after 2nd dose for syncope or GI symptoms).Mean WBC counts were 6.5x10^9/L (range 3.36-11.90) pre- and 6.5 (3.16-12.08) post-IFG.The difference between the two sample was not significant (Mann- Witney U test p =0,8). Basal leukopenia was present in 2/53 pt (3,7%) and resolved in both after IFG, when others 2/50 pt (4%) became leukopenic. Mean PC were 313x10e9/L (range 136-770) pre- and 246x10e9/L post-IFG (r142-429).The difference between the two sample was statistically higly significant (Mann-Witney U test p=0,0004). Pre-IFG, 1/53 pt (1,9%) was thrombocytopenic *vs* 0/50 post-IFG; 5/53 pt (9,4%) had thrombocytosis pre-IFG *vs* 0/50 post-IFG.Mean PC in these 5 pt was 537x$10e^9$/L (r 445-770) pre-IFG *vs* 324x$10e^9$ post-IFG (r 215-411). Conclusions. To the best of our knowledge this is the first prospective study demostrating a significant reduction of PC in pt with IDA without chronic kidney or hearth diseases treated with IFG. About 10% and 2% of pts presented with high and low PC, respectively, with PC normalisation in all cases post-therapy.

Table 1. Hematological and iron parameters in 53 pt with IDA treated with intravenous ferric glucose

	Mean ±DS	
	Pre- Therapy	Post-Therapy
WBC (x$10e^9$/L)	6.5±1.95	6.4±1.8
RBC (x$10e^{12}$/L)	4.1±560.1	4.7±543.2
Hb (g/dL)	9.2±1.34	12.6±0.98
Hct (%)	31.1±3.5	40.2±3.12
MCV (fL)	75.4±10.5	84.9±6.13
MCH(pg)	22.5±4.08	26.8±2.2
MCHC(g/dL)	29.6 ± 1.74	31.3±0.92
Platelets (x10e9/L)	313.9±117.9	246.1±66.15
Serum Iron (pg/mL)	20.1 ± 8,85	82.1±28.9
Transferrin saturation (%)	5.6±2.92	25.1±6.33
Serum Transferrin (mg/dL)	3.4±0.52	2.6±0.41
Serum ferritin (ng/mL)	16.7±24.8	282.5±202.1
Patients, n	53 (40F,13M)	50 (37,13)

Values are expressed as mean ± standard deviation

P069

PYRUVATE KINASE DEFICIENCY: CLINICAL AND MOLECULAR CHARACTERIZATION OF 11 PEDIATRIC CASES

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Pyruvate kinase (PK) deficiency is the most common defect of erythrocyte glycolytic pathway causing hereditary nonspherocytic chronic haemolytic anaemia. The disease as an autosomal recessive transmission and more than 200 mutations have been so far reported in the red cell pyruvate kinase encoding gene (PK-LR). The severity of anemia is highly variable, ranging from mild forms to life-threatening haemolysis requiring continuous transfusion therapy. PK deficiency has a worldwide distribution with the higher prevalence of 1:20.000 in Caucasians. No reports were so far available on patients from Argentina. Here we describe the clinical, haematological and molecular characteristics of 11 PK deficient children from 9 Argentinean families. Seven patients were of Gypsy ethnic group and consanguinity of parents was declared, the remaining 4 had Italian or Spanish origin. Median age of admission was 0.2 yrs (range 0-2.4 yrs). The most important clinical and haematologic data and the results of the molecular characterization are reported in Table 1. All the patients had a severe onset at birth with hyperbilirubinemia and need of phototherapy, and 7 required exchange transfusion. Because of the high transfusion need (median 15/year, range 6-29) nine patients were splenectomised (median age at splenectomy 2.2 yrs, range 8 months-4 yrs); splenectomy did not normalize anaemia, but resulted in stabilisation of the haemoglobin to slightly higher levels in all patients but one (median Hb increase 1.8 g/dL, range 0.2-4.2), with a drastic reduction of transfusions (median 2/year, range 1-4). All patients but one had increased serum ferritin levels (median SF 1013 ng/ml), and 6 underwent chelation therapy. As typically reported in PK deficiency, a conspicuous rise of reticulocytes after splenectomy was observed. PK-LR gene analysis lead to detection of 9 different mutations; 4 of them

were novel: three missense (Ala495Thr, Val506Ile, Gly341Ser), and a 6 nt duplication (c.364_369 ins GGCTCC) resulting in the in-frame duplication of Gly122 and Ser123. All patients of Gipsy origin were homozygous for mutation c.1437-518_1618+440 del 1149 bp (already reported as "Gypsy mutation"). This is the first comprehensive report of molecular characterization in PK deficiency from Argentina. Four novel mutations were identified in the patients of Italian and Spanish origin. For the first time the clinical and haematological parameters of a large group of Gypsy subjects were described.

Table 1.

Pts	Ethnic Group	Age at admission (years)	Splenect. age (years)	Hb g/dL	Retics %	Hb g/dL	Retics %	SF ng/ml	Mutation	Effect
				Pre splenectomy		Post splenectomy				
TM-S	Gypsy	0,2	1,0	5,5	n.a	7,9	75	548,17	Gypsy/Gypsy	del ex11/ del ex11
TM-Y	Gypsy	0,1	0,8	7,1	n.a	7,6	87	392,68	Gypsy/Gypsy	del ex11/ del ex11
CR	Gypsy	0,1	3,9	6,6	24,6	8,5	94	2347,71	Gypsy/Gypsy	del ex11/ del ex11
J-L	Gypsy	0,2	2,2	6,7	8,9	8,1	96,8	1561,39	Gypsy/Gypsy	del ex11/ del ex11
J-D	Gypsy	18 days	0,7	5,5	n.a	7,2	102	1445,2	Gypsy/Gypsy	del ex11/ del ex11
GMH	IT- ES	24 hours	2,0	9,8	5	8,5	60	102,69	**347A / 1232T**	Arg166Gln/ Gly411Val
RJ	IT-IT	0,6	3,5	7,6	17,8	7,8	63	1012,67	1483A / 1516A	Ala 495Thr/ Val506Ile
VJA	ES-ES	0,4	3,8	3,4	20	7,6	22,2	2915,99	1594 T/ **364-369 GGCTCC dupl.**	Arg532Trp/ Gly122-Ser123 dupl
SAM	Gypsy	2,4	2,9	7,9	23,2	11,5	80	785,06	Gypsy/Gypsy	del ex11/ del ex11
CK	Gypsy	0,3	No	10,7	3,2			1800	Gypsy/Gypsy	del ex11/ del ex11
CB	ES-ES	0,4	No	6,9	14			475,66	**1021A** / 1456T	Gly341Ser/ Arg486Trp

IT : Italian, ES: Spanish, SF : Serum Ferritin, n.a.: not available; new mutations are reported in bold.

P070

CLINICAL CHARACTERISTICS AND OUTCOME OF ASYMPTOMATIC SUBJECTS WITH AN ERITHROCYTE AUTO-ANTIBODY

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An increased incidence of cancers and hematologic malignancies has been reported in the follow-up of DAT+ blood donors. On the other hand, a high prevalence of DAT+ cases is described in patients with chronic lymphoproliferative disorders. To better define the outcome of asymptomatic individuals with a positive DAT, between December 1997 and January 2013 all subjects with an anti-erithrocyte auto-antibody (AeAb), normal Hb value, no clinical and laboratory signs of hemolysis, acrocyanosis or associated disease (autoimmune disorder, infection, lymphoproliferative disorder, cancer) were followed at our institution. At study entry, the following tests were performed: blood and reticulocyte counts, bilirubin, LDH, autoimmunity panel (anti-nucleous; ACA, LAC, anti-beta 2-glycoprotein-I; anti-peroxidase and anti-thyroglobulin antibodies), HBV and HCV serology. In addition, a peripheral blood (PB) immunophenotypic characterization was performed by 4 color flow-cytometry (CD5/CD3/CD20; CD23/CD10/CD19/CD20; Ig /Ig ; CD19/CD5; CD3/CD4/CD45/CD8). A total body CT scan or a chest radiography with an abdomen ultrasound were also performed. During the follow-up blood counts and hemolysis investigations were regularly repeated and DAT was performed once a year. Thirty-eight subjects, 19 males and 19 females with a median age of 56.5 years (range: 18-84) were included in the study. A positive DAT was detected in 21 (55%) blood donors, in the pre-surgical screening of 14 (37%) subjects and in 3 (8%) pregnant females. The median Hb value was 12.8 g/dL, the AeAb isotype form was an IgG in 14 (37%) cases and an IgM in 24 (67%; warm, cold in 9 and with a thermal amplitude to 37°C in 15). Despite the persistently positive Coomb's test, no patient developed an AIHA. At the baseline screening, the asymptomatic positivity of one or more autoimmune antibodies was observed in 55% of cases, while the presence of a thymoma was revealed by the CT scan in 1 case. A clonal B-lymphocyte population was detected in the PB of 6 subjects (16%), all with an IgM AeAb. The clonal B-lymphocyte count ranged between 0.04 and 0.478 x10^9/L, and persisted in all cases after a median follow-up of 36 months (range 6-108 months). However, during the follow-up, no patient developed clinical evidence of a lymphoproliferative disease, while a thyroid cancer was diagnosed in 2 cases. These findings suggest the need of a diagnostic screening and a long-term clinical follow-up of healthy subjects with an AeAb.

P071

ORAL FENTANYL AS PAIN-BREAKING TOOL IN EARLY MANAGMENT OF SEVERE ACUTE VASO-OCCLUSIVE CRISIS IN ADULT PATIENTS WITH SICKLE CELL DISEASE

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Acute vaso-occlusive events (VOCs) are one of the major causes of hospitalization of patients with sickle cell disease (SCD). Pain characterized the VOCs and the aggressive treatment of pain during VOCs reduces the more severe SCD related organ complication. We carried out an open double-dummy active clinical trial treating pain during acute VOCs in the same group of SCD patients referred to the Department of Medicine, University of Verona, Italy, between January 2009 to July 2012. Eligible population were patients with SCD aged 18-45 years suffering from painful vaso-occlusive crisis (VOC) for which no other clinical explanation was identified, with a VAS pain level of 7 or more. Each hospitalization was defined as an episode of VOC. The patients received two different types of analgesic treatment during two separate painful VOC event with interval between crisis of at least six months. TK group: continuous intravenous infusion of ketorolac (0.86 mg/kg/day), tramadol (7.2 mg/kg/day) and metoclopramide (0.57 mg/kg/day) for a maximum of 72 hours (TK group). TKF group: administration of the same pharmacological protocol with the addition of Fentanyl Buccal Tablet (FBT: 100 mcg) in single administration as pain-breaking molecule. The following parameters were recorded at the admission and at 3-6-12-18-24 hours: pain intensity, level of anxiety, patient's sedation, patient's mood. The primary efficacy measure was the time-weighted sum of pain intensity differences from 3 to 24 hours after the administration of study drugs (time-weighted SPID24). The PID (pain intensity difference) was calculated at each time interval. The secondary efficacy measures were time-weighted Total Pain Relief (TOTPAR), calculated considering Pain Relief (PR) instead of PID. Changes in patient's anxiety during treatment was evaluated as "time-weighted SAID24 (sum of anxiety intensity difference from 3 to 24 hour) calculated in the same way as SPID24. In TKF treated SCD patients (n=20) we observed increased time-weighted SPID24 and decreased PID compared to the same SCD patients treated with TK. This finding was associated with increased time-weighted TOTPAR with faster pain-relief and reduction of patients' anxiety in TKF treated SCD patients compared to TK group. No significant changes in peripheral oxygen saturation was detected between the TK and TKF treated SCD patients. These data suggest FTB as an interesting new tool in management of pain of VOCs in adult SCD patients.

P072

SINGLE CENTER EXPERIENCE ON 25 PATIENTS WITH AGE OVER 60 CONSECUTIVELY DIAGNOSED WITH SEVERE APLASTIC ANEMIA

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Background. Aplastic anemia (AA) is a rare hematologic disorder. The annual incidence is 2 per million/year in Europe with two peaks from 10 to 25 years and >60 years. Although treatment in pediatric/adult patients is well standardized, few data exist about treatment and outcomes in elderly pts and therefore the best approach to their management is still debated. Aim of Study. To contribute our experience on 25 patients aged >60, consecutively diagnosed with severe aplastic anemia (SAA) at a single center and to evaluate the outcome of different treatment strategies. PATIENTS AND Methods. The diagnosis of SAA was made according to the criteria of Camitta (1986) and Bacigalupo (1988). All consecutive pts diagnosed with SAA at our Center between January 1991 and January 2013 were retrospectively analyzed. Treatment included immunosuppressive therapy, with horse or rabbit ATG according to standard protocols (with/without CSA) or other treatments without ATG (supportive therapy, G-CSF, CSA and PDN).The choice of treatment was based on clinical evaluation. Horse or rabbit ATG was used based on drug availability. Results. Of 25 pts retrieved, 21 were SAA and 4 very-SAA. Elderly pts accounted for 40% of consecutive SAA pts at our Institution in accordance with literature reports. Of 25 pts, 17

(68%) were treated with ATG+CSA (groupA) while the other 8 pts were treated with supportive therapy, GCSF, CSA and PDN only (groupB).In group A median age was 71, M/F 6/11. Five of these pts received a reduced dose of ATG (50%). Six pts died within three months of infection (3), bleeding(2) and acute renal failure(1). Four pts did not respond and died of infection after 4-8 months. Of the remaining 7 pts, 4 are in complete remission, 2 in partial response and 1 is ongoing. Survival at 4-years is 36%, with no significant differences according to ATG dosage. In group B median age was 76, M/F was 3/5. All these patients died within eight months, of infection or bleeding. The overall survival of the two groups differed significantly (P 0.02) (Figure 1) Conclusions. Our study confirms that SAA in elderly pts is not rare and merits specific attention. ATG is feasible and can obtain long term remission in a significant proportion of pts, thus changing the natural history of the disease. ATG dosage may be reduced. Since the frequency of infection and bleeding is higher than in the young, patients management remains challenging and requires careful clinical evaluation.

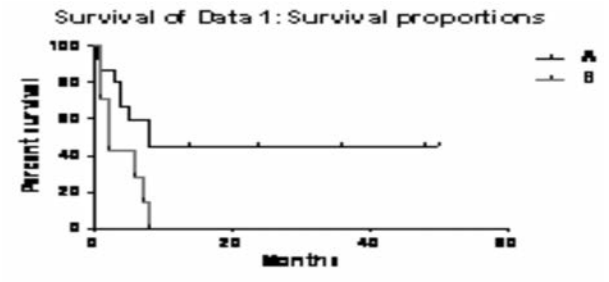

Figure 1.

P073

A RARE HEMOGLOBIN VARIANT [HB CHEVERLY BETA 45 (CD4) PHE>SER] DETECTED IN A CHILD WITH SEVERE OXYGEN DEFICIENCY IN THE TISSUES

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We report the case of a patient, 1 yrs old, hospitalized because of a home accident: during hospitalization, a pulse oximeter was applied and a pulse oximetry (SpO^2) of 80% has been observed. The pO^2 (SaO^2) was normal at the central level by the use of arterial blood gas analysis, but the values, always very reduced at the tissue level could be assumed respiratory disorders of unknown nature, for deficit in the transport of oxygen. An in-depth investigation in a specialized Center (C.S.M. R. of Rome), led to the detection of mild microcytic anemia, with hypochromia and anisocytosis (in the presence of evident iron deficiency) and of an abnormal Hb fraction (20% by cation exchange HPLC, Variant II Bio-Rad) confirmed by reverse phase HPLC. Molecular analysis has shown the presence of a rare hemoglobin variant, already described in association with a moderate chronic anemia: Hb Cheverly (TTC>TCT, Phe>Ser at codon 45 in a beta globin gene) indicated as an Hb with reduced oxygen affinity and Bohr effect and therefore always with decreased levels of oxyhemoglobin. It has been described that the substitution of a Phenylalanine residue at codons 41, 42 or 45 with another amino acid residue results in altered physicochemical properties in the hemoglobin molecule because the residues of Phe are part of the contact with the heme: so, the instability observed in Hb Cheverly is most likely due to the destruction of the bond between Phe and heme.The substitution of Phe with Ser in Hb Cheverly is the same identified in Hb Hammersmith [beta 42 (CD1) Phe>Ser]: therefore, these two variants have properties very similar to each other, even if Hb Hammersmith is more severely unstable because in this case the substitution with Serine leads to a heme pocket opening. A reduced oxygen saturation was also observed in Hb Köln [beta 98 (FG5) Val>Met] carriers, although this variant demonstrates an affinity for oxygen higher than normal, and in two patients with Hb Bonn [alpha1 87(F8) His>Asp] that appear with modest chronic anemia: all these carriers, studied with pulse oximeter, show a decreased oxygen affinity. Tipically, these hemoglobins appear as asymptomatic and do not require special treatment, but we should be able to recognize them, using pulse oximeter analysis, to avoid abnormal or inappropriate responses in case of anesthesia or monitoring of the state of oxygenation in emergency conditions or in pediatric patients with chronic anemia.

P074

HEMATOLOGICAL PARAMETERS IN HIGHLY TRAINED ATHLETES: CORRELATION WITH TRAINING/COMPETITION PERIOD AND SPORT DISCIPLINE

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The hematological parameters of highly trained athletes have been investigated over the last years in order to define exercise-induced changes and identify possible indirect markers of illicit procedures. There is an overall consensus about a slight and variable decrease in hemoglobin concentration induced by the endurance training, mainly attributable to exercise-induced plasma volume expansion. However, conflicting results have been reported and the actual adaptation of erythroid cell population in highly trained competitive subjects is not yet well understood. To address this issue, a comprehensive analysis of hematological parameters has been performed in competitive athletes during an entire sporting season. The study was approved by the Ethical Committee and was partly supported by the Italian Ministry of Health. Main hematological parameters were evaluated in 139 athletes over three seasons, *i.e.*: basal time (T1); intensive training (T2); competition phase (T3). The athletes were from various disciplines, including soccer (36), cycling (27), swimming (29), volleyball (20), rugby (27). Overall, minor variations were detected in both hematocrit (Hct) and hemoglobin (Hb) concentration in the three phases, but values were consistently within normal ranges in all sports, with Hb ranging between 13.7 and 15.6 gr% throughout observation. Statistically significant differences occurred depending on the exercise phase and discipline. In particular, from T1 to T3, a slight but significant increase of Hct was observed in soccer, swimming, volleyball and rugby, not associated with analogous Hb variations; indeed, a small and variably significant decrease of Hb was observed in cycling, swimming, volleyball and rugby. Red blood cell volume (MCV) and hemoglobin concentration (MCHC) also varied, again within normal ranges. The most marked changes were observed for reticulocyte counts, analysed in soccer and cycling: a sharp increase was observed in T2, followed by a rapid drop in T3. In conclusion, the study indicates that: i. hematological parameters show minor though significant variations in highly trained athletes during an entire sporting season; ii. despite variations, Hct, Hb, MCV, MCHC remain well within normal ranges; iii. the small variations, depending on exercise phase and sport discipline, may account for the discrepancies reported in the literature; iv. reticulocyte count is a relevant parameter in the hematological monitoring of competitive athletes.

P075

LONG-TERM FOLLOW UP OF ALEMTUZUMAB-BASED IMMUNOSUPPRESSION FOR THE TREATMENT OF IMMUNE-MEDIATED BONE MARROW FAILURE SYNDROMES

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Aplastic anemia (AA) and pure red or white cell aplasias (PRCA and PWCA) are immune-mediated disorders; thus, immune suppressive therapy (IST) targeting T cells is the main treatment for patients suffering from these diseases. Here we report the long-term follow up of a pilot phase II prospective trial (NCT00895739) investigating the anti-CD52 monoclonal antibody alemtuzumab in combination with low-dose cyclosporine A (CyA; Risitano, BJH 2010;148:791). We enrolled twenty-eight patients:13 SAA, 13 PRCA and 2 PWCA whose 15 (6 SAA, 9 PRCA and 1 PWCA) had not received any previous IST. Median age was 51 years (range 25-87). All patients received alemtuzumab as sub-

cutaneous injection (103 mg for SAA, 73 mg for PRCA and PWCA), followed by oral CyA (1 mg/kg). Anti-infectious prophylaxis included valganciclovir, bactrim, ciprofloxacin, itraconazole, and lamivudine in HBV+ patients. Injection related adverse events were irrelevant; mild hematological toxicity (neutropenia and/or thrombocytopenia) was observed in some patients, while lymphocyte depletion was immediate and long-lasting in all patients. With a median follow up of 39 months (range 3-75), infectious events were infrequent and clinically mild; there were 6 asymptomatic CMV reactivations, all responsive to valganciclovir. The response rates were 77% (38.5% CR) in AA and 84.5% (61.5% CR) in PRCA patients; both PWCA achieved long-lasting CR. Current stable remission was achieved in 38.5% of AA and 23% of PRCA; the majority of long-term responders have received an additional dose of alemtuzumab to sustain the hematological response. Long-term treatment failures were due to refractory relapses (15% for AA and 7.5% for PRCA) or to clonal evolution (15% for AA and 23% for PRCA). Overall survival (OS) in AA was 69%, with all deaths due to refractory disease. Unexpectedly, OS was only 31% in PRCA; the causes of death were disease evolution (3 leukemias and 1 AA), PRCA-associated morbidities (1 thymoma complicated with PML, 1 refractory connective tissue disease) or unrelated cardiovascular comorbidities. Long-term follow up of patients treated with alemtuzumab confirms that this agent has a remarkable efficacy for the treatment of immune-mediated bone marrow failures. Treatment-related long-term toxicity was acceptable, with a low risk of infectious complications; as with standard IS, late treatment failures were mainly due to relapse (alternative maintenance strategies might be considered) or clonal evolution.

P076

AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS): ESPERIENCE OF OUR AIEOP DEPARTMENT

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Autoimmune lymphoproliferative syndromes (ALPS) represent a failure of apoptotic mechanisms to maintain lymphocyte homeostasis, with accumulation of lymphoid mass and persistence of autoreactive cells that often manifest in childhood with chronic nonmalignant lymphadenopathy, hepatosplenomegaly, and recurring multilineage cytopenias. Multiple autoantibodies, positive Direct Coombs test, high serum IL-10, FAS-L and B12, Cytometric expression of lymphocytes CD3+ CD4- CD8- (DNT/double negative) and mutations of genes envolved in FAS pathway, which regulates apoptosis, are useful diagnostic markers. Suggested treatment is immunosuppression and in cases of refractory cytopenias it's necessary AlloBMT. In our AIEOP Onco-Hematology Department (A.O.R.N. "Santobono-Pausilipon" we have retrospectively analyzed a group of 6 patients affected by ALPS, everyone diagnosed in our Unit from 2000 to 2013. Patients had mean age of 52.5 months (range 19 months – 11 years), 1 M/5 F. Firstline treatment was endovenous IgG + corticosteroids in 3/6 (50%), with a CR which lasted 7 months, a PR since 7 months and a non-responder, only endovenous IgG in 1/6 (16%) with a PR for 18 months, and Cyclosporine + corticosteroids in 1/6 (16%) with a PR which lasted 4 months. 5/6 (83%) underwent to a second line treatment. In 1/6 (16%) corticosteroids treatment with a stable CR since 34 months. In 2/6 (33%) secondline was standard-dose Rituximab in 4 administrations with 2 CR: in one case followed by manteinance with endovenous IgG since 6 years and the other lost the response after 6 years and underwent to AlloBMT, with a CR 34 months from the procedure. In 33% (2/6) second line was Sirolimus with a complete response since 16 months and a partial response since 18 months. Mean OS is 58,8 months (range 20 - 154 months), and they're continuing followup in our Unit. Diagnosis and management of ALPS and ALPS-related diseases are a great challange not only for OncoHematologist but also for Pediatric team who often discover the symptoms. In our cases, it was essential for diagnosis the increase in DN Lymphocytes and FASL-induced apoptosis. Therapy was necessary and almost every the patients needed a second line treatment. Rituximab and sirolimus can be considered effective and well tolerated second line treatments in refractory and relapsed patients. ALPS are a chronic disease, with many treatments but without a cure, that's why we need to clarify role of AlloBMT, the only curative treatment.

P077

EVIDENCE OF CLONAL B-LYMPHOCYTES IN PATIENTS WITH "PRIMARY" AUTOIMMUNE HEMOLYTIC ANEMIA

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The detection of an associated disease, in particular, a lymphoproliferative disorder, in patients with an autoimmune hemolytic (AIHA) diagnosis is a relevant issue since these patients may benefit upfront from a more specific treatment approach. Between January 2000 and March 2013, all patients with a AIHA diagnosis defined as "primary" on a clinical basis excluding an associated disease (malignancy, lymphoproliferative or autoimmune disease, infection) were included in this study. Before treatment, a diagnostic work-out was performed including a total body CT scan, or chest X-ray with an abdomen ultrasound, a bone marrow (BM) aspirate and biopsy, a peripheral blood (PB) and BM immunophenotype by 4 color flow-cytometry analysis (CD5/CD3/CD20; CD23/CD10/CD19/CD20; Ig/Ig; CD19/CD5; CD3/CD4/CD45/CD8) an autoantibody panel (anti-nuclear, anticardiolipin IgG/IgM, LAC, anti-beta 2-glycoprotein-I IgG/IgM, antiprothrombin IgG/IgM, anti-thyroid peroxidase, anti-thyroglobulin antibodies); HBV, HCV and HIV serology. Seventy-three patients were included in the study, 29 males and 44 females with a median age of 66 years (range: 18-83 years). The median Hb value was 7.8 g/dL (range: 4.2-11 g/dL) and the median value of indirect bilirubin 2.26 mg/dL. The AeAb isotype form was an IgG in 46 cases (63%), IgM in 26 (36%) and IgA in 1. A primary AIHA diagnosis was confirmed in 44 (60%) cases while a cancer was diagnosed in 2 (breast, 1 case; larynx, 1), an autoimmune disorder in 4 (5%; Hashimoto's thyroiditis,1; athrophic gastritis, 1; autoimmune hepatitis, 1; biliary cirrhosis,1) and an active HCV hepatitis in 1. The presence of clonal B-lymphocytes involving the BM and the PB (median number, 0,310 x10^9/L) displaying most frequently a lymphoma-like phenotype (CD5-/CD20+/CD23±) was detected in 21 (29%) patients while a splenic ago-biopsy revealed the presence of a DLBCL in 1 case. More frequently, patients with the evidence of a lymphoroliferative disorder were characterized by an IgM AeAb (p= .03). The results of this study show that in 30% of patients with a AIHA defined as primary on a clinical basis an extended diagnostic work-out revealed the presence of an associated lymphoproliferative disorder. These patients should be considered for a more appropriate treatment approach including a monoclonal antibody.

P078

A NEW TP53 MUTATION DETECTED AT DIAGNOSIS IN A PATIENT WITH B-CELL PROLYMPHOCYTIC LEUKEMIA WITH DOUBLE PRODUCTIVE IMMUNOGLOBULIN SEQUENCE REARRANGEMENTS

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A 63-year-old male patient was admitted to our Hematology Department for marked leucocytosis (WBC: 28x10^9/L) with an absolute lymphocytosis (90%; 25.500x10^9/L); Hb was 14.9 g/dL and platelet count was 149x10^9/L. No systemic signs were reported. The peripheral blood smears examination showed the presence of 70% of medium-sized lymphocytes, with round nucleus, a prominent central nucleolus and a relatively small amount of faintly basophilic cytoplasm. Flow cytometric analysis was carried out and a population of monoclonal cells (69% of total cells) was detected with the following pattern: CD19+, CD5+, CD23+dim, CD20+, CD10-, CD22+, FMC7-, CD11c+, CD79b++, CD25+dim, CD49d+, CD200+dim, cyCD79a+, CD43-, CD38-, CD138-, CD103-, CD30-, TdT- and kappa light chain immunoglobulin with high intensity. A bone marrow biopsy was performed and the histology evaluation confirmed the presence of a monoclonal k-light chain population, CD5+, CD20 +, CD3 -, CD 10-, Cyclin D1-. Molecular analysis for IgVH genes evidenced a pattern of biclonality with two VDJ produc-

tive functional rearrangements: the first unmutated V3-74*01 D6-13*01 J4*02, the other mutated V4-31*03 D2-2*01 J5*02. Comparing data to known stereotyped CDR3 sequences available on line databases, the unmutated rearrangement resulted stereotyped with subset #53. FISH (Fluorescence In Situ Hybridization) and MLPA (Multiple Ligation Probes Amplification) analysis assessed deletions in EBF1(5q33.3), MET(7q31.2) and p53(17p13) in 70% of cells. As abnormalities of the TP53 gene are associated with a particularly severe prognosis in patients with B- CLL and this tumor-suppressor is mostly inactivated by the deletion of one and mutation of the other allele, we screened for mutations TP53 exons 4-9 by sequencing. An insertion of 22bp in exon 8 was assessed, of these 18bp resulted in a duplication from nt.13850 to nt.13868 disrupting the frame of the sequence. The alteration was validated using IARC TP53 Mutation and UMD TP53 Mutation Databases. The patient persists in an asymptomatic phase of the disease after 6 months of follow-up, without significant modification of blood cell counts and, consequently, no specific therapy was initiated.

P079

QUALITY CONTROL PROCESS IN THE JACIE ACCREDITATION: EXPERIENCES IN THE SPECIALISTIC LABORATORY OF CRIO

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A quality management system JACIE (Joint Accreditation Committee International Society for Cellular Therapy and the European Group for Blood and Marrow Transplantation), was introduced to improve quality of care of hematopoietic stem-cell transplantation (HSCT) in our transplant center from to May 2012 . Implementation of this involves a significant investment of time and resources by applicant centers. Generally the most common deficiencies were in quality management, for this we would like to evaluate improved laboratory quality from introduction of JACIE. Data on 18 allogeneic (39%) and 42 autologous (61%) HSCTs, 98 therapeutic uv treatment, 4 BM treatment for AB0 incompatibility and 85 thaw cell products and relative vitality evaluations were evaluated from Jan. 2010 to Apr. 2013. The parameter introduced in the course accreditation were not previously considered. Control microbiological and dust control in cell handling laboratory, microbiological quality control pre freezing of stem cell with DMSO , vitality control pre freezing and after thawing, bags temperature control during transport cell were evaluated. Were are performed in total 33 Standard Operative Procedure (SOPs),: 3% for specialist laboratory, and 36% for hematology department, operative instructions performed for laboratory were 32% (n= 19). In the accreditation, 23 audit were performed, the 35% involves laboratory.Audits performed have affected the laboratories an improvement performance over time, mainly due to the implementation of the system. From May 2012, the traceability of cell therapy products has been implemented. At 31/12/2012 were carried out 68 freezing stem cell products,1 minimal cell manipulation and 2 allogeneic cell counts.In addition, from July 2012 we introduced the bacteriological control of cell products in pre apheresis phase and pre freezing phase. The 1% of products was contaminated with Staphylococcus epidermis, this survey will implement a timely prophylaxis therapy on the receive before reinfusion of the product. Mapping revealed an uneven distribution of indicators and microbiological results across the different sub processes that contribute at revised and improve these highly specialized diagnostics procedure. The path of accreditation has a positive impact on all staff by raising the professional skills, improving them with training and responsibility and combining them all into one working group.

P080

FLOW CYTOMETRY SCREENING OF LYMPHOCYTE SUBPOPULATIONS IN BRONCHOALVEOLAR LAVAGE FLUIDS: EVALUATION OF PARAMETERS INDICATIVE OF IN-DEPTH IMMUNOPHENOTYPE ANALYSIS IN THE SUSPECT OF PULMONARY T-CELL NON HODGKIN LYMPHOMA

Cesana C,[1] Scarpati B,[1] Scampini L,[1] Zilioli VR,[2] Rusconi C,[2] Klersy C,[3] Barba C,[1] Ferri U,[1] Liga G,[1] Chiericozzi M,[4] Torre M,[5] Morra E,[2] Vanzulli A,[6] Rossini S[1]

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In bronchoalveolar lavage fluid (BALF) analysis by flow cytometry (FC), screening parameters eventually suggesting the need of an in-depth phenotype characterization leading to prompt lung T-cell non Hodgkin lymphoma (NHL) diagnosis are undetermined. Among BALF analyzed by FC between 2002 and 2013 with available follow-up for longitudinal retrospective study to notice definitive diagnosis at the time withdrawal, we selected BALF with sure pulmonary localization of T-cell NHL (cases) and at least 6 BALF per case with definite non hematologic disease (controls), matched for computed tomography findings. FC screening provided for total cellularity and CD3-CD4-CD8-CD16/56-CD19 panel, measurements containing 50,000 cell events in the list mode storage. FC parameters detected in cases were compared with the median corresponding values of those detected in controls by means of Wilcoxon test for paired comparisons. After a median of 5 [interquartile range (IQR): 4-16] months, 6 cases (each, peripheral T-cell NHL, n.o.s.) were identified (enlarged mediastinal lymph nodes, n=1; parenchimal densities, n=2; ground glass pulmonary infiltrates, n=2; parenchimal densities plus ground glass infiltrates, n=1). After a median of 17 (IQR: 8-26) months, 62 (median per case: 11, IQR: 8-12) matched controls (infection, n=40; sarcoidosis, n=6; drug-induced pneumopathy, n=5; bronchiolitis, n=4, graft rejection, n=2; thromboembolism, n=1, lung cancer, n=1) were identified. As compared to controls, BALF cases showed significantly higher median lymphocyte event number [18,761 (IQR 18,611-19,392) *vs* 6,545 (IQR 5,509-10,581)], median % of lymphocytes on total leucocytes [41% (IQR 35%-58%) *vs* 15% (IQR 10%-23%)], median T lymphocyte event number [18,126 (IQR 17,756-18,537) *vs* 6,162 (IQR 5,099-10,112)], and median % of T lymphocytes on total leucocytes [41% (IQR 35%-57%) *vs* 12.5% (IQR 8%-19.5%)](each P, <.05). Total cellularity (193,736/mL *vs* 265,405/mL), leucocyte events (46,378 *vs* 48,186), B (33 *vs* 76) and NK (215 *vs* 146) lymphocyte events, % of B (0.1% *vs* 0.1%) and NK (0.2% *vs* 0.3%) lymphocytes on total leucocytes, % of T (96% *vs* 93%), B (0.2% *vs* 1%), NK (1.4% *vs* 3.7%) lymphocytes on total lymphocytes did not differ significantly between cases and controls. FC evaluation of T lymphocyte as events and as % of leukocytes in BALF seems to strongly suggest the suspect of lung T-cell NHL infiltration, and therefore the need of further deepened FC BALF analysis.

P081

FLOW CYTOMETRY ANALYSIS OF BRONCHOALVEOLAR LAVAGE FLUIDS: EVALUATION OF SCREENING PARAMETERS SUGGESTING THE SUSPECT OF PULMONARY B-CELL NON HODGKIN LYMPHOMA

Cesana C,[1] Scarpati B,[1] Scampini L,[1] Zilioli VR,[2] Rusconi C,[2] Klersy C,[3] Barba C,[1] Ferri U,[1] Liga G,[1] Chiericozzi M,[4] Torre M,[5] Morra E,[2] Vanzulli A,[6] Rossini S[1]

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Flow cytometry (FC) study of bronchoalveolar lavage fluid (BALF) is frequently required in patients with B-cell non Hodgkin lymphoma (NHL)(B-NHL). In an attempt to find screening data indicative of possible B-NHL lung localization, we retrospectively selected BALF analyzed by FC between 2002 and 2013 with definitive diagnosis at withdrawal

[BALF with sure pulmonary B-NHL localization (cases), and at least 6 controls per case with non hematologic disease, matched for computed tomography findings], as detected by reliable follow-up. FC screening included total cellularity and CD3-CD4-CD8-CD16/56-CD19 panel, measurements containing 50,000 cell events in the list mode storage. Data detected in cases were compared with the median corresponding values of those detected in controls by means of Wilcoxon test for paired comparisons. After a median of 18 [interquartile range (IQR): 5-33] months, 6 cases (2 mantle cell NHL, 1 Burkitt-like NHL, 1 B-cell chronic lymphocytic leukemia, 1 diffuse large B-NHL, 1 follicular NHL) were identified (ground glass pulmonary infiltrates, n=2; enlarged mediastinal lymph nodes plus parenchimal densities, n=2; nodules, n=1; parenchymal density plus nodules, n=1). After a median of 21 (IQR: 8-37) months, 56 (median per case: 10, IQR: 9-11) matched controls (infection, n=41; sarcoidosis, n=4; solid tumor, n=3; other, n=8) were identified. As compared to controls, cases showed significantly higher median lymphocyte event number [27,869 (IQR 22,234-40,055) *vs* 12,103 (IQR 8,671-12,924)], median % of lymphocytes on total leucocytes [65% (IQR 62%-69%) *vs* 26% (IQR 19%-34%)], median B lymphocyte event number [997 (IQR 224-2,814) *vs* 110 (IQR 49-160)], median % of B lymphocytes on total leucocytes [2.9% (IQR 0.7%-4.7%) *vs* 0.2% (IQR 0.1%-0.3%)], and median T lymphocyte event number [26,657 (IQR 21,699-36,588) *vs* 11,530 (IQR 7,250-11,964)](each P, <.05). Total cellularity (319,900/mL *vs* 232,053/mL), leucocyte (49,134 *vs* 49,180) and NK lymphocyte (374 *vs* 270) events, % of T (58% *vs* 25%) and NK (0.9% *vs* 0.7%) lymphocytes on total leucocytes, % of T (92% *vs* 93%), B (4.6% *vs* 1%), NK (1% *vs* 2.3%) lymphocytes on total lymphocytes did not differ significantly between cases and controls. FC evaluation of B lymphocytes as event number and as % of leukocytes in BALF seems mandatory to eventually carry on deepened B-cell immunophenotying in the suspect of lung B-NHL, despite the detected accompanying T-cell lymphocytosis.

P082

REAL-TIME MULTIPLEX MOLECULAR DETECTION OF BCR-ABL P190 AND P210 TRANSCRIPTS BY Q-RT-LAMP ON THE LIAISON IAM SEMI-AUTOMATIC INSTRUMENT

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Introduction. molecular detection of BCR-ABL transcripts is routinely performed to confirm diagnosis of suspected Chronic Myeloid Leukaemia (CML) and for risk stratification of B-precursor Acute Lymphoblastic Leukaemia (ALL). RT-PCR is the method of choice for this purpose, consisting in at least 3 hours of complex procedure. Methods. BCR-ABL Q-RT-LAMP is a non-PCR method consisting in multiple primer sets able to selectively recognize both p190 and p210 BCR-ABL transcripts and the Gus internal control gene. Patient total RNA is incubated with the reaction mix at constant temperature for 50 minutes. The reaction can proceed directly from RNA thanks to the employment of a DNA polymerase with reverse transcription activity and can be monitored in real time due to labelled specific probes in the Liaison IAM instrument. Moreover, the Liaison IAM automatically analyses data releasing final result. Results. The assay sensitivity has been analytically determined by testing serial dilutions of mutated p190 and p210 RNA (from TOM-1 and K-562 cell lines respectively) into wild type RNA from HL-60 cell line, resulting down to 10-4 and 10-5 respectively. The assay specificity has been established by testing 275 replicates of wild type RNA from 7 cell lines, all resulted BCR-ABL negative in the presence of the correct amplification of the GUS internal control (100% specificity). The BCR-ABL RT-LAMP assay was finally validated on clinical samples and analyses were compared with conventional RT-PCR method. The results were 100% concordant (35 samples out of 65 were positive for p210, 30 out of 65 were positive for p190 transcript). Specificity was confirmed on 60 additional negative clinical samples (30 healthy donors and 30 Ph negative patients) all resulted BCR-ABL negative as expected. Conclusions. The semiautomatic detection of the p190 and p210 BCR-ABL transcripts by the triplex Q-RT- LAMP performed on the Liaison IAM instrument represents a novel system for efficient diagnosis of CML and Ph positive ALL. The multiplex, one-step format reduces the contamination risks of conventional multi step RT-PCR and simplifies the overall procedure. The validation of negative results through amplification of endogenous GUS mRNA ensure reliability. Finally, the high sensitivity, specificity and rapidity significantly improve the patient management and the diagnostic laboratory routine.

P083

A NEW PROMISING APPROACH TO PERFORM HLA TYPING IN A QUICKLY, SIMPLE AND ACCURATE WAY: THE PYROSEQUENCING

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The HLA genes are the most polymorphic in the human genome and are characterized by a large number of alleles and haplotypes. Nowadays a variety of methodologies are available for HLA typing both at the protein and at nucleic acid level, but ambiguity can affect the possibility of the right call for each HLA-locus. In particular, phase ambiguities arise from the incomplete genomic coverage or the contemporary Sanger sequencing of two heterozygous alleles that determines different haplotypes. The new generation sequencing (NGS) technologies have the potential to perform HLA-typing in a simply, rapid and accurate way without phase ambiguities. In this study we performed high-resolution HLA-typing using an NGS platform based on pyrosequencing and subsequent bioinformatic analysis and we evaluated his feasibility, reliability and robustness in 40 samples. Fourteen amplicons for sample were synthesized using two custom assay. The output file was then uploaded into JSI SeqPilot software to align all sequences with the reference database (ref 3.9 2012). The PCR reactions generate 560 amplicons who correspond to HLA-A/B/C exons 2, 3 and 4, DQB1 exons 2 and 3 and DPB1, DQA1, DRB1/3/4/5 exon 2. Using Multiplex Identifier (MID) tag method, we pooled amplicons from different samples and to analyze them contemporary. We pooled our 40 samples into 8 pools (5 different samples in each one) and performed 8 sequencing runs. We have obtained over of 150 reads for most amplicons. The assignment of unambiguous genotype was possible on 45.5% of alleles. The ambiguities were related to the assay design (above all for class-II). Notably, some ambiguities on the locus B and C have a little biological importance because the genomic differences between the two alleles were located on the transmembrane domain coding region, soboth alleles code for the same peptide binding domain, instead. Ten cases analyzed in this study were also genotyped using conventional strategies resulting in a concordance of 100%. Clonal amplification and pyrosequencing strategy is a feasible and reliable method to perform the HLA-typing. This technology discriminates very well the alleles that determine differences on the peptide binding domain. Using a NGS platform we quickly have obtained a high-resolution HLA-typing for the most important loci of the samples without phase ambiguities. This work was supported by Lions Club Bassa Bresciana, BCC Pompiano e Franciacorta and by Roche Diagnostics.

P084

CLOSE-TUBE, ONE STEP MOLECULAR DETECTION OF AML1-ETO AND CBFB-MYH11 TRANSCRIPTS IN 40 MINUTES BY THE FLUORESCENT DIASORIN RT-LAMP

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Introduction. The AML1-ETO and CBF -MYH11 fusion transcripts resulting from the (8;21) and the inv(16)/t(16;16) aberrations are characteristic of Core-Binding Factor Acute Myeloid Leukemias (CBF-AML), a subclass of AML characterized by a good prognosis. The molecular testing by RT-PCR is therefore routinely performed with the purpose of risk stratification. Methods. here we present 3 novel RT-LAMP (Retro Transcription-Loop mediated isothermal AMPlification) assays aimed to sim-

plify and speed-up the molecular detection of the AML1-ETO and CBF -MYH11 fusion transcripts in AML. The system consists in one duplex fluorescent assay specific for the AML1-ETO transcript and in two fluorescent multiplex assays specific for the most common CBF -MYH11 transcripts (type A and type D,E respectively).The 3 assays simultaneously detect also the endogeneous Gus mRNA, that acts as internal control. Both AML1-ETO and CBF -MYH11 RT-LAMP assays proceed at constant temperature directly from total RNA for 30 and 40min respectively in a fluorescent, close-tube format, monitorable in real time. Results. The analytical sensitivity has been determined by testing serial dilutions of mutated sequence (RNA from Kasumi cell line positive for t(8;21), RNA from ME-1 cell line positive for t(16;16) typeA and plasmids carrying the CBFB-MYH11 type D and E sequences) into wild type RNA from HL-60 cell line. Dilutions containing the AML1-ETO transcript at 10-4 and 10-5 levels have been detected on 112 replicates within 30 minutes in 100% and 80% of times respectively. The 10-4 ME-1 dilution has been detected 98.6% of times on 72 replicates within 40 minutes while the 20 copies of plasmids D and E in wild type RNA have been consistently detected on 100 replicates. The analytical specificity resulted 100% in all the assays on 130 replicates of wild type RNA. The systems have been finally tested on clinical samples derived from CBF-AML positive patients, previously tested by RT-PCR (16 AML1-ETO positive, 15 CBF -MYH11 positive), and on 30 RNA samples from healthy donors demonstrating 100% concordance with the conventional assay. Conclusions. the AML1-ETO and CBF -MYH11 RT-LAMP assays represent a convenient solution for molecular diagnosis of CBF-AML.The close-tube format simplifies the procedure, fastening the set-up and decreasing risk of errors and contamination. The multiplex format monitorable in real time optimizes time and resources and ensures absence of false negative results.

P085

MINIATURIZED AND AUTOMATED FISH APPROACH (AUTOFIND F) FOR SCREENING AND CHARACTERIZATION OF GENETIC LESIONS IN HEMATOLOGICAL MALIGNANCIES

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Introduction. Multiple myeloma (MM) and chronic lymphocytic leukaemia (CLL) are hematologic malignancies characterized by a large clinical, biological and genetic heterogeneity. In particular, genetic lesions associated to these neoplasms are routinely detected by fluorescence in situ hybridization (FISH) for their important prognostic role. Methods. We have used a novel platform (autoFIND F) that performs FISH analyses in a fully automated modality. Based on the miniaturized device microFIND™ (Zanardi A, Biotechique, 2010), the instrument executes automated FISH allowing saving technician time, samples, reagents and probes, providing a ready to use standard slide for imaging analysis. Cytological sample is captured and concentrated in a microchannel, allowing a faster analysis of the required nuclear targets. Being this system a novel approach, we have sought to compare autoFIND F results with standard FISH protocols. Highly purified peripheral mononuclear CD19+ cells from 12 CLL and CD138+ purified bone marrow plasma cells from 12 MM were investigated. Deletions at 11q23, 17p13, 6q21-23 as well as trisomy 12 were investigated in CLLs, whereas structural and numerical abnormalities including t(11;14) t(4;14), t(14;16) translocations and chromosomes 1 and 17 alterations were tested in MMs. All of the probes are commercially available and have been selected from different commercial providers (Abbott Chicago, IL or Cytocell, Cambridge, UK). Results. A complete concordance of diagnostic results for all tested probes was found when FISH and autoFIND F were compared. Notably, the imaging analysis showed a better definition of the specific hybridization signals with respect to standard FISH protocol. This improvement in the quality of hybridization makes easier the interpretation of FISH results especially for MM patients, which are characterized by complex karyotypes. Conclusion: We demonstrated that autoFIND F approach provides high quality FISH tests together with the saving of technician time and reagents, suggesting that its introduction in routinely cytogenetic diagnostic labs could improve turnaround time for FISH results, by performing "high content" FISH tests at affordable costs. In addition, this novel platform, by providing good quality FISH results, makes easier to evaluate the signal patterns distribution in patients showing complex karyotypes.

P086

MRD DETECTION FOR ALL BASED ON THE IKZF1 4-7 DELETION

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Several studies have shown that detection of minimal residual disease (MRD) is a strong and independent prognostic factor in adult ALL. In particular, the presence of MRD appears to be highly informative to identify patients with a high chance of relapse. The gold standard for MRD analysis in ALL is the use of RQ-PCR-based quantification of leukemic immature Ig/TCR gene rearrangements, however, the concept of using disease-related markers for ALL MRD testing has been already established for fusion transcripts such as BCR-ABL, EA2/PBX1 and MLL/AF4. The IKAROS (IKZF1) gene, which codes for the lymphoid transcription factor IKAROS is very commonly involved in copy number alterations (CNAs) in Ph positive ALL , CNAs in IKZF1 were also detected in high risk B-ALL Ph-negative. One quarter to one third of patients with IKZF1 alterations had a deletion of exons 4-7. The deletion breakpoints for these IKZF1 4-7 alterations are usually located within a few nucleotides, and have specific N regions comparable to Ig/TCR gene rearrangement; these characteristics make it a valuable marker for MRD studies. We particularly focused on the comparison of MRD assay performance obtained by IKZF1 marker and Ig/TCR and BCR/ABL standard Methods. We identified, according to Iacobucci *et al* (Blood, 2099) 6 out of 36 B-ALL patients (16%) carrying IKZF1 4-7 deletion, for all patients we had a second MRD standard marker available (3 of them an Ig marker and the others the BCR/ABL marker). We performed MRD analysis using Ig according to EuroMRD protocol and BCR/ABL MRD monitoring according to EAC protocol. For IKZF1 MRD assay we used the reverse primer specific for the unique N regions at the breakpoint fusion site and consensus forward primer and probe as described by Venn *et al* (Leukemia, 2012). Established EuroMRD guidelines were used to assess all MRD test performances; all IKZF1 MRD tests showed high specificity with no levels of background amplification, quantitative ranges between 5X10E-4 and 10E-4 and sensitivities between 10E-4 and 10E-5. A minimum of 3 follow up points were studied for all patients and we obtained a close concordance of MRD results between IKZF1 and standard markers in all patients. According to Venn *et al*, we confirm in our experience that IKZF1 gene deletion will provide an additional marker for MRD detection in ALL.

P087

IMPROVED DETECTION OF THE C-KIT D816V MUTATION USING ALLELE-SPECIFIC ARMS REAL TIME PCR ASSAY ALLOWS A FINER RECOGNITION OF PATIENTS WITH INDOLENT SYSTEMIC MASTOCYTOSIS

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The somatic D816V mutation of the KIT gene is present in more than 90% of Systemic Mastocytosis (SM) and represents one of the minor criteria for its diagnosis by the World Health Organization (WHO) classification. Patients with indolent SM, could have a very low mast cell (MC) burden, especially if lacking skin lesions: in these cases a highly sensitive diagnostic test for D816V detection is necessary. In order to improve detection of D816V c-KIT mutation, we tested BM cells from 110 consecutive adult patients referred with a suspicion of SM to our

Multidisciplinary Outpatient Clinic for Mastocytosis in Verona from May 2009 to July 2011 using both RT-PCR+RFLP (Reverse Transcriptase Polymerase Chain Reaction with Restriction Fragment Length Polymorphism) followed by sequencing, and ARMS-RTq-PCR (Amplification Refractory Mutation System-Reverse Transcriptase and Real Time PCR), respectively in Bologna and Verona laboratories. All patients underwent to a BM evaluation with histology/cytology and flow cytometry. For both D816V c-KIT mutation methods RNA was extracted on BM mononuclear cell fraction and reverse transcribed. Then for PCR+RFLP/sequencing assay a RT-PCR product of 287 bp, spanning exons 16-18, was digested with HinfI to detect D816V (variant cell population must be at least 4% to be detectable). For patient samples that did not harbour D816V mutation, the RT-PCR product was further investigated by direct Sanger sequencing to detect other site mutations (sensitivity of about 25%). These methods revealed D816V mutations in 47 patients. Simultaneously BM samples were analyzed by ARMS-RTq-PCR, setting a cut-off of positivity at 0.001% by MC line HMC-1 RNA dilutions: D816V was identified in 77 patients, corresponding to 100% of cases showing CD25+MC. SM was diagnosed in 76 cases with ARMS-RTq-PCR, Cutaneous Mastocytosis (CM) in 1 and Monoclonal MC activation syndrome (MMAS) in 1. Seventeen out 76 SM patients would not have satisfied sufficient WHO criteria for the diagnosis of SM on the basis of RT-PCR+RFLP and would diagnosed as CM (6), MMAS (10), idiopatic myelofibrosis (1). Patients with discordant D816V results had significantly lower serum tryptase levels, lower amount of CD25 MC and less frequently fulfil major histological criteria (Table 1). We suggest that a ARMS-RTq-PCR technique, along with a highly sensitive flow cytometry assay, could improve the recognition of patients with indolent SM with a very low MC burden; then negativity assessment must be verified by d-HPLC/sequencing.

Table 1. Characteristics of 78 patients with final diagnosis of clonal Mast cell disorder according to the result of D816V detection with RT-PCR+RFLP/sequencing and ARMS-RTq-PCR

	Total	Concordant D816V result	Discordant D816V result	p
Cases of CMD	78	48	30	
Male/female ratio	1.0	1.0	1.0	ns
Median age (range) years	49.1±15.2	50.8±15.6	46.5±14.5	ns
Serum Tryptase ng/mL; mean±SD	30.6±21,4	35.5±27.7	14.5±22.6	0.002
Serum Tryptase < 20 ng/mL	30 (38.5%)	15 (31.2%)	15 (50.0%)	
UP skin lesions; n° (%)	36 (46.1)	25 (52.1)	11 (36.6)	0.18
Major histological criterium; n° (%)	26 (33.3)	20 (41.7)	6 (20.0)	0.048
MCs CD25+, mean % + SD of MNC evaluated by flow cytometry	0.10±0.16	0.15±0.19	0.039±0.065	<0.001

P088

IMPLEMENTING A BIOBANK OF HEMATOLOGICAL MALIGNANCIES

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In recent times, the increasing need to prospectively and retrospectively analyze biological samples in experimental studies have made it necessary to build up a structured centralized biobanking activity in our hematology unit. This project was also supported by the evidence of the usefulness of banking provided by several international study groups. We started to collect samples in a structured biobank of hematological malignancies from 2009. All the patients enrolled signed a dedicated informed consent approved by local IRB. Both patients' bone marrow and peripheral blood are collected at diagnosis and at specific follow up time points. The laboratory performs mononuclear cells separation, serum, plasma and cells isolation by immunoselection. Cells are vitally conserved and samples are preserved in liquid nitrogen vapors in bar-coded tubes cataloged and recorded by easy track software to ensure traceability. The stored samples are recorded for disease status, treatment, cellular amount and volume. The biobank database categorizes patients according to FAB and WHO classifications; the data are available on web-platform to authorized researchers by Cryosmart® software. Additional information (morphological, cytometric, cytogenetic and molecular biology data) are also available on request. To ensure the reliability of the data, a multidisciplinary working group from different diagnostic areas provides the review of new cases enrolled and refines the diagnostic classification according to WHO classification. Access to the stored samples is controlled by the Scientific Committee of the biobank. Fourteen projects have been submitted in the leukemia area and 12 for the myeloma area up to now. From 2009 to April 2013, 660 new patients were enrolled (median 15 admissions/month). About 2300 samples were processed for a total of more than 31.200 vials. Of these, 1500 were used and about 1000 are reserved for active projects. Recently , an area dedicated to healthy volunteers has been activated, also collecting bone marrow and peripheral blood. Up to now, 15 papers have been produced with the assistance of the biobank, others are runnings. Collaboration with other institutions are ongoing, particularly promising is the creation of a network of biobanks (Biorel) for MDS samples under the supervision of Rete Ematologica Lombarda (REL).

Allogeneic and Autologous Transplantation I

P089

COMPARISON BETWEEN BONE MARROW MESENCHYMAL STROMAL CELLS (BM- MSC) AND LUNG MESENCHYMAL STROMAL CELLS (LUNG-MSC) FOR EPITHELIAL REGENERATION

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Developing a therapeutic strategy for lung regeneration still remains complex. Stem cell- based therapeutical approaches have been suggested as a potential tool; among them, human mesenchymal stromal cells (MSC) possess some promising features to this aim. MSC are stem cells residing in many tissue, *i.e.* bone marrow (BM), adipose tissue, cord blood, lung, etc., and they are capable of differentiating into different cell types of mesodermal origin, such as fat, bone and cartilage. To assess MSC epithelial differentiation potential, through a partially known process called Mesenchymal to Epithelial Transition (MET), MSC were collected from bone (BM) aspirates and lung biopsies after informed consent. MSC were characterized by immunophenotyping and clonogenicity assay. MSC mesodermal differentiation potential was assessed by testing their ability to differentiate into adipocytes, osteoblasts and chondrocytes. MSCs at different culture passages were induced to acquire the epithelial phenotype by culturing in presence of retinoic acid. The epithelial differentiation was checked by quantitative RT-PCR, immunofluorescence and a functional assay based on the Trans Epithelial Electrical Resistance Measurement (TEER). In presence of retinoic acid, MSC from BM and, mostly, lung upregulated a panel of general epithelial genes (cytokeratin 18, occludin, tight junction protein and claudin) and downregulated some specific mesenchymal markers (smooth muscle actin, snail2, vimentin, THY1), as detected by quantitative RT-PCR. Immunofluorescence confirmed the presence of E-cadherin4, occludin and cytokeratin 18 in a small number of cells (about 0,2%). Trans Epithelial Electrical Resistance (TEER) measurement confirmed that MSC can acquire *in vitro* partial epithelial polarization after retinoic acid treatment. These data show that MSC can be induced to differentate into cells resembling some morphological, phenotypical and functional properties of epithelial cells. BM-MSC are less prone to acquire an epithelial phenotype as compared to hLung-MSC. Additional *in vivo* studies on mouse model with lung damage are in progress.

P090

PERIPHERAL BLOOD STEM CELL MOBILIZATION WITH BIOSIMILAR GRANULOCYTE COLONY-STIMULATING FACTOR ZARZIO®. A "REAL LIFE" EXPERIENCE FROM A SINGLE CENTER

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Introduction. Recombinant granulocyte colony-stimulating factors (G-CSFs) are widely used to mobilize peripheral blood stem cells (PBSC). Recently, several forms of biosimilar nonglycosylated recombinant human G-CSF have been clinically developed and are now available in Europe. However, there are few data regarding biosimilar G-CSF for PBSC mobilization in hematological diseases. We investigated efficacy and safety of a new biosimilar G-CSF (Zarzio®) in PBSC mobilization in patients (pts) with hematological cancers. PATIENTS AND Methods. Between January 2012 and March 2013, at Hematology Unit of Niguarda Ca' Granda Hospital, 29 pts received Zarzio® following chemotherapy for PBSC mobilization. We treated 29 pts affected by Hodgkin's Lymphoma (5 pts), non Hodgkin's Lymphoma (18 pts) and Multiple Myeloma (6 pts); median age 55 yrs (range 23–64). The mobilizing chemotherapy regimens for lymphomas included: ICE (days 1-3 etoposide 100 mg\mq, day 2 ifosfamide 5 g\mq and carboplatin AUC of 5), IGEV (days 1-4 ifosfamide 2 g\mq, days 1 and 4 gemcitabine 800 mg\mq, day 1 vinorelbine 20 mg\mq) or ID ARA-C (days 1-3 ARA-C 800 mg\mq). All multiple myelomas were mobilized with cyclophosphamide 3-4 g\mq. From the second or the third day after the end of chemotherapy, the pts received subcutaneous Zarzio® (5 ug\kg\day) up to white blood cell (WBC) recovery. Stem cell collection was started when the absolute number of CD34+ was more than 20\uL. PBSC harvesting was considered optimal if >5x10^6\CD34+ cells\kg were collected. Results. WBC recovery of more than 1x10^9\L was observed after a median of 10 days (range 8-14) following mobilizing chemotherapy. The median peak of absolute PB CD34+ cell number was 105,7\µL (range 37,1 – 201,5) reached on day +9 (median, range 8-19). The median time from the first day of stimulation with Zarzio® to the first day of leukapheresis was 9 days (range 5-19). Twenty five patients (86%) harvested a median of 10x10^6\CD34+ cells\kg body weight (range 4,4-43,2), with a single apheresis procedure in 17 cases. Four pts (14%) were unable to mobilize PBSC. The only adverse event induced by Zarzio® was mild bone pain. Sixteen pts were autografted with a median of 10,3 x10^6\CD34+ mobilized cells per kg (range: 5,1 - 18) with rapid and sustained engraftment. Conclusion. The use of Zarzio® in the PBSC mobilization was safe, and resulted in a sufficient stem cell harvest in the majority of patients.

P091

PROPHYLACTIC THERAPY WITH ORAL LOW-DOSE VALGANCYCLOVIR IN CYTOMEGALOVIRUS-POSITIVE ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

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Pretransplant cytomegalovirus (CMV) seropositivity in allogeneic stem cell transplant (HSCT) recipients is associated with the highest risk of CMV reactivation. The efficacy and safety of low dose oral valganciclovir (VGCV) as CMV reactivation prophylaxis was retrospectively evaluated in 32 cytomegalovirus-seropositive HSCT recipients (30 HLA-matched related and 2 unrelated HSCT) with a median age of 40 years (range 18-59). Primary diseases were acute myeloid leukemia (19), acute lymphoblastic leukemia (4), non Hodgkin's lymphoma (3), multiple myeloma (3) and myelodysplastic syndrome (3). Fifteen received a myeloablative conditioning regimen, while 17 patients received a reduced-intensity conditioning regimen. Twenty-one patients received graft-versus-host disease (GVHD) prophylaxis with cyclosporine-A (CsA) and methotrexate (MTX), and the others CsA with MTX and anti-thymocyte globulin. Graft source was mainly mobilized peripheral blood stem cells (80%). Seventeen HSCT recipients were transplanted in first complete remission (CR), the remaining in second CR (6) or with advanced disease (7). The median follow-up post-HSCT was 30±12 months. CMV infection was monitored weekly using polymerase chain reaction (PCR). VGCV was administered orally at dose of 450 mg daily for six months. Six patients (18%) developed asymptomatic early and late positive CMV-PCR on average 56 days after HSCT successfully treated with VGCV at 1800 mg/day, except one who developed fatal gastrointestinal CMV disease. At the time of CMV reactivation, four patients had been affected by grade II-IV acute GVHD and two by an extensive chronic GVHD. None of the patients required discontinuation of the oral VGCV secondary to specific gastrointestinal intolerance. Hematologic toxicity, such as mild anemia, neutropenia and thrombocytopenia was documented in seven cases (22%), but did not required drug discontinuation. The rate of non CMV-related infections was 25% and was similar in both groups with and without CMV reactivation. At the end of the follow-up, 18 of 32 patients were alive with a median follow up of 31 months (range 2-56). Relapsed-related mortality was 20%, transplant-related mortality was 9% and did not differ between group with and without CMV reactivation. Our data suggest that low dose VGCV is safe and effective as CMV reactivation prophylaxis in allogeneic CMV-seropositive HSCT recipients. These results require further validation in prospective randomized studies.

P092

ACCELERATED AND PERSISTENT BONE LOSS AFTER AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANTATION

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Osteoporosis and avascular necrosis (AVN) are long-lasting and debilitating complications of hematopoietic stem cell transplantation (HSCT). We describe the magnitude of bone loss, AVN and impairment in osteogenic cell compartment following autologous (auto) and allogeneic (allo) HSCT, through the retrospective bone damage revaluation of 100 (50 auto- and 50 allo-HSCT) long-term survivors up to 15 years after HSCT. Age at transplantation of HSCT recipients ranged between 18 and 50 years (median, 30) and their post-HSCT follow-up lasted from 1 to 15 years (median, 6). Primary diseases were acute (n=44) or chronic (n=13) myeloid leukemia, Hodgkin disease (n=17), non-Hodgkin lymphoma (n=12), and multiple myeloma (n=14). Bone mass density (BMD) was measured by dual energy X-ray absorptiometry (DEXA) at lumbar spine (LS, L1-L4) and femoral neck (FN), and by quantitative phalangeal ultrasonometry. At the time of testing (mean follow-up after HSCT: 65 months; range: 1-15 years), LS (Z score mean: -0.4 and -0.9 in auto- and in allo-HSCT recipients, respectively; $p<0.05$), FN (-0.6 and -1.4 in auto- and in allo-HSCT recipients, respectively; $p<0.05$) and phalanges (-1 and -1.5 in auto- and in allo-HSCT recipients, respectively; $p<0.05$) BMD were significantly reduced in comparison with BMD of 100 healthy controls ($p<0.001$ in all examined sites), suggesting more prolonged bone damage in cortical than in trabecular bone. BMD at LS, but not at FN, improved 3 years after HSCT in some patients, suggesting more prolonged bone damage in cortical than in trabecular bone. Phalangeal BMD values remained low for even more years, suggesting persistent bone micro-architectural alterations after HSCT. Eight patients developed AVN, 1 to 15 years following HSCT: 6 (12%) after allo-HSCT and 2 (4%) after auto-HSCT ($p<0.05$). Development of acute and chronic graft versus-host disease, was associated with both a more severe BMD reduction in all bone sites (<0.05) and with higher frequence of AVN. Reduced BMD and higher incidence of AVN was partly related to a reduced regenerating capacity of the normal marrow osteogenic cell compartment, measured by growing colony-forming units-osteogenic cells. Our results document that an accelerated bone mineral loss and micro-architectural deterioration occurs during the first years after HSCT and is more severe in the allogeneic setting, suggesting that all patients early after auto-HSCT and allo-HSCT should be evaluated for their bone status

P093

IMMUNOLOGICAL CHARACTERIZATION OF MULTIPOTENT MESENCHYMAL STROMAL CELLS. THE INTERNATIONAL SOCIETY FOR CELLULAR THERAPY (ISCT) WORKING PROPOSAL

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The large number of experimental approaches, culture conditions, qualitative and quantitative methods, and *in vitro* and *in vivo* models employed so far to assess immune regulatory properties of multipotent mesenchymal stromal cells (MSC) has led to an excess of literature data that sometimes are poorly comparable, redundant, and even contradictory. Thus, quite paradoxically, the risk is that pre- clinical literature data may become eventually weak and scarcely useful, in both researchers' and Regulatory Authorities' opinion, for supporting experimentally specific MSC-based clinical trials aimed at treating autoimmune and inflammatory diseases. However, some data in this field appear more solid and reproducible and may be generally accepted to suggest reproducible immunological assays to quantify the differences in immune modulatory properties of MSCs produced according to Good Manufacturing Practice (GMP). The MSC Committee of the International Society of Cell Therapy (ISCT) released a statement paper in 2006 that established the minimal criteria characterizing human MSC, without focusing particularly on their immunological properties. In the 7 years following the publication of this statement paper, more than 10,000 manuscripts on MSC, and many of them deal with immune regulation. To consolidate the scientific research in this field, the MSC Committee of the ISCT is publishing a working proposal paper aimed at stimulating the general discussion about the need of shared guidelines for the immunological characterization of MSCs for clinical use: 1. A standard immune plasticity assay should be implemented by using IFN- + TNF- as model *in vitro* priming agent 2. Functional analysis of an expanded cell product may provide mechanistic insights on intra- and inter- study variance in clinical response amongst patients 3. The use of purified responders would be widely practicable and should provide more generalizable guidance on relative functional potency of MSC and as a companion to clinical trials 4. Interrogating the IDO response as part of an *in vitro* licensing assay should be considered central 5. Conclusions based on xenorecipient animal models on how to conduct clinical trials should be drawn with caution 6. The prospective hypothesis-driven analysis of lymphocyte populations in patients groups treated with MSC should be encouraged 7. Clinical analysis should also include the monitoring of whether injected MSCs are the target of an immune response.

P094

EFFECTS OF A NOVEL CERAMIC BIOMATERIAL ON IMMUNE MODULATIVE PROPERTIES OF BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

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Bone is one of the most frequently transplanted tissues (1 million procedures/year in Europe), with allografts and autografts accounting for more than 80% of total grafts despite their considerable disadvantages. Synthetic biomaterials in association with autologous or allogeneic mesenchymal stromal cells (MSC) represent a valid alternative in orthopaedic and maxillofacial surgery. Aim of REBORNE consortium is to perform clinical trials using standardized protocols based on advanced biomaterials and MSC. Aim of our Immunological Unit inside Reborne is to assess MSC immunomodulatory properties in presence of a novel hydroxyapatite and tricalcium-phosphate biomaterial (HA/TCP - MBCP+®, Biomatlante) used as scaffold for MSC delivery. Bone marrow MSC were provided by REBORNE Production Centres. For proliferation assays, purified T, B and NK cells were stained with CFSE. For quantification of survival of immune effector cells after co-culture experiments caspase-3 cell staining was employed. Differentiation potential was evaluated by culturing MSC with two different media containing either bone morphogenetic protein 4 (BMP4) or dexamethasone (DXM). After 3 weeks, osteogenic differentiation was quantified by qRT-PCR, ALP activity and alizarin red staining. Results. Primed MSC (pre-treated with IFN- and TNF-) displayed HLA-ABC, CD54, CD106 upregulation and HLA-DR de novo expression, both in standard culture conditions and in association with HA/TCP, without significant differences in proliferation of immune effector cells between standard and 3D-coculture conditions. Resting MSC suppressed T and NK cell proliferation, more significantly after priming with inflammatory cytokines. B cell proliferation was inhibited only in co-culture with primed MSCs, with slight differences related to the culture system. Immune effector cell viability was

not affected by the biomaterial and MSC co-culture increased their survival even in presence of HA/TCP.

P095

UNMANIPULATED HAPLOIDENTICAL BONE MARROW TRANSPLANTATION (HBMT) WITH MYELOABLATIVE CONDITIONING (MAC) AND POST-TRANSPLANT CYCLOPHOSPHAMIDE (PT-CY)

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Introduction. The hBMT is available for virtually all pts who urgently need an allograft but lack an HLA- matched donor. Promising results were obtained by the Baltimore group (Luznik L. 2008) with hBMT consisting of non-myeloablative conditioning followed by PT-CY. We now report a series of 9 pts with high-risk hematologic malignancies. 7 of them received an hBMT with a modified MAC Baltimore protocol. 2 pts received the same MAC but with a 5 drugs combination as GVHD prophylaxis (Di Bartolomeo P. 2013). Results. From 2008 to 2013, 7 adult pts (M/F=5/2) and 2 pediatric pts (M/F=1/1) with high risk ALL (1 pediatric; 1 adult) and AML (1 pediatric; 6 adults) underwent hBMT. The median pts age was 33 y (range 5- 62). At time of transplant 7 pts were in CR (CR1=6, CR2=1) and 2 had refractory disease. The MAC consisted of TBF regimen: Thiotepa, Busulfan and Fludarabine. In 7 pts stem cells source was non-primed BM, and GVHD prophylaxis consisted of PT-CY (50mg/kg) iv on day + 3 and +4, CSA 2.5 mg/kg/day and MMF 45 mg/kg/day since day +4. In 2 pts stem cells source was G-CSF-primed BM and GVHD prophylaxis consisted of ATG (Fresenius), CSA, MTX, MMF and Basiliximab. Grafts contained a median of $6.7x10^8$/kg nucleated BM cells (range 0.6-19.8), $2.74x10^6$/kg CD34+ cells (range 0.91-7.09) and $0.4x10^8$/kg CD3+ cells (range 0.1- 0.9). The engraftment rate was 100% for PMN and 98% for plt, with a median time to PMN recovery (>0.5) of 23.5 days (range, 21-41) and to plt recovery (>20.0) of 26 days (range, 23-50). All patients had full donor chimerism by day +30. Non relapse mortality (NRM) was 22%: 1 pt died for sepsis, 1 for aGVHD. 2 pts had grade I and 2 grade II acute GvHD. Only 1 pt had a grade III, and 1 grade IV fatal GVHD. 3 pts had limited chronic GvHD. Up to now, only 3 pts have relapsed and of these 2 have died. 5 pts are currently surviving, 4 of them are disease-free with median time of 114 days (range 23-764 days) from transplant and one has active disease; 2 pts relapsed after 311 and 238 days from hBMT, respectively. Conclusions. These preliminary results confirm that hBMT is a feasible treatment in pediatric and adult pts, and that PT-CY is highly effective as GVHD prophylaxis, with encouraging relapse-free survival.

P096

EARLY EXTRACORPOREAL PHOTOAPHERESIS FOR THE TREATMENT OF ACUTE GRAFT VERSUS HOST DISEASE (AGVHD)

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Extracorporeal photoapheresis (ECP) is an immunomodulatory treatment for steroid-refractory acute and chronic graft versus host disease (a/cGVHD), with a reported response rate of about 60%. In this retrospective monocentric study, we analyzed 12 patients who underwent early-ECP because of steroid-refractory aGVHD grade II/IV and compared the response with that of a cohort of 9 patients with steroid-refractory aGVHD who were treated with other second-line immunsoppressive agents. The response to steroid treatment was evaluated after 5 days. The clinical and the transplant characteristics of these two groups of patients were comparable. The median time from aGVHD onset to ECP initiation was 20 days (range 11-41) and the schedule was twice weekly for 4 weeks, then every 15 days for 4 weeks and then monthly until steroid permanent discontinuation without GHVD. Overall, 10/12 patients (83%) addressed to early ECP and 6/9 (67%) of those treated without ECP showed a complete response according to the standard criteria. The median time to achieve a response with ECP was 51 days (range 3-117). The median number of ECP procedures was 20 (range 8-39) and the median duration of steroid treatment was 96 days (range 63-184). Only one out of 12 patients (8%) treated with ECP experienced mild and reversible adverse reactions (paresthesia and fever). After a median follow up of 16 months: -4/12 patients treated with ECP (33%) were alive (two cases with limited cGVHD) and 8/12 (67%) died due to disease relapse (n=3), infections (n=4) or cGVHD (n=1); -2/9 patients not addressed to ECP (22%) were alive (both with extended cGVHD), and 7 (78%) died due to infections (n=3) or GVHD (n=4) Our preliminary results, show that the response rate in patients treated with early ECP was higher, although not significantly, than that reported in the literature and in patients not addressed to ECP (83% *vs* 60% *vs* 67%). On the contrary, the mortality rate was lower in patients treated with early ECP than in patients not addressed to ECP (67% *vs* 78%). At present, we haven't analyzed yet data of immune reconstitution and the expectation of steroids sparing in patients undergoing ECP. The accrual of patients in this pilot study is continuing with the aim to achieve data to properly design a prospective multicentric trial on the usefulness of early or pre-emptive ECP for treatment of aGVHD. Acknowledgments: Progetto Regione Lombardia, Lions Club Bassa Bresciana and BCC Pompiano/Franciacorta.

P097

CORRELATION BETWEEN HLA-G 14-BP POLIMORPHISM AND RESPONSE TO TREATMENT OF ACUTE GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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The human leukocyte antigen-G (HLA-G) is a non-classical HLA class I molecule characterized by immunomodulatory, anti-inflammatory and tolerogenic activities. The HLA-G expression is regulated by a deletion(del)/insertion(ins) polymorphism of 14 base pair (bp) at the 3' untranslated region of exon 8. The 14bp-insertion is associated with a decreased mRNA stability and a lower HLA-G protein expression. We analyzed the possible implication of HLA-G in stem cell transplantation (SCT) outcome and its correlation with acute graft-versus-host disease (aGvHD) response. We studied retrospectively 76 patients (pts), 32M/44F, with a median age of 45 years (range 10-65) underwent SCT. The underlying diseases were: 2 Aplastic anemia, 1 HL, 19 ALL, 45 AML, 2 CML, 2 NHL, 3 IMF, 1 MDS. A myeloablative SCT was performed in 54 pts, while 22 pts received a reduced intensity conditioning. GvHD prophylaxis was obtained with Cyclosporine A and short course of methotrexate. Stem cell source was bone marrow in 5 pts, cord blood in 4 pts and peripheral blood in 67 pts with a median dose of CD34+ of $6.7*10^6$/Kg (range 1.2-19.7). Thirty pts (39.5%) developed aGvHD at a median time of 23 days (range 2-97). Fourteen pts (46.7%) responded to treatment after a median time of 32 days (range 10- 66), while the others progressed into chronic GvHD. HLA-G polymorphism was determined on genomic DNA extracted by donor and recipient samples before transplant, amplified by standard PCR and visualized on agarose gel electrophoresis.

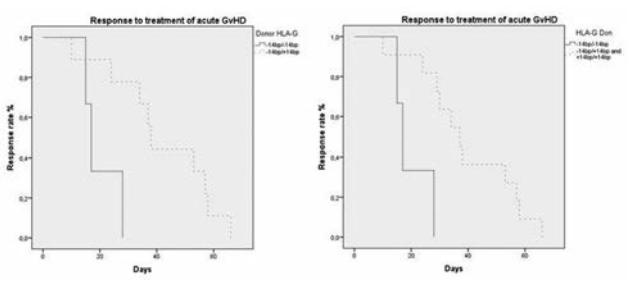

Figure 1.

Data were analysed using IBM SPSS Statistics 20 Core System. Although no correlation between HLA-G polymorphism and the risk of GvHD occurrence was observed, we found that donor HLA-G 14bp del/del genotype was associated with a shorter time to response of aGvHD to therapy (median time of 17 days, IC 95% 13.799-20.201; Kaplan Meier, p=0.007). Since that 14bp-ins is associated to a lower expression HLA-G molecule, pts receiving a graft from an HLA-G del/del donor showed a better aGvHD response to treatment compared to the others genotype (median time of 37 days, IC 95% 28.368- 45.632). Interestingly, transplants performed by donor HLA-G del/del presented a shorter time to response of aGvHD also compared to transplants by donor HLA-G ins/del genotype (median time of 38 days, IC 95% 35.078-40.922; Kaplan Meier p=0.019).

P098

FECAL CALPROTECTIN AS POSSIBLE MARKER OF GRAFT VERSUS HOST DISEASE (GVHD) ACTIVITY AND RESPONSE TO TREATMENT

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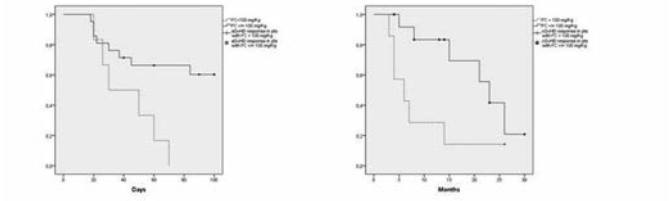
Graft-versus-host disease (GvHD) is one of the main complications after allogeneic stem cell transplantation (SCT). Since there are no validated laboratory test for GvHD, confirmed clinical suspicion requires tissue biopsies. Recently, some authors focused their attention on the proteomic pattern of 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 62 pts (M/F 32/30, median age 51 ys, range 17- 56) receiving SCT. Underlying diseases were: 1 plasma cell leukemia, 1 HL, 1 lymphoblastic lymphoma, 7 ALL, 3 CLL, 32 AML, 7 NHL, 3 IMF and 7 MDS. Stem cells source were peripheral blood in 58 pts, bone marrow in 2 pts and cord blood in 2 pts. Graft was performed by a sibling donor in 36 cases and by a matched unrelated donor in 26 cases. A reduced intensity conditioning was performed in 39 cases and myeloablative regimen was used in 23 cases. GvHD prophylaxis was performed with Ciclosporine A (CSA) + micophenolic acid in 25 cases, only CSA in 4 cases, CSA + short course of methotrexate in 33 cases. Anti- thymocyte globulin and Campath-1H were added in 11 and 4 cases respectively. Twenty-nine patients (46.8%) developed acute GvHD at a median time of 23 days (range 1-97 days) after SCT. The onset of chronic GvHD was detected in 26 patients (51%). Fourteen of them presented classic chronic GvHD at a median time of 145 days (range 100-310 days) after SCT, while the other twelve patients presented an overlap syndrome. Fecal calprotectin was measured in stool sample by a quantitative enzyme immunoassay (Calprest®). Data were analyzed using IBM SPSS Statistics 20 Core System. Fecal calprotectin was higher in patients with GvHD than in the others (p<0.0001 for acute GvHD and 0.002 for chronic GvHD), in particular in patients with gastrointestinal involvement (p=0.004 for acute GvHD and 0.0026 for chronic GvHD). The value of fecal calprotectin at the onset of the symptoms correlates with time to response of GvHD both in acute (p=0.011) and chronic setting (p=0.028), and its level decrease after response (p=0.038). Value of fecal calprotectin was higher in gastrointestinal GvHD than in infective diarrhea (p=0.04) or aspecific colitis (p=0.0086).



A: Time to response of acute GvHD related to fecal calprotectin level

B: Time to response of chronic GvHD related to fecal calprotectin level

Figure 1.

P099

PEGYLATED-GCSF COMPARED TO DAILY G-CSF IN ALLOGENEIC STEM CELL TRANSPLANTATION: SINGLE CENTRE EXPERIENCE

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The effectiveness and safety of pegylated-GCSF after autologous transplant has been demonstrated in several studies while few reports exist in the allogenic setting.We compared 20 patients treated with pegylated-GCSF,6mg in a single dose on day+4,to an historical control cohort of 45patients who were treated with dailyGCSF from day+2 until neutrophil recovery after allogeneic transplantation. Patients' characheristics are listed in Table1. Eight (18%) pts in GCSF and 10(50%) in the pegylated-GCSF treated group received a previous autologous transplantation, (p<0.001). The patients were well matched regarding the clinical and biological characteristics. The primary end points was the time to achieve neutrophil engraftment and incidence of febrile neutropenia.Secondary end points were the duration of febrile neutropenia, days of hospitalisation, days of antibiotic therapy and response to first line antibiotic therapy. The outcome of the transplant are shown in Table2. Graft failure was observed in 15% and 10% of GCSF and pegylated-GCSF group, respectively (p=0.77). Neutrophil engraftment (ANC>1x10^9/litre) was significantly faster (p<0,05) in the pegylated-GCSF group with a median of 12 days compared with 15days in the GCSF group, Figure1. No difference was observed concerning platelet engraftment. The variables statistically significant for a faster neutrophil engraftment were, both in univariate and multivariate analysis, the use of pegylated-GCSF (p<0.05) and the disease status at transplantation (p<0.03), whereas number of infused CD34+ cells (>=4x10^6/Kg) was a significant factor only in univariate analysis. The incidence of neutropenic fever was similar in the two cohorts of pts.The median number of febrile neutropenic days was 3 (1-26) in the GCSF and 4 (1-12) in the pegylated-GCSF group(p=0.422). No difference was observed regarding the duration of intravenous antibiotic (p=0.413) and about the response to first line antibiotic therapy(p=0.55). Incidence of acute or chronic GvHD, mucosites and diarrhea was similar.The TRM at + 100 days was 27% for GCSF and 25% for pegylated-GCSF treated pts (p=0.5).Also,no differences were seen regarding response rate at +90 after transplantation(p=0,71).At a median follow-up of 268 days (range 4-2674) no significant difference was seen in estimated 2yEFS(40%; p=0.9).We conclude that pegylated-GCSF was associated with faster neutrophil engraftment after allogeneic SCT,without any differences observed regarding the outcome of the allogenic transpant procedure.

Table 1. Patients' and transplanations' characteristics

	G-CSF (45)	Pegylated-GCSF (20)	p
Age, median (range) years	53 (15-70)	46 (14-68)	0.3
>60 years, ,n (%)	17 (38)	4 (20)	
Previous autotransplant,n (%)	8 (18)	10 (50)	0.001
Disease, n (%)			
Non Hodgkin Lymphoma / Hodgkin Disease	19 (42) / 5 (11)	7 (35) / 5 (25)	0.22
Multiple Myeloma / AL+MDS	2 (5) / 19 (42)	3 (15) / 5 (25)	
Conditioning regimen, n (%)			
Standard / Reduced Intensity	16 (36) / 29 (64)	2 (12) / 17 (85)	0.12
Status disease			
Responsive / Not responsive	28 (62) / 17 (38)	11 (55) / 9 (45)	0.35
N. previous chemoterapies, median (range)	2 (1-5)	3 (1-5)	0.77
> 2, ,n (%)	22 (4)	16 (80)	
Months to TMO, median (range)	13.8 (4-127)	13.1 (6-154)	0.55
<=12, ,n (%)	17 (38)	6 (30)	
HLA Match, n (%)			
Match / Mismatch	34 (76) / 11 (24)	17 (85) / 3 (15)	0.22
Donor			
Familiar / MUD	35 (78) / 10 (22)	14 (70) / 5 (30)	0.7
Source of Stem cells (PBSC), ,n (%)	36 (80)	15 (75)	0.9
CD34+cells infused, median (range)	5.5 (1-20.10)	4.79 (1.05-14.9)	0.52
>4 x10e6/kg, ,n (%)	29 (78)	15 (75)	

Table 2. Outcomes of allogenic stem-cell transplanations with G-CSF and pegfilgrastim

	G-CSF (45)	Pegylated-GCSF (20)	p
Days to ANC > 1 x10e9/litre, Median (range)	15 (10-23)	12 (11-27)	0.05
<=15 days, ,n (%)	20 (54)	13 (65)	
Days to PLT > 20x 10^9/litre, Median (range)	14 (10-33)	14 (11-30)	0,.66
<=15 days, ,n (%)	18 (58)	10 (50)	
Graft failure ,n (%)	7 (15)	2 (10)	0.77
Acute GvHD, n (%) / **Chronic GvHD,** n (%)	6 (14) / 11 (37)	4 (20) / 3 (15)	0.315 / 0.36
Days hospital stay, Median (range)	28 (11-59)	24 (15-50)	0.191
>24 days, ,n (%)	31 (69)	10 (50)	
Neutropenic Fever, ,n (%)	32 (71)	12 (60)	0.422
N of days of fever, median (range)	3 (1-26)	4 (1-12)	0.433
>5 days, ,n (%)	10 (32)	5 (25)	
N of days of antimicrobial therapies (ABT), median (range)	25 (2-42)	15 (6-33)	
>10 days, ,n (%)	23 (77)	7 (35)	0.413
Response to first line ABT, n (%)	13 (42)	5 (45)	0.55
TRM + 100 days, ,n (%)	12 (27)	5 (25)	0.5
Mucosites, ,n (%) / III-IV grade	35 (83) / 8 (22)	15 (75) / 4 (27)	0.5
Diarrhea, ,n (%) / III-IV grade	27 (66) / 4 (15%)	13 (65) / /	0.07
Response +90, ,n (%)			
Complete Response / Partial response	24 (53%) / 3 (6,7%)	8 (40%) / 4 (20%)	0.741
Progressive disease / Exitus	4 (8.9%) / 14 (31.1%)	1 (5%) / 7 (35%)	

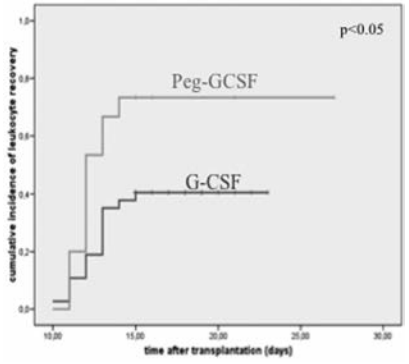

Figure 1. Cumulative incidence of leukocyte recovery

P100

DONOR AND RECIPIENT STR ANALYSIS BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION:POSSIBLE CORRELATION WITH POST-TRANSPLANT OUTCOME

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The chimerism analysis after HSCT is a useful tool to monitor the engraftment of donor cells and to early predict graft failure or disease relapse. Among several available methods to perform chimerism study, multiplex fluorescent short tandem repeat (STR) analysis represent a sensitive, accurate and reproducible technique. It provide a high rate of discrimination between donor and recipient cells since that fluorescent signal is proportional to the cells number, obtaining a quantitative assessment of chimerism. We enrolled 111 patients (pts) affected by oncohaematological diseases,submitted to HSCT at our division between February 2005 and December 2012. Donor and recipient allelic status was performed using a multiplex PCR amplification of ten STR loci: Amelogenin alleles X/Y, D3S1358, FGA, D8S1179, D18S51, D13S317, vWA, D21S11, D5S818, D7S820. We evaluated the donor/recipient (D/R) match or mismatch for each locus and the following variables: GvHD development and onset time, time to neutrophil engraftment, relapse, DFS and OS. Statistical analysis was performed by Kaplan Meier analysis using IBM SPSS Statistics 20 Core System. Pts with D/R mismatch for D8S1179 locus achieved neutrophil engraftment (ANC>1*10^9/L) at a median time of 18 days (CI 95% 17.202-18.798) compared with 21 days (CI 95% 17.328-24.672) of pts with D/R match for the same locus (p=0.007) (Figure 1A). Pts with D/R mismatch for D3S1358 locus developed acute GvHD at a median time of 20 days (CI 95% 15.781-24.219) compared with 29 days (CI 95% 15.421-42.579) of pts with D/R match for the same locus (p=0.031) (Figure 1B). Pts with D/R mismatch for D3S1358 locus showed an OS of 16 months (CI 95% 11.016-20.984) compared with 41 months (CI 95% 25.420-56.580) of pts with D/R match for the same locus (p=0.025) (Figure 1C). Pts with D/R mismatch for D8S1179 locus showed an OS of 16 months (CI 95% 8.528-23.472) compared with 56 months (CI 95% 11.254-78.057) of pts with D/R match for the same locus (p=0.012) (Figure 1D). We found that pts with a D/R mismatch for D8S1179 locus showed an earlier neutrophil engraftment but a worse OS compared with the others. For D3S1358 locus, D/R mismatch was associated with an earlier onset of acute GvHD after HSCT and with a shorter OS than the D/R match ones. Whether or not discrepancies between donor and recipient basal allelic status could be considered useful in predicting post-transplant outcome will require validation in a large sample of pts.

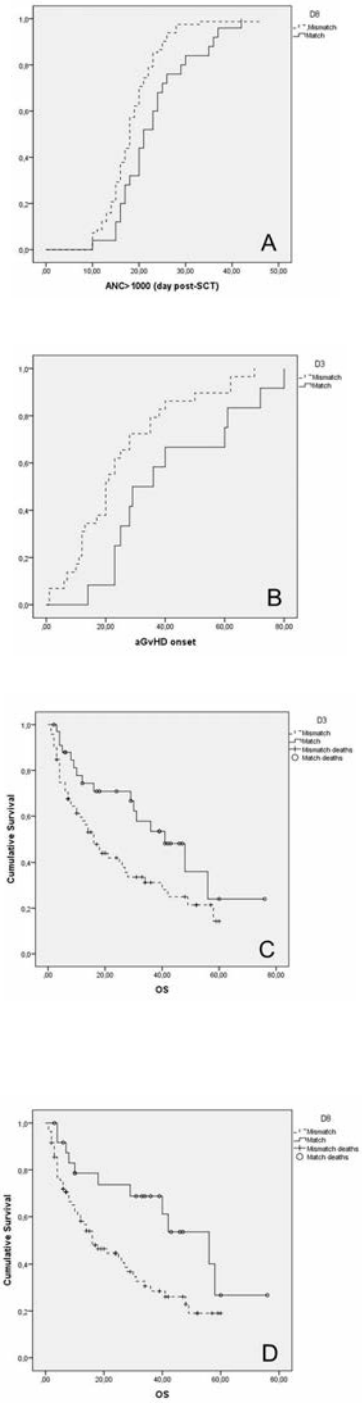

Figure 1.

P101

COMPARATIVE STUDY OF IMMUNE REGULATORY PROPERTIES OF STEM CELLS DERIVED FROM DIFFERENT TISSUES

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Allogeneic stem cell-based therapy is a promising tool for the treatment of a range of human degenerative and inflammatory diseases. Many reports highlighted the immune modulatory properties of some stem cell (SC) types, such as mesenchymal stromal cells (MSCs), but a comparative study with SCs of different origin, to assess whether immune regulation is a general SC property, is still lacking. To this aim, we applied highly standardized methods employed for MSCs characterization to compare the immunological properties of bone marrow- MSCs, olfactory ecto-mesenchymal stem cells, leptomeningeal stem cells, and three different c-Kit-positive SC types, *i.e.* amniotic fluid SCs, cardiac SCs, and lung SCs. We found that all the analyzed human SCs share a common pattern of immunological features, in terms of expression of activation markers, modulatory activity towards immune effector cells, immunogenicity and molecular inhibitory pathways, with some SC type-related peculiarities. In addition, we found that the inhibitory behaviour is not a constitutive property of SCs, but is acquired as a consequence of immune effector cell activation, as previously described for MSCs. Thus, immune regulation is a general property of stem cells and the characterization of this phenomenon may be useful for a proper therapeutical use of SCs.

P102

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

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Background. We have shown that high dose post- transplantation cyclophosphamide (PT-CY) following a myeloablative conditioning (MA) and a haploidentical related transplant is safe and effective in patients with hematologic malignancies (BBMT 2013; 19:117). Aim of the study. In this study we analyze 100 consecutive patients, for hematologic engraftment, non relapse mortality (NRM), acute and chronic GVHD, infections, leukemia free survival (LFS), overall survival (OS). Patients. All patients were first transplanted with a median age of 48 (range 16-74). The diagnoses were AML (n=41), ALL (n=24), MPD (n=17), LPD (n=13) MDS (n=4), SAA (n = 1). Thirty seven (37%)% were in early (CR1/CR2), whereas 63% had active disease at the time of transplant; Fifty four patients were prepared with Thiotepa, Fludarabine and Busulfan (TBF), and 46 patients were prepared with TBI (9.9-12 Gy) and Fludarabine. High dose of Cy was given at 50 mg/kg on day+3, day+5. Cyclosporin and mycophenolate were given from day 0 and + 1 respectively. All patients received unmanipulated bone marrow with a median cell dose of $3x10^8$/kg (range 1.4-7.7); Results. Four patients died before day 10 of haemorrhage and infections. Three patients had autologous recovery and died with progressive disease (3%). Hematologic recovery was complete in all other patients, with full donor chimerism. The median time to neutrophil (>500/L) and platelet recovery (>20,000/L) was 17 days (range, 13-32 days) and 25 days (range, 12 - 126 days), respectively. GvHD was scored as grade I in 21 patients (21%), grade II in 9 patients (9%) and grade III – IV in 4 patient (4%). The incidence of grade II-III acute GvHD was 13%. Chronic GvHD was scored as limited in 15% of patients and extensive in 12%. With a median follow up of 10 months (2-31months), NRM is 15% (5% and 21% for early or active disease), LFS was 52% (76% *vs* 39% for early *vs* active disease, p=0.001) and OS 57% (76% *vs* 47% for early *vs* active disease p=0.0003). Causes of 15 NRM were: septic shock (4), pneumonia (8: IFI 3, adenovirus 1, legionella 1, idiopathic pneumonia syndrome 3), haemorrhage (3). Conclusions. This data confirm that myeloablative HLA-haploidentical BMT with T cell replete bone marrow and PT-CY is associated with high rate of engraftment, low rate of acute GVHD and NRM. Leukemia free survival is very encouraging in a setting of high risk patients.

P103

HAPLOIDENTICAL DONOR LYMPHOCYTE INFUSION (DLI) FOR RELAPSE POST HAPLO BONE MARROW TRANSPLANTATION (BMT) WITH HIGH- DOSE OF CYCLOPHOSPHAMIDE POST-TRANSPLANT AS GVHD PROPHYLAXIS: WHICH DOSE?

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Background. In the halpoidentical setting, the use of DLI is associated with high risk of acute and chronic graft versus host disease (GVHD) (Huang X, BBMT 2009; Huang X, Clin Transpl 2012). Aims. We tested the feasibility and occurrence of GVHD, following 61 DLI administered in 27 patients relapsed after unmanipulated haploidentical T-cell replete BMT, with high dose post-transplant cyclophosphamide (PT-CY), cyclosporine and micophenolate as GVHD prophylaxis. Patients and Methods. Patients were transplanted from haploidentical related donor, after myeloablative (n=21) or non myeloablative conditioning (n=6). The diagnosis were Hodgkin's disease (HD) (n=6), acute lymphoblastic leukemia (ALL) (n=5), acute myeloid leukemia (AML) (n=13), chronic myeloid leukemia (CML) (n=2), multiple myeloma (MM) (n=1). The median interval BMT-relapse was 198 days (range 45-697); the median interval DLI-relapse was 44 days (range 8-339) and DLI-BMT 227 days (range 99-726). GVHD prophylaxis were discontinued if still ongoing. The doses of CD3+/kg were $1x10^3$(n=2), $1x10^4$ (n=13), $5x10^4$ (n=3), $1x10^5$ (n=23), $5x10^5$ (n=6), $1x10^6$ (n=7), $5x10^6$ (n=4), $1x10^7$ (n=3). The median number of DLI/patient was 1 (range 1-5). In 17 cases (4 molecular and 13 hematologic relapse) DLI followed chemotherapy: gemcitabine or bendamustine in HD; fludarabine, ARA-C, antracycline or mitoxantrone, ARA-C, etoposide or azacitidine in AML and ALL; bortezomib in MM; tirosino-kinase inhibitors in CML. Median interval between chemotherapy and DLI was 17 days (range 5-33). Nine patients (8 molecular and 1 hematologic relapse) received DLI alone. Only 3 cases were mixed chimerism (94%, 88%, 63% donor). Results. No major adverse effect was observed. Acute GVHD grade I, II and III was observed respectively in 11%, 4% and 7%. No patient developed chronic GVHD neither DLI-related aplasia. Fifteen patients responded: 8 complete and 7 partial response; 4 patients maintain stable disease. Nine patients died with progressive disease, 18 are alive (3 disease-free, 6 partial response, 4 stable disease, 5 progressive disease). The median response duration is 104 days (range 40-660); the median survival is 490 days from BMT (range 145-1280) and 165 days from first DLI (range 60-810). Conclusions. This study suggests that patients grafted with haplomismatched BMT and PT-CY, can be treated with DLI at doses up to $1x10^7$/kg, with low risk of developing acute or chronic GVHD and with significant response rates.

P0104

FEAM COMPARED WITH BEAM AS CONDITIONING REGIMEN IN AUTOLOGOUS TRANSPLANT: SINGLE CENTRE EXPERIENCE

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BEAM is the standard conditioning regimen for lymphoma patients undergoing autologous transplant. Recently, the availability of BCNU has became increasingly difficult and Fotomustine could be used as substitute of BCNU in this regimen.Here we present the results in 34 consecutive patients treated with FEAM compared with 54 patients previously treated with BEAM. Patients were similar in terms of diagnosis,median age,number of previous treatments,disease status at transplant,number of CD34+ cells infused (3,6x10^6/kg). Hematopoietic engraftment and treatment related toxicity are shown in Table1. Thirty-four (77%)BEAM patients required more than 20days to recovery platelets (>50000 x10^9/L) *vs* 11 (46%) FEAM patients, (p=0,01). Mucositis (98% *vs* 82%, p=0.019) and diarrhea (98% *vs* 85%, p=0.044) seem occurred hardly more frequently in the BEAM group. No differences in terms of renal, hepatic and neurologic toxicity were observed. Almost all patients in the two groups had fever during neutropenia. We documented mostly FUO (45% in BEAM *vs* 62% in FEAM, p=ns). The median days of hospitalization were 22 (6-59) in FEAM and 25 (20-60) in BEAM group: 28(54%) patients treated with BEAM and 12(35%) with FEAM needed more than 24 days of hospitalization,p=0.06.The incidence of TRM was 9% in BEAM and 12% in FEAM cohort, p=0.9.At univariate analysis, older age was only factor influenced negatively TRM. In patients <65years, TRM was 7% in BEAM and 4% in FEAM, p=0.05. The 90-day overall response rate (ORR) was 85.7% (71.4% CR) and 84.8% (65.2% CR) in patients treated with FEAM and BEAM,respectively. No difference was observed regarding 90-dayORR between the two groups,Table2. After 31 months of median follow-up (1-98), the estimated 2yEFS was 57%,without difference among the two groups, Figure1. In our experience FEAM ensured a reduction of mucositis,diarrhea and a more rapid platelets engraftment with a reduced hospitalization. We observed a higher incidence of TRM in both groups,being 32% (FEAM) and 26% (BEAM) of our patients older 65years. In younger patients the TRM did not differ from that reported in the literature and in patients treated with FEAM the TRM occurred less frequently.In terms of efficacy,ORR and EFS of FEAM was comparable to BEAM, however longer follow-up is needed to evaluate fully its efficacy and long term safety.

Table 1 Hemopoietic engraftment and toxicity

	FEAM 34	BEAM 54	P
Hematopoietic engraftment			
Neutrophils (> 500 x 10e 9/L)	10 (8-22)	10 (7-21)	0,9
>12 days	2 (6%)	3 (6%)	
Neutrophils (> 1000 x 10e9/L)	10 (8-25)	11 (8-26)	0,59
>15 days	3 (50%)	3 (50%)	
Plts (> 20000 x 10e9/L)	13 (9-32)	13 (8-60)	0,44
>15 days	7 (27%)	15 (32%)	
Plts (> 50000 x 10e9/L)	18 (14-40)	17 (11-180)	0,01
>20 days	11 (46%)	34 (77%)	
Febrile neutropenia	29 (85%)	42 (84%)	
Days of onset, median (range)	4 (-1; 7)	5 (1; 22)	0,87
Median duration days, range	3 (1-10)	3 (2-23)	
FUO	18 (62%)	19 (45%)	
Mucositis	27 (82%)	42 (98%)	0.019
Diarrhea	29 (85%)	42 (98%)	0.04
Renal Toxicity	1 (3%)	4 (9%)	0.3
Hepatic Toxicity	1 (3%)	2 (5%)	0.7
Neurological Toxicity	2 (6%)	3 (7%)	0.43
Hospitalisation median day, range	22 (6-59)	22 (20-61)	
> 24 days	12 (35%)	28 (54%)	0.06

Table 2. TRM and +90 response rates

	FEAM 34	BEAM 54	
TRM	4 (12%)	5 (9%)	0.74
TRM (<=65years)	1 (4%)	3 (7%)	
RC	20 (71.4%)	30 (65.2%)	0.9
RP	4 (14.3%)	9 (19.6%)	
PD	4 (14.3%)	7 (15.2%)	
Not Valuable	2	3	

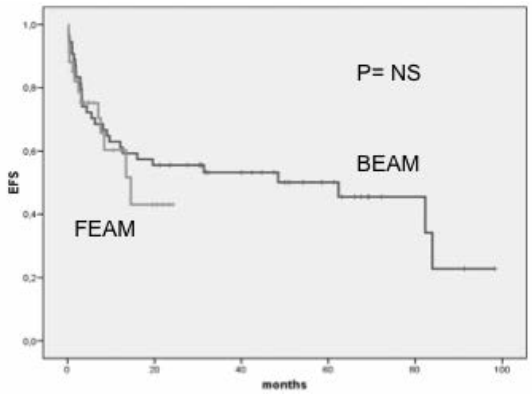

Figure 1. EFS stratified for conditioning regimen

P105

MULTI-GENOTYPING OF MINOR HISTOCOMPATIBILITY ANTIGENS (MHAGS) TO EVALUATE THEIR ROLE IN DETERMINING GRAFT VERSUS HOST DISEASE (GVHD) AND GRAFT VERSUS LEUKEMIA (GVL) EFFECTS IN ALLOGENEIC STEM CELL TRANSPLANTATION

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The outcome of allogeneic stem cell transplantation (Allo-SCT) is closely related to graft versus host disease (GvHD) and graft versus leukemia (GvL) effects which, in part, are mediated by mHAgs. Twenty-six mHAgs (Table 1) have been identified and reported to be differently and variably correlated with GVHD or GVL, but a simultaneous method to genotype a so large panel of mHAgs has never been employed. The aim of this work has been to develop a feasible method to genotype all the 26 mHAgs described so far and to test them for their correlation with GVHD and GVL in a group of donor/recipient pairs submitted to allo-SCT. For the multi-genotype of 23 mHAgs we used a Maldi-Tof IPlex Gold technology through the design of 3 multiplex. This assay is relatively fast and requires a small amount of DNA. For the other three mHAgs we performed other three assays: two based on capillary sequencing of PCR products (for LB-MR1-1R and LRH1) and the last based on PCR alone (for UGT2B17). By these methods, we tested the 26 mHAgs in 70 donor/recipient pairs at least 6/6 matched at 4-digit high resolution typing, that underwent allo-SCT (sibling or MUD)

because of Philadelphia positive CML (n=46) or ALL (n=24). Maldi-Tof IPlex Gold technology proved a high degree of efficiency. Out of a total of 3220 SNPs a good genotype was obtained in 3176 (98.6%). Also the other assays were efficient (417/420, 99.3%). As expected, sibling pairs showed most identity of MUD pairs. Notably, donor/recipient mismatch on ACC-5, UGT2B17, DPH1, LRH1 can induce the pathogenetic mechanism of GvHD ($p<0.05$). Next we identified that LB-ADIR1 can improve RFS ($p<0.05$) as GvL effect. This is potentially important (p=ns, but there is a trend) especially for ALL-Ph+ patients because this mismatch can enhance GvL in a subgroup that is otherwise un-responsible to allo-immunotherapy. Our data generated by a multi-genotype technique confirm the role of mHAgs in addressing GvL (in some cases without GvHD) and suggest that a study of restricted mHAgs (ACC-5, UGT2B17, DPH1, LRH1 and LB-ADIR1) could be perfomed before transplant in order to better and prospectively investigate the role of the known and new mHAgs involved in GvHD and GvL effects. Work supported by Lions Club "Bassa Bresciana" and BCC di Pompiano e Franciacorta Founds.

Table 1.

mHAgs correlated with GvHD	mHAgs correlated with GvL	mHAgs correlated with both GvHD and GvL	mHAs with clinical significance to be determined
UGT2B17	ACC-1, ACC-2, ACC-6, C19orf48, HB-1, LB-ADIR-1, LB-LY75-1K, LB-MR1-1R, LB-MTHFD1-1Q, LB-PTK2B-1T, LRH1	HA-1, HA-2, HA-8, CD31	ACC-4, ACC-5, CTL7A7, DPH1, DRN7, HA-3, HEATR-1, P2RX7, LB-ECGF-1H, UTA2-1

P106

SPONTANEOUS SPLENIC RUPTURE FOLLOWING STEM CELL MOBILIZATION WITH LENOGRASTIM AND PLERIXAFOR IN AL AMYLOIDOSIS

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Spontaneous splenic rupture is a rare complication in both systemic AL amyloidosis and even in healthy donors during stem cell mobilization with G-CSF. On September 2011, a previously healthy 50-year-old-woman was admitted to the hospital for heart failure and diagnosed with systemic amyloidosis AL (l) with cardiac involvement. Lambda serum Free Light Chain (sFLC) was 235 mg/L; the k/l ratio was 0.089. The patient was started on a bortezomib–dexamethasone regimen but she did not tolerate it. An orthotopic heart transplantation was planned and carried out on June 2012. The patient was then treated with cyclophosphamide-bortezomib-dexamethasone regimen achieving a complete haematological remission. Since her therapeutic program included autologous stem cell transplantation in case of disease relapse, a peripheral stem cell mobilization with granulocyte colony stimulating factor (G-CSF) was carried out in a semi-intensive care unit. The spleen was normal at an abdominal ultrasound examination. On January 2013 the patient received lenograstim 5 g/kg twice daily. Before the administration of G-CSF her CBC showed WBC $7.61x10^9$/L, Hb 98 g/L, Plts $235x10^9$/L. After 4 days the WBC raised to $50x10^9$/L; hemoglobin was unchanged (98 g/L), and the CD34+ cell count was 15 cells/mL. On this basis, she was considered a proven "poor mobilizer" and received Plerixafor 0,24 mg/kg at midnight. On day 5, WBC count was $70x10^9$/L, Hb was 88 g/L. The patient underwent stem cell collection. During the apheresis procedure she experienced low blood pressure and complained of mild nausea. After a few hours she developed a mild pain on her upper left quadrant (no abdominal guarding), tachycardia, hypotension and oliguria. Hemoglobin fell to 65 g/L. The abdominal ultrasound showed splenic rupture with abdominal free fluid. The patient underwent a successful laparotomic splenectomy and recovered uneventfully. On gross examination, the spleen was normal in weight and dimensions; microscopic examination revealed mild, diffuse amyloid deposition. To the best of our knowledge, this is the first report of splenic rupture following stem cell mobilization with G-CSF and Plerixafor in AL amyloidosis. The role, if any, of either drugs is uncertain. Anyway, the decision to perform peripheral stem cell mobilization with G-CSF and/or both G-CSF and Plerixafor in patients with AL amyloidosis requires careful evaluation and needs monitoring of CBC, signs and symptoms of spontaneous splenic rupture.

P107

PEG FILGRASTIM VERSUS DAILY GRANULOCYTE-COLONY STIMULATING FACTOR: A COMPARISON AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION. SINGLE CENTRE EXPERIENCE

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The high-dose chemotherapy used as a conditioning regimen prior to transplantation of autologous stem cells (ASCT), places the patient to a high risk of complications determined by prolonged neutropenia. Granulocyte-Colony stimulating factor (G-CSF) administration after ASCT has been reported to reduce the time of neutrophil recovery. We present retrospective comparison of the effects of Pegfilgrastim (PEG) and G-CSF on 123 patients with lymphoproliferative disorders (47% non Hodgkin Lymphoma, 43% Multiple Myeloma and 10 % Hodgkin's Disease) who received an ASCT between March 2005 and October 2012. 85 patients received a single dose of PEG (group-1) and 38 patients received a daily dose of GCSF (group-2). There were no statistically significant differences between the two groups for baseline characteristics of the patients in term of age, sex, disease status, conditioning regimens and doses of CD34 + cells transplanted. In group-1 the median age was 51ys (19-74) with 68% under the age of sixty; in group-2 the median age was 62 ys (18-71) with 67% over sixty ys (p=0.005). The patients who receveid PEG required fewer days of hospitalization compared to the patients in the group-2 (median days 22 *vs* 24 respectively), without statistical difference (p=0.06). Median time to engraftment, as the time to reach an absolute neutrophil count (ANC) greater than 0.5x10^9/L was 10 days, the same for both groups, with a better trend in the group-1 (graphic n.1). There were no statistically significant differences regarding the gastrointestinal toxicity. Mucositis grade III-IV was observed in 23 patients of the group-1 and 11 of the group-2 (p = 0.26). Diarrhea of grade III-IV was observed in 8 and 7 patients respectively in the two groups (p=0.09). Not significant differences were observed in the incidence of febrile neutropenia and in the median duration of febrile neutropenia. In our experience, we found a statistically significant difference (p = 0.04) about infections documented on blood cultures and other microbiological analysis: 34% in PEG group and 59% in G-CSF group (graphic n.2). In summary both PEG and G-CSF groups, have similar outcomes in terms of ANC engraftment, toxicity and to prevent febrile neutropenia after ASCT. Our experience showed a lower rate of microbiologically documented infections in the PEG group. These preliminary data, although limited, suggest that PEG is safe and efficacious alternative to daily dose of G-CSF after ASCT.

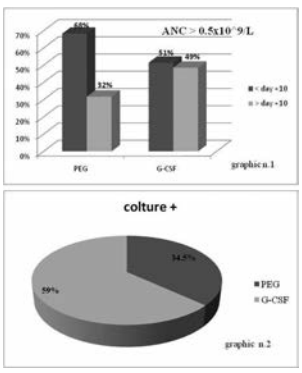

Figure 1.

P108

ARA-C VERSUS CYCLOPHOSPHAMIDE FOR HAEMATOPOIETIC STEM CELLS MOBILIZATION IN LYMPHOMA PATIENTS: CAN WE PICK A WINNER?

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Introduction. The optimal protocol for mobilization of haematopoietic stem cells (HSC) in lymphoma patients has not been determined so far. Mobilization protocols may either be based on the use of cytokines alone, most frequently G-CSF, or in combination with chemotherapy. Chemomobilization was demonstrated to increase CD34+ cell yield and it could have potential effect of *in vivo* purging. Some studies indicate that a higher number of CD34+ cells reinfused are associated with a better outcome after autologous HSC transplantation. We retrospectively analyzed the mobilization efficacy of Ara-C in our Department compared with cyclophosphamide (CTX), both combined with G-CSF. Methods. We analyzed results of 125 patients affected by lymphoma (112 non Hodgkin lymphoma and 13 Hodgkin lymphoma), 36 treated with Ara-C and 89 with CTX between 2001-2012. Ara-C was administered 1-2 g/mq twice daily for 3-6 days (total dose 6-24 g/mq), while CTX was given at a dose of 3-7 g/mq on one day. G-CSF (5 g/kg) was started 48 hours from the end of chemotherapy and continued until last leukaphaeresis. The number of circulating CD34+ cells was first evaluated on the first day of white blood cells >1 10*3/ L and HSC collection was started when the CD34+ cell peak was ≥10/ L. The peak number of circulating CD34+ cells was primary study end point. The differences between the groups were evaluated with the use of Mann-Witney or Student's t tests for quantitative and Chi-square test for qualitative variables (p values <0.05 were considered significant). Results. Age, sex, weight and diagnoses were comparable between the two groups. The peak number of circulating CD34+ cells was significantly higher after Ara-C treatment (median 129/L; 15–775) compared with CTX (77/ L; 10-752) (p<0.05). If we account for the day at collection, the CD34+ cell peak for the Ara-C treatment was higher of 155% with than the CTX treatment(p<0.01). HSC collections were started in mean 3 days later in Ara-C cohort (median 16 day; 13–22) than in CTX cohort (13; 7–23)(p<0.01). A single leukaphaeresis was sufficient to collect optimal numbers of CD34+ cells (>5 10^6/Kg) in 69% patients in the Ara-C group compared with 59% in the CTX group(p=n.s.). Conclusion. in our experience Ara-C gives a higher CD34+ cell peak in comparison with CTX, especially considering the day at HSC collection. Furthermore Ara-C allows collecting an adequate HSC harvest with a single leukaphaeresis in the majority of lymphoma patients.

P109

PEGYLATED-FILGRASTIM AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION: SINGLE-CENTRE EXPERIENCE

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Introduction. Recombinant granulocyte colony-stimulating factors (rG-CSF) are currently used as standard of care to accelerate cell count recovery after autologous stem cell transplantation (auto-SCT). Pegylated-filgrastim (Peg-GCSF) is a long acting form of rG-CSF with the advantage of a single subcutaneous injection. Aims: Several studies have shown activity of Peg-GCSF after conventional chemotherapy, while few data are available about auto-SCT; therefore we performed a retrospective analysis of use of Peg-GCSF after auto-SCT in our centre. Patients and methods. From July 2001 to March 2013, 151 patients (96 Multiple myeloma-MM-, 55 Non Hodhkin Lymphoma-NHL-), with median age of 45 years (21–65), received auto-PBSCT. Conditioning regimens in NHL were BEAM in 28 patients, TEAM in 4, FEAM in 4 and BTM (BCNU, ThioTepa and Melphalan) in 19 of them, while in MM were Melphalan (MEL) 200 in 73 patients, MEL140 in 9, and MEL-based in 14 of them. Patients received Peg-GCSF at day +1, with levofloxacin 500 mg once daily and fluconazole 400 mg once daily as antimicrobial prophylaxis, and acyclovir 5 mg/kg from day +1 and a single infusion of immunoglobulin on day +1 as antiviral prophylaxis. Febrile neutropenia (FN) was defined as body temperature≥ 38°C on 2 consecutive readings, or ≥ 38,5°C on single reading. Empirical antibiotic therapy, red blood cell and platelet transfusions were given according to our istitutional guidelines; neutrophil (ANC) and platelet (PLT) recovery was considered when counts were higher than $1x10^3$/mmc and $20x10^3$/mmc, respectively. Results. Median time to ANC and PLT recovery was 10 days (5–21) and 13 days (4–48), respectively. Median number of erythrocyte and PLT transfusion per patient was 2 (1-8) and 2 (1–11) units, respectively. FN was observed in 53(35%) patients. Median duration of fever was 2 days (1–8), with a median duration of intravenous antibiotic therapy of 6 days (3-21). Two patients affected by NHL died within 100 days from auto-SCT (1 for pulmonary embolism and 1 for sepsis due to E. Coli). Oral and gastrointestinal mucositis was observed in 23(15%) and 74 (49%) patients, respectively. Median time of hospitalization was 15 days (7-50). Conclusions. In our hands Peg-GCSF was safe and effective: incidence of infectious episodes and time to ANC recovery seems similar to what already described in literature, but prospective randomized trials are needed to finally asses its role in auto-SCT.

P110

XM02 PLUS CHEMOTHERAPY IS SAFE AND EFFECTIVE IN STEM CELL MOBILIZATION FOR PATIENTS CANDIDATED TO AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. Recombinant granulocyte colony-stimulating factors (rG-CSF) are currently used, alone or in combination with chemotherapy, to mobilize peripheral blood stem cells (PBSC) in patients affected by Multiple Myeloma (MM) or Hodgkin and Non Hodgkin Lymphoma (HD, NHL) who are candidated to autologous stem cell transplantation (auto-SCT). Even if biosimilar forms of rG-CSF are now registered for the same uses, few data are still available, even if recent reports suggest that stem cell mobilization with these drugs could bring to a significant reduction of costs with comparable safety profile. Aims: To assess efficacy and safety of XM02, a new biosimilar agent, in stem cell mobilization and subsequent auto-SCT, we prospectively analyze data from a cohort of patients who received chemotherapy plus subcutaneous XM02 as stem cell mobilization regimen. Patients and methods: From May 2011 to March 2013, 27 patients were treated (8 male; 19 female) with median age of 48 years (18-69); 12 patients were affected by MM, 8 by NHL and 7 by HD. All patients received disease specific mobilizing chemotherapy (12 received intermediate dose of Cyclophosphamide, 4 R-IEV regimen, 4 high dose Ara-C and Methotrexate and 7 IGEV regimen) followed by subcutaneous XM02 at dose of 5 mcg/kg/daily until stem cell collection. Cell dose was targeted at least to $2x10^6$ CD34+/kg for a single transplant. In patients who received auto-SCT, colony growth assay of CD34+ cell reinfused, and evaluation of neutrophil (ANC) and platelets (PLT) recovery, were perfomed to assess quality and safety of stem cell collection. Results. All but one patient enrolled (26/27; 96%) collected the planned number of cells, with an average number of apheresis of 1,5. Median number of days of XM02 administration was 7 (4-10), median number of CD34+ cell peak before starting apheresis was 47,5/l (20-190), and median number of CD34+ collected was $5,1x10^6$/kg. Seventeen patients (62%) received auto-SCT. Engraftment occurred in all patients, with median number of days to PMN≥500 and to PLT≥20000 respectively of 10 days (r.7-11) and of 12 days (9-18). Colony growth assay was performed for 10 of 17 transplanted patients, with median number of Colony Forming units Cells observed of 174(102–469). Conclusion. In our hands XM02 resulted in a safe and effective strategy for PBSC mobilization that may replace older rG-CSF, even if larger series studies are needed to finally assess its role and its costs.

Hemostasis and Thrombosis - Platelet Disorders

P111

SURGERY WITH TUROCTOCOG ALFA: EFFICACY AND SAFETY IN BLEEDING PREVENTION DURING SURGICAL PROCEDURES – RESULTS FROM THE GUARDIAN TM TRIALS

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Introduction. Novo Nordisk is developing turoctocog alfa, a human third generation recombinant FVIII for treatment of hemophilia A. During the pivotal trial in adult and adolescent previously treated patients with severe hemophilia A (guardian TM 1), subjects in need of surgery were able to participate in a subtrial to document efficacy and safety of turoctocog alfa in prevention of surgical bleeding. Pediatric (<12 years of age) previously treated patients in the guardian TM 3 trial were allowed to undergo minor surgery if needed during the trial. In addition, after completing these initial trials subjects were allowed to continue treatment with turoctocog alfa in the extension trial (guardian TM 2) which also includes a subtrial to document efficacy and safety of turoctocog alfa in prevention of surgical bleeding. Methods. We here describe surgeries performed within the guardian trials. For the ongoing guardian TM 2 extension trial, only cases included in the interim analysis (data cut-off 21NOV2011) are included. Results. In all, results from 10 major and 3 minor surgeries are included. Surgery indication was related to hemophilia joint disease in 8/13 cases. The hemostatic efficacy during and after surgery was rated on a 4-point scale (excellent, good, moderate and none) by the Investigator and/or Surgeon. Details and outcome of the individual surgeries performed are presented in Table 1 (attached). In addition, there were no safety concerns. Discussion: Prevention of surgical bleeding is an important aspect of hemophilia treatment. In the present 13 surgeries, including all surgeries performed with turoctocog alfa in the phase 3 guardian TM trials, hemostatic efficacy during and after was rated as either excellent or good in each case. The results support that turoctocog alfa has an excellent safety and efficacy profile for use in hemophilia A.

Table 1. Details and outcome of surgical procedures in the guardian™ trials using turoctocog alfa for prevention of surgical bleeding

	Description of surgery	Surgery indication	Type of surgery (major/minor)	Duration of surgery (hh:mm)	Hemostatic response during surgery	Hemostatic response after surgery	No of blood transfusions	Age	Exposure days since first exposure to turoctocog alfa at the time of surgery
1	Left knee replacement	Arthropathy and chronic pain in left knee	Major	1:30	Excellent	Excellent	0	36	27
2	Arthroscopy and synovectomy, partial menisectomy	Chronic synovitis	Major	1:33	Good	Excellent	0	30	154
3	Right knee synoviectomia with extirpation of osthesosynthetic graft	Arthropathy	Major	1:00	Good	Excellent	0	25	83
4	Circumcision	Religious	Major	0:30	Excellent	Excellent	0	14	106
5	Left total hip arthroplasty	Hemophilic arthropathy	Major	1:40	Excellent	Excellent	3	25	38
6	Synovectomy, right ankle	Recurrent hemarthrosis	Major	1:21	Excellent	Good	0	29	16
7	Right ankle synovectomy	Hemophilic arthropathy	Major	1:05	Excellent	Good	0	24	9
8	Right ankle synovectomy	Hemophilic arthropathy	Major	1:19	Excellent	Excellent	0	19	65
9	Arthroscopy of left ankle	Pain in left ankle	Major	0:40	Excellent	Excellent	0	24	421
10	Left hip arthroprosthesis, reduction finger fracture	Polytrauma	Major	3:25	Good	Excellent	1	55	437
11	Removal of a central venous access port (guardian™ 3)	SVC thrombosis	Minor	0:27	Excellent	NA	0	6	29
12	Dental extraction (guardian™ 3)	Tooth A caries	Minor	0:10	Excellent	NA	0	11	9
13	Surgical extraction of tooth 48 and radix of tooth 12	Semi-impacted tooth 32 and rest radix of tooth 7	Minor	0:46	Excellent	Excellent	0	23	141

P112

ATYPICAL THROMBOTIC THROMBOCYTOPENIC PURPURA MAY NOT BE SO UNCOMMON IN YOUNG AND MIDDLE-AGED WOMEN WITH RECURRENT THROMBOSIS AND THROMBOCYTOPENIA

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A 42-year-old woman with a clinical history of coronary and cerebral ischemic events was admitted to the Internal Medicine ward of our local hospital following another transient ischemic attack (TIA). From a hematological point of view, the patient had progressively developed severe anemia and thrombocytopenia with laboratoristic signs of intravascular hemolysis and mild renal impairment. ADAMTS-13 activity was measured, revealing a value at the lower limits of the normal range (6%). ADAMTS-13 inhibitors were also found. Plasma exchange (PEX) and corticosteroid treatment were thus started and maintained until a clinical and hematological remission was achieved. The patient experienced a recurrence of thrombotic thrombocytopenic purpura after a few days, which was controlled by weekly administration of rituximab. A second relapse occurred one month later. At least 5 similar cases have been reported by other Authors and all patients had similar characteristics: women aged 25 to 68 years (median=45) with a history of recurrent arterial thrombosis but without important cardiovascular risk factors, presenting with mild thrombocytopenia or minimal microangiopathic hemolytic anemia at the onset. ADAMTS-13 activity was > 5% in 33% of cases. The classic pentad of symptoms was generally not observed. All the subjects showed a tendency to relapse and the majority recovered after the administration of rituxmab or other immunosuppressive agents; one patient subsequently underwent splenectomy. In our opinion, when dealing with this subset of patients characterized by recurrent thrombosis and no apparent risk factors, a diagnosis of TTP should be considered even when there is little evidence of hematological manifestations.

P113

PRIMARY MIXED-TYPE AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) ASSOCIATED WITH ACUTE SPLANCHNIC VEINS THROMBOSIS (SVT) OF IDIOPATHIC ORIGIN: A CASE REPORT

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The association of primary mixed AIHA with idiopathic acute SVT, as recently observed by us, represents an exceptional observation which has not be described so far. A 19-year-old presented with severe abdominal pain. Her past medical was unremarkable. Clinical pictures and laboratory parameters were consistent with an acute and severe hemolytic disorder; hemoglobin had decreased until to 3.9 g/dl; an elevated reticulocyte count, indirect bilirubin concentration, and lactic dehydrogenase concentration were present. A strongly positive direct antiglobulin test (IgG3 and C3d) was found; indirect antiglobulin test was positive at 4°C and negative at 22 and 39°C, being these findings due to the presence of cold panagglutinant antibody (IgM). A diagnosis of mixed AIHA was made and 1 mg/kg body weight/day prednisone was promptly started. From the admission, the patient received as compatible as possible transfusions (12 units of red blood cells package). A comprehensive radiological work-up, which included an abdominal echography and a body CT scan, revealed the complete thrombosis of the portal and splenic veins as well as a partial occlusion of superior mesenteric vein. Full dose fraxiparin was initiated concomitantly with warfarin. Additional laboratory investigations revealed no other causes of anemia and ruled out autoimmune disorders as well as infections and liver diseases. Investigations into possible causes for the development of the thrombosis, including hypercoagulability, as well as those aimed to identify a possible underlying neoplastic etiology were unrevealing. The patient was discharged home on warfarin with a fraxiparin bridge. However, soon after, the patient requested to discontinue warfarin for which fraxiparin only was maintained for six months when a careful revaluation showed the complete SVT resolution and the full vein recanalization in the splanchnic area. The prednisone dose was adjusted according to clinical

response; this agent was suspended after a very gradual dosage reduction after six months of treatment, the patient being in excellent conditions and after that the immunohematological studied, repeated several times, were completely negative. Today, after about a year of diagnosis, the patient is doing well and is not receiving any therapy. Therefore, we have reported a rare association of primary mixed AIHA responsive to prednisone with acute SVT of idiopathic origin for which fraxiparin was effective to obtain full vein recan.

P114

LONG TERM FOLLOW-UP IN PATIENTS WITH ACQUIRED HEMOPHILIA: THE ROLE OF IMMUNOSUPPRESSIVE THERAPY

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Background. Acquired hemophilia is a rare but potentially life-threatening bleeding disorder caused by development of auto-antibodies against FVIII. Recent data of European registry (EACH 2) and from United States (Boles *et. al*) confirm the efficacy of immunosuppressive therapy with corticosteroid alone or in association with Rituximab at standard regimen (375mg/m^2 1 dose per week for 4 weeks) to obtain complete remission (CR) of AHA (defined as the proportion of patients who are in CR without relapse during follow-up). However, the reported duration of patients' follow-up (<2 years) does not allow to make firm conclusions about the long-term effect of such therapy. Now we report data on AHA patients followed-up for a median time of 3.4 years. Aims. To assess the long-term response after immunosuppressive therapy. Patients and Methods. We retrospectively analyzed data from 15 consecutive patients diagnosed with idiopathic and secondary AHA, evaluated between June 2001 and December 2012. Primary objective was to assess the role of immunosuppressive drugs (steroids with or without Rituximab) after a follow-up of at least 3 years, by comparing its effectiveness with published data. All patients was treated first with steroids regimen while Rituximab was added in refractory cases. Refractory has been defined as the lack of clinical response, maintenance of severe FVIII deficiency (<1%) and inhibitor. Results. Among our population, the median follow-up was 3.4 years. Two patients (13%) had spontaneously inhibitor disappearance, without immunosuppressive treatment, 5 (33%) were treated with regimens containing rituximab (in combination with steroids), 8 (53%) with steroids alone. Rituximab was administered according to the standard regimen above reported. All Rituximab-based regimens obtained CR as well as steroids-based regimes. However, the average time to obtain the CR was shorter in steroids-based regimen than that in rituximab-based regimes (4.8 *vs* 1.5 months, respectively). The rate of relapse of patients treated with rituximab was similar to that of patients treated with steroids (60% *vs* 50%, respectively). Seven (46.6%) patients (3 treated with Rituximab, 4 with steroid alone) have experienced relapse after a mean time of 2.6 years. All relapsed patients had idiopathic AHA. Conclusions. In our population, refractory patients treated with Rituximab based regimes, obtain a CR in all cases. This is clinical relevant since our median follow-up is longer than previously reported (1147 vs 262 days of EACH2 registry). However, this response was not maintained in long-term follow-up. With a median time to relapse of 708 days, 60% of patients treated with Rituximab experienced a relapse. The efficacy of subsequent Rituximab after the first relapse deserve further investigation. In our population, all relapsed patients were primary treated with steroids, 4 of them with good response and a short time to remission (1.3 months); three patients was re-treated with Rituximab, with complete and stable remission.

P115

THROMBOEMBOLIC EVENTS IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Introduction. Primary immune thrombocytopenia (ITP) is an immune-mediated disorder that is paradoxically associated with thrombotic complications. We report two patients with ITP who presented once acute ischemic stroke other acute myocardial infarction. Patient#1. A 66 year old woman was admitted with right-sided arm prickle and weakness. Computerized Tomography scan demonstrated acute right thalamic infarct. She reported moderate thrombocytopenia from about 15 years for which she had never done investigations. Peripheral blood tests were normal except low platelet count (32.000/mmc). Blood smear revealed normal findings. Homocysteine, antiphospholipid and anticardiolipin antibody, antibodies for human immunodeficiency and hepatitis were normal or negative. Bone marrow examination demonstrated only increased number of megakaryocytes. A carotid ultrasound showed a diffuse thickening parietal without significant stenosis. Patient#2. A 78 year old man presented to us for a severe thrombocytopenia (13.000/mmc) with mucocutaneous bleeding. He was also affected by hypertension. Secondary causes of thrombocytopenia and thrombophilia were excluded. Blood smear revealed normal findings. Bone marrow examination was normal with no dysplasia or malignant features. Therefore he started treatment with prednisone (1 mg/Kg/die) with a prompt recovery of platelets; after twenty days, he began cortisone tapering. After a month, when his platelet count was 156.000/mmc, he went to the emergency room for recurrent angina. Coronary angiography showed occlusion in right coronary artery and he was diagnosed with acute myocardial infarction. Percutaneous transluminal coronary angioplasty (PTCA) was performed successfully. Combined anti-platelet therapy (aspirin 100 mg and clopidogrel 75 mg) was performed. Cortisone was reduced until suspension in four weeks. After a year without specific treatment, the platelet count is greater than 100.000/mmc. Conclusion. Thromboembolic events are not uncommon in patients with ITP, but there is very little published data on this association. Prospective studies would be useful to assess the incidence of this relationship, to identify ITP patients with increased risk of thrombosis and to assess its implication in ITP management.

P116

A CASE OF ACUTE VENOUS THROMBOEMBOLISM DURING TREATMENT WITH THROMBOPOIETIN RECEPTOR AGONIST IN A PATIENT WITH SEVERE THROMBOCYTOPENIA

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Thrombopoietin receptor (TPOr) agonists (Romiplostim and Eltrombopag) increase platelet counts and restore platelet function in patients with primary immune thrombocytopenia (ITP). These drugs may represent a risk for thromboembolic complications for increase on platelet count and platelet activation. The present reported describes a case of pulmonary embolism and deep venous thrombosis in a patient with severe thrombocytopenia being treated with TPOr agonists. A 30 year-old woman with primary immune thrombocytopenia (ITP) diagnosed five months before was treated with corticosteroids as initial therapy (firstly prednisone and then high dose dexamethasone) with poor response. At the onset bone marrow aspirate was compatible for ITP, abdominal ultrasound was normal, she was positive for antinuclear antibodies (ANAs) testing (1:640, nuclear homogeneous pattern) with mild complement consumption. Thrombophilia testing were normal. She has used oral estrogen-progestin hormone therapy from six months. She started romiplostim three months before (maximum dose 7 mcg/Kg) and then swicthed to eltrombopag for poor response (PLT 4000/mm^3). A week after she presented at our hospital with shortness of breath and chest pain, she performed a ventilation-perfusion lung scan that revealed "high probability" of pulmonary embolism. Laboratory data showed severe thrombocytopenia (2000/mm^3). She immediately suspended hormone therapy and TPOr agonist. She was transfused with platelets and treated with intravenous immunoglobulins with increase of platelet count and started promptly anticoagulant therapy with low molecular weight heparin (LMWH). Computerized Tomography Scan revealed abdominal and axillary lymphnodes enlargement (extreme diameter 35x18 mm) and hepatomegaly. A week after platelets dropped and the patient became unresponsive to IV Ig. She was treated with Romiplostim (10 mcg/Kg) to perform lymph node biopsy with appearance of leg pain the evening after the somministration. Doppler ultrasound revealed deep vein thrombosis, platelets count was 2000/mm^3. She was transfused with platelets since platelets count increase and restarted LMWH. Lymphnodes and bone marrow histological analyses were compatible for autoimmune disease. The present case supports reports in literature that increase on platelets count is not the only risk factor for thrombotic events during TPO-agonists treatment and thrombosis can also occur in patient with severe thrombocytopenia.

P117

UPSHAW-SCHULMAN SYNDROME AND OMOZYGOUS FOR V LEIDEN MUTATION IN PREGNANCY: A CASE REPORT

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Upshaw-Schulman Syndrome (USS) is a rarely congenital form of thrombotic thrombocytopenic purpura (TTP) that results from mutations in ADAMTS13 gene. Pregnancy can be the trigger event for 5-25% TTP cases (late-onset USS forms or acute acquired TTP). During pregnancy TTP may be particularly challenging because of its difficult differential diagnosis with other thrombotic microangiopathies (TMAs), such as preeclampsia and HELPP syndrome. We report a case of a 28-year old white pregnant woman, gravida 2 para 0, at 20 weeks of gestation, who presented at our Department for worsening fatigue in the last week. Two years before she underwent an urgent cesarean section at 29 weeks of gestation for an atypical form of HELLP syndrome with perinatal death of a growth restricted fetus. Thrombophilia evaluation revealed omozygous for V Leiden mutation. In the actual pregnancy thromboprophylaxis with LMWH was adopted. At hospital admission at 20 weeks laboratory data were: Hb 10.7 g/dl, platelets 73x10^9/L, schystocytes 12/1000 and LDH 265 U/L. Serum haptoglobin levels, DAT, coagulation test, transaminase, creatinine and blood pressure were normal. The patient was found to have an ADAMTS13 activity of <6% with the presence of a weakly positivity for antibodies anti- ADAMTS13. She started oral prednisone. No clinical sign of TTP or fetal compromise were noted, whereas platelets slowly decreased. Plasma ex-change (PEX) was initiated at 24 weeks' gestation for progressive worsening of laboratory data obtaining a prompt increase of platelet count. A cesarean section was performed without complications at 30 weeks' gestation, after nine PEXs, for progressive onset of allergic reactions to the procedures. A female neonate of 1440 grams was born in good health condition. After the delivery, a spontaneous progressive normalization of the blood count of the patient was observed. LMWH was continued for six weeks post-partum. Repeated analysis of ADAMTS13 confirmed level <6% with no antibodies. We performed mutational analysis of the ADAMTS13 gene and Upshaw-Schulman syndrome was diagnosed. We supposed that the atypical form of HELLP syndrome reported in her first pregnancy could be a manifestation of USS and its prompt recognition and treatment could have been able to avoid the perinatal death that had occurred. It is therefore critical to create a multidisciplinary team to follow patients with TMAs and to prevent unnecessary mortality both of the mother and the baby.

Figure 1

P118

SUCCESSFUL PREGNANCY OUTCOME IN WOMEN WITH BAD OBSTETRIC HISTORY AND RECURRENT FETAL LOSS DUE TO THROMBOPHILIA: EFFICACY OF COMBINATION OF ASPIRIN AND HEPARIN

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Backgound. Recent investigations highlight the potential role of thrombophilia for determining unfavorable pregnancy outcome including recurrent fetal loss. However, the results so far published furnish discordant Results. There are differences of opinion whether these patients need to be treated with aspirin, unfractionated heparin, low—molecular weight heparin or corticosteroids. Purpose. To evaluate the safety of anticoagulant agents, such as aspirin and heparin, in women with a history of at least two spontaneous miscarriages or one later intrauterine fetal death without apparent causes other than inherited thrombophilias. Material and Methods. We studied, for the common tests for acquired and congenital thrombophilia, 108 women with previous adverse (*i.e.*,preeclampsia, intrauterine growth restriction (IUGR), placental abruption, intrauterine fetal death and recurrent pregnancy loss). Low molecular weight heparin was given at 4000 IU subcutaneously once daily, started at positive pregnancy testing and followed until delivery. Aspirin, 100 mg daily, was given in addition to enoxaparin to women with and without antiphospholipid syndrome. The anticoagulation was continued 6 weeks in postpartum period. Results. All the women were positive either for a solitary or for a combination of acquired and heritable thrombophilia markers (FVLeiden, 13%,FII mutation 12%, MTHFR C677T 49% MTHFR A1298C 34.5% Combined defects 32.7% Fasting homocysteine levels 14.8%, LAC 22%); 52 out of 108 patients (48.1%) had subsequent pregnancies. They were treated with low—molecular weight heparin plus aspirin, and all of them had successful pregnancy outcome (live birth rate of 100%). None of the patients had any adverse reactions such as heparin-induced thrombocytopenia, thrombosis, or fracture. None of the patients had to interrupt the therapy for any adverse treatment-related complications. Conclusions. Thromboprophylaxis with aspirin and heparin in women with bad obstetric history and recurrent fetal loss due to thrombophilia seems to be safe in prevention of pregnancy loss in women with inherited and acquired thrombophilia. Its efficacy should be tested in properly designed clinical trials.

P119

PERSISTENT REMISSION OF CHRONIC IMMUNE THROMBOCYTOPENIA AFTER ROMIPLOSTIM DISCONTINUATION

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Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count of <100x10^9/L in the absence of an identifiable underlying cause of thrombocytopenia. Increased platelet destruction by antiplateled autoantibodies plays a key role in the pathogenesis. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction. TPO-receptor agonists are currently used for patients at risk of bleeding, who relapse after splenectomy or who have a contraindication to splenectomy and who have already failed at least one other therapy. As far as we know an interruption of treatment with TPO mimetics is not feasible. In our study we evaluated the feasibility of stopping treatment with Romiplostim. We evaluated treatment course with Romiplostim in 27 patients with chronic ITP referred to our institution between 2008 and 2013. Diagnosis of ITP was made according to established guidelines. Median age was 72 years (range 52-93 years), 13 were male and 14 female. Prior starting treatment with TPO mimetic, all patients demonstrated severe ITP and platelet counts were below 20 109/ l. Romiplostim was started at 1 g/kg per week and the dose was adjusted to a maximum of 10 g/kg per week to reach a target platelet-count range of 50-250 10^9/L. At the last follow-up, 22 patients (81%) showed a clinical benefit consisting in achieving a platelet count ≥ 50 10^9/L. 4 of 27 (15%) patients were able to maintain a stable platelet response when Romiplostim was stopped. Two patients with a stable platelet count >250x10^9/L discontinued Romiplostim without no dose reduction. At time of interruption one patient was currently treated with 1 g/kg and the other one with 3 g/kg per week. With a follow up of 17 and 3 months respectively, they are still off- treatment. 2 patients discontinued treatment after a progressive dose reduction and they showed a stable platelet count >100x10^9/L at 22 and 8 months of follow-up respectively. In our limited experience we were able to discontinue Romiplostim in selected patients observing stable platelet counts >100 10^9/L during the period of observation. Romiplostim seems a very promising therapy for the treatment of refractory forms of ITP; further investigations and specific clinical trials are warranted to explore the feasibility of stopping treatment.

P120

SUCCESSFUL TREATMENT OF A REFRACTORY CASE OF HIV-RELATED THROMBOTIC THROMBOCYTOPENIC PURPURA WITH RITUXIMAB IN ASSOCIATION WITH PLASMA EXCHANGE

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A 41-year old African, HIV-positive man was admitted to the Infectious Disease Department for nuchal headache and recurrent left-sided paresthesias involving upper and lower limbs. He had been treated with HAART for 15 years. On admission, HIV viral load was undetectable and absolute CD4 count was 259 cells/mm^3. Thrombotic thrombocytopenic purpura (TTP) was diagnosed based on the findings of anemia, thrombocytopenia, significant increase in schistocytes on peripheral blood smear examination and markedly elevated lactate dehydrogenase (LDH). ADAMTS13 activity was reduced (<5%) and anti-ADAMTS13 IgG antibodies were positive at high titer. The patient was promptly started on daily plasma exchange (PEX) associated with steroids (Metilprednisolone 1 mg/kg/day) while continuing HAART (darunavir, ritonavir, raltegravir, lamivudine). On day 4 after initiation of PEX, he was transferred to the Intensive Care Unit due to neurological deterioration (generalized tonic-clonic seizures, left hemiparesis, drowsiness progressing to coma). Head CT-scans were repeatedly negative. No increase in platelet count had yet been observed. Immunosuppressive treatment with monoclonal anti-CD20 antibody (Rituximab) was initiated at the dose of 375 mg/m^2, administered once weekly for 4 doses in association with PEX and steroids. Anti-infective prophylaxis with co-trimoxazole was also given. Following the administration of the second dose of Rituximab, significant neurological improvement was observed in parallel with progressive increase in platelet count. Unexpectedly, despite evidence of complete normalization of LDH (on day 20) and ADAMTS13 activity, and suppression of anti-ADAMTS13 IgG antibodies, platelet count failed to raise above 70.000/mcl. Tests for viral infections were positive for CMV reactivation (CMV-DNA 2.99x10^2). The patient was treated with Gancyclovir 5 mg/kg BID for 20 days resulting in clearance of CMV viremia and complete normalization of blood counts on day 40 after treatment initiation. Currently, four months after PEX discontinuation (total number of sessions: 29), the patient is asymptomatic with neither clinical nor laboratory evidence of TTP relapse. HIV-RNA is persistently undetectable on continuous HAART, as well as CMV-DNA. In conclusion, Rituximab in association with PEX is a feasible option in HIV-related refractory TTP. Suppression of HIV viral load by HAART and monitoring of CMV and other herpes virus reactivation is advisable.

P121

PREVALENCE OF COMBINATION OF TRIPLE ALLELIC MUTATIONS ASSOCIATED WITH THROMBOPHILIA IN PATIENTS WITH VENOUS THROMBOEMBOLISM

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Background. Triple thrombophilic abnormalities have been rarely reported and most patients carried polymorphisms nowadays not included in the recommended laboratory investigation for thrombophilia, such as those in the MTHFR gene. Aim of the study. To assess the prevalence of triple allelic mutations associated with thrombophilia in patients with venous thromboembolism (VTE). Patients. We selected from a cohort of 2,199 individuals with VTE who were referred to our Thrombosis Center those with triple thrombophilic abnormalities (*i.e.* deficiency of antithrombin [AT], protein C [PC], protein S [PS], factor V Leiden [FVL], prothrombin [PT] 20210A) and/or triple allelic mutations (*i.e.* triple heterozygosity [Het], homozygosity [Hom] plus He). Results. We identified 10 patients (M/F 5/5) (0,45%) (Table). Four patients had triple Het, and the remaining carried a combination of Hom and single Het. No patient in the cohort carried double Hom neither Hom for deficiency of natural anticoagulants, nor triple deficiency of natural anticoagulants. Het for deficiency of natural anticoagulants was present in 5 patients (AT=2, PC=1, PS=1, PC+PS=1), associated with FVL or PT20210A (Table). Four patients had triple abnormalities (various combinations of AT,PC,PS deficiency, and FVL or PT20210A) (0.18%), and 6 had double abnormalities (AT deficiency and Hom for FVL in one case, FVL and PT20210A in the remaining ones). The first VTE was deep vein thrombosis (DVT) of one leg in 9 cases, in 2 of them with pulmonary embolism (PE), and superficial vein thrombosis (SVT) in 1 case. The median age of first VTE was 27 years (range 2-73), in 8 cases <45 years. The first event was provoked in all cases. Two of them had an additional thrombophilic acquired abnormality (hyperhomocysteinemia and lupus anticoagulant, respectively). Four patients had recurrent VTE events. Eight patients received lifelong treatment with vitamin K antagonists (VKA). Conclusions. In patients with VTE triple allelic mutations associated with thrombophilia are uncommon but not exceedingly rare (0.45%), and diagnosis of a single thrombophilia abnormality should not discourage from an exhaustive laboratory investigation. Abnormality can be a combination of Hom and Het or a combination of triple Het. The clinical onset occurs in young age in the large majority of cases. Surprisingly, such conditions seem not associated with unprovoked events, and recurrence occur in a minority of subjects.

Table 1.

Patient	Sex	Genotype	Family history	Age of onset	First VTE	Risk factors	Recurrence	VKA
P.M.	F	Triple Hetero (AT+ FVL+ FIIA)	Yes	2	DVT	Viral infection (measles)	Yes	Ongoing
G.B.	M	Triple Hetero (PC+ FVL+ FIIA)	Yes	41	DVT+PE	Surgery	Yes	Ongoing
L.G.	F	Triple Hetero (PS+ FVL+ FIIA)	Yes	73	DVT	Pneumonia	No	Ongoing
A.R.	F	Triple Hetero (PC+ PS+ FIIA)	Yes	28	DVT	Oral contraception	Yes	Ongoing
C.P.	M	Hetero AT + Homo FVL	Yes	57	DVT	Obesity Bed rest	No	No
E.D.	M	Homo FVL + Hetero FIIA	Yes	26	DVT	Viral infection (mononucleosis) Bed rest	No	Ongoing
M.D.	F	Homo FVL + Hetero FIIA	Yes	26	DVT+PE	Oral contraception HyO	No	No
V.O.	M	Homo FVL + Hetero FIIA	Yes	16	DVT	Trauma	No	Ongoing
G.G.	F	Hetero FVL Homo FIIA	No	24	DVT	Puerperium	No	Ongoing
G.S.	M	Hetero FVL Homo FIIA	No	28	SVT	Trauma Lupus anticoagulant.	Yes	Ongoing

P122

OBSERVATIONAL ANALYSIS OF GENETIC RISK FACTORS IN PATIENTS WITH A DOCUMENTED DIAGNOSIS OF CEREBRAL SINUS THROMBOSIS

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Background. In industrialized countries, the cerebral sinus vein thrombosis (SCVT) is most often aseptic and it can be observed in several settings. SCVT can particularly occur in postoperative period and puerperium, both contexts characterized by hyperfibrinogenemia and thrombocythemia. In addition, in SCVT the use of birth control pills is often imputed as a risk factor. However, the etiology of this disease remains indefinite in 25-35% of cases. AIMS The objective of this study was to retrospectively analyze the relationship between factor V Leiden, prothrombin G20210A mutation (PT 20210A), MTHR and the occurrence of SCVT. METHODS The study has included 20 patients with a diagnosis of SCVT instrumentally performed between 2002-2012. We determined the mutational status of factor V Leiden, Prothrombin (PT) G20210A and C677T and A1298C of MTHFR in each enrolled patient. We performed a comparative analysis of mutations found in patients with a diagnosis of cerebral sinus thrombosis compared with a population of unselected patients without venous thrombosis. Results. The distribution of mutational status in the genetic analysis performed in the enrolled patients was as follows: Wild type for Factor V Leiden, Factor II and MTHFR: 19%. Factor V Leiden: 15% heterozygote and 0,5% homozygote in cases and only 2- 3% and 0,02% respectively in control group (European unselected patients for venous thrombosis). Heterozygous for PT G20210A mutation: 20% in patients and 3-5% in control group. Mutation of the MTHR C677T and A1298C: 45% heterozygote

for C677T and 10,5% double heterozygote for C677T and A1298C in cases. In the control group, the mutation C677T was not statistically different from cases, on the contrary a double heterozygote mutation for C677T and A1298C was 1.5%. Conclusions. The presence of factor V Leiden and PT 20210A are known risk factors for SCVT. The single mutation of C677T of MTHFR does not represent a risk factor for the disease because it has the same prevalence in the normal population. Instead, the coexistence of a double mutation in MTHFR (C677T and A1298C) can be considered a risk factor, as the mutations of the factor V and factor II. The coexistence of a double mutation of MTHFR is associated with elevated homocysteine levels and an increased relative risk for venous thromboembolism in the examined population.

P123

RITUXIMAB AND THROMBOPOIETIN RECEPTOR AGONIST ASSOCIATION IN HIGH RISK REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA

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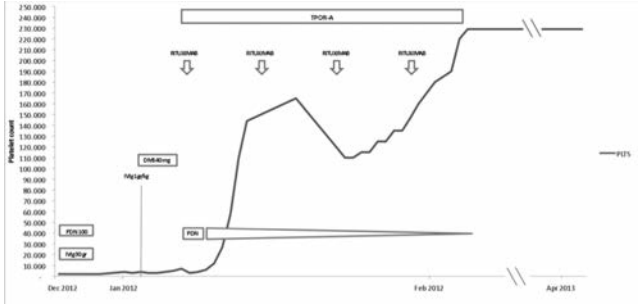
Case description. On December 2011 a 31-year-old man was admitted to his territorial hospital for diffuse petechiae and purpura, gingival and nose bleeding. The hemogram showed severe thrombocytopenia ($2x10^9$/L). The remaining blood cell counts and differential were normal. The blood smear confirmed thrombocytopenia without other abnormalities. At bone marrow examination megakaryocytic hyperplasia in the absence of additional abnormal findings was observed. On this basis, the diagnosis of ITP was done. In spite of prompt treatment with steroids (prednisolone 1 mg/kg/day), IVIG (400 mg/kg/d for 5 days) and daily platelet infusions, the platelet count remained far below $10x10^9$/L and haematuria also occurred on the following days. On January 4th 2012 the patient was admitted to our unit, when platelet count was $3x10^9$/L associated with severe haemorrhagic manifestations. One more cycle of IVIg (1 g/kg for one day) and high-dose dexamethasone (40 mg/d for 5 days) were administered without any improvement of the platelet count and of the haemorrhagic syndrome. Splenectomy did not appear feasible due to the risk of the procedure with such a low platelet count. Appearing the patient at high risk for fatal bleeding, a contemporary treatment with rituximab and thrombopoietin receptor agonist was considered. Our aim was to obtain a rapid increase of platelet count promoted by TPO-R agonist which could act as a bridge therapy until the later response eventually obtained by rituximab. This therapy was applied from January 11th (rituximab 375 mg/m^2 weekly for 4 weeks and romiplostim 1 mcg/kg weekly for 6 weeks). Over the next 7 days the platelet count increased until $110x10^9$/L, with progressive resolution of haemorrhagic manifestations.



Changes in the platelet count according to treatment. Corticosteroids and intravenous immunoglobulin (IVIg) failed to increase the platelet count. Subsequent combination therapy of Romiplostim (TPO-R) with Rituximab rapidly increased the platelet count.

One month later the platelet count reached the normal range ($284x10^9$/L) and remained stable over time after the discontinuation of TPO-R receptor agonist. At last control February 15th 2012) platelet count was 224 $x10^9$/L. Considerations. Thrombocytopenia in ITP might be associated either with increased platelet destruction and/or insufficient platelet production. Therefore, the association of an immunomodulatory agent with a TPO-R receptor agonist may exhert an enhanced efficacy. The rapid increase of platelet count might safely allow to wait for response to other immunomodulatory agents, like Rituximab, or to safely perform splenectomy, if feasible.

P124

RITUXIMAB MAINTENANCE THERAPY IN PATIENT WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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TTP is a disease characterized by the triad: microangiopathic haemolytic anemia occurring with alteration of erythrocytes, consumptive thrombocytopenia, neurological signs, renal damage, fever. It's a rare disease even if in recent years is diagnosed with a certain frequency and with a head of TTP equal to a case on 50,000 hospital admissions.In june 2012, a 48 years old woman was referred to emergency department of a hospital peripheral for hemorrhagic manifestations form of purple generalised and vaginal bleeding after review the cavity uterine and presenting a severe anemia and thrombocytopenia. The patient was sent to our hospital. with thrombocytopenia, hemorrhagic anemia and mainly hyperbilirubinemia indirect, increase LDH and normal coagulation parameters, Coombs test negative, reticulocytosis and schistocytes in the peripheral blood, functionality kidney in accordance with clinically neurological symptoms floating up to the state of coma with TAC negative and fever. On the basis of all these data was diagnosed TTP and was started therapy plasma exchange (PEX) daily (7 procedures in 8 days) associated to infusion vincristine 2 mg. After 24 hours was shooting the daily PEX, with increase platelets, reduction LDH, improvement consciousness, we decided to do with consolidation PEX other day and with stable blood chemistry parameters, disappearance of fever and complete recovery of conscience of the patient is carried to the administration of weekly rituximab for a total of four weeks and stopped PEX. Improved clinical status, the patient was discharged invited to make monthly checks in day hospital and maintenance therapy with rituximab at a dose of 375 mg/m^2/monthly. After 9 monthly rituximab therapy, the pathological and clinical data of patient and blood chemistry parameters were normal.This communication wants to put your attention on utility of continuing the infusion of the monoclonal antibody monthly in order to stabilizing the results obtained with the first four treatments.

P125

TREATMENT WITH ROMIPLOSTIM IN ITP PATIENTS: A MULTICENTER EXPERIENCE FROM "RETE EMATOLOGICA PUGLIESE" (REP)

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Romiplostim is a thrombopoietin receptor agonist recorded for treatment of primary immune thrombocytopenia (ITP). Aim. To report experience with romiplostim in persistent/chronic ITP patients followed in 9 Centers of "Rete Ematologica Pugliese" (REP). Patients and Methods. We evaluated 62 patients (37 female, 25 men; median age 57,5 years, range 18-82) with persistent/chronic ITP treated with romiplostim (initial dose 1 µg/Kg/week). The dose of romiplostim was adjusted on the basis of the patient's platelets count. Median time between ITP diagnosis and romiplostim start was 3 years. Median number of previous lines of treatment was 2 (range 1-7); namely, 31/62 patients had received ≤2

lines of treatment, 31/62 >2 lines, including splenectomy (13/62). Results. Fifty-seven out of sixty-two patients (92%) were responders (doubling of baseline platelets count), 5/62 (8%) were non responders. Seventeen out of fifty-seven responding patients (30%) discontinued romiplostim: 6 patient for loss of response, 4 for consent withdrawn, 2 for physician decision and 2 after "bridge" to splenectomy; three patients were lost to follow-up. After a median follow-up of 17,5 months (range 9-37), 40/57 (70%) responding patients continue treatment with romiplostim at median dose of 5 µg/Kg/week (range 1-10), maintaining a platelet count ≥ 30.000/mmc. The median romiplostim dose to achieve a response was 2 µg/Kg/week (range 1-10). At the start of romiplostim 18/40 patients (45%) received concomitant ITP medication, that was discontinued in 10/18 patients. Nine out of sixty-two patients reported the following adverse events: headache, rush, arthralgia, peripheral neuropathy, infectious event (toxoplasmosis), bleeding (epistaxis, gastrointestinal hemorrhage, retinal hemorrhage), thromboembolic event (pulmonary embolism). Conclusions. Our multicenter retrospective study confirms that romiplostim is effective and safe treatment of refractory/relapsed ITP and has a positive impact on patient-quality of life.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH BUDD-CHIARI SYNDROME TREATED WITH COMPLEMENT INHIBITOR ECULIZUMAB; A CASE REPORT

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Paroxysmal Nocturnal Haemoglobinuria (PNH) is a rare, acquired haemolytic anaemia caused by somatic mutation in phosphatidylinositol glycan-complementation class A gene, resulting in absence of two key complement regulatory proteins CD59 and CD55. Thrombosis occurs in up to 40% of PNH patients; it commonly involves abdominal and cerebral veins and is the leading cause of disease related death. We describe response to Eculizumab (Soliris, Alexion) in a 28 year old male with PNH, Budd-Chiari Syndrome, acute liver dysfunction, haemolytic anaemia and thrombocytopenia. The patient was admitted to the gastroenterology department with acute abdominal pain, haemolitic anaemia, thrombocytopenia and transaminitis. Abdominal doppler ultrasound (US) was immediately performed, detecting of venous sovrahepatic thrombosis (Budd-Chiari Syndrome), portal vein thrombosis, portal hypertension and ascites. He was started on low dose low molecular weight heparin (platelets <40x10^9/L), but despite anticoagulation progressive liver failure occurred, with poor pain control and worsening ascites. We observed worsening thrombocytopenia and haemolysis, with lactate dehydrogenase (LDH) reaching 1766 IU/L, unresponsive to steroids administration. Bone marrow biopsy showed increased red cell turnover, and peripheral blood flow cytometry characterized a large PNH clone (85% total red blood cells). Liver biopsy revealed advanced stage idiopathic cirrhosis. Eculizumab therapy was then started at the dose of 600 mg weekly for 4 weeks and then 900 mg every 15 days. During the first month clinical conditions improved and progressive reduction in abdominal pain was observed; transaminases progressively normalized, LDH dropped to 518 IU/L and platelets reached 40x10^9/L, allowing therapeutic anticoagulation with warfarin. Recanalization of the portal vein thrombosis was found at the Doppler US after 6 weeks' anticoagulation, but recanalization of sovrahepatic veins was not achieved. Currently, after 12 Eculizumab administrations, the patient is well and pain free, platelets are stable >40x10^9/L, Hb 11.9 mg/dL, AST 36 IU/dL, ALT 60 IU/dL, GGT 169 IU/dL, LDH 649 IU/L. No further thrombotic episode has occurred. This case shows that Eculizumab can block intravascular haemolysis and platelet consumption and can improve hepatic failure, allowing full dose of anticoagulants as therapy for current thrombosis or as prophylaxis for future events.

P127

ACQUIRED HAEMOPHILIA A (AHA): CLINICAL FEATURES AND MANAGEMENT OF PATIENTS FROM A SINGLE CENTER

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Background. AHA is a rare bleeding disorder with an incidence of 1.5/million/year. Mortality rate is high (9-22%), if diagnosis is delayed and treatment is not promptly established. Aims. Description of clinical presentation and management of AHA patients followed at our Institution. Patients. Thirty four patients (14M; 20F), were diagnosed with AHA. Diagnosis median age: 70.2 years (25-89); median time from the first bleeding symptoms and diagnosis: 48.5 days (4-264); median follow-up (FU): 19.7 months (2.6-215.4). Results. At diagnosis, bleeding symptoms were present in 33/34 patients (97%); median inhibitor titer was 9.6 BU/mL (2.5-138), median FVIII:C level, 2% (0.01-21). Clinical conditions triggering the inhibitor appearance were present in 17 cases (50%): previous delivery in 9, autoimmune diseases in 4, cancer in 4. First-line eradication therapy was prescribed in all patients: prednisone (PDN) (median dose 1 mg/kg day, range 0.5-2) for 4 weeks, in 23(68%), dexamethasone (DXM) (median dose 24 mg/day, range 24-40) for 4-day courses, in 7 (20%), azathioprine (AZA) 100 mg/day for 3 months, in 2 (6%), cyclophosphamide (CTX) (0.5 and 1.5 mg/kg/day) plus PDN for 2 months, in 2 (6%). Thirty-one patients are evaluable for response. Inhibitor eradication, was obtained in 24/31 patients (77%) (22/27 [81%] on PDN/DXM, 2/4 [50%] on other immune suppressants). Second-line therapy was administered in 4/7 no responder patients: CTX+PDN in 2, DXM in 1, PDN in 1. Inhibitor eradication was obtained in 1.Third-line therapy was performed in 2/3 second-line no responders: 1 CTX+PDN, 1 AZA+PDN. No one responded. One of these two patients was treated with Rituximab, obtaining persistent inhibitor eradication. Three patients relapsed after first-line treatment (3/24, 12.5%). At last control, 23 patients maintained persistent inhibitor eradication (median FU: 25.9 months [2.6-150.9]). Bypassing agents (rFVIIa or FEIBA) were used in 21 patients with a high efficacy to control bleeding symptoms. During FU, occurrence of diseases possibly related to the inhibitor presence (cancer) was recorded in 2/17 idiopathic cases (11.7%). Conclusions. We confirm literature data as regard as idiopathic AHA cases percentage (50%). We observed a high response rate after steroid administration (81%). Bypassing agents were efficacious in all treated patients. Relapse rate was relatively low (12.5%). A good management of AHA reduce the mortality risk bleeding related.

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ESSENTIAL THROMBOCYTHEMIA PATIENTS PRESENT AN INCREASED PLATELET ADHESION TO COLLAGEN UNDER FLOW CONDITIONS

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Background. The myeloproliferative neoplasm essential thrombocythemia (ET) presents a high incidence of arterial and venous thromboembolic events. The acquired activation of leukocytes and platelets (PLT) has been suggested to play a role in these complications. AIM. We aim to assess whether the increased activation of PLT from ET patients reflects in an increase in the thrombus formation potential *in vitro* under flow. Methods. Nine ET patients (mean PLT count 755x10^9/L, range 240-1,409) and 9 healthy controls (mean PLT count 244x10^9/L, range 216-323) were enrolled into the study after informed consent. Peripheral venous whole blood samples, withdrawn in sodium citrate, were recalcified, anticoagulated with heparin and perfused over a collagen coated surface at a shear rate of 1000/s. PLT adhesion and thrombus formation was evaluated with EVOS microscope system. PLT were stained with an anti-P-selectin-FITC antibody as an index of PLT activation, and annexinA5-Alexa Fluor 647 as a measure of PLT surface procoagulant expression (*i.e.* phosphatidylserine). After staining, images of adherent PLT in random fields were taken using phase contrast and flu-

orescence imaging. Results are expressed as mean±SD of the percentage of area covered by all or fluorescently-labeled platelets. Results. After 4' of blood perfusion, the area covered by adherent PLT was found significantly greater in ET patients compared to controls (38.7±5.1 *vs* 20.4±3.1 % coverage, p<0.05). No statistically significant correlation between PLT count and percentage of coverage was found. However, in the 4 ET patients with a PLT count >700x10^9 plt/L, the % coverage was significantly higher compared to the 5 ET patients with a PLT count <700x10^9 plt/L (*i.e.* 54.9±17.5 vs 25.8±11.3 % coverage, respectively, p<0.05). Regarding the specific markers, the area covered by P-selectin positive PLT was significantly (p<0.05) higher in ET patients versus controls, while no statistically significant differences were found in the coverage by annexinA5-positive adherent PLT. Conclusions. These preliminary results show that blood from ET patients has an increased tendency of shear-dependent PLT adhesion at a collagen surface, indicative of a greater thrombus formation capacity, without increase in PLT-dependent coagulation. These results support an active role of PLT adhesiveness in the prothrombotic state of patients with ET.

P129

BETA-THALASSEMIA AND BLOOD TRANSFUSIONS: AN *EX VIVO* STUDY OF HEMOSTATIC PARAMETERS

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Background. In the last decades clinical trials report a high rate of thromboembolic events in ß-thalassemia intermedia and in splenectomized patients. Regular transfusions seem to significantly reduce the relative risk of thrombosis. Aims: To describe the post-transfusional modifications of hemostatic parameters in ß-thalassemia. Patients & Methods. Seven thalassemia carriers (trait) were enrolled at the Center of Immunohematology and Transfusion Medicine of Bergamo hospital. Nine healthy donors acted as control group. Blood samples were obtained before the monthly scheduled blood transfusion and 60' post-transfusion. The following tests were performed: hemocromocytometric analysis; platelet function by Multiplate with ADP, arachidonic acid (ASPI), COLLagen or Thrombin Receptor Activating Peptide 6 (TRAP); flow cytometry to detect platelet surface Tissue Factor (TF) before and after ADP-stimulation. In addition, thrombin generation (TG) was performed by calibrated automated thrombography in platelet-rich plasma spiked with 1 pM Tissue Factor or ADP (1.6 and 8.3 M). Results. Patients were enrolled from May to August 2012 (mean age 28.9, age range 5-48, ßthalassemia intermedia 33%, splenectomy 44%, prior history of thrombosis 22%). Before blood transfusion, splenectomized patients had a higher increase of platelet surface TF expression upon ADP-stimulation compared to healthy controls (p<0.05). In the same patients the platelet aggregation response was significantly higher than healthy donors (p<0.05 for ADP, COLL, TRAP and ASPI), thalassemic trait subjects (p<0.05 for ADP, COLL and ASPI) and non-splenectomized patients (p<0.05 for ADP and COLL). In splenectomized patients we observed a decrease (p=n.s.) in platelet aggregation response to all agonists after blood transfusion. In the same patients, TG lag-time and time-to-peak performed in the presence of TF or ADP were significantly (p<0.05) shorter than in controls both before and after blood transfusion. Differently, the hemostatic profile of non-splenectomized patients was similar to controls. Conclusions: In routinely transfused ß-thalassemia patients, splenectomy is associated with a prothrombotic shift of hemostatic parameters (*i.e.* thrombocytosis, enhanced platelet reactivity and function, and earlier thrombin generation), which are not influenced on the short term by blood transfusions.

P130

MANAGEMENT AND OUTCOME OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN PREGNANT WOMEN: A RETROSPECTIVE SINGLE CENTER CASE SERIES

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Background. Management of ITP in pregnancy (1:1000 to 1:10000 pregnant women) requires treatment based mostly on the risk of maternal bleeding and the possible harm due to pharmacological therapies. We report a single center experience of ITP and pregnancy management. Patients. Since January 2010 we observed 17 pregnant women with ITP (mean age 32.9 yrs, range 20-43), 4 of them had a previous history of pregnancy and treated thrombocytopenia (6 total pregnancies). 12/17 pregnant patients required treatment: steroid alone (4 patients), Intravenous immunoglobulin (IVIG) alone (1 pt), combination of steroid and IVIG (4 cases), steroid + IVIG + platelet transfusion (1 pt), steroid + IVIG + cyclosporine-CSA (2 pts). One of these woman conceived during chronic immunosuppressant therapy (azathioprine) and was switched to CSA at 8th gestational week because of a better safety profile of the drug. However CSA has been stopped in both the treated women (1 patient developed hyperthension and gain of weight, and 1 patient lost the response). 15 patients had vaginal delivery without complications. 2 women required caesarean section (CS): 1 to optimize the timing of delivery after the rising of platelet count, 1 for obstetric reason. Among treated patients, median platelet count at delivery was 95x10^9/L (3 to 163x10^9/L). 1/17 newborns had severe asymptomatic thrombocytopenia (6x10^9/L) and she was treated with IVIG with complete recovery of platelet count after 11 days. After delivery 7/17 patients need to continue a pharmacological treatment: 2 Rituximab (375 mg/mq for 4 weekly administration) with complete response sustained after 13 and 25 months respectively; 2 slow steroid tapering until stop; 1 lost response during steroid tapering and eltrombopag was started three months after delivery with complete response; 1 is continuing IVIG one month after delivery. Conclusions. In most ITP patients, pregnancy has a limited and acceptable risk for women and babies. No evidence support specific "safe" platelet threshold during gestation, labour and delivery. Most patients showed a good recovery of platelet count after delivery. The management of ITP pregnant patients requires full collaboration among haematologist and obstetrician to select a tailored treatment before and after delivery.

Table 1.

Mean maternal age (years)	32.9 (20-43)
Median plt at 1st presentation (109/L)	55 (3-97)
Mean gestational age at start of therapy (weeks)	17 (2-37)
Mean gestational age at delivery (weeks)	38 (35-40)
Mean platelet count at delivery (109/L)	95 (3-163)
Require therapy after delivery (n/N)	7/17
Newborn M:F	7:8
Thrombocytopenia in newborn (n/N)	1/17
Treatment:	
No therapy	5
Steroid only	4
IVIG only	1
Steroid + IVIG	5
Steroid + IVIG + Plt transfusion	1
Steroid + IVIG + CSA	2

P131

SUCCESSFUL PREGNANCY AFTER RITUXIMAB PROPHYLACTIC TREATMENT IN A PATIENT WITH CHRONIC RELAPSING THROMBOTIC THROMBOCYTOPENIC PURPURA

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Thrombotic thrombocytopenic purpura (TTP) is an acute life-threatening microangiopathic disorder associated with a deficiency in the ADAMTS13 metalloprotease. The majority of cases are mediated by inhibitor antibodies to ADAMTS13. The severe reduction of ADAMTS13 activity are predictive of risk of relapse; moreover, pregnancy may precipitate a relapse in women with a history of TTP. Clinical data suggest that the Rituximab may be useful in treating acute refractory or chronic relapsing TTP and may be given as prophylaxis in selected cases to prevent relapse. We describe a successful pregnancy after prophylactic treatment with Rituximab in a patient with chronic relapsing TTP. The patient's first episode of TTP occurred when she was 26 years-old; because of the unresponsiveness to plasma-exchange (PEX) plus steroids, 5 doses of Vincristine were administered to obtain a complete remission (CR). The second and the third TTP episode occurred when she was 28 and 30 years-old, and were successfully treated with PEX and steroids alone. At the time of the third acute episode, monitoring of the ADAMTS 13 activity was performed and a reduced ADAMTS13 activity with inhibitors was documented. After the achievement of the CR, immunosuppressive treatment with Azatioprine was administered for two years which was associated with a normalization of the ADAMTS13 activity. One year later, a reappearance of inhibitors with reduced ADAMTS13 activity was detected and due to the patient's pregnancy desire, Rituximab treatment (375 mg/m^2 for 4 weekly doses) was started, which was followed by a rapid normalization of the ADAMTS13 activity and antibody disappearance within 10 months. One year later she became pregnant. Monthly ADAMTS13 monitoring was carried out during the pregnancy; a normal ADAMTS13 activity was detected until delivery and a caesarean section was performed at the 38th week of gestation. A fit baby was delivered. One month after delivery, ADAMTS13 activity was normal and anti-ADAMTS13 inhibitors were absent. Based on our experience, a successful pregnancy can be planned in women with a history of TTP who wish to have a baby despite the high risk of TTP relapse. Evaluation of the ADAMT13 activity levels and the search of ADAMTS13 inhibitors before planning the pregnancy are essential. A deficient ADAMTS13 activity pre-pregnancy predicts a high risk of relapse and could identify patients for whom the risk/benefit ratio justifies the prophylactic use of Rituximab.

P132

THROMBOTIC THROMBOCYTOPENIC PURPURA AND PREGNANCY. EXPERIENCE ON 7 CASES

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Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening microangiopathic disorder caused by the absence or severe depletion of the metalloproteinase ADAMT13. Pregnancy can precipitate the onset of the disease or it can exacerbate its recurrence in patients with known prior TTP. We report hereby on 8 pregnancies in 7 patients. Three patients presented with a first episode of TTP during pregnancy, while 4 suffered from a chronic relapsing form of the disease. From this latter group, 1 patient developed a relapse during pregnancy. Cases 1 and 2 presented an acute single episode of TTP during the first pregnancy at 9 weeks of gestation and during the second pregnancy at 5 weeks of gestation, respectively. Both obtained a complete remission (CR) after daily plasma-exchange (PEX) plus methylprednisolone (MP). After remission, in case 2, as per request of the patient, an elective abortion was carried out. Case 3 developed TTP when the first pregnancy was complicated by placental abruption and intrauterine foetal death. Two years later a TTP relapse was documented during the second pregnancy at 20 weeks of gestation; ADAMTS13 activity was <5% with the presence of ADAMTS13 inhibitors. After PEX plus MP, a CR was obtained. Case 4 presented a TTP relapse during the first pregnancy at 18 weeks of gestation and obtained a CR after PEX plus MP. Prophylactic PEX were performed until delivery in cases 1, 3 and 4. Cases 5, 6 and 7 with chronic relapsing TTP in remission at the time of pregnancy, maintained a normal ADAMTS13 activity throughout pregnancy, requiring no specific therapy. All patients received low-dose aspirin and prophylactic low molecular weight heparin (LMWH) throughout pregnancy until delivery and during the postpartum for six weeks. Six healthy babies were delivered in the third trimester of gestation. Our data suggest that pregnancy-related TTP or TTP relapse during pregnancy should be treated with PEX, and PEX should be continued until delivery. When ADAMTS13 inhibitors are present, MP can be used because it does not cross the placenta. Evaluation of the ADAMT13 activity levels and the search of anti-ADAMTS13 antibodies before planning a pregnancy is essential in the management of pregnancy. ADAMTS13 activity should be monitored throughout pregnancy so that prompt PEX can be implemented. Due to the multifactorial thrombotic risk, a prophylactic treatment with aspirin and LMWH was initiated during gestation and continued in the postpartum.

P133

SIRT-1 AS A NOVEL THERAPEUTIC TARGET FOR IMPROVING NITRIC OXIDE RELEASE IN HETEROZYGOUS AND HOMOZYGOUS PATIENTS FOR MTHFR MUTATIONS

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Hyperhomocysteinemia is associated with increased risk of atherosclerosis, stroke, myocardial infarction, and possibly Alzheimer's disease. The C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) is a polymorphism responsible for its decreased enzymatic activity leading to a mild to moderate increase in plasma homocysteine concentrations. Homocysteine accumulation on the vessel wall induces vascular dysfunction through mechanisms not yet known. In our experimental model, we have shown that endothelial dysfunction in MTHFR heterozygous mice is associated with both reduced phosphorylation of sirtuin-1 (Sirt-1) and decreased endothelial nitric oxide synthase (eNOS) dimer-to-monomer ratio, marker of enzymatic dysfunction and abnormalities in the production of nitric oxide (NO). Sirt-1 is an important modulator of NO production and resveratrol is one of its major activators. We evaluated the effect of both acute (25 microM) and chronic (10mg/Kg/die for 21 days) administration of resveratrol in heterozygous MTHFR mice and related controls. In both experimental conditions, resveratrol administration reduced endothelial dysfunction by increasing Sirt-1 phosphorylation and eNOS dimer/monomer ratio. Noteworthy, after Sirt-1 inhibition by EX527, resveratrol was no longer able to exert its endothelial protective action, suggesting Sirt-1 as a potential therapeutic target to reduce endothelial dysfunction during hyperhomocysteinemia. In order to translate the results obtained in experimental models, we have extended our study to platelets from heterozygous and homozygous patients for MTHFR mutations and healthy controls, evaluating the vascular effects of NO released by platelets. The treatment of platelets with resveratrol increased Sirt-1 and eNOS (in serine 1177) phosphorylation as well as NO-mediated vasodilation. This protective action of resveratrol was reverted by inhibiting Sirt-1. Our data demonstrate that Sirt-1 is responsible for decreased NO production in heterozygous and homozygous patients for MTHFR mutations suggesting Sirt-1 as a novel therapeutic target to reduce cerebro- and cardiovascular accidents in patients with hyperhomocysteinemia.

Quality of Life and Support Therapy - Infections

P134

ANTI-MÜLLERIAN HORMONE AND ANTRAL FOLLICLE COUNT REVEAL A LATE IMPAIRMENT OF OVARIAN RESERVE IN PATIENTS UNDERGONE LOW GONADOTOXIC REGIMENS FOR HAEMATOLOGICAL MALIGNANCIES

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The impact of cancer therapy on the reproductive potential of patients is increasingly being recognized as survival rates of patients have clearly improved over the recent years. Different fertility preservation methods, either generally accepted or still experimental, are currently available, and counseling of patients requires a delicate balance between efficacy and side-effects of the proposed method and the characteristics of both the tumor and the therapy. A deeper knowledge of the effects of cancer therapy on the reproductive potential of patients over time is required to identify the most appropriate fertility preservation method. Here, we report a case-control study in which 63 female patients diagnosed with haematological malignancies (44 Hodgkin Lymphoma; 13 non-Hodgkin Lymphoma; 6 Acute Myeloid Leukemia) and treated with chemo- and/or radiotherapy were compared to 64 age-matched controls in terms of ovarian reserve, as measured by ultrasound examination (antral follicle count) and hormonal status (follicle-stimulating hormone (FSH), anti-müllerian hormone (AMH), Inhibin-B). By stratifying patients for gonadotoxicity of the therapy received and time elapsed from the end of the therapy, we report that patients treated with low gonadotoxic therapies, while being similar to age-matched controls in their ovarian reserve when evaluated within few years from the end of the therapy, show a clear impairment over longer times. We also report that AMH is the most sensitive hormonal parameter in detecting changes in ovarian reserve when compared to FSH or Inhibin-B. This study stresses the importance of accurate counseling at the time of diagnoses of cancer and emphasizes the risks of infertility with low gonadotoxic therapies that may reduce the reproductive window of survivors.

P135

BLOOD MANAGEMENT AND TRANSFUSION REQUIREMENTS IN HOSPITALIZED HEMATOLOGICAL PATIENTS: A "REAL LIFE" ANALYSIS

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Introduction. Transfusions of packed red blood cells (PRBC), platelet concentrates (PLT) and, although less frequently, fresh frozen plasma (FFP), are commonly required in hospitalized hematological patients (pts), mainly due to bone marrow failure and secondly, to bleeding and increased consumption. The demand of PRBC and PLT frequently exceed the availability of these hemocomponents; so that, administration delays are not rare occurrences. Strategies on predicting blood transfusion requirements at the patient's admission could drive selective and planned donor recruitment, thus limiting the discrepancy from demand and offer; however, data about this issue are scarce. Aim. To analyze blood requirements, features and predicting factors during inpatient hematologic management. Methods. Data of pts hospitalized in our hematology ward from 1-January and 1-March 2011 were analyzed; weekly transfusions data, as well as pts data, were extracted; relation between transfusion intensity (transfused unit /day (u/d)) and pts data at entry were explored, and factor predicting transfusion requirements were identified. Results. During the 2 months period, 46 pts (see Table) were managed (newly or previously admitted); admissions were 50, median duration 18, 5 days, range 3-73 (1314 hospitalization days). We transfused 119 PRBC and 696 PLT concentrates (0.11 PTBC and 0.63 PLT u/d). Among the 50 admissions, median transfusion intensity was 0.06 RBC and 0.14 PLT u/d, respectively. Trend over time of transfusion intensity markedly change, being 0.08 *vs* 0.14 PRBC u/d (p=0.015) and 0.41 *vs* 0.83 PLT u/d (p=0.010) < day 14 *vs* ≥ day 14 from admission, respectively. Statistical analysis is reported on Table; in particular, higher blood demand (both hemocomponents) was observed among myelo-proliferative *vs* lympho-proliferative disorders and among pts with PLT count <100 *vs* >100x10^9/L. Conclusions. By using simple parameters at entry, patients at risk for higher transfusion requirements could be individuate and selective donor recruitment could be planned in order to increase blood availability for the period of maximal requirement. Transfusion requirements increased over time, mainly being allocated after week 2 from admission, so that physicians and transfusionists have enough time to plan donor recruitment interventions. Lastly, physicians should consider strategies to improve PLT count in order to reduce transfusion requirements.

Table 1. Patients data

	Rbc transfusions			Plt transfusions		
	n	mean	p	n	mean	p
gender						
f	22	0,11	0,31	22	0,42	0,22
m	28	0,09		28	0,60	
age						
<=median (56)	27	0,09	0,28	27	0,59	0,25
>median (56)	23	0,11		23	0,44	
diagnosis						
Lym/CLL	19					
AL	14					
MM	7					
MDS	5					
CML	1					
Other	4					
neoplasm						
myeloid	17	0,15	0,02	18	0,81	0,05
lymphoid	29	0,08		29	0,40	
active treatment						
no	13	0,11	0,37	13	0,42	0,30
yes	37	0,10		37	0,56	
wbc						
<=6,1	25	0,09	0,35	25	0,57	0,32
>6,1	25	0,11		25	0,47	
hb						
<=10	25	0,12	0,10	25	0,43	0,22
>10	25	0,08		25	0,61	
plt						
<=100	25	0,13	0,02	25	0,81	0,01
>100	25	0,07		25	0,23	

Lym/CLL: lymphoma/chronic lymphocytic leukemia; AL: acute leukemia; Multiple Myeloma; MDS: myelodysplastic syndrome; CML: chronic myeloid leukemia.

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LOW DOSE CHEMOTHERAPHY AT HOME IN AGGRESSIVE LYMPHOMAS OF VERY OLD PATIENTS: AN ECONOMIC AND EFFECTIVE CHOICE THAT PRESERVE AUTONOMY AND QUALITY OF LIFE. DICENTRIC STUDY

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Aim. Aim of this study is to verify if metronomic therapy is not inferior and less toxic than standard chemotherapy in treatment of aggressive lymphomas of very old patients. Patients and Methods. In 26 patients to calculate frailty of patients CHARLSON,CIRS-G,CRASH and GISL score were used. In groupA patients were treated at home with metronomic therapy with cyclophosfamide 50 mg days 1 to 5, etoposide 50 mg days 1-3-5, prednisone 25 mg days 1 to 7, lenalidomide10 mg days 1 to 21, all orally, every 28 days for 9-12 cycles (Large B Cell Lymphoma and Mantle Cell Lymphoma), or with cyclophosphamide 50 mg days 1 to 3, fludarabine 25 mg days 1 to 3, etoposide days 4 to 6, prednisone 25 mg days 1 to 15, all orally, methotrexate 15 mg in day 15, every 28 days for 9-12cycles (T cell Lymphoma). In group B patients received at hospital i.v. Rituximab 375 mg/sqm day1, Cyclophosphamide 750 mg/sqm day 1, adryamicin50mg/sqm day1, prednisone 50 mg/sqm orally day 1 to 5(Large B Cell Lymphoma,T cell lymphoma and Mantle Cell Lymphoma). In group A M/F:8/8,median age was 85.5 years(R85-94), TNHL/DLBCL/MCL: 5/4/1, median IPI 4 (R2-5), median follow-up was 6 months (R2-13), 9 patients showed 1 comorbidity (56%), 7 patients 2 or more (44%; CHARLSON>5:12pat (75%), CIRS-G=4:9pat. (56%), CRASH>9:7pat (43%), GISL FRAIL:12pat. (75%). In group B M/F:4/6, median age was 85 years (R85-91), TNHL/DLBCL: 2/8,median IPI 4 (R2-5), median follow-up was 6 months (R1-24), 2 patients showed 1 comorbidity (20%), 2 patients 2 or more (20%), 6 patients no comorbidities (60%), CHARLSON>5:4pat (40%), CIRS-G=4:5pat. (50%), CRASH>9:3pat (30%), GISL FRAIL:5pat. (50%). SF8 questionnaire was used to evaluate quality of life of patients. Results. In group A median hospitalization was 0 weeks (R0-12), complete remission 4 patients (25%), partial remission 8 patients (50%), progression of disease 4 patients (25%), G3/G4 toxicities (hematologic 25%, not hematologic 25%, infection 37%, transfusion 19%,death 37.5%), days of hospitalization/days of global survival 5%(R0-25), cost per month of survival € 5000 (R250-9100), SF8 60 (R40-100). In group B median hospitalization was 9 weeks (R3-17),complete remission 5 patients (50%), partial remission 2 patients (20%), progression of disease 3 patients (30%), G3/G4 toxicities (hematologic 70%, not hematologic 50%,infection 40%, transfusion 80%,death 60%), days of hospitalization/days of global survival 33% (R20-100), cost per month of survival €21000 (R5000-37000), SF8 40 (R20-50). Median survival at kaplan-mayer was 18 months for both groups. Conclusion. Metronomic therapy is cost-effective and warrants a good quality of life.

P137

LAMIVUDINE PROPHYLAXIS AND RESCUE THERAPY OF HEPATITIS B VIRUS OCCULT INFECTION REACTIVATION IN ONCOHEMATOLOGIC IMMUNOSUPPRESSED PATIENTS

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Background. Hepatitis B virus (HBV) overt and occult infection reactivation in oncohematologic patients (OHPs) can lead to severe hepatitis and to liver acute failure even. Reactivation can occur from 5 to 36 months (m) after the start of chemotherapy. Patients with serological markers of resolved HBV infection (HBsAg-, HBcAb+, HBsAb -/+) which receive highly immunosuppressive chemotherapy are at high risk of viral reactivation. Antiviral prophylaxis is recommended but the optimal length and monitoring are uncertain. Aims. 1) to evaluate the efficacy and safety of lamivudine (Lam) prophylaxis given for 18 m after discontinuation of chemotherapy; 2) we also report 11 cases of HBV reactivation who were treated with antiviral rescue therapy. Patients. Group A: 39 OHPs (M/F:26/13; median age yrs:65; range 29-82) were studied: 26 non Hodgkin lymphoma (NHL), 6 chronic lymphocytic leukemia (CLL), 7 multiple myeloma (MM). All were screened for HBsAg, HBsAb, HBcAb, HCV-Ab, HAV-Ab and ALT values. HBsAg and ALT were monthly monitored and serum HBV-DNA was tested every 3 m after the start of chemotherapy. Group B: 11 OHPs (3 NHL, 4 LLC, 2 MM, 1 Waldenstrom's disease, 1 lymphoproliferative syndrome) with ongoing HBV reactivation. All patients received standard highly immunosuppressive chemotherapic protocols. Results. Group A: all were HBsAg negative, 9/39 (23%) presented isolated HBcAb positivity and 29/39 (74%) HBsAb/HBcAb positivity. Five of 39 (13%) were HCV-Ab positive and 38/39 (99%) HAV-Ab (IgG) positive. Group B: 11 pts. (M/F: 7/4; median age 68 yrs), 6 with severe clinical reactivation (jaundice and high ALT levels) and 5 with mild/moderate disease. 4 pts. were HBsAg neg/HBV-DNA pos. Lam prophylaxis: Group A pts. started Lam 100 mg/d for 18 m after the last chemotherapy cycle. Twenty of 39 (51%) pts. completed 18 m of Lam prophylaxis and, among these,14/20 (70%) passed 12 m after discontinuation of Lam prophylaxis. Median time after discontinuation of chemotherapy and Lam is 30 (1-58) and 19 (1-54) m respectively. None case of HBV reactivation has been observed. Rescue therapy: Group B pts. received entecavir 0.5 mg/d (6) and Lam (5). One died because liver failure, 3 because hematologic disease but were still HBV-DNA pos. Conclusions. HBV reactivation is life threatening condition and must be prevented. Preliminary data show that 18 months Lam prophylaxis is safe and effective in preventing HBV reactivation and in permitting the completion of chemotherapy.

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NEW PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) POWER GROSHONG DEVICE, FOR HAEMATOLOGICAL PATIENTS MANAGEMENT: A SINGLE CENTRE EXPERIENCE

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PICCs are inserted bedside, via peripheral vein such as cephalic, basilic or brachial veins, without general anesthesia, sedation and surgical procedures. The only single lumen silicone 5 FR Power Groshong PICC (Bard Access, Usa), is a new device recently available, that showed to be efficacious and safe. We describe our experience in the management of 40 haematological patients. Forty PICCs were inserted in 40 patients (20 F and 20 M; median age 63.5, range 25-86) and remained in place for an overall period of 3,031 days (median time 57 days, range 3-208). All PICCs were positioned by a team of specifically trained physicians and nurses and used by trained nurses in our Haematology Unit. We inserted PICCs in 13 pts with Acute Myeloid Leukemia (AML), 14 with Non Hodgkin Lymphoma (NHL), 7 with Multiple Myeloma (MM), 1 POEMS syndrome, 1 Castleman Disease, 1 Myelodysplastic Syndrome (MDS), 2 Autoimmune Haemolytic Anemia (AHA), 1 Chronic Lymphocytic Leukemia (CLL). Of note, 4 patients received autologous stem cell transplant (ASCT) and 8 patients collected peripheral blood stem cells (PBSCs) using Power Groshong PICC. We didn't observe any insertion-related major complication. Late complications were: 1 accidental catheter removal (2.5%, 0.32 per 1000 PICCs days), 1 symptomatic catheter-related venous thrombosis (2.5%, 0.32 per 1000 PICCs days) and 1 catheter-related bloodstream infection (CRBSI 2.5%, 0.32 per 1000 PICCs days). Reasons for PICCs removal were:1 accidental removal (2.5%), 4 end of therapy (10%), 9 deaths (22.5%), 1 CRBSI with positive tip culture (2.5%);the others 25 are still in place. Only 1 patient developed thrombosis in left basilic and axillary veins, following multiple venous punctures during the insertion, he was treated with LMWH with complete recanalization and carried on chemotherapy by PICC. In our experience this new device is a safe and effective alternative particularly compared to tunnelled central venous catheters (CVC). Furthermore, the device due to Power Groshong system and larger diameter, could be used in a increased number of patients. We used this device to

collect PBSCs, to perform ASCT and to give intensive chemotherapy for AL and other onco-haematologic diseases. We observed a lower rate of complications, compared to previous literature data. We believe Power Groshong PICC can be easily and cost-effective employed and could replace tunnelled CVC. Larger series of patients are needed to confirm our data.

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CHRONIC BENIGN NEUTROPENIA IN ADULTS: CLINICAL FEATURES IN A 4-YEAR PROSPECTIVE STUDY

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Chronic benign neutropenia (CBN) is a rare acquired hematological condition, defined by an absolute neutrophil count (ANCs) lower than 1800/µl in white and 1500/µl in black people for more than 3 months, in the absence of any underlying disease; CBN may be idiopathic or autoimmune based on the absence/presence of anti-neutrophils antibodies. In this prospective study we followed up 52 patients with CBN (19 males and 33 females, median age 55 years, range 25-86 years) for a median time of 48 months from January 2009, focusing on 1) ANCs variations (by general estimating equations GEE models), 2) positivity for anti-neutrophil antibodies (by direct and indirect granulocyte immunofluorescence test), 3) bone marrow features, and 4) incidence of infectious episodes. As shown in Figure 1, the mean ANCs were stably under the normal range (1500-6500/µl) at all the time points considered; by GEE analysis, a great inter-subject variability was observed during the follow-up (p=0.012), whereas no significant intra-subject variations were found. The mean ANCs observed during the follow up were significantly lower in males than in females (p=0.023) and in cases with mild splenomegaly, although not significantly (10 cases, 20%, mean maximal diameter 11,4 cm by ultrasonography), independently from gender (multivariate analysis). Anti-neutrophil antibodies were detected in 19/52 patients (37%), and mean ANCs values over the follow up were significantly lower in positive versus negative cases (p=0.027). Bone marrow evaluation (N=27) showed features of dismyelopoiesis in 15 cases (56%), hypocellularity in 3 (11%) and normal morphology in 9 (33%), and flow cytometry demonstrated increased Natural Killer cells in 13 patients (25%). Finally, 9 patients (17%) showed monocytosis, and 5 (10%) a MGUS. An infection needing oral antibiotic or antiviral therapy occurred in 13 patients (25%) (2 pneumonias, 7 upper respiratory tract, 3 Herpes Zoster Virus and 1 urinary tract infections), without relationship with the patient's mean ANCs value, the nadir of ANC value, nor with the presence of anti-neutrophil antibodies. In conclusion, CBN in adults is a benign disease, with an infectious rate not superior to that of the general population and a great variability in ANCs values. Bone marrow evaluation shows abnormal findings in half of patients, without reaching the criteria for clonal hematological diseases, but suggesting that this condition deserves clinical follow up.

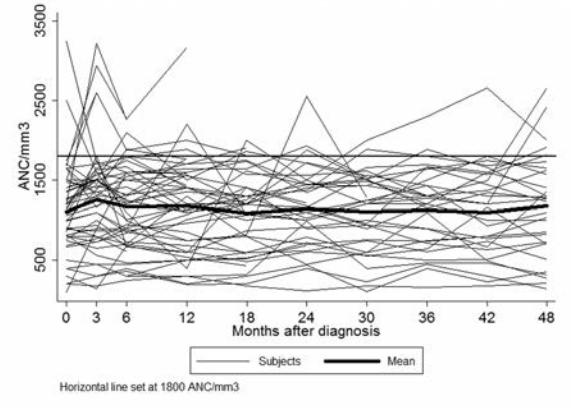

Figure 1.

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IMPORTANCE OF CYTOMEGALOVIRUS MONITORING IN PATIENTS WITH ACUTE MYELOID LEUKEMIA DURING FIRST-LINE CHEMOTHERAPY

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Cytomegalovirus (CMV),infection is an important cause of morbidity and mortality in acute myeloid leukemia (AML) patients undergoing allogeneic hematopoietic stem cell transplantation, but little is known about the incidence of CMV infection and disease in patients with AML during first-line chemotherapy. The aim of the present study was to assess prospectively the incidence of CMV infection in patients with AML at diagnosis and during first-line therapy, and to describe the outcome of treatment. Between 2002 and 2012, 117 consecutive adult patients with AML at diagnosis were enrolled in the study. CMV-DNA and/or pp65-antigenemia were monitored at diagnosis, post-induction and post-consolidation chemotherapy, and whenever CMV reactivation was clinically suspected. Patients with CMV positivity received preemptive treatment. No patient was CMV positive at baseline. Among 105 patients whose serology results were available, 92 had anti-CMV IgG (88%). Overall, out of 90 patients achieving complete remission (CR), 27 (30%) showed CMV positivity; 11/90 (12%) after induction and 16/82 (20%) after consolidation. Twenty-one of the 27 CMV positive patients received anti-CMV treatment (19 pre-emptive, 1 for gastrointestinal disease, 1 for interstitial pneumonitis). Six patients with a low viral load (<5 cells/350 cp/ml) were not treated, because of hematological toxicity or renal damage, but none of them developed CMV disease. Five-year projected probability of overall survival and disease-free survival are 45% *vs* 49% (p:0.9) and 41% in both groups, respectively for CMV positive and CMV negative patients. CMV positive patients had more hospital admissions (2.59 *vs* 2.10; P=0.001) and a longer median hospital stay (82 days *vs* 66 days; P=0.008). In particular, 9/11 patients with high levels of pp65-antigenemia (>10 cells) or CMV-DNA (>900 copies/ml) were admitted to hospital at least once because of clinical complications. Only 2/15 patients with lower levels of pp65-antigenemia/CMV-DNA required hospitalization. In AML patients, modern induction chemotherapy schedules are associated with a significant incidence of CMV infection, which in some cases displays the clinical picture of CMV disease. Close monitoring of the commonly used markers of reactivation is essential and future studies are warranted to assess the cost-effectiveness of pre-emptive antiviral therapy and the standard thresholds necessary to start specific treatment.

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PROSPECTIVE SURVEILLANCE FOR INVASIVE FUNGAL INFECTION IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (HCT): EPIDEMIOLOGY, RISK FACTORS AND OUTCOME IN A SINGLE CENTER

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Invasive fungal infection (IFI) has emerged as a leading cause of morbidity and mortality among HCT recipients. In this study we report an overview on the incidence of IFI by analyzing 10-year data collected prospectively on 350 consecutive patients who received an allogeneic HCT in our center between 2001 and 2011. Patient, donor and transplant characteristics are described in Table 1. We included only IFI defined as probable or proven in accordance with published guidelines. We identified 39 cases of IFI (prevalence 11,1%), of which 30 were due to invasive aspergillosis (IA) (22 probable and 8 proven (Aspergillus flavus 5, Aspergillus fumigatus 3)), 1 to Fusarium solanis infection, and 8 to Candidemia (C. albicans 3, C. krusei 4, C. parapsilosis 1). The median day of onset for IA and Candidemia was day 55 (12-3023) and day 35 (11-187) respectively, whereas the Fusariosis infection occurred on day 140 post-transplant. IA involved lung in 29 cases and brain in 1; Fusariosis involved skin and blood; Candemia occurred in blood (n=6) or in blood, liver and spleen (n=2). Imaging findings in pulmonary IA on CT scan included: macrododules > 1 cm in 13 patients, consolidation in 7, clus-

ters of small nodules in 7, cavitary lesion in 2, and non specific ground-glass opacification in 2. The 5-yr and 10-yr cumulative incidence (CI) of IFI was 11.3% (IA 8.9%, Candidemia 2.4%) and 12.5% (IA 10.1%, Candidemia 2.4%) respectively. In univariate analysis there was no significant correlation between recipient factors (age, gender, ferritin, underlying disease, previous transplant, disease risk) or transplant factors (type and intensity of conditioning regimen, stem cell source), and occurrence of IFI, whereas the use of antithymocyte globulin (ATG) and the development of both acute and chronic graft-versus-host disease (GvHD) were significantly associated with an higher CI of IFI. In multivariate analysis we found 4 risk factors significantly associated with an higher CI of IFI: recipient age >50 years (Hazard Risk (HR) 1.65 (p=0.05); acute GvHD (HR 1.59, p=0.01); chronic GvHD (HR 1.62, p=0.001; CMV infection (HR 1.37, p=0.003). Five of the 39 patients with IFI died for this complication, in all cases from IA. The 10-yr overall survival for the 311 patients with no evidence of IFI was 42% as compared to 17% of the 39 patients with IFI (p=0.006). These results emphasize that IFI represent a significant complication in HCT recipients.

Table 1. Patient, donor and transplant characteristics

N. of patients	350
Male / Female (%)	204 / 146 (58 / 42)
Median age, years (range)	40 (1-71)
Malignant / non malignant disease (%)	314 / 36 (90 / 10)
Disease risk at HCT	
Standard / intermediate / high (%)	84 / 135 / 131 (24 / 38 / 27)
Previous transplant, N. (%)	52 (15)
Median ferritin ng/ml (range)	1125 (14-9513)
Donor median age (range)	37 (2-70)
Type of donor, N. (%)	
HLA identical sibling	187 (53)
HLA haploidentical family donor	59 (17)
Matched unrelated donor	104 (30)
Type of conditioning	
Myeloablative conditioning (%)	259 (74)
Reduced intensity	91 (26)
Stem cell source (%)	
Bone marrow	190 (54)
Peripheral blood stem cells	151 (2)
Cord blood	7 (2)
GvHD prophylaxis (%)	
Cyclosporine (CSA)	28 (8)
CSA / Methotrexate (MTX)	246 (70)
CSA – MTX – Basiliximab – Mycofenolate - ATG	42 (12)
T-cell depletion	17 (5)
Other	16 (5)
Anti-thymocyte globulin (%)	161 (46)

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POSACONAZOLE PROPHYLAXIS IN ACUTE MYELOID LEUKEMIA. REAL LIFE EXPERIENCE IN FOUR HEMATOLOGICAL CENTERS

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Introduction. Acute Myeloid Leukemia (AML) patients (pts) are at high risk of Invasive Fungal Diseases (IFD). Posaconazole (POS), an oral azole with a broader spectrum, was approved for the prophylaxis of IFD in high risk hematologic pts. We report a multicenter real-life experience with POS prophylaxis in AML. We also compare the performance of POS with an historical, well matched, control group of AML pts who received prophylaxis with Fluconazole (FLUCO) or Itraconazole (ITRA). Patients and Results. 120 unselected and consecutive AML pts (96 with active disease and 24 in CR) received POS prophylaxis (600 mg daily), between Jan 2009 and Dec 2012, in 4 Hematological Centers. Median age was 52 yrs (range 18-69). All cases were given intensive chemotherapy. 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 16 days (range 7-71); 27/120 (23%) of cases had an oral mucositis grade II-III CTC and 74% of these pts received a proton pump inhibitor. An active diagnostic work up was made in all cases with GM assay, standard chest X-ray and thoracic CT scan in case of FUO lasting over 48 hours. The median duration of POS was 16 days (range 7-41). Only 17/120 (14%) of pts required parenteral empiric or pre-emptive antimycotic therapy and only 6/120 (5%) experienced a probable or proven IFD. Mortality IFD related was 0%. POS was well tolerated and only 8% of pts experienced mild drug related side effects. No cases of POS discontinuation were reported. When we compare the 120 pts who received POS with an historical control group of 120 AML pts who received FLUCO or ITRA prophylaxis, no significant differences were observed for underling disease status, age, IFD risk factors, days of severe neutropenia and days of prophylaxis. Instead, there were significant differences in number of cases who required parenteral antymicotic therapy (14% in POS group *vs* 25% in the control group; P=0,01), and in days of parenteral antymicotic therapy (151 *vs* 374, with a saving of 223 days). Conclusions. This real-life experience confirms that POS prophylaxis is safe, well tolerated and effective in unselected AML pts. Only 14% of these high risk pts required parenteral antimycotic therapy and only 6/120(5%) experienced probable (5/120) or proven (1/120) IFD. We also confirm that POS, in real life setting, is more effective than FLUCO/ITRA as antifungal prophylaxis in AML pts.

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CIDOFOVIR TREATMENT FOR HAEMORRHAGIC CYSTITIS AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HIGH RISK HAEMATOLOGICAL MALIGNANCIES

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Hemorrhagic cystitis (HC) is a common cause of morbidity after allogeneic stem cell transplantation (allo-SCT), often associated with BK virus infection. Cidofovir (CDV) is an acyclic nucleoside analogue with a broad range of antiviral activity, including BK virus and thus it has been proposed as treatment for HC after allo-SCT. We retrospectively evaluated 59 consecutive patients (median age 45 years) who received CDV for HC after allo-SCT from 2008 to 2012. All pts were affected by hematologic malignancies, 39 had active disease at allo-SCT. Donors were HLA identical sibling (8), family mismatched (39), unrelated volunteer (11), cord blood (1). All pts received a myeloablative conditioning regimen. BK viral load was determined on urine by TaqMan PCR.

CDV was administered at the dose of 5 mg/kg/week intravenously (i.v.) in 41 pts and intravescically in 21 pts (4 double treatments). The median dose number of CDV doses was 2 (range 1 to 8). HC occurred in median 14 days after allo-SCT (range -4 to 330). Median duration of symptoms was 34 days (range 6 to 166). At treatment onset 13 pts had grade 0-I HC, 13 grade II, 24 grade III and 9 grade IV. In 55 cases (93%) high BK viral load was detected in urine (BK virus median load 10^7 cp/ml) before treatment. After treatment the reduction of BK viruria was documented in 29 out of 33 evaluable cases (87%), with a 1-log reduction of BK viruria median load. Improvement of HC grade was observed in 41 pts (70%) and a complete clinical response within 7 days from last CDV dose was observed in 30 cases (51%). Worsening of HC leading to urological intervention occured in 5 treated pts. Four pts died with an ongoing uncontrolled HC: 2 of acute Graft-versus Host Disease, 1 of pneumonia and 1 of relapse. During i.v. CDV treatment 22 pts (54%) had a viral reactivation (Cytomegalovirus n=14, Epstein-Barr Virus n=8, Herpes Simplex-1 n=3, Herpes Simplex-6 n=4) and 2 pts developed acute renal failure requiring drug discontinuation. CDV treatment after allo-SCT is associated with a reduction of BK viruria load and HC clinical responses in more than half of cases. Viral reactivations under CDV treatment should be better investigated in the allo-setting, since the long-half life of the drug and its potential nephrotoxicity limits the possibility of concomitant antiviral therapy with other agents. Our data warrant prospective randomized trials to investigate the role of CDV in allo-SCT.

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HAEMATOLOGIC HOME-CARE: THE EXPERIENCE IN VERONA SINCE 1999

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The Haematologic Home Care was introduced in Verona in April 1999, in order to treat old patients and to provide them with an adequate assistance outside the hospital, for the purpose of reduce their discomfort. Considered at the beginning only as a transfusional support for patients affected by chronic haematologic diseases, this activity soon after enhanced its service with other treatments formerly carried out in the hospital. In regard to the logistical and financial organization, the service is totally supported by the AIL (Associazione Lotta alla Leucemia Linfomi e Mielomi) of Verona and implies the full time work of one doctor, one nurse and one secretary, besides the contribution of other five doctors and three nurses. Activity is conducted under a free agreement with the hospital of Verona and with the supervision and consultation of one doctor employed in the Haematologic Department. The service manages costs and procedures as a traditional hospital unit. The distribution of drugs is carried out in the same way as for outpatients.

Table 1: Provided Services

Year	Transfusion	Blood samples and lab. tests	Chemotherapy	Other + visits	Tot
1999	37	33			70
2000	252	309	70		631
2001	387	419	90		896
2002	286	369	120		775
2003	322	382	144		844
2004	380	401	116		897
2005	314	370	118		802
2006	319	327	108		754
2007	363	370	127		860
2008	242	355	131	9	737
2009	315	360	167		842
2010	392	510	275		1177
2011	429	669	340	15 +25	1478
2012	438	811	306	21 + 116	1693

The service is dedicated to patients affected by hematological diseases (not in terminal phase when home care begins), disabled for comorbidity or in unsuitable clinical conditions or in need of familiar help to go to the hospital and underwent the outpatient treatments. At this moment 73 patients are followed, among which 30 are men and 43 are women. The average age is 79 years; 46% of them are affected by multiple myeloma, 30% by myelodisplastic syndrome, 16% by myeloid neoplasms, 6% by non Hodgkin lymphomas and 2% by amiloydosis. The total number of provided services shows a constant and progressive increase in time, as summarized in Table 1. The increased number of physical examinations and the number of blood samples collected at home to monitor the disease caused the reduction of the number of admissions to our divisional ambulatory and day hospital. Moreover, the significant portion of patients affected by multiple myeloma and assisted in day-care reflects our service ability to adapt to old patients' needs, which further on reduces the need of support therapies and admissions to hospital.

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POLYMICROBIAL BLOODSTREAM INFECTIONS IN PATIENTS WITH HEMATOLOGICAL CANCER

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Background. Bloodstream infections (BSI) often complicate the course of hematologic diseases and may negatively impact patients' survival. BSI mortality depends on patient-related factors (age, comorbidities, type of hematologic disorder), and microbiological features (*i.e.*, type of pathogens, antimicrobial susceptibility). We aimed to characterize the differences between BSI caused by a single pathogen and polymicrobial BSI. Patients and Results. Patients population characteristics, infectious source, severity of infection (sepsis, septic shock, IRA), and outcome were collected in a group of 193 BSI involving 147 patients with various hematological disorders. Polymicrobial sepsis (pBSI) were observed in 43 (22%) cases and non-polymicrobial BSI in 150 (78%) cases collected at our Centre over a 4–year period (2008-2012). Non-pBSI were equally distributed between Gram-negative and Gram-positive pathogens (53% and 47%, respectively), with most commonly isolates represented by E. coli (56%), S. epidermidis (52%), E. faecium (18%), and P. aeruginosa (17%). Occurrence of pBSI followed a non-pBSI in 53% of cases. Overall, pBSI involved more frequently Gram-positive and Gram-negative bacteria association (20/43, 47%), particularly E. coli and Enterococcus spp or Staphylococcus spp (14% and 16%, respectively). or Enterococcus spp. and P. aeruginosa (5/43, 12%).

Table 1.

Parameter	Non-pBSI Gram-postive (79)	Non-pBSI Gram-negative (71)	pBSI (43)
Multiple infections site (%)	34(43)	34 (48)	32 (74)
Pneumonia	3 (4)	14 (20)	5 (12)
Mucositis	7 (9)	2 (3)	1(2)
Abdominal infection	3 (4)	11 (15)	8 (19)
Catheter related	19 (24)	0	10 (23)
Urinary tract	1(1)	4 (6)	2 (5)
Skin and soft tissue	1 (1)	3 (4)	6(14)
SEPSIS/SHOCK (%)	2(3)	15 (21)	9 (21)
MORTALITY RATE(%)	3 (4)	16 (23)	7 (16)

The most common underlying disease was acute myeloid leukaemia (AML) in both groups without significant differences. Risk factors correlating with pBSI *vs* non-pBSI included the presence of another contemporary site of infection (74% *vs* 45% respectively, p<.0001) with the exception of pneumonia that was more frequent in non-pBSI (20% *vs* 12%, p value not significant).Other infection sites in pBSI included catheter-related BSI (31%) followed by abdominal infections (25%) and skin and soft tissue infections (19%). Overall severe sepsis and mortality rates were not significantly different in pBSI and non pBSI groups (21% and 16% *vs* 17% and 13%, respectively) although non-pBSI due to Gram-positive agents had significantly lower rates compared to Gram-negative pathogens (3% and 4% *vs* 21% and 23%, p<0.0001, respectively). Conclusions. pBSI frequently occur after a previous infectious episode of BSI and are often associated with multiple sites of infection. Although they do not represent a frequent event in haematological patients, their occurrence may have high impact on severe sepsis occurrence and mortality.

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PATIENT-RELATED QUALITY OF LIFE IN ONCO-HEMATOLOGICAL SETTING. A SURVEY FROM A SINGLE INSTITUTION

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Background. Life-expectancy of cancer patients is increased due to new treatments and to the improvement of supportive therapies. The evaluation of the Quality of Life (QOL) in patients with cancer has become an important item in several Oncological as well as Hematological Institutions. In particular, in the setting of onco-hematological patients, the availability of new drugs (*i.e.*, targeted therapies, biological modulators) and of a better supportive treatments carried out to an improvement of the QOL and to a prolonged survival of patients. Aim. To evaluate the QOL in a cohort of onco-hematological patients attended to a single Institution. Patients. Between September 2012 and March 2013, 170 patients suffering from different onco-hematological diseases received the SF-36 questionary, that is a short-form health survey structured by 36 questions regarding 8 scale profile of functional health. 124 patients, 67 males and 57 females, median age 65 years, 9 of them fully hospitalized, and 115 in Day Hospital regimen, accepted to attend to the survey. 40 patients suffered from NHL, 23 from MDS, 20 from MM, 10 from CLL, 10 from HL, 8 from AML, 7 from MPS and 6 from ALL; all patients were in-treatment. The Karnofsky Perfomance Status was ≥50% for the entire population. 64 patients had an higher education, while 60 had a low education profile. Results. More than 70% of the patients (92/124) affirm a satisfactory level of health; moreover, 34 patients (27,4%) declare a better health status than one year ago. 30% of patients had some limitation in their personal care. 94 patients (76%) complain to be unable to physically demanding activities as well as 88 (71%) of them have reduced the time devoted to their job. The fatigue is also responsible for the reduced social life while the emotional state appeared good enough in more than 60% of the patients. The pain is a less relevant item in this cohort of patients. A large majority of patients appreciated their relationship with the medical as well as the nursing staff. Conclusions. Fatigue resulted the most relevant problem in this hematological-based patients population. Therefore, fight the fatigue seems to be the real challenge treating onco-hematological patients.

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AMPHOTERICIN B AND POSACONAZOLE: COMBINATION THERAPY IN INVASIVE MUCORMYCOSIS. AN ITALIAN MULTICENTRIC EXPERIENCE

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Background/Aims. Despite the recent advances in the treatment of invasive fungal diseases in hematological patients, invasive mucormycosis (IM) continues to be a severe infection, with high mortality. Aim of the present study was to evaluate the efficacy of combination therapy (combo) with posaconazole (POS) and liposomal amphotericin B (L-Amb) in hematological patients affected by IM. Methods. We collected a retrospective, multicentre registry of all cases of proven/probable IM treated with L-AmB+POS in 8 Italian Hematology centers. The variable analyzed were: age, sex, hematological malignancy and phase of treatment, site of infection, neutropenia and recovery from neutropenia, antifungal prophylaxis (AF), kind and duration of AF, aetiology, and outcome. Every patient received a follow up of at least 90 days. Results. 13 cases of IM were enlisted and analyzed. More than half (8/13, 61%) occurred in acute myeloid leukemia, followed by lymphoproliferative diseases (4/13, 31%) and severe aplastic anemia (1/13, 8%).

Table 1. Characteristics of 13 patients with IM

		OUTCOME	
	Caes 13 (100%)	**Favorable 11 (85%)**	**Unfavorable 2 (15%)**
SEX (M/F)	7/6	7/4	0/2
AGE (median, years)	49	47	60
NEUTROPENIA at onset			
Yes	9 (69%)	8 (89%)	1 (11%)
No	4 (31%)	3 (75%)	1 (25%)
PROPHYLAXIS			
Fluconazole	5 (56%)	4 (80%)	1 (20%)
Itaconazole	3 (33%)	2 (66%)	1 (33%)
Voriconazole	1 (11%)	1 (100%)	
SITE OF INFECTION			
Lung	2 (15%)	2 (100%)	
Rhinocerebral	3 (23%)	3 (100%)	
Other*	4 (31%)	3 (75%)	1 (25%)
Multiple	4 (31%)	3 (75%)	1 (25%)
AGENT			
Mucorales spp	4 (31%)	3 (75%)	1 (25%)
Rhizopus	3 (23%)	2 (66%)	1 (33%)
Absidia	4 (31%)	4 (100%)	
Rhizomucor	2 (15%) 2 (100%)		
COMBO (dose of L-Amb)			
3mg/kg	4 (31%)	3 (75%)	1 (25%)
5mg/kg	9 (69%)	8 (89%)	1 (11%)
SURGERY			
Yes	2 (15%)	2 (100%)	
No	11 (85%)	9 (82%)	2 (18%)
RECOVERYCFROM NEUTROPENIA			
Yes	6 (67%)	6 (100%)	
No	3 (33%)	2 (67%)	1 (33%)

*1 liver/duodenum, 1 tracheobronchial, 1 skin/soft tissues, 1 CNS

Most of cases were observed during the induction/consolidation phase of treatment (5/13, 38%), while the remaining occurred during relapse/salvage (3/13, 23%), before any treatment was given (supportive therapy, 3/13, 23%) and in two cases as a complication of allogenic hematopoietic stem cell transplantation (15%).
As showed in Table 1, 69% of patients had received AF prophylaxis. In 2 (15%) cases the IM was pulmonary, in 3 cases was rhinocerebral (23%) and was multiple (according to EORTC criteria) in the rest of cases (4, 31%). Eleven patients achieved a partial/complete response (11/13, 85%). In 2 cases combo was administered as first line therapy (15%) while it was a second/third line therapy for the remaining 11 patients. In 9 cases combo was a sequential therapy (69%). The average duration of combo was 35 days (range 10-72). Surgery was performed in 2 cases only (15%). Combo therapy was well tolerated. Conclusions. Despite the current improvement of antifungal therapy, a high mortality persists. Notwithstanding the several limits of the present study (the low number of patients, the retrospective design), our results suggest a possible role for combination therapy with L-Amb+POS in IM. This strategy appears to be more successful when compared to current monotherapy standards.

P148

PREDICTIVE PLATELET RECOVERY AND QUALITY OF LIFE IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS TREATED WITH AZACYTIDINE: RESULTS OF A RETROSPECTIVE SINGLE CENTRE ANALYSIS

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Objectives. Acute leukemia in elderly patients is characterized by a poor prognosis,especially in those patients with several comorbidities or poor performance status (PS). Azacitidine(Aza) treatment is well tolerated and might be an ambulatory alternative to standard intensive chemotherapy in those frail patients. Methods. We treated in our institution from 2009 to 2013,twenty AML patients >65 years old (15 de novo, 5 secondary) with performance status 2 or 3.Aza was administered subcutaneously (75mg/m/d) for 7 days every 28-day cycle until progression of disease. Of 20 patients (7 female and 13 male) the median age was 76.5 (range 65-83),median white blood cells was 3.3 (range1.1-48), median hb was 7.85 (range6.2-12.2), median platelets 70(17-245), median bone marrow blasts counts was 47% (range 21-90%). The comorbidities were:hepatopathy HCV correlated, ischemic cardiopathy, chronic renal failure, BPCO, diabetes), Cytogenetic analysis was normal risk in 11 patients(55%), intermediate risk in 3 patients (10%) and unfavourable risk in 6 patients (35%). The fever infections that requiring IV antiobiotics were in 7 patients (35%) and 5/7 (71%) of infected patients not reduced rate and were not hospitalized.15 patients in Aza group achieved response to treatment in fact overall response rate (ORR) was 75%(complete remission 45%+ partial remission 30%)with a median duration of 9 months (range1-29), the median overall survival (OS) was 12 months (range 2-29). The transfusion independence was in 5 (25%) patients, transfusion dependence was reduced in 9 (45%) and stable in 6 (30%) patients. 12 patients of 20 showed platelet recovery (>100) within 3 months of treatment and 18/20 (90%) were transfusion indipendence. Adverse events were limited to grade 1: neutropenia (60%) and thrombocytopenia (20%),gastrointestinal as constipation or diarrhoea (10%), injection–site reaction,gastrointestinal toxicity as nausea, vomiting (10%),corrected only with home medical treatment. Results. Platelet recovery within 3th cycle of Aza treatment is predictive of complete response to the therapy ($p<0.0005$). Discussion/conclusion. AZA may improve quality of life by reducing requirements for transfusions platelets and red cells symptoms and hospitalization in unfit/frail AML patients.

P149

EPIDEMIOLOGY OF FEBRILE EVENTS IN ACUTE LYMPHOBLASTIC LEUKEMIA: PRELIMINARY RESULTS FROM THE SEIFEM 2012 B STUDY

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Objectives. To investigate the different causes of fever in patients (pts) affected by Acute Lymphoblastic Leukemia (ALL) and their role in the outcome. Methods. From April 2012 to April 2013, in 13 Italian participating centers, all consecutive pts admitted in hematological wards with a diagnosis of ALL were enlisted. All febrile events (FEs) were analyzed in the different phases of treatment. Pts undergoing autologous and allogeneic HSCT were withdrawn from the study. We considered some risk factors connected to ALL itself (*i.e.* WBC count, lineage, Ph+ chromosome, ECOG score) and factors linked to treatment (high-doses therapy -HD-, use of tirosyne-kinases inhibitors -TKI-, phase of treatment). We also collected data on well-known conditions that increase the risk of infection (neutropenia, use of steroids, prophylaxis). We evaluated the outcome at 30 days from the event.

Table 1. Characteristics of 47 patients presenting febrile events.

	All	Favorable	Unfavorable
Cases	47 (100%)	44 (94%)	3 (6%)
Sex (M/F)	27/20	24/20	3/0
Lineage			
B	32 (68%)	30(94%)	2(6%)
T	15(32%)	14(93%)	1(7%)
Ph+ chromosome	11 (23%)	11 (100%)	/
Prior admissions			
0	32 (68%)	30 (94%)	2 (6%)
>1	15 (32 %)	14 (93%)	1 (7%)
Phase of treatment			
First induction	18 (38 %)	18 (100%)	/
Relapse	12 (26%)	11 (92%)	1 (8%)
Consolidation/ maintenance	12 (26%)	12 (100%)	/
Terminal disease	3 (6%)	2 (67%)	1 (33%)
Prior to treatment	2 (4%)	/	1 (100%)
Therapy			
High doses (HD)	36 (76 %)	34 (94%)	2 (6%)
TKI	4 (9%)	4 (100%)	/
HD+TKI	2 (4 %)	2 (100%)	/
Other	5 (11%)	4 (80%)	1 (20%)
Steroids			
Yes	37 (79%)	34 (92%)	3 (8%)
No	10 (21%)	10 (100%)	/
Neutropenia			
Yes	37 (79%)	34 (92%)	3 (8%)
No	10 (21%)	10 (100%)	/
Type of infection			
Bacterial	18 (38%)	17 (94%)	1 (6%)
Fungal	9 (19%)	9 (100%)	/
Viral	1 (2%)	1 (100%)	/
FUO	19 (41%)	17 (89%)	2 (11%)
Site			
Blood	13 (28%)	12 (92%)	1 (8%)
Lung	5 (10%)	5 (100%)	/
Other	10 (21%)	10 (100%)	/
Unknown	19 (41%)	17 (89%)	2 (11%)

Results. We enrolled 103 pts; 47 FEs were recorded over a number of 156 admissions to hospital for ALL (incidence 30%). Median age was 49 years (range 18-78). B phenotype was expressed in 68% of cases (32/47), T phenotype in 32% (15/47). Ph+ chromosome was found in 23% of pts (11/47). For the most part (68%, 32/47) FEs occurred in pts at first admission to hospital, except in 2 pts where fever onset happened before any treatment was started. In 38% patients (18/47) FEs onset arose when first induction therapy was administered, in the remaining cases occurred during relapse (26%, 12/47), consolidation or maintenance (26%, 12/47), and in terminal phase (6%, 3/47). In 36 cases (76%) arose after HD therapy, other 2 (4%) received TKI also. 4 cases during TKI only (9%) and in the remaining 5 patients other less aggressive strategies were utilized (11%). At the onset of FEs 37 cases were neutropenic (79%) and 37 cases were under steroid treatment. A known etiologic agent was found to be bacterial in 38% of FEs, fungal in 19%, viral in 2%. In 19 FEs (41%) it was classified as fever of unknown origin (FUO). 28% infections originated in blood, 10% in lung, 21% in other sites and in 41% of cases primary site was unknown. Only 3 patients died for a FE (6% of FEs, 3% of all ALL cases), specifically 2 for FUO and 1 for bacterial sepsis (2% of FEs, 1% of al ALL cases). Conclusions. Although performed with a restricted number of pts, due to preliminary data, our experience suggests that ALL pts can develop frequently a FE, however the mortality risk for infectious complication seems truly low.

P0150
COMPLICATIONS RELATED TO CENTRAL VENOUS CATHETERS IN PATIENTS WITH HAEMATOLOGICAL DISEASE IN A SINGLE CENTRE: THE EXPERIENCE OF 3 YEARS

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Background. In haematology divisions using central venous catheters (CVC) for the administration of chemotherapy is become indispensable even if they are associated with a range of complications. We verify the incidence of complications both for the short-term CVC (Certofix) and for tunnelled CVC (Hickman). Methods. A retrospective study was conducted on a series of 220 consecutive patients hospitalized in the Division of Adult Hematology and Bone Marrow Transplant of San Gerardo Hospital in Monza during the period between January 2008 and February 2011 with hematological diseases undergoing to intensified cycles of chemotherapy. Results. Patient characteristics and CVCs are shown in Table 1.

Table 1.

Gender:	
Male	120
Female	100
Age:	
< 65 years	156
> 65 years	64
Disease:	
Acute myeloid leukemia	159
Lymphoproliferative disease	48
Others	13
CVCs type:	
Single lumen Certofix line	1
Bilumen Certofix line	345
Triple lume Certofix line	17
Single lumen Hickman line	116
Bilumen Hickman line	1
Peripherally inserted central catheter (PICC)	3
Porth-a-cath	3
CVCs duration of positioning (days):	
bilumen Certofix line	27,6
Triple lumen Certofix line	20,5
Single lumen Hickman line	178,9
PICC	16
Porth-a-cath	352,7

There were 9 mechanical complications and 14 thrombotic complications. The total time of catheterisation was 9836 days for Certofix CVC (CC) and 16270 days for Hickman lines (HC). 95 (19.6%) CVC were removed for infectious diseases (76.8% CC and 20% HC). The removal rate of each type of CVC for an infective cause was 21.2% for CC and 16.4% for HC. In 58 cases it was possible to demonstrate microbiologically a CVC-related infection (CRI). The remaining CVC were removed by clinically proven infections.The main diagnostic criteria for CRI was the differential time (more than 2 hours) from positive CVC-blood culture (BC) and peripherical BC (33 cases)In 8 cases the infections were dimostrated thanks to a quantitative BC and in 20 cases thanks to CVC-tip colture when the line was removed. CRI were caused by 31 Gram positive (22 Staphilococcus Epidermidis), 25 Gram negative (3 Escherichia Coli, 7 Pseudomonas Aeruginosa) and 2 Candidae.We have calculated the rate of CRI in our population: CC infections rate for 1000 days of catheterization is 4.3 instead HC infections rate is 0.9 (including only the microbiologically proven CVC-related sepsis). These rates increased if were included clinically suspected infections (respectively 7.5 and 1.2 for 1000 days). There was 16 insertion site infections of CC and 3 tunnel infection in HC (respectively 4.4% and 2.6%). Conclusions. This work shows that the positioning of a CVC in an haematological patient is a feasible and safe procedure. The main complication remains CRI (20% CVC was removed because of infection in our series). Even though our infections rate is much lower than what is reported in literature the relatively high infectious rate highlights the need to improve CVC management, vital part of care for this population.

P151
217 BLOOD STREAM INFECTIONS IN HAEMATOLOGICAL PATIENTS: IMPACT OF RESISTANCES

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Background. Mortality rate of bloodstream infections (BSI) among neutropenic cancer patients ranges between 5-10%. Multi-drug-resistant (MDR) pathogen has been associated with increased mortality. We want to determine the impact of BSI (in particular Pseudomonas Aeruginosa -Pa-) on the outcome of haematological patients in our Institution. Materials and Methods. From January 2008 and Februay 2011 we followed up 220 patients (pts) treated with high-dose chemotherapy and hospitalized at S. Gerardo Hospital of Monza. All strains were isolated in Haematological Department. All pts were neutropenic and didn't receive any antibiotical prophylaxis. MDRPa and Extensive Drug Resistent Pa (EDRPa) are defined as literature reported (Falagas 2008). Results. During the surveillance we have found 217 BSI from 220 pts. Mortality rata in BSI was 21/193 sepsis (10.8%). Twenty-four BSI were presented as a septic shock and in this group 15 pts were died (62.5%). BSI-etiology and related mortality rate were: Candida 7 (1 death: 14.3%), Clostridium 6 (0), Corynebacterium 7 (0), Enterobacter 13 (1 death: 7.7%), Enterococci 32 (8 deaths: 25%), Escherichia Coli 59 (4 deaths: 6.8%), Klebsiella 4 (2 deaths: 50%), Staphilococci coagulasi negative 45 (6 deaths: 13.3%), Staphilococcus Aureus 5 (1 death: 20%), Stenotrophomonas 10 (4 deaths: 40%), Pa 29. Death related to Pa were 9 (31%) but splitting different Pa with resistence profile, MDRPa had a mortality rate of 1/1 (100%) and EDRPa of 7/7(100%). Picture 1 shows the relationship between mortality and incidence of pathogens.

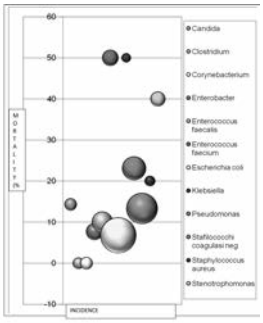

Figure 1.

All non MDRPa responded to first line therapy except 1 pt that died for multiorgan failure.Other Pa were resistant to fluoroquinolones and third generation cephalosporine. All EDRPa showed a resistance in carbapenemics and positive metallobetalattamasis, in all cases colimicin was the only active antibiotic.The lack of temporal and physical proximity and the strains' genetic analysis had excluded the presence of a Pa out-break. Both state of disease and number of previous hospitalization were not correlated with a different profile of resistence. Conclusion: BSIs were still the major cause of morbidity and mortality in this setting of pts. Pa mortality in our series is worsen than what is reported in literature (40% mortality for MDRPa in Trecarichi 2011). Even with small numbers, the impact on survival is relevant with no active combination antimicrobial therapy on EDRPa (100% mortality for MDRPa-EDRPa).

P152

ANTIFUNGAL DRUGS IN THE MANAGEMENT OF HEMATOLOGICAL DISEASES IN A HOME CARE PROGRAM

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Introduction. Antifungal drugs are frequently used in hematology, due to relatively high frequency of fungal infections, affecting both quality of life, management complexity and disease outcome variables. The literature data about the use of antifungal therapy in patients with hematological diseases are very limited and completely absent in the home care setting. Materials and Methods. Electronic health records of home care patients were retrospectively scanned, looking for brand names and active components of the following antifungal drugs: nystatin, fluconazole, amphotericin B, caspofungin, itraconazole, voriconazole, posaconazole; we extrapolated frequency, type, indication for use and duration of each identified antifungal treatment. Results. From 15 September 2011 to 15 September 2012, we cared 106 patients, median age 83 years, affected by cancer in 82 (77,4%) (hematologic cancer in 73 (68,9%) and solid tumors in 9 (8,5%)), and other diagnosis in 24 (22,7%) patients (Table). Eighteen out of 106 (17%) patients (18/73 hematological cancer patients (24,7%)) were treated with antifungals, 12 (11.3%) with systemic antifungal (fluconazole 6, itraconazole 5, sequential voriconazole and itraconazole 1) and 6 (5.7%) with local antifungal. The indications for nystatin use were: oral candidiasis treatment in 5 and prophylaxis in 1; the indications for systemic antifungals use were: prophylaxis during neutropenia in 7, oropharyngeal or esophageal candidiasis in 3, possible invasive fungal infection in 2. Administration was: acute (short-term) in 12 (median duration of acute treatment 22 days (1-134)) and chronic in 6 patients. Treatment response among patients receiving local (n=5) or systemic (n=3) antifungal treatment for oropharyngeal or esophageal candidiasis was complete resolution in all cases (8/8); treatment response among patients receiving antifungal treatment for possible invasive fungal infection (n=2) was clinical improvement without resolution in both pts. Total antifungals treatment days were 2,182 days; antifungals use intensity during the 1 year observation period (antifungals treatment days / patient care days) was 11.3 % (17.7% among hematological cancer patients). Discussion and Conclusions. According to our results, the use of antifungals in home care hematological patients is frequent, especially in hematological cancer patients, but limited to certain molecules and indications, probably due to the particular population composition.

P153

PNEUMOCYSTIS JIROVECI PNEUMONIA IN HEMATOLOGICAL PATIENTS: OUR EXPERIENCE

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Pneumocystis jiroveci pneumonia (PJP) is usually documented in in HIV infection. PJP also occurred in others groups of subjects with compromised immune system such patients with hematological malignancies. Aims of this study are to establish frequency and clinic characteristics of PJP in a group of patients with hematologic neoplasms and to analyze the relationship between PJ occurrence and main features of hematologic diseases. HIV negative adult cases with a clinical doubt of PJP was studied. The diagnosis was done using PCR for PJ performed on oral wash. All the cases with microbiologically documented PJP were analyzed considering the following variables: demographic data, underlying hematological malignancy, relationship between chemotherapy and diagnosis of PJP, clinical parameters and radiological reports related to pneumonia and lymphocyte count. Among 86 subjects with hematologic malignancies, 24 resulted affected by PJP. Mean age was 59 ±18.8 years; 14/24 (58.3%) of them were male and 10/24 (48.7%) female. The underlying hematologic diseases were: 16 (66.7%) NHL, 5 (20.8%) HL, 2 (8.3%) AML and 1 (4.2%) aplastic anemia. All the patients were treated with antiblastic drugs. 48% of the patients received PJP diagnosis after that the treatment finished, 37.5% during chemotherapy and the other cases before the beginning of the therapy. Only in 5 cases of the PJP patients TMP-SMZ prophylaxis was effectuated. Mean value of lymphocyte count was $0.86x10^9$/L (r. 0.10 -$2.36x10^9$/L ; ±0.615). Oral wash test became negative after PJ therapy with a mean of 27.6 days (±30.9). In our series, a broad spectrum of hematologic diseases can be the complicated by PJP. Interestingly we described the infection in Hodgkin lymphoma, and, in this case, the diagnosis was performed before starting chemotherapy. Further studies could suggest if TMP-SMZ could be started before immunosuppressing therapies are administered, assuming that hematological diseases are themselves conditions that cause alterations in immune system that could lead to a PJ infection. No one of these patients died or was transferred to intensive care unit: our point of view is that the discrepancy is due to an early diagnosis thanks to non-invasive oral washes examination.

P154

A LYMPHOMA SURVIVORS CLINIC: A NEW PARADIGMA OF CANCER CARE. AN EXPERIENCE OF SINGLE CENTER

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In recent years, the medical and scientific progresses make possible long disease free periods after lymphoma treatments, and long survivorships has become a distinct phase of cancer care that includes surveillance for recurrences, evaluation of medical and psychosocial consequences of treatment, evaluation of the quality of life after treatment, recommendations for screening for new primary cancers and for health promotion. The impact of the lymphoma and treatment on the long-term health of the survivors is substantial. After treatment, the patients are at risk for physical and psycological sequelae depending on stage and treatment modalilies. Late effects include organ damage and functional disabilities like cardiorespiratory system dysfunction, neurocognitive problems, premature menopause, gastrointestinal system dysfunction, sexual impairment, infertility, chronic fatigue and secondary malignancies. A multidisciplinary team formed by Medical hematologists/oncologists and psychologists was established within the Department of Medical Oncology at the National Cancer Institute of Aviano (PN) in 2008. This cancer survivors clinic (O.RA project-Oncologia RiabilitativA) is devoted to long term cancer survivors (all persons who are lymphoma or solid cancer and treatments free since at least 5 years), in addition to standard oncological follow-ups through a both clinical and rehabilitative standard. This projetc involves a multidisciplinary team: oncologist/hematologist, psychologist, nurse, other physician if necessary (*i.e.* cardiologist, gynecologist, etc.) and offers an unique clinical approach. In the last five years we have evaluated (now we consider only hematologic patients) 56 HD, 49 NHL, 2 LMA. The cardiorespiratory system dysfunction, thyroid disfunction, osteoporosis are the main late effects found. We have registred in our population 15 pregnancies in 8 women, 16 conceptions in 9 men, and 3 new primary cancer (1 thyroid carcinoma, 2 skin cancer). About psychosocial consequences of treatment, the lymphoma survivors seem to be more fatigued than normal population. Quality of life appears to be poorer in survivors than in normal population in many aspects (*i.e.* physical and social function, mental health). In conclusion this program could favourably impact on the new evaluation of hematological follow-up with re-evaluation of the lifestyles and the reintroduction in social life and working activities.

Myeloproliferative Disorders I

P155

MYELOPROLIFERATIVE NEOPLASMS (MPN) WITH NEGATIVE JAK2/V617F, MOLECULAR STUDY OF MPL W515L/W GENE

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Background. In the last years there has been a improved interest in the molecular characterization and classification of myeloproliferative neoplasms (MPN) and targeted therapy. This interest began after the finding of MPN onset in patients positive for MPL W515W/L mutation but negative for JAK2 V617F and BCR- ABL1. It's important to emphasize that patients with negative JAK2V617, are often associated with mutations in the MPL gene, in particular in the receptor binding region for thrombopoietin. Therefore, detecting the MPLW515W/L mutation in patients JAK2 negative can easier provides a correct diagnosis of MPN (chronic myeloproliferative neoplasms). Aims of the study. In this study, we plan to qualitatively and quantitatively analyze JAK-2 and MPL somatic mutations in a group of Ph-negative (MPL) chronic myeloproliferative neoplasm patients. A second purpose is to identify a possible association between type of mutations and clinical phenotype, highlighting a possible correlation between the mutational load percentage and the risk of hematologic malignancy progression. Materials and Methods. We have been examined 44 patients, 24 men and 20 women afferent to the Hematology Department of Cagliari Cancer Hospital for diagnostic hypothesis of MPN, from January 2011 to January 2013. Peripheral blood samples were used for DNA and RNA molecular studies. BCR/ABL transcript and JAK-2 mutation analysis had already been done for all samples. Only samples negative for JAK-2 mutation were subjected to MPL mutation screening. To assess whether samples carried JAK-2 mutation, qualitative PCR and allelic discrimination assay were done. Cases involved in the present study were patients JAK-2 negatives that have been analyzed for W515 L/K MPL mutation using a commercial kit. Results. Patients positive for W515L MPL mutation were in total 18%. Following clustering for pathology, in the group of patients with essential Thrombocythemia only 5% resulted with W515L/K MPL mutation, while 29% had mutated 515L/K MPL alleles in the patients with Myelofibrosis. Conclusions. The presence of the genetic variant W515L MPL can be indicated as specific molecular disease marker. The quantitative allelic discrimination evaluation (ratio and cut-off) can be used in the monitoring of MRD disease. An evolution of the present study would be to quantitatively monitoring the MPL RNA transcript levels, as number copy for the study of MRD.

P156

SERUM EXPRESSION OF INTERLEUKIN-15 IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: WHICH SIGNIFICANCE?

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The essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by a surplus of inflammatory cytokines that mediates differentiation and proliferation. It is reported that the JAK2V617F expressing cells are more stimulated by inflammatory environment than the JAK2V617F not expressing cells. Interleukin-15 (IL-15) is a cytokine that controls the expansion of the hematopoietic precursors. Therefore, we evaluated platelets, red blood cell (RBC), haemoglobin (Hb) concentration, hematocrit (HCT) and white blood cell (WBC), as myeloproliferative markers, fibrinogen (Fg), as inflammatory indicator, JAK2V617F mutation and IL-15. We recruited 82 patients with ET who fulfilled WHO criteria. Their mean duration of disease was 10 years (range, 3-20 years). Of 82 ET patients, 45 were JAK2V617F mutated (15 males and 30 females, mean age 65 years) and 37 were JAK2V617F WT (17 males and 20 females, mean age 62 years). All patients were on aspirin. Eighty-seven healthy subjects served as controls. Platelets, RBC, Hb, HCT and WBC were measured by automated analyzer. Fg and IL-15 were measured by Clauss and ELISA assay, respectively. The JAK2 mutated patients had higher platelets, RBC, Hb, HCT and WBC ($920\pm282x10^9$/L, $5.64\pm0.88x10^6$/L, 14.8±1.6 g/dl, 45±4%, $10\pm2.8x10^9$/L) than JAK2 WT patients ($802\pm204x10^9$/L, $4.48\pm0.93x10^6$/L, 13±1.8 g/dl, 40±4%, $7\pm2.7x10^9$/L) (p=0.031, p<.0001, p<.0001, p<.0001, p<.0001) whereas the JAK2 WT patients had higher Fg (411±98 mg/dL) than JAK2 mutated patients (285±61 mg/dL) (p<.0001). The JAK2 mutated patients and JAK2 WT patients had elevated IL- 15 (18±6 pg/mL and 18±5 pg/mL, respectively, *vs* 2.5±1 pg/ml) (p<.0001). These results suggest that IL-15 may promote the clonal cell growth in JAK2 mutated patients leading us to speculate that IL-15 may serve as a negative prognostic marker.

P157

FISH TO REVEAL NEW TYROSINE KINASE (TK) GENE FUSIONS IN CHRONIC MYELOPROLIFERATIVE DISORDERS (CMPD)

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CMPDs are an heterogeneous group of hematopoietic stem cell disorders resulting from genetic and epigenetic alterations that perturb key processes. Constitutive activation of TK genes due to mutations or chromosomal translocations, plays an important role in pathogenesis. (Koopmans SM *et al*, 2012). FISH with probes specific for TK genes was applied in order to assess the incidence of alterations involving TK genes and to identify uncommon TK translocation partners. The 36 patients (pts) analysed (7 females-29 males, median age 45 yrs and median follow-up 29 mos) were 20 atypical CML, 16 CEL and one T-LL. FISH probes were obtained from Kreatech (Amsterdam, NL), Abbot Molecular Inc. (Chicago, Il, USA) and from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA). The commercial probes were: ON FIP1L1-CHIC2-PDGFRA (4q12) Del, Break; ON PDGFRB (5q33) Break; ON FGFR1 (8p12) Break; ON JAK2 (9p24) Break; LSI BCRABL. The BAC probes labelled and applied as previously described (Dambruoso I. *et al*, 2012) were RP11-484L21 and RP11-880I16 covering the PCM1 gene. An abnormal FISH pattern was revealed in 9 pts: 3/20 aCML, 5/16 CEL and one AML/T-LL. Two aCML pts presented a trisomy 8 and the last one a t(9;13)(q34;q14) which involved the ABL gene and a partner gene not yet identified. Two CEL pts showed a JAK2 rearrangement: one with a t(8;9)(p22;p24) on CC presented the PCM1-JAK2 gene fusion, the other with a t(3;8)(?;p24) not revealed by CC presented a fusion between JAK2 and a partner not yet identified. Two CEL pts showed a PDGFRB rearrangement, one with a t(1;5)(?;q33) and the other with a t(5;8)(q33;?); the PDGFRB translocation partner has not been yet identified. One CEL pt showed a PDGFRA deletion. The AML/T-LL pt carried a t(8;13)(p11;q12) which produced the classical FGFR1-ZNF198 gene fusion. Thus, in 3 pts FISH with BAC probes is still on-going in order to search the unknown translocation partners of JAK2 and PDGFRB. Despite these TK positive pts presented a relevant eosinophilia (≈65%), FISH performed on peripheral blood smears always provided negative Results. From a clinical point of view no aCML/CEL pt showed a lymphoid neoplasms. So our data suggest that FISH i) effectively reveals cryptic TK translocations in about 25% of chromosomally normal CMPDs; ii) these TK rearrangements are more common in CELs than in aCML; iii) peripheral blood eosinophils may show a normal FISH pattern.

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ESSENTIAL THROMBOCYTHEMIA AND NEUROLOGICAL SYMPTOMS: IMPACT OF JAK2V617F MUTATION AND RESPONSE TO THERAPY

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Patients (pts) with Essential Thrombocythemia (ET) may present a wide spectrum of neurological symptoms (NS) at onset or during the course of disease, only in part resulting from previous thrombotic cerebral events (TCE). Sometimes NS may indicate a micro-vascular occlu-

sive event however the risk factors and specific therapeutic strategy have not completely defined yet. We retrospectively described NS that occurred in 300 ET referred in our center from 1995 to 2012. We separately analysed NS secondary to TCE by the remaining functional ET-related NS, in order to identify clinical and biological features associated to NS and to assess the response to therapy. 119/300 pts (39%) present whatever NS; 15/119 pts (12,6%) develop NS after TCE (transient ischemic attack or stroke). The remaining 104 pts report subjective and often transient and fluctuating NS, by frequency order: cephalalgia (55), chronic paresthesias (49), dizziness or hypotension (18), blurry vision (9), tinnitus (4), erythromelalgia (8) which we defined as ET-related NS. Median age of NS pts is 54 years, with prevalence of female sex. Analysing JAK2 mutational status in 104 ET-related NS respect to 166 pts without NS, we find a significant higher prevalence of JAK2V617F mutation in ET-related NS pts respect to pts without NS ($p = 0,01$). All pts receive anti-platelet agents (AP) at diagnosis; a cytoreductive treatment, instead, is reserved to TCE-pts and selected ET-related NS pts. In most TCE-pts, anti-platelet associated to cytoreductive treatment is ineffective on resolution of NS; in ET-related NS group, 34/104 pts (33%) achieve a complete response after AP, alone (23) or associated with cytoreduction (11). 67% of ET-related NS pts doesn't respond to AP; 31 pts of whom (46%) improve NS after addition of cytoreductive therapy. Overall, 87% of pts with NS are alive with a median follow-up of 49 months (range: 1-212 months). We observe that 39% of pts present NS. In the 87% of them (104/119), NS aren't secondary to TCE, so these are interpreted as ET-related NS. Mostly of these pts are females and seem to present a significant higher prevalence of JAK2V617F mutation. Only 33% of ET-related NS responds to AP. The addition of cytoreductive therapy should be considered in AP refractory pts with ET-related NS, suggesting the involvement of different mechanisms from micro-vascular disturbances, not fully understood yet, in pathogenesis of these NS.

P159

DEFERASIROX AND TRASFUSION IRON OVERLOAD IN PRIMARY MYELOFIBROSIS

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Transfusion-induced iron overload is a frequent problem that clinicians have to face in the treatment of patients affected by myelodysplastic syndrome (MDS), resulting in multiple organ failures, with significant hepatic and cardiac involvement. Deferasirox (DSX) is the principal option currently available for chelation therapy, but the expertise in the management of iron overload in patients with primary myelofibrosis (MF) is limited. We analysed our initial experience in 10 patients with MF treated with oral DSX from September 2010 to March 2013, starting to dose of 10 mg/kg/day, up to the maximum tolerated dose. The median values of ferritin pre-treatment was 2280 microg/l and the median red blood cells (RBC) transfused was 2 units/month. The median dose tolerated of the DSX was 750 mg/day (10 mg/kg/day), with 3 transient interruption of treatment for grade 2 of drug-related adverse events (AE), in particular 1 rash, 1 diarrhea and 1 transaminitis; 3 patients experienced a definitive discontinuation of the drug for grade 3 AE (1 hepatitis, 2 renal failure). 2 patients interrupted DSX for leukemic evolution of disease. Overall, only 4/10 patients (40%) continued permanently the oral iron-chelation. According to IWG criteria, erythroid responses with DSX, comprising reductions in transfusion requirements or increases in hemoglobin levels, were observed in 4/10 patients (40%), with 2 patients (20%) who obtained transfusion independence. Median serum ferritin reductions were greater in hematologic responders compared with non-responders. The median time to response was 102 days (range 45–900 days). Our preliminary data open new insights regarding the benefit of iron chelation therapy not only in MDS, but also in MF patients with the possibility to obtain a partial or complete erythroid response, overall in 40 % of patients. However, the tolerability of the drug seems to be lower compared to MDS patients, both in terms of lower median tolerated dose (10 mg/kg/day) that for higher frequency of discontinuance for drug related AE (40%). The biological mechanism of action of DSX in this specific myeloproliferative setting, through an independent NF- B inhibition and not based on the reactive oxygen species scavenging properties of the drug, could explain a direct action on the malignant clone during *in vivo* therapy, inducing a hematopoietic improvement, but further investigations are required.

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THROMBOTIC CEREBRAL EVENTS IN ESSENTIAL THROMBOCYTHEMIA

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Essential Thrombocythemia (ET) may present with thrombosis in different sites. Severe thrombosis in central nervous system (CNS-T) are rare, but neurological symptoms secondary to microvascular involvement are common. We retrospectively described CNS-T occurred in 310 patients (pts) with ET referred in our centre from 1990 to 2012, in order to analyse the prevalence, clinical and biological features and impact of JAK2V617F mutation in this setting. 33/310 pts (10.6%) present CNS-T. The median age of CNS-T pts is 71 years, with prevalence of females (21 *vs* 12 pts). Only 4 pts are high risk for 2 o more cardiovascular risk factors (hypertension, diabetes, obesity, dyslipidemia, smoking, thrombophilia). 54,5% of pts develops CNS-T before diagnosis of myeloproliferative neoplasm, with a median time of 16 months of "latent phase". Of the 22 CNS-T preceding diagnosis, 18 are arterial events (8 transient ischemic attack, 9 strokes), only 2 events are venous (2 ocular thrombosis). 7 pts (21,2%) develop CNS-T as presentation of disease. Therefore, 11 pts (33,3%) develop CNS-T after diagnosis of ET, despite of antiplatelet and cytoreduction, with 15% of pts (5/33) with recurrent neurological thrombosis. There aren't any significant difference in count of platelets (PLT) and white blood cell (WBC) at time of diagnosis and at onset of CNS-T. The median values of hematocrit, WBC and PLT count at time of CNS-T are 41%, 7.720/mm^3 and 650.000/mm^3, respectively. JAK2V617F mutation is evaluated in 30/33 pts, with significant prevalence of JAK2 positive versus negative pts (83,3% *vs* 16,7%). All pts with recurrent CNS-T are JAK2 mutated. Overall, 91% of pts (30/33) are alive, with a median follow-up of 72 months. We observed that 10,6% of ET pts present CNS-T, with high prevalence (75,7%) of cerebral events at diagnosis or before diagnosis, as first sign of a subclinical disease. The majority of CNS-T are arterial. The higher prevalence of JAK2V617F mutation in CNS-T may provide an early diagnosis of MPN in pts with latent phase of disease. Moreover, a subgroup of patients (33,3%) developed CNS-T after ET diagnosis, in despite of antiplatelet and cytoreductive treatment, with high risk of thrombotic CNS-T recurrence. This seems to suggest that in selected setting, in presence of JAK2 mutation or other significant cardiovascular risk factors or thrombophilia, an enhancement of thrombotic prophylaxis could be proposed, for example with oral anti-coagulant therapy.

P161

IDIOPATHIC MYELOFIBROSIS: DISEASE CHARACTERISTICS AND PERIPHERAL BLOOD CD34+ CELLS

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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. 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 31 patients affected by IMF, according to WHO criteria (M:18, F:13; median age: 57 years, range: 48-68 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 Philadelphia-negative chronic myeloproliferative neoplasms. 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 36-48 months we observed an increase of myeloid precursors (CD34+/CD117+: 45,7%) compared with a reduction of endothelial precursors (CD34+/CD133+:15,3%) in patients who showed clinical and laboratory signs of disease progression. 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. Immunophenotypic profile of PB CD34+ cells is associated in IMF with patients' clinical characteristics and may have potential prognostic application.

P162

ERYTHROCYTE DEFORMABILITY IN POLYCYTHEMIA VERA

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Polycythemia vera (PV) is associated with a hyperviscosity syndrome, traditionally considered to be induced by the increased number of circulating cells. As there is a relationship between a high haematocrit and the incidence of vascular occlusive episodes, and thrombosis is a major cause of death in PV, a strict control of hematocrit has a central role in the disease management. However, evidence has long emerged that a raised red cell count is not the only mechanism of hyperviscosity in PV, and that a reduced erythrocyte deformability should be also taken into account. Erythrocytes from PV patients show metabolic and biochemical abnormalities, including enhanced activity of some glycolytic enzymes, increased lactate output, low concentration of ATP and 2,3-diphosphoglycerate. Fetal haemoglobin percentage can be increased in PV red cells, and their membranes seem to be altered in lipid composition and sialic acid pattern. Some of these findings may theoretically account for a reduced erythrocyte deformability, adding a "sclerocythemic" mechanism to the blood hyperviscosity in PV. In last years the evaluation of erythrocyte deformability has employed laser diffractometry. By this technique, erythrocytes are suspended in an isotonic medium of known viscosity and subjected to a variable shear stress, resulting in a measurable cell deformation. We enrolled a group of PV patients with clinically stable PV, 80% positive for JAK2 mutation, whose hematocrit was within the range 34-53%, and a group of healthy controls. The evaluation of erythrocyte deformability was performed using the diffractometer Rheodyn SSD of Myrenne, at the shear stresses of 6, 12, 30 and 60 Pascal. The red cell deformability was expressed as elongation index (EI), calculated from the length and width of the cell under the deforming force. In PV patients the erythrocyte deformability was reduced, in comparison with control subjects, at all the shear stresses, with the more significant difference at the lower ones. EI was not related to hematocrit in PV patients nor in controls. The impaired deformability of red blood cells has been confirmed by other studies. An abnormal erythrocyte-endothelium interaction has been recently observed in PV, and JAK2 mutation has been involved in it. In conclusion, the role of erythrocytes in the trombotic risk of PV patients seems to be more complex than simply related to hematocrit, and its clarification may be useful to prevent vascular occlusive events.

P163

SPLEEN ENLARGEMENT AT DIAGNOSIS OF ESSENTIAL THROMBOCITEMIA IS A NEW PROGNOSTIC FACTOR FOR THROMBOSIS?

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A mild to moderate spleen enlargement is present in about 15-20% of Essential Thrombocitemia (ET) at baseline. The clinical characteristics and prognosis of these patients are still unknown. We report in the present retrospective analysis data from 1,141 patients affected by ET and diagnosed from January 1979 to December 2010 in 11 haematological Centers (5 universitary Institutes and 6 community-based Hospitals) of our regional cooperative group. The diagnosis was made according to PVSG criteria or WHO 2001 and 2008 criteria, based on the date in which the diagnosis was made. Among these 1141 pts, 1097 were evaluable for spleen enlargement at baseline, defined as palpable spleen under costal margin or ultrasound longitudinal diameter longer than 12,5 cm. On the whole, 213 patients (19.4%) had a spleen enlargement (sET), while 884 (80.6%) had a normal spleen size (oET). We compared clinical characteristics and evolution of the two groups. The main clinical features are reported in the Table. As shown in the Table, the most significant differences between the 2 groups are referred to gender, WBC and PLTs count, rate of positivity and allelic burden of Jak-2 V617F mutation. Two patient populations with a different disease phenotype seem to be defined: the first group without spleen enlargement (female gender prevalence, low WBC and PLTs count, low rate of positivity and allelic burden of Jak-2 V617F mutation); the second group with spleen enlargement and features more similar to early MF (M/F ratio 1/1, high WBC and PLTs count, higher rate of positivity and allelic burden of Jak-2 V617F mutation). The Thrombosis-free Survival (TFS) and the Overall Survival (OS) of the two groups were also evaluated and a significant higher risk of thrombotic events was shown in patients with spleen enlargement (p= 0.007), while no difference was reported as to OS (p= 0.11): moreover, the role of spleen enlargement as independent additional risk factor for thrombosis was retained at multivariate analysis (p= 0.0203, HR= 1,7833, 95%CI= 1,0967–2,8998). Spleen enlargement at diagnosis seems to be associated with a peculiar "prefibrotic" clinical phenotype but differently from early/prefibrotic MF subtype it seems to be a new important feature to individuate ET patients with high risk of thrombosis during follow-up.

Table 1.

Item	No spleen enlargement (0ET)	Spleen enlargement (sET)	p (IC 95%)
N of patients	884	213	--
Median age (yrs)	59,7	58,2	0,23
Gender (M/F), Male %	(294/590), 33	(105/108), 49,3	*0,04*
Median Hb (g/dL)	13,9	14,0	0,45
Median WBC (x 10^9/L)	9,3	9,8	*0,0385 (30,3 -110,83)*
Median PLTs (x 10^9/L)	879	933	*0,04 (2,28 – 107,52)*
LDH (Higth/Normal)	246/456	75/117	0.35
JAK-2 V617F mutated/performed (%)	235/566 (41.5)	79/129 (61,2)	*0,02*
Median JAK-2 V617F burden (%)	24,3	33,5	*0,016 (1,73-16,67)*
Antiplatelet Therapy, N° (%)	814 (94.7)	196 (93.8)	0.98
Cytoreductive treatment, N° (%)	681 (77.7)	159 (75.4)	0.83

P164

SINGLE-AGENT LENALIDOMIDE IS EFFECTIVE FOR TRASFUSION INDEPENDENCE IN A PATIENT WITH REFRACTORY ANEMIA WITH SIDEROBLASTS, THROMBOCYTOSIS AND JAK (V617F) MUTATION

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Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) is a rare myelodysplastic/myeloproliferative disorder proposed as a provisonal entity in the 2001 and 2008 WHO classification. JAK2-V617F mutation is found in the majority of these patients. Lenalidomide is effective in patients with myelodysplastic syndromes with or without the del (5q) cytogenetic abnormality to reach transfusion independence, while no efficacy data of Lenalidomide are available in essential thrombocythemia to reduce platelet count. The efficacy of single-agent Lenalidomide in RARS-T has been recently reported in only two cases. We report the clinical outcome of Lenalidomide in the treatment of one patient with RARS-T and JAK2 V617F mutation. A 58-year-old caucasian man was admitted at our hospital in September 2006 for anemia (Hb 9.8 g/dL) and thrombocytosis (platelet count 1163x10^9/L). The bone marrow showed 80% cellularity, increased atypical megacaryocytes with often lobulated nuclei and erythroid dysplasia with 30% ring sideroblasts. Cytogenetic analysis showed a normal karyotype, FISH examination was negative, while PCR revealed the presence of JAK2 V617F; a diagnosis of RARS-T associated to JAK2 mutation was made. Due to increase platelets count, hydroxyurea was started after 11 months from diagnosis, with worsening of anemia (Hb 8.3 g/dL *vs* Hb 9.6 g/dL pre-therapy). Mild steroid therapy showed a transient efficacy while treatment with recombinant erytropoietin was no successful. Thus, blood red cells transfusion treatment was started, after 40 months from diagnosis. Because of a high transfusion need, Lenalidomide 10 mg daily for 21 days consecutive was started and Hydroxyurea was concurrently interrupted. After 3 cycles of Lenalidomide, anemia improved (Hb 9 g/dL) and the platelet count decreased to 700 x10^9/L. After 6 cycles, the hemoglobin reached a stable value at >9 g/dL without need of transfusion and the platelet count reached the stable value of 5-600.000x10^9/L. Currently, the patient successfully continues 21 days- cycles of 10 mg lenalidomide and he maintains the transfusion independence, after a total of 30 cycle during 2 year of follow-up. No adverse event have been recorded until now. We confirm that Lenalidomide as single agent is efficacy in the treatment of RARS-T and JAK2 V617F mutation, to reach transfusion independence and to control the platelet count after a long follow-up.

P165

DIAGNOSTIC CLASSIFICATION OF 170 UNSELECTED PATIENTS WITH POLYCYTHEMIA/ERYTHROCYTOSIS

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The term polycythemia/erythrocytosis defines a group of diseases characterized by persistent increase of erythrocytes and hematocrit, which can be expression of a clonal disorder (polycythemia vera, PV) or caused by congenital or acquired factors (secondary polycythemia, SP). A diagnostic classification is important for therapeutic choice, in particular to determine the target hematocrit for each patient (McMullin *et al*, 2005), as recently confirmed (Marchioli *et al*, 2012). From 2006 to 2012 we saw 170 unselected patients (pts) for polycythemia/erythrocytosis in our Center. The newly diagnosed pts were evaluated according to three levels of investigations: 1st level: history/physical examination, full blood count, serum erythropoietin level, arterial oxygen saturation, arterial blood gas analysis (with COHb in smokers), abdominal ultrasound, chest x-ray and lung function tests, research of Jak-2 V617F mutation. 2nd level: bone marrow biopsy with cytogenetics, research of Jak-2 exon 12 mutation, evaluation to rule out sleep apnoea syndrome. 3rd level: BFU-E culture and oxygen dissociation curve (p50) plus any other test, if available, to rule out most rare causes. The diagnosis of PV was placed according to 2001 and 2008 WHO criteria. Of 170 pts, 36 (21%) had abnormalities of blood tests without clinical significance. In 18 pts (10.5%) further investigations were recommended, but they have been lost at follow up. 60 pts (35.5%) had a SP: 26 chronic respiratory diseases, 14 sleep apnoea syndrome, 15 heavy smokers, 3 multicystic kidneys, 1 iatrogenic SP, and 1 right-to-left vascular shunt. 46 pts (27%) met the criteria for the diagnosis of PV; 1 of them was initially classified as SP in pulmonary disease, then revaluated for the occurrence of splenomegaly and reclassified as PV. The search for mutations of Jak-2 was available in 39 of 46 pts and was found in 36 cases (92%): the V617F mutation in 34 cases, the exon 12 mutation in 2 other. Cytogenetics was normal in all cases undergoing bone marrow biopsy. Among pts who have run the complete diagnostic course, in 10(6%)it was not possible to clarify the cause of polycythemia which was classified as "idiopathic". In a series of unselected pts, the diagnostic classification of polycythemia/erythrocytosis may not be easy and in some cases you can not get to a safe diagnosis with resulting in uncertainty about therapy, in particular in identifying the most appropriate target hematocrit for each patient.

P166

CD30 EXPRESSION IN SYSTEMIC MASTOCYTOSIS PATIENTS

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Introduction. Systemic mastocytosis (SM) is one of the myeloproliferative neoplasms recognized in the 2008 World Health Organization (WHO) classification. SM is characterized by the multiorgan infiltration by clonal mast cells (MC). The hallmarks of neoplastic MC are usually the CD25 expression and the D816V KIT mutation. SM patients follow mostly an indolent clinical course showing a life expectancy comparable to that of healthy people. Nevertheless, few patients develop an aggressive disease that is poorly responsive to current therapies leading rapidly patients to death. During the last decade, many efforts have been made to allow a clear-cut distinction between indolent and aggressive forms of the disease. CD30 expression by BM MC has been recently reported as prognostic marker in SM patients. The aim of this study was to investigate the potential diagnostic and prognostic value of CD30 expression in SM as assessed by multiparameter flow cytometry. Methods One hundred sixty-three consecutive BM samples corresponding to 142 SM patients and 21 non-mastocytosis cases were studied. BM samples were obtained and studied at the Mast Cell Unit of the Hospital Virgen del Valle, Toledo (n=92) and at the Multidisciplinary Outpatient Clinic for Mastocytosis of Verona (n=50). For all cases, a complete BM examination was performed according to WHO criteria. Moreover, the multiparameter flow cytometry immunophenotype of BM samples was performed for contemporaneously analyzing the CD25 and CD30 expression on BM MC. Results. CD30 was expressed in most SM patients (80%), while detected in only one non-mastocytosis case (4.8%). The combination of CD30 and CD25 led to improvement of accuracy over that of CD25 alone (98% *vs* 93%) mainly because most (8/9) of well-differentiated SM (WDSM - a provisional SM subtype), who lacked CD25, were CD30+. Among all different subgroups of SM, except mast cell leukemia, similar levels of expression of CD30 were observed. Among indolent SM patients, no significant association was observed between the levels of CD30 expression and the other clinical and biological features of the disease. Conclusions. The increased expression of CD30 contributes to the diagnosis of WDSM and its distinction from other subtypes of SM. By contrast, CD30 expression did not contribute neither to prognostic stratification of ISM nor to the differential diagnosis between ISM and aggressive SM cases.

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MYELOFIBROSIS AND FOLLOWING CHRONIC MYELOID LEUKEMIA: IMATINIB AND RUXOLITINIB TREATMENT IN TWO CASES

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Myelofibrosis (MF) is a stem cell derived disorder characterised by bone marrow fibrosis, extramedullary hematopoiesis, anemia, splenomegaly, constitutional symptoms, leukemic progression and shortened survival. It can be primary or secondary to evolution from polycythemia vera (PV) or essential thrombocythemia. JAK2 V617F mutation is present in almost 50% of cases and recently Jak-2 inhibitors have been successfully used in some of these patients. Very few MF cases are reported to develop chronic myeloid leukemia (CML) during the follow-up. Herein we report two cases of post-PV MF that developed CML 7 years after their initial presentation. Case 1: a 48 year old man was diagnosed with PV in 1992; he was treated with hydrossiurea, antiplatelet therapy and phlebotomy. Thirteen years later, after increasing of splenomegaly and a decrease of haemoglobin level, a new bone marrow evaluation was performed and according to WHO criteria, post-PV MF Jak2 positive was diagnosed. He continued cytoreductive therapy at a low dose. On December 2011 t(9;22)(q34;q11.2) [2/30] was detected on bone marrow, suggesting the presence of a Philadelphia positive CML clone. PCR analysis showed a BCR-ABL transcript (b2a2). Consequently, cytoreductive therapy was stopped and imatinib started at a daily dose of 400 mg. A complete cytogenetic and major molecular response were achieved after 10 months, but this treatment had no effect on MF (increase splenomegaly, constitutional symptoms); therefore we added ruxolitinib at 15mg BID. Case 2: a 49-year old man was diagnosed with PV in 2000, after a recovery for TIA. He started an anticoagulant and a cytoreductive therapy with hydrossiurea. Five years later after increasing of splenomegaly and decrease of haemoglobin level, evolution to MF was observed. On March 2012 bone marrow evaluation confirmed a myelofibrosis (MF-3), but at cytogenetic evaluation, t(9;22)(q34;q11.2) was detected. PCR analysis on peripheral blood showed the BCR-ABL transcript (b3a2). He stopped the previous therapy and started imatinib at a daily dose of 400 mg. Six months later, a complete cytogenetic response was achieved. After observing an increase of splenomegaly and Jak-2 allele burden and the presence of constitutional symptoms, ruxolitinib at 15mg BID was started. Conclusion: these rare cases of MF and following CML, obtained improving clinical conditions and reduction of splenomegaly with imatinib and ruxolitinib unusual association.

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THROMBOTIC EVENTS IN MYELOFIBROSIS: RELATIONSHIP WITH CLINICAL AND MORPHOLOGICAL CHARACTERISTICS, JAK2V617F MUTATIONAL STATUS AND AUTOIMMUNE PHENOMENA

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The clinical course of myelofibrosis (MF) may be complicated by both arterial and venous (sometimes in unusual sites) thrombotic events, whose relationship with clinical risk, grade of bone marrow (BM) fibrosis, JAK2V617F mutational status and/or mutational burden, as well as with autoimmune phenomena (anti-phospholipid, anti-RBC, antiplatelets, organ and non-organ specific antibodies) is still undefined. To address this issues, we evaluated retrospectively 100 MF patients (diagnosed from 1996 to 2010, and followed at our Institute for a median of 5.7 years until September 2012). Patients, 51 males and 49 females, median age 72 years, were classified as follows: 58 primary, 32 post-thrombocythemia and 10 post-polycythemia; according to clinical risk (IPSS), 14 were LR, 60 IR-1, 14 IR-2, and 12 HR; the grade of marrow fibrosis was MF-0 in 18 cases, MF-1 in 65, MF-2 in 15, and MF-3 in 2 cases. Thrombotic events were observed in 25 patients, 17/25 venous events (12 deep venous of lower limbs and 5 splancnic), and 8/25 arterial thrombosis (2 acute myocardial infarction and 6 strokes); thrombosis was equally distributed among IPSS groups (31% in LR, 24% in IR-1, 27% in IR-2 and 18% in HR), and no relationship was found with primary and secondary MF; 84% of thrombotic events occurred in MF-0/MF-1 patients, even without a clear relationship with marrow fibrosis. Likewise, no association was found with Jak2 positivity (56% of cases) and allele burden (34% homozygous and 66% heterozygous), even if homozygosity was more frequent in post-PV MF (6/9, 67%) than in post-ET MF (6/15, 40%) (P=0.02). No relationship was observed between thrombosis and the presence of anti-phospholipid antibodies (found in 30% of cases), nor with other markers of autoimmunity [anti-RBC (45% of cases), anti-platelets (15%), and serologic antibodies (57%)]; anti-phospholipid antibodies were mainly observed in MF-1 (19/30, 63%) and in IR-1 group (20/30, 67%). Given these results patient were prospectively followed for a median of 2 years, when we observed that thrombosis was associated with progression of clinical risk (p=0.026); unexpectedly, JAK2 allele burden was related to clinical risk progression (p=0.020) and leukemic evolution (p=0.041), although the short follow-up does not allow definite conclusions. Our results suggest that thrombotic events are not infrequent in MF, also in early clinical stages, and are not related with JAK2 V617F positivity nor with autoimmune markers.

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MUCOCUTANEOUS TOXICITY IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS TREATED WITH HYDROXYUREA: A RETROSPECTIVE MONOCENTER COHORT STUDY

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Background. Hydroxyurea (HU) is the mainstay treatment in high-risk patients with myeloproliferative neoplasms (MPN). Mucocutaneous (MC) toxicity HU-related was 4.8% and 8.3% in two retrospective studies on MPN patients, respectively (Antonioli *et al*, Am J Hematol 2012; Latagliata *et al*, Cancer 2012) and 11.1% in a prospective trial in patients with essential thrombocythemia (ET) (Harrison C *et al*, N Engl J Med 2005). Aim of the study: To assess the probability over time of MC toxicity HU-related in a retrospective monocenter cohort of MPN patients. Patients and methods: The cohort included 217 patients (males 90, 41%) with MPN (polycythemia vera [PV]=78, ET=135, myelofibrosis [MF]= 4) who started HU treatment from 1994 to 2013. Patients were followed by an unique medical team and 91% of them started HU from 2000. All cutaneous or oral lesions of clinical significance, *i.e.* requiring consultation with dermatologists and/or discontinuation of treatment, were recorded. The interval between the start of HU and discontinuation due to unacceptable MC toxicity (uncensored observations) or discontinuation due to other causes or the last visit to the center (censored observations) was analysed. Moreover, the total HU dose intake was calculated in patients having had MC toxicity. Results. The median time of exposure to HU was 35 months (range 1-188). Overall, 44 patients (20.1%) had MC toxicity (leg ulcers=17, oral aphtosis=12, dermatitis=4, keratosis=4, basalioma=4, melanosis=2, squamous skin cancer=1). In 13 of them (10 with aphtosis) toxicity was mild and allowed continuation of HU; 31 patients (14.2%) discontinued treatment. The median interval between start of HU and discontinuation for severe toxicity was 37 months (range 7-150). The cumulative probability of toxicity requiring discontinuation was 1.6% at 1 yr, 9.0% at 3 yrs, 18.7% at 5 yrs, and 28.7% at 10 yrs. Out of the 31 patients with severe toxicity 14 were males (45%), 17 had PV (55%), 13 ET (42%), 1 MF (3%); 29 patients carried JAK2V617F (94%), at variance with those without MC complications (130/173, 60%, p=0.01). The median total HU dose intake was 766 gr (range 69-4536) in patients who discontinued HU and 1865 gr (range 226-6166) in those with skin cancer (p=0.4). Conclusions. In this cohort the probability of MC HU-related toxicity was higher than previously reported and seems time-dependent and associated with JAK2V617F; interruption of treatment was required in 14% of the cohort.

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ERK1/2 IS THE SIGNALING PATHWAY PRIMARILY ACTIVATED IN MYELOID NEOPLASMS CARRYING T(8;9)/PCM1-JAK2 FUSION: A TWO CASES REPORT

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Myelodysplastic/Myeloproliferative neoplasms resulting from an acquired t(8;9)/Pericentriolar material-1 (PCM1)-Janus activated kinase 2 (JAK2) fusion are rare diseases with a widely heterogeneous clinical presentation. Although recent studies showed an activation of the JAK/STAT axis in a PCM1-JAK2-transformed murine fibroblast (Lierman, Blood 2012) or human lymphoma (Ehrentraut, PLOSone 2013) cell lines, the status of JAK/STAT signaling in primary cells from PCM1-JAK2 patients has not been assessed yet. Given this background, we analyzed, in primary cells from two patients presenting with a t(8;9)(p22;24)/PCM1-JAK2-related myeloid neoplasm, the activation pattern of the main signaling cascades associated to Receptor tyrosine kinases that are most commonly activated in cancer: Mitogen-activated protein (MAP) kinase, JAK/STAT and Phosphatidylinositol 3-kinase (PI3K)/AKT pathway. Clinical presentation of the diseases was different: atypical chronic myeloid leukemia (aCML) and myelofibrosis (MF), respectively. In both cases, FISH analysis documented the t(8;9)(p22;24) and RT-PCR revealed the PCM1-JAK2 fusion transcript. The first patient underwent allogenic bone marrow transplant from HLA-matched sibling donor, while the second was treated with the JAK1/2 inhibitor Ruxolitinib, achieving only partial cytogenetic response. Circulating myeloid progenitors isolated from patients' peripheral blood (PB) were subjected to western blot analyses with antibodies that separately recognize the total and phosphorylated forms of JAK2, STAT3 and 5, AKT and ERK1/2. PB mononuclear cells from 4 healthy subjects (HS1-4) and K562 cells were utilized as controls. We found reduced levels of phosphorylated STAT3, JAK2, STAT5 and Akt in our patients compared to the four HS. Furthermore, while levels of total ERK were comparable among all conditions, only PCM1-JAK2 cells displayed a robust increase in ERK1/2 phosphorylation. These results demonstrate a peculiar "signaling signature" of these two PCM1-JAK2 fusion cases typified by a selective activation of the ERK pathway. The lack of phosphorylation of both STAT3 and STAT5 suggests that PCM1-JAK2 fusion protein is incapable of activating the JAK/STAT signaling axis. Our molecular data provide a biological rationale for the poor clinical response of the MF patient to Ruxolitinib, and immediately suggest that more efforts need to be done to elucidate molecular mechanisms underlying myeloid neoplasms carrying JAK2 translocations.

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SETBP1 OVEREXPRESSION IN CLASSICAL BCR/ABL1-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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SET binding protein 1 (SETBP1) gene encodes a protein able to bind the SET nuclear oncogene and to inhibit the activity of protein phosphatase 2A tumor suppressor. SETBP1 represents the first gene identified to be recurrently mutated in atypical Chronic Myeloid Leukemia (aCML), accounting for about 25% of all cases. SETBP1 mutations were also detected in 10% of "unclassified MDS/MPN" and in 4% of chronic myelomonocytic leukemia (CMML) cases. The occurrence of SETBP1 point mutations has also been investigated in classical BCR/ABL1-negative myeloproliferative neoplasms (MPN) but no mutation was identified. To date the possible activation of SETBP1 gene expression in classical BCR/ABL1-negative MPN has never been explored. In this study, a cohort of cases affected by polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) was examined. In detail, 47 MPN patients at diagnosis (including 24 PV, 12 ET, and 11 PMF cases) were analyzed by quantitative real-time PCR (qRT-PCR) experiments using the LightCycler 480II System. This analysis revealed a high SETBP1 expression level in PV and PMF bone marrow samples when compared with healthy matched controls. In fact, SETBP1 gene expression in PV and MPF was more than 5 ($p < 0.0001$) and 9 ($p < 0.0001$) fold when compared to controls, respectively. Regarding ET cases, not statistically significant gene overexpression was revealed. Considering a cut-off of >5 fold-change, expression values >5 were revealed in 58% (14 out of 24), 33% (4 out of 12), and in 90% (10 out of 11) of PV, ET, and PMF cases, respectively. No association between SETBP1 overexpression and JAK2 mutational status (homozigosity/heterozygosity) was detected in PV whereas all analyzed JAK2V617F-negative PMF cases (3 out 11) showed high gene expression level. Recently, several acquired mutations in genes such as TET2, ASXL1 and IDH1/2 were identified suggesting that, in cooperation with JAK2V617F, additional molecular alterations are involved in the MPN pathogenesis and in the initiation of a leukemic transformation. Our data revealed that SETBP1 gene dysregulation is a recurrent event in MPN. It is noteworthy to note that in our study SETBP1 overexpression was revealed in more than half of the analyzed cases; moreover, SETBP1 was upregulated in almost all PMF cases included in our study. Further analysis are needed to verify the association between SETBP1 gene expression and clinical factors in MPN cases.

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IS CONSIDERED ESSENTIAL THROMBOCYTHEMIA (ET) IN YOUNG (AGE<45 YRS) ADULTS A NON MALIGNANT DISEASE? RESULTS ANALYSIS OF A RETROSPECTIVE STUDY OF INCIDENCE AND RISK FACTORS FOR THROMBOTIC COMPLICATIONS, MYELOFIBROTIC PHASE (MP) OR BLASTIC PHASE (BP) EVOLUTION IN 257 PTS

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Background. In ET pts older age (>65yrs) and previous thrombotic events are associated with an increased incidence of vascular complications. As a consequence, young adults are usually included in low risk group for thrombotic events, Myelofibrotic Phase (MP) or Blastic Phase (BP) evolution. The true risk for such events had not still been yet completely stated in this subset of pts. Aims. To assess the incidence of vascular complications and MP or BP evolution among young ET pts and to identify factors associated with the development of such events. atients and Methods. Among 1141 ET pts - from January 1978 to December 2011- collected in the retrospective database of the "Gruppo Laziale SMPC Ph negative", we revised 257 consecutive young pts (M79/F178, median age 35,3 yrs, range: 17,9-44,9) diagnosed according to PVSG or WHO criteria based on the date of onset. The main clinical and laboratory characteristics are listed in the Table 1. All 23 but two pts who had had a previous thrombotic event were considered in high risk group and started a cytoreductive therapy (13 HU, 3 anagrelide, 3 IFN-alpha and 2

pipobroman). Results. In this series of pts the 25-year overall survival (OS) and event free survival (EFS) are 98.1% (C.I. 95%: 95.2-100.0) and 60.4% (C.I. 95%: 42.6-85.7), respectively. The 25-year cumulative incidences of thrombotic complications, MP and BP evolution are 12.2% (C.I. 95%: 5.8-18.6), 20.4% (C.I. 95%: 0-47.1) and 14.4% (C.I. 95%: 0-40.0), respectively. In univariate analysis, age, gender, prior thrombosis, JAK2 status, leukocyte count, spleen enlargement, cardiovascular risk factors proved not to be associated with increased risk of thrombosis, MP and BP evolution. Furthermore, thrombosis free survival (TFS) is not correlated with cytoreductive therapy (HR 1.427; C.I. 95%: 0.457-4.460; p=0.5403). Conclusions. Data from our cohort of pts seem to confirm that: a) age <45 years is associated with good long-term OS and low disease propensity to transform into MP or BP; b) vascular events in younger pts is lower than in older ones, but it appeared higher compared with general population; c) neither gender, prior thrombosis, splenomegaly, cardiovascular risk factors, nor leukocyte count and JAK2 status were found to influence the occurrence of events during the disease out come. Because treatment options in ET are tailored according to pt thrombotic risk, we need a prospective long term study in this subset of ET pts in order to identify.

Table 1. Clinical and laboratory characteristics of 257 young ET patients.

Median age (yrs, range)	35.3 (17-44.9)
Male/Female (%)	79/178 (31-69)
Median haemoglobin (gr/dl, range)	14 (12.6-18.6)
Median WBC (mm3, range)	8.96 (3.9-22.35)
Median PLTs (mm3, range)	876 (237-2800)
V617F JAK2 mutation(%)	102/210 assessable pts (48.9)
Splenomegaly (%)	59 (23.4)
Previous thrombosis(%)	23(9.0)
Arterious(%)	15 (6.0)
Venous(%)	8 (1.0)
MP evolution (%)	5(1.95)
BP evolution (%)	2 (0.78)
Thrombotic complications(%)	16 (6.2)
Arteriosus (%)	6(2.3)
Venous (%)	10(3.9)
Cytoreductive therapy (%)	136 (53.1)
Antithrombotic treatment(%)	233 (92.1)
Median follow-up (months, range)	84,7 (0.7-365)

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FUNCTIONAL AND GENETIC ABERRATIONS OF *IN VITRO* CULTURED BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS OF PATIENTS WITH CLASSICAL PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Genetic alterations of bone marrow-derived mesenchymal stromal cells (BM-MSCs) have been detected in patients affected by myeloid malignancies, like myelodysplastic syndromes and acute leukemia. Since little is known about BM-MSCs derived from patients with classical Philadelphia-negative myeloproliferative disorders (Ph-neg MPNs), we functionally and genetically characterized the BM-MSC of 38 patients (28 with MPN-associated myelofibrosis, and 10 with essential thrombocythemia or polycythemia vera), and we compared them with those obtained from healthy donors (HDs). Mononuclear cells were obtained either from BM aspirates or, in case of dry tap, from BM biopsy bone fragments digested with collagenase. Clonogenic efficiency and morphology of patients derived BM-MSC were similar to those obtained from healthy donors; however, the former displayed a significant lower proliferation rate and earlier senescence than the latter, as proven by morphology, cell counting and beta-gal staining. Moreover, patients derived BM-MSC were less efficient in osteoblastic differentiation, showed a significantly reduced capacity of sustaining long-term hematopoiesis at the LTC-IC assay *in vitro*, and exhibited a higher expression of the filamentous protein nestin, a putative regulator of homing and differentiation of hematopoietic stem cells. We confirmed that in patients with the JAK2V617F mutation in the hematopoietic compartment, BM-MSCs displayed only the wild-type allele. However, at the comparative genomic hybridisation array, we originally documented that 17% of patients, regardless of the type of MPN, showed genetic abnormalities in their BM-MSCs, whereas MSCs derived from healthy donors never showed any genetic abnormalities, irrespectively of the passage tested. These abnormalities, which included random deletions or duplications of chromosomes 1, 3, 5, 7, 11, 17, and Y, were never detected in patient hematopoietic cells. Moreover, 4 out of 7 female patients with polymorphic alleles of the HUMARA locus, had MSCs with a skewed X-chromosome inactivation pattern. These results document that i) functionally altered BM-MSCs are present in classical Ph-neg MPNs; ii) a proportion of them is genetically altered; and iii) they may undergo clonal selection. How these aberrant MSCs contribute to support clonal hematopoietic stem cell disorders, or drive their phenotype, is a matter of further investigation.

Chronic Myeloid Leukemia I

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EXTREME THROMBOCYTOSIS IN CHRONIC MYELOID LEUKEMIA (CML) IN THE ERA OF TYROSINE KINASE INHIBITORS (TKIS)

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Background. Thrombocytosis is a common feature in chronic myeloproliferative diseases disorders. The incidence of thrombocytosis in CML is reported to be around 30 to 50%. Extreme thrombocytosis defined as a platelet count > $1.000x10^9$/L is uncommon in CML as well as isolated thrombocytosis. Aims: To analyze the behavior of CML with extreme thrombocytosis and the problems associated to the clinical management and the therapeutic response. Methods. From November 1997 to February 2013 we treated 100 consecutive patients (pts) with CML. Results. Only 11 pts (11%) presented at diagnosis an extreme thrombocytosis. There were 8 females and 3 males with a median age of 42 years. At diagnosis, median hemoglobin level was 12.2 g/dl, median WBC 19.240/mmc and platelets count $1.160x10^9$/L. The Sokal score was high in 5, intermediate in 3 and low in 3 pts. In all cases PCR analysis showed the presence of p210 and absence of JAK2 V617F mutation. Bleeding time (Ivy test) was prolonged with a median of 10.68 minutes. aPTT was within the normal range in all but two pts. Iron levels were normal in all but one female patient. Only one patient developed thrombosis of the caephalic vein at diagnosis while no patient reported history of bleeding. All but three pts received initial treatment with hydroxyurea and allopurinol. One patient underwent plateletpheresis. Platelet count was largely unresponsive to initial treatment. Low dose aspirin (100mg/day) was administered in 5 out of 11 pts. Upfront treatment was imatinib in 8 pts and nilotinib in 3 pts. Platelet count normalization was rapidly achieved after introduction of TKIs. Haematological response was reached at a median of 1 month, complete cytogenetic response after 3 months and major molecular response in 9 out of 11 pts after 9 months. One patient was in suboptimal molecular response at 18 months of imatinib and he was shifted to dasatinib, achieving MMR. One patient, without bcr-abl mutation, lost MMR after 5 years of imatinib, and shifted to nilotinib achieving MMR. All pts are alive and in optimal response at a median follow up of 67 months. Conclusion: Extreme thrombocytosis in CML is unfrequent. Prolonged bleeding time was detected in all pts although it was not accompanied by bleeding diathesis. Cytoreduction with hydroxiurea was not able to achieve normalization of platelet count. The role and optimal treatment of extreme thrombocytosis, that was easily and rapidly achieved by TKIs, should be codified.

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BODY MASS CHANGES IN WOMEN WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB

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Increased body mass index (BMI) has been associated with increased incidence of hematologic cancers. Recently, our group demonstrated a relationship between increased BMI and suboptimal cytogenetic and molecular responses during imatinib treatment. We retrospectively assessed weight and BMI in 339 CP-CML patients treated with imatinib; 142 first received interferon and then switched to imatinib for failure, while 197 patients were treated with imatinib first-line from January 2000 onward. For all patients, BMI and body weight data were collected at the time of start of imatinib. According to WHO, patients were stratified into four categories: underweight (BMI <18.5), normal weight (18.5-<25), overweight (25-<30) and obese (≥30). Median age of whole population was 48 years, with a male prevalence; 167 patients were low Sokal risk, 133 intermediate and 39 high risk. Stratification according to Eutos score identified 304 patients as low and 19 high risk (in 16 patients the score was not applicable). Our results showed that BMI increased with age (median age 29 years in underweight category, 43.4 years in normal weight, 54.9 years in overweight and 62.4 years in obese patients, p=0.001), there was an association between increased BMI at baseline and sex (higher percentage of males in overweight/obese categories, p=0.002). Median BMI at baseline of the whole cohort was 25.1; this did not change at 12 months (BMI=26) and 24 months of imatinib treatment (BMI=26, p=ns). Furthermore, we considered BMI modifications during therapy according to sex: BMI at baseline in males was 25.7 and only a slight increase was detected during therapy (26 both at 12 and 24 months, p=ns). On the contrary, BMI at baseline in females was 24.4 and increased to 25.7 at 12 months and to 27 at 18 months (p=0.02). BMI changes in women were probably due to increased body weight not related to fluid retention or changes in alimentary habits. We also prospectively collected BMI data in 35 patients, which received nilotinib at the dose of 400 mg BID and we did not find significant changes during treatment (p=ns). It is possible to hypothesize that imatinib induces metabolic and hormonal modifications, which in turn translate into body weight gain. No evidences exist about effect of nilotinib on adipogenesis. Further studies are needed to establish potential pathogenetic mechanisms and to clarify the reasons of preferential body weight gain in female patients treated with imatinib.

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CYTOGENETIC RESPONSE, MOLECULAR RESPONSE AND OUTCOME OF ELDERLY PH+ CML PATIENTS WITH STABLE COMPLETE CYTOGENETIC RESPONSE AFTER 4-YEARS OF INTERMITTENT TREATMENT WITH IMATINIB (INTERIM)

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Imatinib (IM) significantly changed the prognosis of Philadelphia (Ph+) chronic myeloid leukemia (CML). With imatinib at the standard dose of 400 mg daily, 80-90% of patients are alive at 8 years but only a small proportion of patients (about 5%) can discontinue the treatment without having a molecular recurrence. Thus, the great majority of responsive patients would be destined to continue the treatment indefinitely, at the same standard dose. Although elderly patients have cytogenetic and molecular responses comparable to younger ones, they tolerate imatinib worse and this may reduce the benefit of therapy. We report on a study where an alternative intermittent (one month on and one month off) treatment schedule of imatinib (INTERIM) was tested in seventy-six Ph+ CML patients aged 65 years or older, who had been treated with IM/daily (standard) for more than two years and who were in stable complete cytogenetic response (CCgR) and in major molecular response (MMR). At least three characteristics of these patients are worth noticing: the long duration of IM treatment, with a median of more than 60 months; the high proportion (81%) of the patients on a 400 mg dose, in spite of the age and the long treatment duration; and the low proportion of high-risk patients, in spite of the advanced age. According to the treatment plan of the study, the patients who lost the CCgR resumed the pre-study, daily imatinib treatment. The patients who lost the MMR alone within the first year had to continue the intermittent schedule. After the first year, the patients who lost MMR alone were allowed to go back to

the pre-study continuous treatment. With a minimum follow-up of four years, 13 patients (17%) have lost CCgR and MMR, and 14 (18%) have lost MMR only. All these patients resumed imatinib continuously, and all but one (lost to follow-up) regained CCgR and MMR. No patients progressed to accelerated or blastic phase, or developed clonal chromosomal abnormalities in Ph+ cells, or BCR-ABL mutations. No patients complained of new or more severe side effects during the months "on". In elderly Ph+ CML patients carefully selected for a stable CCgR (lasting > 2 years), the policy of intermittent imatinib treatment affected the markers of residual disease, but not the clinical outcomes (overall and progression-free survival). Acknowledgments: EuropeanLeukemiaNet (contract LSHC-CT-2004-503216) through the EUTOS supported by Novartis Oncology Europe, and COFIN 2009.

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IRF5 IS A TARGET OF BCR-ABL KINASE ACTIVITY AND REDUCES CML CELL PROLIFERATION

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Interferon Regulatory Factor 5 (IRF5) modulates the expression of genes controlling cell growth and apoptosis. Previous findings have suggested a lack of IRF5 transcripts in both acute and chronic leukemias. However, to date, IRF5 expression and function has not been investigated in Chronic Myeloid Leukemia (CML). We report that IRF5 is expressed in CML cells where the BCR-ABL kinase lowers its expression and induces its tyrosine phosphorylation. Tyrosine phosphorylated IRF5 displayed reduced transcriptional activity that was partially restored by Imatinib Mesylate (IM). Interestingly, a mutant devoid of the BCR-ABL consensus site (IRF5Y104F) still presented significant tyrosine phosphorylation. These findings, coupled with the lack of IRF5 phosphorylation in BCR-ABL-negative cells, suggest that the oncoprotein regulates additional signaling pathways leading to IRF5 phosphorylation on other tyrosine residues. We also found that ectopic IRF5 decreases the proliferation of CML cell lines by slowing their S-G2 transition and synergizes with inhibition of BCR-ABL signaling observed after IM, alpha-2-IFN and a DNA-damaging agent. Furthermore, IRF5 overexpression successfully reduced the clonogenic ability of CML CD34-positive progenitors before and after exposure to the above-indicated cytotoxic stimuli. Our data identify IRF5 as a downstream target of the BCR-ABL kinase, suggesting that its biological inactivation contributes to leukemic transformation.

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PROGNOSTIC IMPLICATIONS OF COMORBIDITIES SCORES IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB

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Prognostic importance of comorbidities in CML has never been evaluated in relation to active treatment. Aim of our study was to compare and validate 3 different comorbidities scores, Charlson Comorbidity Index (CCI), Cumulative Illness Rating Scale (CIRS) and Adult Comorbidity Evaluation 27 (ACE-27) in a cohort of CML patients treated with imatinib. All patients were diagnosed and followed between 1995 and 2010 at the Sapienza University of Rome, outside clinical trials. Of 343 patients enrolled, 142 first received interferon outside clinical trials and then switched to imatinib for failure. The remaining 201 patients were treated consecutively with imatinib first- line, from January 2000 onward. Risk evaluation at baseline was performed with Sokal and, retrospectively, with EUTOS score. All patients were followed according to ELN guidelines. Cytogenetic analysis was performed at 3 and then at 6 and 12 months from start of imatinib, while RQ-PCR on peripheral blood was performed every 3 months, and then every 6 months after MMR or complete molecular response (CMR) was achieved. Molecular results were expressed according to the International Scale (IS). We considered as primary resistance lack of any cytogenetic response, and as secondary resistance the achievement with subsequent loss of cytogenetic or molecular response. Application of CCI showed 182 patients as score 0-2 (50%), 101 patients as score 3 (29%), 47 patients as score 4 (13.7%) and 13 patients as score 5 (3.7%). We found a significant correlation between CCI stratification and cumulative incidence of CCyR (p=0.02), MMR (p=0.03), primary and secondary resistance (p=0.01). Application of ACE-27 index in 338 evaluable patients, identified 170 patients (50%) as having score 2 and 168 patients (49.7%) as score 3: we did not reveal any substantial difference according to this stratification in terms of CCyR, MMR or rate of resistance. Finally, we applied CIRS score in 334 patients, which identified 155 patients as having score 1 (46%), 96 patients as score 2 (29%), 61 patients as score 3 (18%), 20 patients as score 4 (6%) and 2 patients as score 6 (0.6%). Again, we did not find any significant association between CIRS and cumulative incidences of CCyR, MMR and resistance. In our study we showed that not all comorbidity indexes were able to evaluate the power of comorbidities at baseline in CML patients treated frontline with imatinib, but only CCI maintains its predictive value in terms of efficacy.

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CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WHO FAILED INTERFERON ALPHA AND SWITCHED TO IMATINIB: LONG-TERM FOLLOW-UP

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Few data are available about the long-term outcome of patients treated with imatinib after interferon-alpha (IFN) failure. Recently, MDACC group reported a 10-year survival rate of 68%. We refer here on the outcome of 134 adult Ph+ CML patients who switched to imatinib after IFN failure. Response criteria used were in accordance to 2009 ELN guidelines; survival was calculated from start of imatinib until death for any cause, progression-free survival (PFS) was calculated from start of imatinib to advent of accelerated/blastic phase or death for any cause and event-free survival (EFS) was calculated from start of therapy to development of any event leading patient to discontinue the drug. There were 74 males and 60 females, median age at diagnosis was 47 years. Sokal score identified 66 patients as low risk, 49 patients as intermediate and 17 as high risk, whereas Eutos score, retrospectively applied, identified 107 patients as low risk and 10 patients as high risk. Median global follow-up was 96 months. After a median time of 52 months of imatinib therapy, 103 patients (76.8%) achieved a CCyR as their best cytogenetic response and 68 (51%) achieved a MMR,. Complete molecular remission (CMR, undetectable transcript according to ELN recommendations) was obtained as the best response in 60 patients after a median follow-up of 76 months. The estimated 9-year overall survival rate was 74%, the PFS rate was 71% and the EFS was 54%. Progression to accelerated/blastic phase was detected in 19 patients. Due to resistance or intolerance to imatinib, 22 patients switched to dasatinib (12 for cytogenetic resistance, 5 for intolerance, 2 for molecular resistance and 3 for BC) and 17 patients switched to nilotinib (11 for cytogenetic resistance and 6 for molecular resistance); 18 patients (64%) achieved or maintained CCyR and 11 patients (35%) achieved MMR. Achieving at 12 months a CCyR or an MMR was associated with a significantly better OS in the long-term (82%) as compared to achieving less than PCyR (70%) or only CHR (12%). Baseline factors that correlated with a poor OS were clonal cytogenetic evolution, higher percentage of basophils and blast cells in peripheral blood, high Sokal and Eutos scores, whereas age did not correlate with a worse OS. The results of our study support the validity of rescuing with imatinib patients failing IFN, with an estimated 9-year survival rate of 74%, that is similar to that reported in other recent studies.

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CHRONIC MYELOID LEUKEMIA DEVELOPED IN A PATIENT WITH PRIMARY IMMUNE THROMBOCYTOPENIA: A CASE REPORT

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Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by immune- mediated platelet destruction and suppressed platelet production. Different immunosuppressive agents have been used in ITP as second-line therapy. The long-term exposure to immunosuppressive medications may increase the risk of hematologic malignancies. There are reports in literature of patients having therapy-related chronic myeloid leukemia (CML). We report CML developing 24 years after the diagnosis of ITP. The patient, a 59-year-old male, had been diagnosed of ITP in 1989 and was treated firstly with corticosteroid and then underwent splenectomy in 1991, which was not effective too long in increasing platelet counts. The patient continued to have fluctuating thrombocytopenia for several years in treatment with corticosteroids. From 2002 he was mantained on Cyclosporin A, that was interrupted in June 2010 for renal toxicity, abnormal growth of gum tissue and redness of the skin. Subsequently, the patient was treated with TPO-agonist (Romiplostim) since June 2012 when he was admitted to Cardiology Unit for acute non-ST myocardial infarction (NSTEMI) and underwent direct percutaneous coronary intervention and stenting. In July 2012 he was treated with Rituximab weekly for four doses with a good response for six months. In February 2013 platelets again dropped and he developed mild fever and asthenia. Laboratory data were: WBC 17717/mm^3 (monocytes 12%, blasts 0%), Hb 12.3 g/dl and platelets 6000/mm^3. CT total body was negative. The increase in WBC count ranging between 15000 to 25000/mm^3 with monocytosis was present since 2006, when we first suspected myeloproliferative neoplasm, but laboratory data didn't confermed it (cytogenetic data of January 2012 were negative for t[9;22]). Actually his bone marrow shows hematologic characteristic typical of CML. Cytogenetic data confirmes the diagnosis with karyotype of 46XX, t(9;22)(q34;q11) [15/40]. BCR-ABL transcript levels by RT quantitative(Q)-PCR analysis is 1613/10000. Since the patient had been splenectomized, the tumor load might have been small since there could be no pooling in the spleen. We decide to treat the patient with the tyrosine kinase inhibitor imatinib mesylate at a daily dose of 400 mg. Furthermore, since our patient received immunosuppressive agents for ITP (Cyclosporin A, Rituximab) for a long period, we think that he developed therapy-related CML, that was diagnosed in early chronic-phase.

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DASATINIB EFFICACY AND SAFETY IN VERY ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA RESISTANT TO IMATINIB

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Background. Dasatinib (DAS) has shown to be effective and safe in a large retrospective cohort of Chronic Myeloid Leukemia (CML) patients aged >60 years resistant to imatinib (IM) Aim To evaluate the impact of DAS in very elderly patients failing IM, we retrospectively collected data from 46 patients aged >75 years treated with DAS from 5/2005 to 6/2012 in 27 Institutions Patients and Methods 25 males and 21 females began DAS, at a median age of 79.5 years (IR 77.3 – 83.0). Median time from diagnosis to DAS treatment was 84.9 months (IR 50.9 – 117.1). Thirteen patients (28.2%) received IFN ± Ara-C before IM, 29 patients (63.0%) were treated with IM at standard dose (400 mg/day) while 17 (37.0%) with IM at a reduced dose (≤ 300 mg/day), with an overall median period of IM treatment of 49.5 months (IR 27.3 – 66.6) Results Twenty-four patients (52.1%) were primary resistant to IM while 22 (47.9%) had secondary resistance. Starting DAS dose was 140 mg/day in 12 patients, 100 mg/day in 19 and <100 mg/day in 15, respectively. Grade 3 – 4 haematological and extra-haematological toxicities were reported in 11 (23.9%) and 16 (34.7%) patients, respectively. Pleuro-pericardial effusions occurred in 21 patients (45,6%) [grade 1-2 in 14 subjects (30.4%) and grade 3 in 7 individuals (15.2%)]. Overall, 3/46 patients (6.5%) permanently discontinued DAS due to early toxicity. A dose reduction was needed in 25/46 patients (54.3%). As to the best cumulative response, 45/46 patients were considered evaluable (≥6 months of treatment) and 1 too early. Four patients (8.8%) did not have any response (including the 3 patients with early discontinuation for toxicity) and 41 (91.2%) achieved a Complete Haematological Response (CHR). Furthermore, 25/45 patients (55.5%) obtained Cytogenetic Responses (CyR) [Partial CyR in 3 (6.6%) and Complete CyR in 22 subjects (48.9%)] with 13/45 patients (28.8%) achieving a major molecular response. After a median period of 24.9 months (IR 15.2 – 46.5) from DAS start, 17 patients have died (1 from disease progression, 14 from unrelated causes and 2 from unknown causes) and 29 are still alive (19 still receiving DAS treatment). Two-year and 5-year overall survival were 73.4% (95%CI 59.6 - 87.2) and 50.3% (95%CI 31.6 - 69.0), respectively. Conclusions DAS seems effective and well tolerated also in very elderly and heavily pretreated subjects. These results encourage DAS use in chronic phase elderly patients beyond any age limit.

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SCREENING AND DEVELOPMENT OF NEW 14-3-3 SIGMA INHIBITORS DISPLAYNG ACTIVITY IN PH+ CELL LINES

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The standard treatment of CML is based on tyrosine kinase inhibitors (TKIs) which target the constitutively activated TK fusion protein Bcr-Abl. Resistance to Bcr-Abl inhibitors has stimulated many efforts to develop key functional compounds able to bind Bcr-Abl protein or, alternatively, downstream kinase effectors. The 14-3-3 sigma protein is an adapter protein implicated in the regulation of a large spectrum of signaling pathways (cell cycle progression, DNA damage response, apoptosis). In normal cells 14-3-3s forms a cytoplasmatic complex with c-Abl. In response to DNA damage, the phosphorilated 14-3-3s releases c-Abl that shuttles into the nucleus and promotes the apoptosis. In CML cells the presence of Bcr-Abl interferes with the dissociation of the 14-3-3s/c-Abl complex. The 14-3-3s inhibitors could enable the c-Abl cytoplasmatic release, its nuclear translocation and consequently its apoptotic effect. Our aim is to identify new molecular compounds which block 14-3-3s protein and to study their cytotoxic effect in CML cells. Computational studies let us to screen a virtual database of molecules on the basis of X-ray 14-3-3s structure. The best 15 compounds were selected using different molecular modeling approaches. In order to study the cytotoxic effect of 14-3-3s inhibitors, we incubated Ph+ K562 cell line with different compound concentrations and times. The inhibitors showed a decrease of cell viability in a range of 10-50% in a proliferation assay. The most active compound was GV2-20, that induces a

reduction of cell viability of 30% and 50% after 72 or 96h, respectively, with daily 15 microM drug additions. GV2-20 activity seemed to be higher than BV01, already known as 14-3-3s inhibitor. The analysis of the cell cycle showed a significant increase of G0/G1 phase cell-cycle in treated cells compared with non treated cells. The result was confirmed in a second Ph+ cell line (JURL-MK1). We did not observed apoptosis in both K562 and JURL-MK1 cells after treatment. In conclusion, we have identified a new molecule which binds 14-3-3s and demonstrated that the GV2-20 causes a cell cycle arrest in K562 and JURL-MK1 cell lines. The identification and study of the cell cycle protein interaction with 14-3-3s are in progress. From this study we expect to characterize the role of 14-3-3s in CML patients and to provide experimental evidences supporting the possibility of using this protein as a new molecular target in CML treatment.

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HIGH FREQUENCY OF SMALL INSERTIONS AND DELETIONS IN THE BCR-ABL KINASE DOMAIN REVEALED BY ULTRA-DEEP SEQUENCING

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The spectrum of Bcr-Abl kinase domain mechanisms that confer resistance to tyrosine kinase inhibitors (TKIs) in Philadelphia-positive (Ph+) Leukemia is quite heterogeneous. Not always molecular events underlying drug-resistance can be explained by presence of mutations; Bcr-Abl KD insertions/deletions can be an alternative mutational mechanisms. The recent development of "deep-amplicon sequencing" (DS) technologies has opened the way to a more accurate characterization of molecular aberrations in Ph+ Leukemia. We took advantage of a DS approach in order to fully characterize the spectrum of insertions and deletions in CML and Ph+ ALL patients who had developed resistance to one or multiple lines of TKI therapy.We set up a Bcr-Abl KD mutation screening assay on the Roche GS Junior instrument that allows to reliably detect sequence variants with a lower detection limit of 1%. A total of 88 samples from 34 CML and 15 Ph+ ALL patients who had developed resistance to one or multiple TKIs were selected for this analysis. In order to reconstruct the dynamics of growth of mutations we evaluated their presence in a serial follow-up samples collected during TKI therapy. DS revealed a 35-base insertion (35INS) in 27/34 (79%) CML and 13/15 (86%) ALL Ph+ patients with an abundance from 1% up to 96% of all Bcr-Abl transcripts. Interestingly DS highlighted an increased expression of 35INS over time in 6 patients (growth ranged from 2% to 96% within a few months). In addition DS detected 1 in-frame deletions in 9 samples, with an abundance from 2% to 19% . This variants include a 72-nt deletion (1233-1304) between the junction of Abl exon 6 and 7 that causes the loss of 24 amino acids (aa 359-383). Our results show that DS technologies on the GS Junior instrument allow a more accurate characterization of mutational status of patients. The higher sensitivity of DS approach allowed to highlight a frequency of 35INS higher than previously reported. The 35INS thus seems to be very frequent in CML and Ph+ ALL patients who develop resistance to one or multiple lines of TKI therapies but its abundance is dynamic in individual patients and seems not to be related to TKI therapy. Although this insertion does not predict for a specific TKIs-resistance its role in Ph+ Leukemia merit additional studies to better understand its biological and clinical relevance . Supported by Fondazione CARISBO, PRIN 2009, IGA MZCR NT11555, AIL e AIRC.

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IN VITRO AND *IN VIVO* CHARACTERIZATION OF BOSUTINIB AS SUBSTRATE OF THE P-GLYCOPROTEIN EFFLUX TRANSPORTER

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Bosutinib (Pfizer) is a dual SRC/ABL tyrosine kinase inhibitor (TKI) recently approved for the treatment of Philadelphia positive leukemias. The efficacy of BCR-ABL inhibitors for the therapy of chronic myeloid leukemia (CML) might be impaired by the development of resistance. The alteration in the expression levels of transporters involved in both drugs uptake and efflux has been already described as resistance mechanisms for other TKI such as imatinib. Our aim was to determine which carriers are responsible for bosutinib transport. The Bcr-Abl positive cell line K562S was selected for this study since it shows low endogenous expression of all the transporter under investigation. K562S overexpressing the drug transporters P-gp, BCRP, OCT1 were produced. FACS analysis performed with fluorescent substrates specific for each transporter confirmed that the proteins were active. The Intracellular Uptake and Retention (IUR) assay performed using C-14 radiolabelled bosutinib showed that only P-gp was responsible for active bosutinib transport. In the same assay, K562DOX cells (overexpressing P-gp) treated with the P-gp inhibitor Verapamil showed a significant increase in intracellular bosutinib concentration, comparable with parental K562S. In a proliferation assay, K562DOX cells demonstrated an increased level of resistance to bosutinib compared to the parental K562S (IC50 values: 36.4nM and 6.5nM, respectively). Cotreatment with verapamil restores the sensitivity of the cells. To confirm our *in vitro* results, we performed *in vivo* experiments with a xenograft model. Nude mice were injected with K562S, K562DOX or K562DOX silenced for P-gp (K562DOX/sh P-gp). When tumors were measurable, mice were treated with either bosutinib (150 mg/kg daily/14days) or vehicle. K562DOX mice initially responded to bosutinib, but tumours eventually relapsed upon treatment stop. In contrast, K562S as well as K562DOX/sh P-gp remained tumour-free for all the follow-up period. In conclusion, our *in vitro* and *in vivo* data strongly suggest that P-gp is responsible for bosutinib transport and that analysis of P-gp expression levels might be helpful in the treatment decision for patients that exhibit resistance to bosutinib therapy.

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THE C.480C>G POLYMORPHISM OF HOCT1 TRANSPORTER INFLUENCES IMATINIB CLEARANCE IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA

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Background. It has been reported that the highest therapeutic benefit from imatinib is expected when minimal plasma concentrations (Cmin) are ≥1000 ng/mL. However, imatinib pharmacokinetics (PK) is influenced by several different factors. Aims. 1) Application of a therapeutic monitoring protocol for imatinib in order to evaluate its PK characteristics. 2) evaluation of a possible correlation between the pharmacogenetics of hOCT1 and imatinib pharmacokinetics in CML patients. The TIKlet study was approved by the Pisa University Hospital Ethics Committee (ref. n. 46013, 08/01/2011) and supported by Regione Toscana. Methods. Sixty-two patients affected by CML and treated with imatinib were enrolled. A commercially-available HPLC assay kit (Chromsystems, Munich, Germany) was used. The hOCT1 c.480C>G (rs683369) SNPs was evaluated by a specific kit from Applied Biosystems (Life Sciences).

Results. After at least 3 determinations/patient, the average of imatinib Cmin was 1064±280 ng/mL. In the PK model, age, sex, height, weight, body mass, alpha1-acid-glycoprotein, smoking, renal and hepatic parameters were inserted and only the alpha1-acid glycoprotein significantly correlated with imatinib plasma levels. The introduction of hOCT1 SNP into the model led to a significant decrease in interindividual variability in the clearance of imatinib. In particular, imatinib clearance was significantly higher in patients homozygous for the wild-type C allele (12.2+/-2.3 L/h) with respect to other patients (9.4+/-1.6 L/h). The 20 months-EFS was shorter in patients carrying at least 1 G allele of the hOCT1 gene with respect to the AA individuals (48% *vs* 86%, respectively), even if this difference was not statistically significant (p=0.14). A significant difference was observed in terms of CL (11.22±2.35 *vs* 9.08±2.15 L/h) and Cmin,ss values (1054±329 *vs* 1383±502 ng/mL) according to the severity of adverse drug reactions (0-2 *vs* 3-4 CTC-NCI grade, respectively). Conclusions: The present results confirm that variability of imatinib pharmacokinetics is not negligible among CML patients. The present PK model is characterized by a good performance, allowing the prediction of Cmin in the present patients regardless the time of blood withdrawal. Finally, results from the present study suggest that patients' genotype with respect to the hOCT1 c.480C>G SNP may predict imatinib clearance.

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EXCELLENT THERAPEUTIC RESULTS ACHIEVED IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH FRONT-LINE IMATINIB AND EARLY TREATMENT MODIFICATIONS FOR UNSATISFACTORY RESPONSE: A RETROSPECTIVE STUDY ON 91 UNSELECTED PATIENTS

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Second generation tyrosine kinase inhibitors (TKI) have been claimed to represent now the first-choice therapy for chronic myeloid leukemia. Indeed, they generally induce faster and deeper molecular responses compared to imatinib that, however, is equally effective in at least 50% of patients. Therefore, an imatinib-based treatment with early shift to 2nd generation TKI for patients with slow/incomplete response might be as effective as front-line 2nd generation TKI. We retrospectively evaluated 91 CML patients, diagnosed in chronic phase from January 2005 and March 2012, treated front-line with standard-dose imatinib and early therapy modifications (at 3-12 months) in case of unsatisfactory response or intolerance. Median age at diagnosis was 61 years (range, 18-86 years). Sokal score, available for 83 patients, was low risk in 36, intermediate risk in 34, high risk in 13. Median follow-up of living patients was 57 months (range, 15-98 months). Thirty-six per cent of the patients (33/91) changed therapy, 9 for intolerance and 24 for unsatisfactory response, either by increasing imatinib dose (11/91) or by switching to 2nd gen TKI (22 directly, 4 after high dose imatinib). Globally, our "early-switch" strategy led to a complete cytogenetic response (CCyR) in 89 patients(98%), with a cumulative incidence (CI) of CCyR of 86% at 12 months and 93% at 24 months. Major molecular response (MMR) was achieved by 80 patients (88%) and 56 of them reached a complete molecular response 4 logs too (BCR-ABL ≤0,01%). CI of MMR was 44%, 69% and 87% at 12, 24 and 48 months, respectively. Three patients (3%) suddenly progressed to lymphoid blastic phase (one was in CCyR and 2 were in MMR too). No cytogentic relapse in chronic phase was observed among patients who achieved MMR. At the last follow up 9 patients died, 7 of CML-unrelated causes and 2 only of CML progression (after 28 and 80 months from diagnosis). Five years overall survival was 92%. These results suggest that our strategy could be as effective as front-line 2nd generation TKI, with the majority of patients still receiving imatinib, a drug of better known long-term side effects and lower cost. However, the issue of the optimal up-front strategy in CML therapy should be evaluated in a prospective, possibly randomized, study.

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SENSITIVITY, REPRODUCIBILITY AND CLINICAL UTILITY OF ULTRA-DEEP SEQUENCING FOR BCR-ABL KINASE DOMAIN MUTATION SCREENING: RESULTS FROM THE IRON II (INTERLABORATORY ROBUSTNESS OF NEXT-GENERATION SEQUENCING) INTERNATIONAL STUDY

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In chronic myeloid leukemia (CML) and Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL) patients receiving tyrosine kinase inhibitors, capillary Sanger sequencing (SS) is the gold standard for BCR-ABL KD mutation screening despite several technical limitations – it cannot robustly identify mutated populations <20%, it provides only rough estimates of mutated clone abundance and it cannot discriminate between polyclonal and compound mutations, unless it is preceded by cloning. Benchtop next-generation sequencers like Roche GS Junior, Illumina Miseq or Ion Torrent PGM have been developed as potential diagnostic platforms and there is growing interest in their clinical application. In the framework of the IRON II (Interlaboratory RObustness of Next-generation sequencing) consortium, 13 laboratories from 7 countries (Italy, Germany, United Kingdom, Spain, Austria, Turkey, Czech Republic) enganged in the set up, standardization and initial validation of a diagnostic assay for BCR-ABL KD mutation screening based on the Roche 454 Titanium technology. Fusion primers were designed to generate four partially overlapping amplicons by nested RT-PCR, the first amplification step needed to select for the translocated ABL allele. The assay was designed in a ready-to-use plate format allowing to analyze twelve samples/patients in a single ultra-deep sequencing (UDS) run by barcoding fusion primers with twelve different MIDs. Serial dilutions of a T315I+ BaF3 cell line in an unmutated one showed high linearity and accuracy of mutation detection down to 1% abundance. Intra-run and inter-run reprodubility were confirmed by resequencing a set of samples in the same and in independent runs, respectively, with and without repetition of the RT and PCR steps. Reproducibility was maintained over wide dynamic range of coverage (100-5000 independent reads). One hundred and fifty CML or Ph+ ALL samples were analyzed in parallel by UDS and SS; twenty of them were also analyzed by pyrosequencing. 99%(148/150) concordance in variant detection and quantification was observed. The higher sensitivity (1%) allowed to backtrack mutations in samples scored as wild-type by SS. A control round to test inter- laboratory reproducibility of mutation detection is about to start. Our results indicate technical feasibility and reliability of UDS for BCR-ABL KD mutation screening and represent an important step forward towards its routine diagnostic application.

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CLINICAL AND BIOLOGICAL FEATURES OF PH+ CHRONIC MYELOID LEUKEMIA (CML) LONG SURVIVOR PATIENTS (MORE THAN 15 YEARS)

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There are limited data on the follow-up of CML long-survivor patients. We analyze the outcome of 98 patients with Ph+ CML,with a follow-up of 15 years or longer (median 211 months): in 30 patients follow-up is >20 years (group A),median 267 months,and in 68 patients follow-up is 15-20 years (group B),median 198 months. Sokal risk evaluation at baseline showed that 74 patients (75.5%) were low

risk,14(14.3%) intermediate and 10(10.2%) high risk.By the EUTOS revised risk score,94 patients (96%) were low risk.All patients were treated with IFN- + Cytarabine as first line treatment for a median time of 85 months.Imatinib was started as second line therapy in 77 patients (78.6%) and median time on Imatinib therapy was 96 months;52 patients continued Imatinib therapy while 25 patients were switched to second line TKIs for resistance (n=21) or intolerance (n=4).One patient developed Myeloid Blast Crisis (BC) after 239 months from diagnosis and one Lymphoid BC,after 161 months.Six of 98 patients (6%) were transplanted:one patient died 15 years after HSCT of disease relapse (lymphoid BC); 5 are alive: 2 (receiving Imatinib) are in Complete Molecular Response (CMR),3 patients (1 on Imatinib, 2 off therapy) are in Major Molecular Response (MMR). In our patients cohort,overall survival after a median follow-up of 211 months is 97%; 44 patients (46.3%) are in CMR (26 receiving imatinib,2 dasatinib,5 nilotinib,4 IFN-,7 off therapy);35 patients (37%) are in MMR(21 receiving imatinib,5 dasatinib,2 nilotinib,3 IFN-, 4 off therapy);8 patients (8.4%) are in CCyR(3 receiving dasatinib,2 nilotinib,2 IFN- ,1 imatinib);2 patients (2%) are in miCyR (1 receiving imatinib,1 nilotinib);5(5.3%) are in CHR (4 patients receiving dasatinib and 1 IFN-);1 patient (1%) receiving nilotinib is in PCyR.Three patients (3%) died; 2 due to disease progression:1 after 172 months (transplanted patient) and 1 after 272 months; 1 patient died on dasatinib therapy from a non-CML cause. In group A, 6 patients (20%) are off therapy,and in group B,6 patients (8.8%) are off therapy.CML management changed dramatically with TKIs but the biological role of IFN- remains crucial; our data from this subset of 98 long-survival patients show that 20 of them (20.4%) never took TKIs: 12(7 CMR, 5MMR) are off therapy and 8(4 in MMR and 4 in CMR) are still under IFN- treatment. Longer follow-up in larger series of patients is warranted to determine peculiar biological clinical features of this subset of patients.

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LOW DOSES OF DASATINIB ARE SAFE AND EFFECTIVE IN ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA RESISTANT/INTOLERANT TO IMATINIB

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The approved dose of dasatinib (DAS) for patients with chronic phase Chronic Myeloid Leukemia (CML) is 100 mg once daily. However, in the real-life setting, some patients considered too frail for standard doses receive l<100 mg per day. To evaluate the efficacy and safety of low-dose DAS (<100 mg/day) in CML patients deemed unsuitable for standard dose, we collected data from 29 patients resistant/intolerant to imatinib (IM) followed by 27 Institutions. All 29 patients (M/F 16/13) received previous IM for a median period of 46.6 months [Interquartile range (IR) 14.3 - 75.7]. Median age at DAS start was 77.1 years (IR 69.0 - 81.5), with a median interval from diagnosis of 80.2 months (IR 41.1 - 100.4). The reasons for the DAS dose reduction were i) age >75 years (7), ii) severe comorbidities (9), iii) the coexistence of age >75 years and severe comorbidities (8), iv) previous intolerance to IM (2) or v) physician's decision based on other clinical considerations (3). At DAS start, 9 patients were primary resistant to IM, 16 developed secondary resistance and 4 were intolerant to the drug. Starting DAS dose was 80 mg/daily in 2 patients, 50 mg/daily in 22 patients and 40 mg/daily in the remaining 5 individuals. Grade 3 - 4 haematological and extra-haematological toxicities were reported in 3 (10.3%) and 12 (41.3%) patients, respectively. Pleural effusions occurred in 8 subjects (27.5%) [grade 1-2 in 6 (20.6%) and grade 3 in 2 patients (6.9%)]. On the whole, 5/29 patients (17.2%) permanently discontinued DAS due to early toxicity (<6 months since drug start). As to the best cumulative response, 2 patients were too early (<6 months of treatment) and 27 were considered evaluable. Aside from the 5 patients with early discontinuation, 22 individuals (81.4%) achieved a Complete Haematological Response (CHR). Among patients in CHR, 16 (59.2%) obtained a Complete Cytogenetic Response, with 13 (48.1%) also achieving a major molecular response. After a median period of 24.3 months (IR 14.0 - 37.9), 8 patients have died (all from causes unrelated to CML progression) and 21 are still alive (13 of them still receiving DAS). Two-year and 5-year overall survival were 72.9% (95% CI 55.4 - 90.4) and 66.8% (95% CI 47.1 - 86.4), respectively. Low-dose DAS could be useful for the treatment of chronic phase CML patients considered too frail for standard 100 mg/day dosage: however, a randomized study is warranted to define with more accuracy the role of such approach.

Acute Leukemia II

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ACUTE MYELOID LEUKEMIA(AML) WITH BLASTS MIMICKING M3 VARIANT (M3V) LEUKEMIC CELLS, LACKING THE PML/RAR REARRANGEMENT A PITTFALL IN MORPHOLOGICAL DIAGNOSIS OF HYPOGRANULAR ACUTE PROMYELOCYTIC LEUKEMIA (APL): A NEW CLINICAL-BIOLOGICAL SUBTYPE OF AML, WITH TRANSITIONAL ASPECTS?

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Acute Promyelocytic Leukaemia (APL) is a unique clinical biologic entity and should be distinguished from other subtypes of Acute Myeloid Leukemia (AML) because of the increased risk of disseminated intravascular coagulation (DIC) and its response to retinoic acid (ATRA). Recently a few cases of AML were described, which were characterized morphologically by blast cells similar to atypical hypogranular promyelocytes (M3v subtype) but lacked of t(15;17) and PML/ Rar rearrangement.We report four AML cases - 3 females, 1 male, aged 49, 72, 74 and 59- with M3v morphological features, who were observed between January 2005 and March 2013 at our institution. At diagnosis, 3 case had WBC count >50.000/mmc (range 69.000-289.000/mmc), while in all cases coagulophaty was present. Peripheral blood blast cells had bilobed, convoluted nucleus and hypogranular cytoplasm, mimicking M3v blast cells. A low percentage of atypical promyelocytes was also observed. Whereas in bone marrow specimens a high percentage of granular and agranular myeloblasts were encountered, thus the diagnosis of AML with maturation subtype(FAB M2) was done. Cytochemistry showed high and intense expression of Myeloperoxidase (MPO>90%) in all cases. As to immunophenotypic features, blast cells stained positive for CD13, CD33, CD9, but negative for HLA-DR, CD34. In 3 out of 4 cases, CD56 was expressed. In none cases t(15;17)(q22;q21) and PML/RAR rearrangement were detected. NPM1 gene mutation was demonstrated in 2 cases(pts 2 and 4), while FLT3 ITD in case 4 only. Three patients died early: 2 during chemotherapy-induced aplasia. (cases 1 and 4 treated according to an intensive induction AML-GIMEMA schedule) and case 3 after one day of the admission because of pulmunary thromboembolism. Interestingly, in case 2 (NPM1+), who was treated with epigenetic therapy (Low doses ARA-C+ATRA), blast cells differentiation was observed during ATRA therapy, as in APL. This patient maintained stable disease for 10 months. In conclusion,these four cases suggest the existence of a distinct and non yet classifie subtype of AML,with transitional aspects between the AML with maturation (FAB M2subtypes)and M3v characterized by :leucocytosis(>50.000/mmc), laboratory evidence of DIC, morphological and immunophenotype findings similar to APL but lacking the t(15;17) and PML/ Rar rearrangement. Further studies are need to confirm our hypothesis.

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SUCCESSFUL SALVAGE TREATMENT WITH CLOFARABINE AND CYTARABINE (ARA-C) IN RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

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Introduction. Relapsed/refractory AML patients have a poor prognosis, with CR rates of 1%-30%, unless allogeneic hematopoietic stem cell transplantation (HSCT) is an available option. It was previously established the activity of clofarabine plus cytarabine in AML relapse (clofarabine dosed once daily for 5 days with 40 mg/m^2 followed 4 hours later by ara-C at 1 g/m^2 per day). However, modifications of this combination in AML therapy of relapsed/refractory patients warrant further evaluation. Aim. To determine the efficacy and safety of clofarabine and cytarabine (Ara-C) in adult patients with relapsed or refractory acute myeloid leukemia (AML). Methods. Patients aged (30-67) years with refractory/relapsed AML were treated at the dose of clofarabine 30 mg/mq on days 1-5 + cytarabine 1000 mg/mq gg on days 1-5. We evaluated the complete remission rate (CRR), duration of remission (DOR) and overall survival (OS). Minimal residual disease (MRD) by molecular targeting was considered in all patients. Results. Twenty-five (25) patients received clofarabine 30 mg/mq on days 1-5 + cytarabine 1000 mg/mq gg on days 1-5 (followed by gentuzumab therapy in only three patients). All patients had relapsed/refractory myeloid leukemia and had received multiple priors therapies. Six pts had received a prior hematopoietic stem cell transplant (HSCT). Fourteen patients achieved a complete remission (CR); nine patients went on to receive allogeneic transplants after clofarabine/ARA-C salvage. The complete remission rate (CRR) was (56,00 %) The Median of Overall survival for all patients was (149) days (range 12-1152), while the media of Overall survival (OS) was (221.52) days, and we estimated a duration of remission (DOR) as (195.00) days in median (range 41-1131), and (311.36) days in media (we calculated from the first day of remission). Treatment was complicated by neutropenic fever (n=16), grade III-IV mucositis (n=2), skin rush (n=1) grade II- III, hepatic transaminase elevations (n=2). Two (n=5) patient died before their disease status could be evaluated Conclusion: Combination treatment with clofarabine 30 mg/mq and ARA-C 1000 mg/mq in adults pts with refractory or relapsed AML resulted in an ORR of (56,00 %) and of the (14) patients who achieved a CR, nine (64.29%) proceeded to HSCT (Five are still alive). The safety profile is acceptable in this relapsed/refractory population, and our results are very similar to previous regimes using higher clofarabine.

Table 1.

N	Age	Refractory/relapsed	Karyotype and molecular genetyc	Previous regimen (n.)	Response after Clofarabine/ARA-C	Toxicities	HSCT
1	53	refractory	47 XY,+8;	1	Refractory	Severe febrile neutropenia	n
2	42	refractory	48 XY, -21,+ 3 der(21)	1	Complete Remission	Severe febrile neutropenia	y
3	47	relapsed	46,XX/FLT3 ITD mutated	2	Refractory	Severe febrile neutropenia	n
4	30	relapsed	46,XY (5 metafasi)/ 46,XY,del(10q) (7 metafasi)FLT 3Mutated	1	Complete Remission	neutropenia	y
5	54	relapsed	46, XY	2	Complete Remission	Severe febrile neutropenia	y
6	61	refractory	Complex karyotype, monosomy 7	1	Refractory	skin rash	n
7	67	refractory	47,XX,+8	1	Refractory	Severe febrile neutropenia	n
8	56	relapsed	46, XX	1+HSCT	Complete Remission	Severe febrile neutropenia	n
9	58	relapsed	46,XY,inv(16)	2+ HSCT	Complete Remission (I)	Severe febrile neutropenia	n
10	41	relapsed	47,XY,+8 (10metafasi)/ 47,XY,+8,5q- (4 metafasi)	1+HSCT	Complete Remission	Severe febrile neutropenia	n
11	50	refractory	46 XX	2	Complete Remission	Severe febrile neutropenia	y
12	45	relapsed	46,XY	1	Complete Remission	Severe febrile neutropenia	y
13	63	relapsed	46, XY	2	Died in induction	Severe febrile neutropenia	n
14	51	relapsed	46, XYNPM-1 mutated	2	Complete Remission	Nausea, vomiting	n
15	62	refractory	46,XY+8	1+HSCT	Complete Remission	Nausea, vomiting	n
16	46	relapsed	46, XX	1	Died in induction	Mucositis	n
17	41	refractory	46, XY	1	Died in induction	Mucositis	n
18	51	refractory	46,XY,inv(3)sfavorevole	1	Refractory	Severe febrile neutropenia	n
19	40	relapsed	46, XX/FLT3/ITD mutated	1	Complete Remission	Severe febrile neutropenia	y
20	52	refractory	46, XX	1	Refractory	Severe febrile neutropenia	y
21	43	relapsed	46, XX	1	Refractory	Severe febrile neutropenia	n
22	54	relapsed	46, XY	1+HSCT	Died in therapy	Sepsis	n
23	61	relapsed	46, XX	1	Complete Remission	Severe febrile neutropenia	y
24	36	relapsed	46, XX/FLT3/ITD mutated	1	Complete Remission	hepatic transaminase elevations	y
25	39	relapsed	46, XX/FLT3/ITD/NPM1 mutated	1+HSCT	Complete Remission	hypertransaminase mia	n

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ABERRANT PHENOTYPIC EXPRESSION OF CD15 AND CD56 IDENTIFIES POOR PROGNOSTIC ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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Few observations have been reported on the relationship between expression of some additional aberrant phenotypic features and outcome of acute promyelocytic leukemia (APL) patients. Here, we set out to assess the frequency of CD15 and CD56 expression, and their prognostic value in a large series of APL patients. One hundred and fourteen adult patients consecutively diagnosed with PML/RAR -positive APL and homogeneously treated with the AIDA induction schedule at a single institution were included in the study. The biological and clinical features, as well as the prognostic impact with respect to CD15 and CD56 expression, were investigated. Twelve (10.5%) and 9 (8%) of the 114 patients expressed CD15 and CD56, respectively. CD15 expression identified a subset of patients with a classic morphologic subtype (92%), a prevalent association with a bcr1 expression (67%) and a low (5) or intermediate (7) relapse risk at baseline. Only 2 patients expressed concomitantly CD34, associated in both with CD2 without the classic features of high-risk patients. In this group, we observed only 1 case of differentiation syndrome, but a higher frequency of relapses (42% *vs* 20% in the CD15- patients, p=0.03) and a low overall survival (median OS at 5 years 58% *vs* 85% in the CD15- patients, p=0.01). On the contrary, CD56 expression was detected only in patients with a classic morphologic subtype, a prevalent bcr3 expression (67%), intermediate risk in 7/9 patients, and a high incidence of differentiation syndrome (55%) and CD34 expression (isolated in 3 patients and associated with CD2 in 3 patients). CD56 expression in our series identified a significantly higher frequency of relapse (34% *vs* 20% in the CD56- population, p=0.04) and a low OS (60% *vs* 85% in the CD56- population, p=0.02). CD15 and CD56 expression appears an independent adverse prognostic factor for relapse in patients with APL treated with all-trans-retinoic acid plus idarubicin–derived regimens. These aberrant markers may be considered for refining risk-adapted therapeutic strategies in APL patients.

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NEGATIVE PROGNOSTIC VALUE OF ISOLATED LOW CD34 EXPRESSION IN ACUTE PROMYELOCYTIC LEUKEMIA

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The early hematopoietic antigen CD34 is expressed on the leukemic cells of approximately 30% patients with newly diagnosed acute promyelocytic leukemia (APL). However, its potential clinical significance has not been deeply investigated. We hereby analyzed the clinico-biological features and treatment outcome of APL patients in relation to CD34 expression, even when partially expressed (<10%). One hundred and fourteen PML/RAR -positive APL adult patients homogeneously treated with the AIDA schedule at a single institution were included in the study. Biological and clinical features, and their correlation with treatment outcome with respect to CD34 expression, both when expressed in association with CD2 and as isolated expression (cut-off ≥2-<10% or ≥10%), were investigated. CD34 was associated to CD2 in 30 patients and was isolated in 19 patients. When compared to the CD34-negative population (65 patients), CD34/CD2 expression identified a subgroup with characteristic features: M3 variant subtype (26% *vs* 7% in the negative group, p=0.02), bcr3 transcript subtype (73% *vs* 32%, p=0.001), high risk according to the risk of relapse (66% *vs* 17%, p=0.002), high incidence of differentiation syndrome (26% *vs* 12%, p=0.01). CD34/CD2 expression identified a subset of APL patients with a trend towards a lower overall survival (88% *vs* 95%) and a significantly higher rate of relapse (22% *vs* 13.8%, p=0.05). We then evaluated the prognostic value of isolated CD34 expression, not associated to CD2: it was detected in 9 patients with a cut-off of expression ≥10% and in 10 patients with a low cut-off, ≥2% but <10%. Isolated CD34 identified a subgroup with a classic morphology (79%) and with bcr1 prevalence (53%), similar to the CD34-negative subgroup, but with a significantly worse prognosis: a higher rate of relapse (37% *vs* 13.8% in the negative group, p=0.002), higher incidence of differentiation syndrome (55% *vs* 12%, p=0.03) and lower overall survival (60% *vs* 95%, p=0.001). The results of our study confirm that CD34/CD2 expression characterizes a subset of APL with a high WBC count and a variant morphologic subtype, associated with an unfavourable clinical course. We also show that the isolated expression of CD34, even at a low cut-off, identifies a group of classic APL with a negative prognosis. Further studies aimed at identifying other molecular signatures in CD34-positive patients are needed in order to optimize the therapeutic strategy for this subset of patients.

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ACUTE MYELOID LEUKEMIA (AML) RESISTANCE TO STANDARD CHEMOTHERAPY: RETROSPECTIVE ANALYSIS OF PATIENTS TREATED WITH "3+7" FIRST INDUCTION AND "FLAIRG" SECOND INDUCTION

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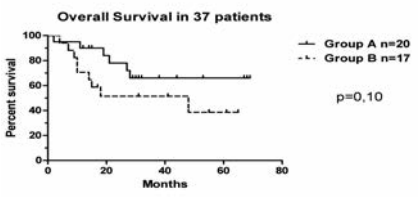
Standard first induction therapy for AML still is the 3+7 regimen (Ara-C 100-200 mg/sqm/d for 7 days, Daunorubicin 45-60 mg/sqm/d or Idarubicin 12 mg/sqm/d for 3 days). This treatment can obtain 60-80% complete remissions(CR). For refractory patients several second-line regimens are available, mostly combinations of high dose Ara-C (HIDAC) with Mitoxantrone and Etoposide (MEC) or Fludarabine and Idarubicine or Mitoxantrone (FLAIRG, FLAG, FLANG). The beneficial role of these regimens in first induction is matter of debate mainly because of their toxicity. We evaluated 48 consecutive patients aged ≤60 yrs with de novo non M3 AML, treated in 2006-2010. The analysis compares clinical and biological features of patients responsive to 3+7 induction (20 pts. group A) with those who did not achieve CR after 3+7 and treated with FLAIRG (17 pts. group B). Patients in CR were consolidated with HIDAC (group A) or a second course of FLAIRG (group B); 8/20 pts (40%) (group A) and 14/17 pts (82%) (group B) were allotransplanted. We focused on early CR (ECR) after one course 3+7 and late CR (LCR) after one (LCR1) or two (LCR2) courses of FLAIRG and on overall survival (OS). Results. 3+7 obtained an ECR rate of 42% (20/48); partial remissions (PR) were 21% (10/48) and refractory patients were 35% (17/48). One patient died in induction. Second-line chemotherapy was administered to 26 patients (10 PR and 16 refractory). FLAIRG was given in 17/26 cases (65%; 1 PR and 16 refractory). The LCR1 rate was 59% (10/17) and PR was 41%(7/17). LCR2 was obtained in 3/17 (17%). The overall LCR rate after FLAIRG was 76%. The distribution of FAB subtypes and cytogenetic risks were similar in groups A and B. The molecular adverse phenotype FLT3+ NPM1- was present in 0/20 cases in group A and in 5/17 cases in group B (p=0,0142) (see Table 1):

Table 1.



5ysOS Group A = 66% , Group B= 38%

Molecular phenotype	Group A (n=20)	Group B (n=17)	p
NPM1+, FLT3-	1	0	NS
NPM1-, FLT3+	0	5	0.01
NPM1+, FLT3+	4	2	NS
NPM1-, FLT3-	15	10	NS

LCR was obtained with FLAIRG in 4/5 (80%) of these pts. OS was not statistically different between group A and B (p=0,10), although it was better in group A rather than B (66% *vs* 38%) (see Table 1). Conclusions: after one course of 3+7 the ECR rate was only 42%, therefore second induction was given to a high percentage of patients (54%). The FLAIRG regimen obtained LCR rate of 76%. Patients in ECR with 3+7 showed an excellent OS at 5ys (66%), possibly for positive prognostic selection. The FLT3+ NPM1- phenotype may identify patients at high risk of induction failure with 3+7 possibly candidates to more intensive regimens.

P195

BRUGADA SYNDROME IN MYELOID NEOPLASMS

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Brugada Syndrome (BS) is an inherited arrhythmogenic disease that may cause syncope and sudden cardiac death in young individuals with a structurally normal heart. The typical electrocardiographic pattern is characterized by a coved ST-segment elevation >2 mm followed by a negative T wave in more than one right precordial lead (V1 to V3), a so-called type 1 electrocardiogram. Diagnosis of the syndrome is difficult because it could manifest for the first time as cardiac arrest without any previous symptom and the electrocardiographic pattern could be intermittent. We report 2 cases of a Brugada electrocardiographic pattern diagnosed in 2 patients with a Myeloid Neoplasm. Case 1. A 45-year old male with Acute Myeloid Leukemia M5b was admitted to our hospital because of disease relapse. During the aplasia phase post-salvage therapy, his temperature was 39.5°C and heart rate 110 beats/min. The EKG revealed, for the first time, a type I Brugada pattern. An echocardiogram showed a normal left ventricular dimension and function (E.F. 65%). The medical history was negative for syncope or a family history of sudden cardiac death and conservative management was indicated. Probably fever unmasked the BS but, despite the presence of this cardiological disease our patient underwent standard dosage chemotherapy. Four months after discharge the patient remains well and free of cardiac events. Case 2. A 69-year old male with Chronic Myeloid Leukemia on dasatinib therapy was admitted to the emergency room of our hospital with syncope; echocardiogram showed a normal left ventricular dimension and function (E.F. 67%) but EKG showed, for the first time, a type I Brugada pattern. After an electrophysiological study, that induced ventricular fibrillation, an implantable cardiac device was inserted. Despite the presence of BS, dasatinib therapy was restarted after a short period of interruption, at standard dosage, and certainly dasatinib did not increase arrhythmic events in our patient, since no ventricular fibrillation or ventricular tachycardia has been observed after a follow-up of 47 months. In conclusion, the balance between cardiological risk and delay (or lack) of antineoplastic therapy should be carefully evaluated to ensure appropriate hematological treatment in patients also suffering from this rare cardiological disease.

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A SINGLE CASE STUDY OF CBFB-MYH11 POSITIVE ACUTE MYELOID LEUKEMIA AND WT1 OVER-EXPRESSION

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Background. Despite acute myeloid leukemia (AML) with inv(16) fusion gene CBFB-MYH11 is considered to have a relatively favorable prognosis when treated with intensive chemotherapy, disease relapse remains the most important cause of treatment failure, occurring in up to 30% of patients. Several prognostic factors have been proposed to identify patients at increased risk of relapse, including older age, high white blood cell count (WBC), receptor tyrosine kinase gene mutations, and minimal residual disease (MRD). In addition, high WT1 expression either at diagnosis or post-induction has been recently associated to higher rate of relapse and is directly related to CBFB-MYH11 burden. Here we report a case of AML with inv(16) and discuss the importance of a comprehensive prognostic approach. Case report. A 36-year-old man presented in April 2011 with hypeleukocytosis (WBC: 20^9x10^9/L), anemia and thrombocytopenia. Bone marrow examination revealed 90% of myelomonocytic blast infiltration consistent with AML-M4. Both CBFB-MYH11 (670%) and WT1 (24215 copies) were overexpressed. First line treatment included standard induction and high-dose cytarabine consolidation, leading to complete remission (CR) with a consistent reduction of CBFB-MYH11 (>3 log reduction to 0.03%) and suboptimal decrease of WT1 transcripts (18 copies). First relapse was observed 11-months later, with parallel increase in CBFB-MYH11 (523%) and WT1 (9172 copies) levels. The patient was treated with timed sequential therapy with fludarabine, cytarabine and mitoxantrone. Second CR was observed in September 2012, with CBFB-MYH11 MRD of 0.66% and 240 circulating copies of WT1. Allogenic peripheral blood stem cell transplantation was then performed in October 2012. To date the patient maintains CR, with undetectable CBFB-MYH11 and WT1. Discussion Currently, the most important prognostic factors for AML are based on cytogenetics and molecular abnormalities, which are assessed at diagnosis. Although these factors play a critical role in risk stratification, the treatment outcome of patients within the thus-defined risk groups is highly variable. Despite standard-risk cytogenetics and consistent MRD reduction, our patient experienced disease relapse after intensive chemotherapy. The present report underline the need to include additional, dynamic prognostic factors, such as initial WBC count and WT1 expression levels, in stratification models for risk adapted-therapy.

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CLOFARABINE IN THE TREATMENT OF RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA: THE RETE EMATOLOGICA PUGLIESE (REP) EXPERIENCE

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After achieving the complete remission (CR), majority of patients (pts) with Acute Myeloid Leukemia (AML) will experience a relapse. Conventional salvage therapies show unsatisfactory results in Relapsed/Refractory (R/R) AML and the allogeneic stem cell transplantation (alloSCT) remains the only viable therapeutic option in this setting. Clofarabine is a second-generation purine nucleoside analogue which demonstrated encouraging results in published studies on newly and R/R AML. Pts of all 9 Hematology Centers from REP were included in this retrospective study with the aim to verify efficacy and the safety of clofarabine regimens. From January 2007 to February 2013, 48 pts (M/F: 27/21) with a median age of 53 years (range: 19-78) were enrolled. Twenty pts (42%) had secondary AML and unfavorable cytogenetics and/or molecular markers were found in 54% of cases. When clofarabine was administered, 24 (50%) pts were in relapse and 24 (50%) had primary refractory AML. Ten out of 24 relapses occurred after autologous or allo-SCT. Clofarabine was used alone (2 cases) or in combination with intermediate-dose cytarabine (IDAC, 1000–2000 mg/m^2/d for 5); in 2 cases the dose of cytarabine was lower than 500 mg/m^2/d. Clofarabine was administered at different doses: 10 mg/m^2/d (n=1), 20 mg/m2/d (n=15), 25 mg/m^2/d (n=10), 30 mg/m^2/d (n=15), 40 mg/m^2/d (n=7). Twenty-one (43.7%) pts achieved a CR independently of the previous treatment provided. Treatment-related deaths were observed in four (8.3%) pts. No predictive factors of response were found among age, sex, de novo *vs* secondary AML, cytogenetics and molecular genetics, relapsed *vs* refractory AML and dose of clofarabine. When previous number of treatments were considered, relevant results were observed: CR was obtained in 19

out of 28 (68%) when clofarabine was used as first line of salvage therapy and in 2 out of 20 pts (10%) when was used as second or subsequent line of salvage therapy. Median Overall Survival was 11 months for responding pts and 3 months for no responders. All pts experienced a grade 4 of hematologic toxicity, 35% of pts grade 1, 2 or 3 of liver toxicity and 40% of pts grade 1 or 2 of gastrointestinal toxicity. Our results showed that clofarabine is safe and effective in the treatment of R/R AML. In particular, a significant increased efficacy was observed when clofarabine regimes were used earlier.

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MINIMAL RESIDUAL DISEASE AND CLEARANCE OF BLASTS IN ACUTE MYELOID LEUKEMIA: OPTIMAL TIMING AND CUT OFFS IN THE ANALYSIS BY FLOW CYTOMETRY AND WT1 EXPRESSION

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Multiparameter flow cytometry (MFC) and WT1-mRNA expression were widely used to monitor minimal residual disease (MRD) in acute myeloid leukemia (AML). Anyway, the agreement on cut-off values and timing of the analysis remain the challenges of these. Methods. Clearance of blasts may represent an important solution to the problems of MRD but more studies are needed to confirm recent published data . Indeed , timing, cut-off values and the impact of clearance on the outcome of AML patients (pts) were investigated. Fresh bone marrow samples from 45 AML pts in CR were evaluated by MFC and WT1 to study MRD and clearance at different time points: after induction (T1), after consolidation (T2) before - (T3) and after -transplant (T4). ROC curves were studied to determine area under curve and optimal cut-off values at each time. A multivariate Cox regression was used to study disease free survival (DFS) and overall survival (OS) at different time points. The assessment of MRD by MFC and WT1 at T4 predicted the recurrence better than other time points. The more reliable thresholds resulted 0.1% at T1, T2 and T3 and 0,055 % at T4 by MFC while 90.0 copies/104ABL at T1 and T3, 71.0 copies/104ABL at T2 and 54.0 copies/104ABL at T4 by WT1 expression. When the clearance was considered, the best prediction of relapse was evidenced at T4 with cut off values of 3.07 and 1.65 by MFC and WT1, respectively. Pts with MFC-values above 0.10% at T1 had a significantly poorer DFS compared to pts with lower levels ($p<0.01$). This difference was preserved after adjusting for age, gender, Hb levels and stem cell transplant ($p<0.01$). Results from MFC clearance showed that patients with log clearance at T1 equal to or below 2.81 had a significantly poorer DFS compared to pts with higher levels ($p<0.01$). Pts undergone to stem cell transplant showed a better DFS at multivariate analysis ($p=0.02$). At T1 statistically significant correlations were observed between WT1 values and DFS ($p=0.023$). No significant correlations between MRD and DFS were obtained at T2 and T3 times. From our study, the most predictive evaluation of MRD was performed after the transplant while the post-induction evaluation stratified high risk patients better than other pre-transplant times. Cut-off values needed to be lower after the transplant compared to previous times and the clearance of LAIP positive blasts predicted significantly the outcome.

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GOOD SURVIVAL IN PATIENTS WITH VERY HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA WHO PRESENT A LOW MOLECULAR MINIMAL RESIDUAL DISEASE BEFORE AND IMMEDIATELY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Acute lymphoblastic leukemia (ALL) carrying genetic abnormalities t(9;22) and t(4;11), with the bcr-abl and MLL-AF4 fusion genes respectively, represent a very high risk subtype of disease. Although the introduction of tirosin-kinase inhibitors (TKI) seems to improve the prognosis of t(9;22) ALL, the only curative option is still hematopoietic stem cell transplantation (HSCT). In the latest years some studies revealed that low molecular level of minimal residual disease (MRD) before HSCT is one of the best favourable index for long term disease free survival in ALL. In this report we observed that also patients with very high risk ALL had a favourable outcome if they present very low molecular level of MRD before HSCT. In the last 5 years we submitted to HSCT 12 patients with very high risk ALL, 8 of them with t(9;22) and 4 with t(4;11). All of them have been transplanted as soon as possible in first remission. MRD was evaluated with quantitative PCR and results were expressed as number of molecules of the fusion gene respect to 104 molecules of a reference gene (ABL housekeeping/control gene). Immediately before HSCT, 5 patients presented a complete molecular remission (defined as undetectable MRD level), 6 a MRD level lower to 10-3, and 1 a MRD level lower to 10-2. Three months after HSCT 6 patients were in complete molecular remission, 4 of them with MRD lower to 10-3 and 2 with MRD lower to 10-4. Despite the fact that all patients who presented detectable MRD were also t(9;22) positive, none of them has been treated with TKI after HSCT; they only subwent a rapid tapering of immunosuppressive drugs. All of them presented a complete molecular response in the months after, with a median time of 10 months (5-16). 11/12 patients are actually alive and disease free, overall survival is 92 % with a median follow-up of 45 months (6-84 months) from HSCT. 1 patient who presented complete remission before HSCT died 6 months after for transplant related events (sepsis and GvHD). Our data agree with the consideration that a deep remission status followed by early HSCT can revert a severe prognosis in a better outcome.

P200

BENEFITS OF THE USE OF AZACITDINE IN PATIENTS WITH AML REFRACTORY OR RELAPSED AFTER INTENSIVE CHEMOTHERAPY

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Our aim is to evaluate if Azacitidine (AZA) could prolong OS, reduce adverse events and improve quality of life in patients with Acute Myeloid Leukemia (AML) refractory/relapsed after intensive chemotherapy (IC). Median OS in this subset of patients is about 3 months when treated with CCRs, with few chances to achieve complete remission and a high risk to experience toxicity. Here we report our experience with AZA in 17 patients (8 MDS, 9 AML).6 MDS patients were Int-2, 2 high, 2 AML were de novo, 2 secondary to MDS, 3 refractory and 2 relapsed after IC; 3 refractory patients have been treated with Clofarabine and Aracytin (Ara-C), Fludarabine, Ara-C and Idarubicine and low dose Ara-C respectively; 2 patients relapsed one after Ara-C and Daunorubicine therapy and 1 after Ara-C, Ethoposide, Daunorubucine and Au-BMT. All AML patients were unfit for other CCRs. Median age was 77 y and BM blasts count was 34% (range 3,5-96%); all patients were transfusion dependent (median Hb 8,5 g/dL, r. 7,0-10 g/dL), granulocyte count was 1896/mmc (+/-3142)and Plt 92400/mmc (+/-73800). AML and MDS patients received AZA 75 mg/m^2 subcutaneously for 7 days every month until disease progression. As expected OS in MDS was 16 months. 1 patient with denovo AML obtained a CR that persists after 7 months, the other one obtained a PR and died after 9 months. 2 patients with secondary AML achieved PR after 3 c, 1 showed disease progression after 12 months and one is still in PR after 4 months. After three c. of therapy all refractory/relapsed AML patients (5) showed increased Hb concentration (median 9,1 g/dL, range 8-10,6 g/dL) with reduction of transfusion requirements and two of them became subsequently transfusion independent; all patients also achieved improvement in Plt count (median 138000/mmc,r.39000-350000/mmc). Median BM blasts count after 3 c. of AZA therapy was 6%(r.0-21%): 1 patient obtained CR that persists after 8 c.; 4 patients achieved PR and 2 of them are still alive in good PR after 4 c., while 2 patients showed disease progression after 3 and 9 c., respectively. During AZA no patient had relevant infections and none needed hospital admission. After a median follow-up of 7 months, 7 patients are still alive(1 denovo AML, 2 secondary AML, 4 refractory/relapsed AML). AZA is effective safe for the treatment of patients with refractory/relapsed AML unfit for IC. AZA provides a clin-

ical benefit reducing transfusion requirement, improving quality of life and extending OS.

P201

CLINICAL OUTCOME OF T-ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOMA (T-ALL/T-LBL): THE BOLOGNA EXPERIENCE

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Background. Precursor T cell ALL/LBL occurs most frequently in late childhood, adolescence, and young adulthood, with a 2:1 male predominance; it comprises 15 and 25 percent of childhood and adult ALL, respectively, and 2 percent of adult non-Hodgkin lymphoma. In adult patients prognosis is poor, due to the high incidence of relapse even after allogenic stem cell transplantation. Design and Methods. We retrospectively analysed and currently report clinical outcome results about 52 newly diagnosed and younger than 60 years T-ALL patients (median age 30 years, range 14-73 years; 37/15 M/F; 41/52 T-ALL e 11/52 T-LBL) treated, from 1991 to 2011 according to standard chemotherapy regimen, including adapted pediatric-like schedule (10 pts), BFM protocol (3 pts) and adult schedules (39 pts). After induction, all the patients underwent consolidation for at least one course. All the patients shared the same strategy for intensification, that consisted, when available, in allogenic or autologous stem cell transplantation. Detailed data about cytogenetics and molecular biology will be provided on site. Durations of complete remission (CR) and overall survival (OS) were estimated according to the Kaplan-Meier method. The CR duration was calculated from start of CR to first evidence of disease recurrence. Results. Informed consent was obtained; after a single induction course 41/52 patients obtained a CR (78.8%) and 2 patients a partial remission (PR) (3.8%) for an overall response rate (ORR) of 82.6%. Seven patients (13.4%) had resistant disease, and 2 (3.8%) died during induction. After a median follow-up of 22.7 months, 19 patients (36.5%) are still in CR. The median CR duration and OS were 12.3 and 23.15 months, respectively. The most common grade 3 adverse events included gastro-intestinal toxicities (*i.e.* nausea, vomiting, mucositis and diarrhoea) and liver dysfunction. Conclusions. Our analysis confirms, in line with literature data, that, despite intensive chemotherapeutic treatments and stem cell transplantation, T-ALL and T-LBL adult patients still show a bad prognosis. The relatively satisfying induction remission rate is followed, in most cases, by a high relapse rate. Therefore, a molecular stratification approach, based on gene-expression profile analysis (Ferrando *et al*, Cancer Cell 2002) should be routinely performed, in order to identify new targets, to optimize therapy, to reduce toxicity and to improve clinical outcome. Supported by: European LeukemiaNet, AIRC, AIL, PRIN 2010-2011, Fondazione del Monte di Bologna e Ravenna.

P202

OUTCOME ANALYSIS IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES FOLLOWED IN A SINGLE-CENTER OF ROMAGNA (2001-2012)

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Background. Survival of hematologic neoplasias is generally calculated on the basis of therapeutic protocols including selected patients, or on the basis of Tumor Registries structured without "high resolution encode" as defined in (1, 2). Patients and Methods. All the patients with a first diagnosis of either aggressive non-Hodgkin Lymphoma (HG NHL), or Acute Non-Lymphoblastic Leukemia (ANLL) or Multiple Myeloma (MM), living in the province of Ravenna, come to our observation between 2001 and 2012 were considered for overall survival, age and gender, irrespectively of other comorbidities. Cut-off between young (>18 years) and old patients was 60 years for all the categories, apart from MM (65 years). Patient were included in the study, according to histologic diagnosis, independently of the treatment received. Results. Three hundred fifteen NHL HG (204 old and 111 young) pts were observed. At 10 years 82% of young females and 60% of young males are alive (median not reached) (p=0,0137), *vs* 40% of old females and 25% of old males. Old patients had a median survival of 54 months (females) and 43 months (males). (Figure 1A). One hundred ninety AML (144 old and 46 young) pts were observed. Median survival was 9 months for old and 16 months for young pts. At 5 years, 36,2% of young patients and 11,6% of old ones are alive. No difference with respect to gender. (Fig.1B). Many old patients received only supportive therapy. The 362 MM (244 old and 118 young) pts showed a median overall survival of 70 months. Young females and young males did'nt reach the median survival (at 10 years 70% and 56%, respectively, of them are alive) *vs* 29% and 19% of old ones. The latter had a median survival of 50 and 48 months, respectively (Fig. 1C). Part of them, particularly in stage I, have never been treated. (Figure 1C). Conclusions. Young females had a consistently longer survival with respect to young males in all the diseases. Old patients had the same outcome independently of gender. This study will be useful in standardizing the inclusion of the patients into Tumour Registries according to high resolution encode criteria. The autors thank Ravenna A.I.L. References 1.Parkin DM,*.et al*. Cancer incidence in five continents. Vol. VIII IARC Sci Public 2002;155:1-781. 2.AIRTUM Working Group Italian survival cancer report 2007 Epidemiol Prev. Jan. - Feb 2007 Suppl. 1 pag. 71-76.

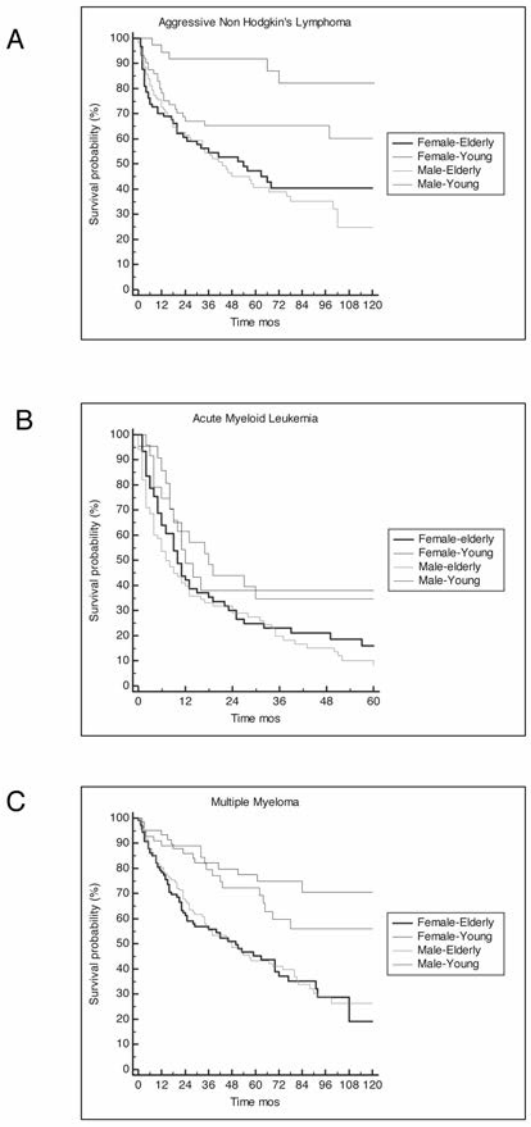

Figure 1. Overall survival observed

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OUTCOME AND TOXICITY OF 286 FLUDARABINE BASED INDUCTION REGIMENS IN POOR RISK ACUTE MYELOID LEUKEMIA PATIENTS YOUNGER THAN 65 YRS

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Introduction. Fludarabine (Fluda)–Cytarabine combinations are commonly used as salvage chemotherapy in AML. The role and efficacy of Fluda based induction regimens in high risk AML younger than 65 yrs are still poorly understood. Patients and Methods. We retrospectively evaluated the efficacy and toxicity following induction chemotherapy with Fluda based regimens in 286 pts, with previously untreated Acute Myeloid Leukemia, followed over a 10 years period (2002-2012) at our Center. All patients were younger than 65 with a median age of 52 years. Diagnosis of AML was confirmed in all cases and Cytogenetic, MDR, FLT3 mutation analysis was performed in all patients; 73% of these were poor-risk at diagnosis [high risk factors: WBC>30x10^9/L, secondary AML, FLT3 mutation, poor-risk karyotype]. The 286 Fluda based induction courses included: 235 FLAI courses (Fludarabine, Cytarabine, Idarubicine) and 51 FLAIE courses (FLAI course plus Etoposide). Patients were evaluated for response rate and treatment related adverse events. Toxicity was evaluated and graded according to National Cancer Institute criteria. Results. After Induction with a Fluda based regimen 213/286 (74%) of patients achieved a Complete Remission (CR). Only a 3% of Death During Induction (DDI) was reported. Non hematologic toxicity was acceptable with 54% of pts presented FUO, 48 % bacteremia or sepsis and 20% grade II-III oral mucositis. As expected, all pts experienced grade IV hematological toxicity and (in CR pts) the median time to PMN (>1000/mL) and platelet (>50.000/mL) recovery was 24 (range 17 to 40) and 26 days (range 18-43), respectively. Supportive treatment consisted of a median of 13 RBC (range 5-26) and 7 platelet units (range 3-18). Median time to hospital discharge was 31 days (range 22-61). 1 yrs Overall Survival was 68%. Conclusions. These data, from a large serie of cases, confirm that Fluda based induction regimens are effective and well tolerated in poor risk AML patients younger than 65 yrs with a high CR rate, favourable safety profile and low DDI.

P204

PROGNOSTIC RELEVANCE OF WT1 MOLECULAR LEVELS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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WT1 is a transcription factor highly expressed in the majority of Acute Myeloid Leukemias (AML). Monitoring WT1 levels at different time points in both peripheral blood (PB) and bone marrow (BM) samples is very useful to assess disease status, particularly to detect minimal residual disease. The present study aimed to investigate the correlation between WT1 levels before transplantation and the risk of relapse in patients with AML after Allogeneic Stem Cell Transplantation (Allo-SCT). Since January 2010 to present 17 patients with AML underwent Allo-SCT in our Transplant Unit. WT1 molecular levels were monitored in both BM and PB at diagnosis, after induction therapy, before transplantation and at 3, 6, 9 and 12 months after Allo-SCT. The samples have been analysed with Real Time PCR as described by Cilloni *et al* (JCO,2009). Levels of WT1 are expressed as WT1 copies/ABL copies x10^4. Less than 250 and 50 WT1 copies/ABL copies x10^4 are considered normal values for BM and PB, respectively. Numerical data are expressed as median (range). The Mann-Whitney U test was used to perform statistical analysis. Our preliminary results showed that molecular levels of WT1 in BM samples are associated with leukemia burden. At diagnosis all patients had high levels of WT1, with a median value in BM samples of 5218 WT1 copies/ABL copies x10^4 (659-18338). After induction therapy WT1 levels in BM decreased in all patients. We calculated the ratio between WT1 levels at diagnosis (WT1d) and after induction therapy (WT1i) as readout of response to the treatment. We found that patients who relapsed after Allo-SCT (7 out of 17 patients) had a lower WT1d/WT1i ratio compared to the ones that did not relapse (4 *vs* 83 fold of change; p=0.048). Furthermore, the molecular levels of WT1 at pre-transplantation time in BM were higher in the 7 patients who relapsed than in subjects who maintained complete remission (122,20-986 *vs* 18,4-760; p=0.037). We performed the same analysis on PB samples but in this case we did not find a correlation between the WT1d/WT1i ratio or WT1 levels at pre-transplantation time and the risk of relapse. Our preliminary data show that WT1 molecular levels measured in BM and PB samples correlate with leukemia burden. However, only the WT1d/WT1i ratio as well as levels of WT1 at pre-transplantation measured on BM samples may be predictive of post-transplantation outcome. (Work supported by "Lions Club Bassa Bresciana" and BCC di Pompiano Franciacorta founds).

P205

5'-AZACITIDINE (AZA) AS MAINTENANCE TREATMENT FOLLOWING STANDARD CHEMOTHERAPY FOR ELDERLY UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background. AML in the elderly is a disease with a dismal prognosis due to frequent coexistence of myelodysplasia and extra-hematological comorbidities, with high incidence of life-threatening complications. In recent trials on patients over 60 yrs of age, reported CR rate not exceed 50% with an OS of 20% after 5 yrs of follow-up. Hypomethylating agent 5'-Azacitidine is approved in the EU for the treatment of patients with diagnosis of AML with 20%-30% of blasts and multilineage dysplasia. Aim. To describe the feasibility and the efficacy of Azacitidine as maintenance therapy in elderly-unfit patients with AML in CR after a standard induction therapy. Patients. Between November 2005 and December 2012, 60 patients aged >60 years received diagnosis of AML. Median age was 75 years (range, 61-89); 80% of them had a pre-existing myelodysplasia. The majority of patients (37/60) received a palliative treatment including either hydroxyurea, etoposide, low-dose Ara-C or BSC, while 23 patients were treated with an induction cytotoxic drug schedule (ICE, 3+7, FLAN, Fludarabine+Cytarabine). Because of age, comorbidities or poor compliance to cytotoxic treatment, patients who did not achieve at a least a PR were included in a palliative program. Results. The ORR (CR+PR) was 65% (15/23). Median OS of entire population was 4,5 months with 5 patients still alive. These patients were diagnosed in the last two years and received one or two courses of standard chemotherapy alone because the presence of comorbidities. After the cytotoxic treatment all patients achieved the CR with a mean bone marrow leukemic cells value of 0,5% (range, 0-4%). These patients were allowed to receive 5'-AZA, 75 mg/m^2 for seven days every 28 days, as maintenance treatment. Four patients are still in CR after a mean time of 20 months from chemotherapy and received a mean of 15 azacitidine courses (range,11-20), while leukemic progression was observed only in a patient after 16 courses. Main toxicity was hematological for the development of a moderate and reversible thrombocytopenia. Conclusions. Azacitidine is a well tolerated treatment in elderly patients also after a standard cytotoxic chemotherapy. A more large number of elderly-unfit patients should be addressed to receive azacitidine as maintenance treatment to draw more information on the efficacy of AZA in this setting. Azacitidine should become the drug of choice for the treatment of maintenance in a subset of AML patients.

P206

CLOFARABINE FOLLOWED BY CYCLOPHOSPHAMIDE FOR TREATMENT OF RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULT PATIENTS

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Background. Relapsed or refractory adult acute lymphoblastic leukemias (ALL) have poor prognosis. The strategy for treating these patients is through reinduction chemotherapy followed by allogeneic stem cell transplantation, provided that the toxicity of the salvage regimen is acceptable. Clofarabine, a next-generation deoxyadenosine analog, has demonstrated significant activity in children and adults with refractory lymphoid and myeloid leukemia in early clinical trials and was granted approval for use in children with acute lymphoblastic leukemia in second or higher relapse. Rationale: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. (Judith E.Karp *et al*. Blood 2007). Aim: To assess response to salvage therapy with clofarabine combined with cyclophosphamide in adult patients with relapsed or refractory acute leukemia. Methods. Patients aged 26–60 years with refractory/relapsed ALL were treated at the dose of clofarabine 10mg/m^2 + cyclophosphamide 400g/m^2 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. The median of Overall survival (OS) for all the patients was 95 days, the media was 169 days. The overall remission rate (ORR) was 44.4%, and we estimated a duration of remission (DOR) as 261.75 days in media (we calculated from the fir st day of RC until relapse). Conclusion: Combination treatment with clofarabine and cyclophosphamide in adults pts with refractory or relapsed ALL resulted in an ORR of 44%, two pts proceeded to HSCT.The safety profile is acceptable in this relapsed/refractory population. More studies with this combination in adults are warranted.

P207

HDAC INHIBITORS TRAIL INDUCTION IS MEDIATED BY 323K-MYC ACETYLATION FOLLOWED BY MYC DOWNREGULATION IN ACUTE MYELOID LEUKEMIAS

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Despite its silencing in acute myeloid leukemias and many other tumors, we demonstrated that the promoter region of TRAIL is highly H3K4 mono-, di- and tri-methylated in AMLs, thus suggesting a complex mechanism to block of transcription, partially independent from histone marks. Furthermore, we demonstrated that c-MYC sits on the TRAIL promoter region is essential for its silencing in AML cells. HDACis such as SAHA or MS275 induce cMYC-k323 acetylation, cMYC down-regulation both at the RNA and protein levels, thus displacing the block for TRAIL transcription. Our findings indicated that this regulatory event can be reproduced on several HDACis-induced apoptotic genes, thus suggesting that this is a common mechanism of action. Finally, we demonstrated that cMYC-k323 acetylation and following down-regulation is selective for HDAC inhibitors action in AMLs ex vivo primary samples, given that in normal progenitors the MYC-TRAIL is not present. cMYC acetylation and down-regulation upon HDACis treatment might represent a useful biomarker for response and follow-up of AMLs.

P208

SOLUBLE FORMS OF UPAR REGULATE ADHESION, PROLIFERATION AND SURVIVAL OF KG1 LEUKEMIC CELLS

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The receptor (uPAR) of the urokinase-type plasminogen activator (uPA) serine-protease is crucial in cell migration processes, since it concentrates uPA proteolytic activity at the cell surface, thus allowing localized ECM degradation. uPAR also binds vitronectin (VN) and interacts with integrins, regulating their activity. uPAR is formed by three domains (DI, DII, and DIII) and is anchored to the cell surface through a glycosyl phosphatidylinositol (GPI) tail. The N-terminal DI can be removed by proteolytic cleavage, generating a shorter uPAR form (DIIDIII-uPAR). Both full-length and cleaved uPAR can be released by the cell surface as soluble forms (suPAR and DIIDIII-suPAR, respectively), which have been detected in human plasma and urine. Full-length suPAR is still able to interact with integrins, unlike DIIDIII-suPAR, which, however, stimulates cell migration by activating the receptors (fMLF-R) for fMLF. The levels of soluble uPAR forms increase in acute myeloid leukemia (AML) and decrease rapidly during chemotherapy. Thus, we investigated whether full-length and cleaved suPAR are able to affect adhesion, proliferation or survival of the AML KG1 cell line. *in vitro* adhesion assays showed that KG1 cells adhered efficiently to plastic-bound fibronectin (FN), which is largely present in bone marrow stroma, whereas they did not bind to plastic-bound vitronectin (VN). Cell treatment with suPAR induced a significant increase in KG1 cell adhesion to FN, whereas cell treatment with DIIDIII-suPAR did not exert any effect. Viceversa, proliferation *in vitro* assays showed that DIIDIII-suPAR significantly increased proliferation of KG1 cells, with an effect peaking at 48h, whereas suPAR did not show any influence. Interestingly, both full length and cleaved forms of soluble uPAR protected KG1 cells from apoptosis induced by serum starvation. Altogether, these results suggest that the soluble uPAR forms identified in sera from AML patients could play a role in the adhesion, proliferation and survival of leukemic cells, probably through their capability to interact with integrins and fMLF-Rs.

P209

DISCOVERY OF NEW SMALL MOLECULES TARGETING THE VITRONECTIN BINDING SITE OF THE UROKINASE RECEPTOR THAT BLOCK NORMAL AND LEUKEMIC CELL MIGRATION

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Besides focusing urokinase (uPA) proteolytic activity on the cell membrane, the urokinase receptor (uPAR) is an adhesion receptor able to bind vitronectin (VN), via a direct binding site. Furthermore, uPAR interacts with other cell surface receptors, such as integrins, receptor tyrosin kinases and G-protein coupled chemotaxis receptors, triggering cell-signalling pathways that promote cell proliferation and migration. The ability of uPAR to coordinate binding and degradation of extracellular matrix and cell signalling makes it an attractive therapeutic target. We used a structure-based virtual screening (SB-*VS*) approach to search for small molecules that might target the uPAR binding site for VN. 41 compounds were identified and further tested on uPAR-negative HEK-293 epithelial cells transfected with uPAR (uPAR-293 cells), using the parental cell line transfected with the empty vector (V-293 cells), as a control. Two compounds, 6 and 37, selectively inhibited uPAR-293 cell adhesion to VN and the resulting changes in cell morphology and signal transduction, without exerting any effect on V-293 cells. 6 and 37 inhibited uPAR-293 cell binding to VN at micromolar concentrations, showing IC50 values of 3.6 and 1.2 mM, respectively. Compounds 6 and 37 targeted S88 and R91, key residues for uPAR binding to VN but also for uPAR interaction with the f-MLF family of chemotaxis receptors (fMLF-Rs). As a consequence, 6 and 37 impaired uPAR-293 cell migration toward FCS, uPA and f-MLF, likely by inhibiting the structural and functional interaction between uPAR and FPR1, the high affinity fMLF receptor. Both compounds blocked *in vitro* cell migration of normal blood cells as well as of several acute myeloid leukemia cell lines; therefore they represent new promising leads for pharmaceuticals in inflammatory diseases and leukemia.

Acute Leukemia III

P210

FLUDARABINE, ARA-C AND LIPOSOMAL DAUNORUBICIN (FLAD) PLUS HEMOPOIETIC STEM CELL TRANSPLANT (HSCT) AS SALVAGE THERAPY IN PATIENTS WITH RELAPSED-REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

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Patients with relapsed or refractory acute lymphoblastic leukaemia (ALL), have a poor outcome. Usually in such patients the achievement of a second remission is followed by hemopoietic stem cell transplant (HSCT). As is known, the absence of active disease before transplantation and the low toxicity of the previous salvage treatment positively affect HSCT outcome. The use of daunoxome may reduce drug-induced cardiotoxicity and increase delivery of daunorubicin to leukemic cell. The association of fludarabine, Ara-C and Daunoxome (FLAD) is well tolerated and effective in both poor risk AML and ALL. The aim of this study is to review our overall experience with FLAD as salvage therapy of ALL. The regimen consisted of three-days treatment with Fludarabine 30 mg/sqm followed 4 hours later by Ara-C 2 g /sqm and DNX 100 mg/sqm. Patients in CR or PR after FLAD induction received a further identical consolidation course or underwent HSCT if aged 60 or less and an HLA matched or haploidentical donor was available. Thirty-five patients with refractory (n. 12) or relapsed (n. 23, 4 after allogeneic BMT) ALL have been included in the trial. Median age was 34 years (range 13-76) and patients had received a median of 3 prior regimens (range 1-7). Three patients died of infection during therapy (8%). FLAD was well tolerated by most patients; eleven cases of fever with seven sepsi were observed; none cardiac complications and severe mucositis were recorded. Sixty per cent of relapsed and 66% of refractory ALL achieved CR. Six out of 11 ALL patients who were refractory to HYPERCVAD regimen achieved CR. Neither disease status before FLAD, nor karyotype or age affected the probability of obtaining a CR. Fourteen patients out of 30 aged 60 or less (46%) underwent HSCT (13 in CR, 1 in PR). In recent years we have observed that the percentage of patients who can receive salvage HSCT is more than doubled (31% and 72% before and after 01-1-2010, respectively). Median DFS was 7 months (range 2-109); median OS was 8 months (range 1-120). Our experience in a series of patients with very severe prognosis shows that FLAD is a feasible and effective salvage treatment for patients with relapsed and refractory ALL. The high remission rate, the low toxicity of the regimen with fast haematological recovery and the progress in haploidentical HSCT allows an increasing proportion of these patients to benefit of BMT in CR, thus improving survival.

P211

CLINICAL OUTCOME OF PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA TREATED OUTSIDE OF CLINICAL TRIALS

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Prognosis of patients with acute promyelocytic leukemia (APL) has dramatically improved, with a reported cure rate of 80%; however these results mostly refer to patients treated in the setting of clinical trials, therefore deriving from selected patient populations. Poorer survival, mainly in terms of higher early death rates has been reported in surveys based on real life data. In this study we describe patient characteristics and treatment results from a series of 40 patients with APL who, due to different reasons, were not accrued into clinical trials. The median age was 67 years (range 25 - 86), while 6 patients were below the age of 60 (2 were pregnant at the time of the diagnosis, 2 had a liver cirrhosis, 1 had a severe dilated cardiomyopathy, 1 had an hepatocellular carcinoma). Overall 85% of the patients in this group had a WHO performance status 2. The most frequent reason for exclusion was age over 60 years, associated with various comorbidities (34/40, 85%). According to Sanz

risk score 11 patients had low-risk (27,5%), 19 (47,5%) intermediate-risk, 10 patients (25%) high-risk. Our approach consisted in administering treatment on the basis of the GIMEMA AIDA2000 protocol, tailoring therapy according to patient's comorbidities, age or any other contraindication to specific drugs. Doses of all-trans retinoic acid (ATRA) were reduced in 2/40 patients (5%); on the contrary, only 20/40 patients (50%) received full dose of Idarubicin (IDA) and 4 patients received ATRA only because of underlying severe cardiac comorbidity. 30 % of patients received reduced consolidation chemotherapy and one was consolidated with single agent gemtuzumab-ozogamicin. Maintenance with ATRA, 6-mercaptopurine and methotrexate was given to 30 of 36 patients (83%). Overall, molecular CR rate, as evaluated at the end of consolidation, was 90%; at the time of writing , median disease free survival (DFS) and overall survival have not yet been reached, after a median follow-up of 80 months (range 33-107); 31 patients are still alive. Four patients (10%) died during induction phase from cerebral hemorrage. Five patients relapsed and four achieved a second CR. These results are poorer that those reported by in controlled trials and, even though APL is a highly curable disease, clinicians are often faced with difficult to treat patients, mainly because of advanced age or comorbidities. These patients are less likely to be cured and require a tailored treatment approach.

P212

DISEASE CHARACTERISTICS AND THERAPEUTIC RESULTS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA WITH CHROMOSOME 3 ABNORMALITIES

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Abnormalities of long arm of chromosome 3 are infrequent but recurrent in acute myeloid leukemia (AML). The 2008 World Health Organization classification recognizes AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) as a distinct subtype characterized by a poor prognosis. Other chromosome 3 abnormalities are less frequent and clinical and their prognostic relevance is yet still defined. The aim of this study is to assess the prognostic impact of chromosome 3 abnormalities on disease characteristics and treatment outcome in AML. Here we describe a series of 42 AML patients, bearing a 3q abnormality detected by conventional cytogenetic analysis. There were 24 male and 18 females, (median age: 58 years, range 22– 81); 23 patients had de novo AML while 19 evolved from a previously diagnosed MDS. Karyotype from MDS phase was available in 2 patients; both acquired 3q rearrangement at time of progression to AML. At time of diagnosis median leucocyte count was $45.3x10^9$/L (1.9 – 431) and median platelet count was $155x10^9$/L (8 – 670). Regarding cytogenetic features, 5 patients had t(3;3)(q21;q26), 7 patients had inv(3) (q21; q26), 8 patients showed a balanced rearrangement involving 3q26, while 15 patients harboured a del3q. Five patients showed monosomy 3. Additional chromosomal changes were demonstrated in 18 patients, 14 of them had a complex karyotype. Thirty-six patients received aggressive induction chemotherapy. Complete remission (CR) rate was 44% (16/36), resistance to induction (including salvage chemotherapy) 46% and death in induction 10%. Among remitters, 4 patients underwent autologous stem cell transplantation (ASCT), while 9 were allo-transplanted. Median overall survival (OS) in this series is 7.2 months, mean disease free survival (DFS) was 6.7 months. Median OS for patients achieving a CR was 17.4 months, median OS for allografted patients was 20.1 months. Overall, the incidence of 3q abnormalities was 2.4%, in keeping with previous studies. Our findings confirm the association between 3q abnormalities and thrombocytosis at diagnosis, as well as higher incidence of secondary AML, association with additional chromosomal abnormalities and poor response to conventional chemotherapy (CR rate 44%). The most interesting data emerging from our observation is that patients who receive ALLO-SCT achieved a better outcome in comparison to those treated with chemotherapy only or ASCT.

P213

EARLY REDUCTION OF BAALC GENE EXPRESSION DURING INDUCTION CHEMOTHERAPY PREDICTS FOR OUTCOME IN ACUTE MYELOID LEUKEMIA

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Age, cytogenetics and secondary versus de novo disease are the most important factors predicting CR in AML patients. These factors provide a pre-treatment stratification in risk groups with different probabilities to obtain CR but they are not sufficient to predict the individual response to induction treatment that is variable. Moreover, to collect the necessary information in time to allow stratification at diagnosis is not generally possible. Recently, a variety of novel molecular markers have refined the risk stratification of intermediate-risk AML; in addition altered genes expression have been proposed as important prognostic marker. BAALC (Brain And Acute Leukemia, Cytoplasmic) is a gene located on chromosome 8q22.3, it encodes a protein with unknown function. BAALC expression was found mainly in CD34+ cells but is restricted to the compartment of progenitor cells, while no expression was detected in mature bone marrow or circulating white blood cells. High BAALC expression levels were shown to be associated with treatment outcome in AML. We investigated whether assessment of BAALC transcript levels in peripheral blood the first days during standard induction therapy could provide information on the chemosensitivity of leukemic blasts and predict for clinically relevant end points.Quantification of BAALC gene expression was carried out by real time quantitative PCR using BAALC ProfileQuant Kit from Ipsogen. We explored the kinetics of BAALC transcript in peripheral blood samples collected on day 1 (immediately before starting therapy) and on day 5 (the fifth day after start of treatment, immediately before cytarabine infusion) in 143 adult patients with AML. We calculated BAALC ratio as the ratio of copy number measured on day 1 and on day 5. We used median BAALC ratio as the cut-off to divide the patients into two groups.The median BAALC ratio in overall cohort was 3.065 (range 0.08-711.72). The median BAALC ratio was greater in patients attaining CR as compared to non responders (4.25 *vs* 0.96, respectively; p=0.004). Furthermore, DFS (Fig. 1A) and OS (Fig. 1B) were significantly longer in patients displaying a BAALC > 3.065 compared to patients with BAALC ratio ≤ 3.065 (p=0.011 and p<0.034, respectively). These data confirmed that PBC assessment by BAALC kinetics is an early predictor of disease outcome and it allows accurate stratification of patients since the very first days of therapy; as such, it entails potential implications for the management of AML, specifically in order to customize treatment since the outset.

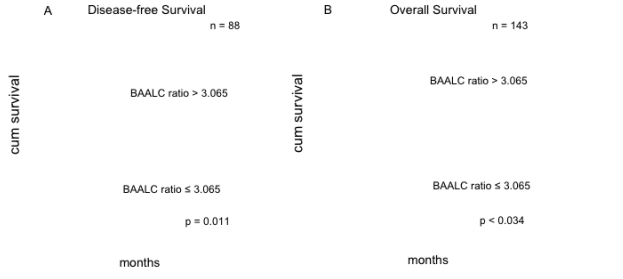

Figure 1.

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NELARABINE FRONT-LINE THERAPY FOR ADULT T-LYMPHOBLASTIC LEUKAEMIA/LYMPHOMA (T-LBL/ALL): PRELIMINARY RESULTS OF BOLOGNA EXPERIENCE

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Background. Precursor T cell LBL/ALL occurs most frequently in late childhood, adolescence, and young adulthood, with a 2:1 male predom-

inance; it comprises 15 and 25 percent of childhood and adult ALL, respectively, and 2 percent of adult non-Hodgkin lymphoma. Nelarabine is an anticancer prodrug of arabinofuranosylguanine (ara-G); it inhibits DNA synthesis and leads to high molecular weight DNA fragmentation and cell death. Nelarabine has showed relevant efficacy in phase II clinical trials, both in pediatric and in adult LBL/ALL populations. Design and Methods. We report clinical outcome results of 9 newly diagnosed and younger than 60 years T-ALL patients (median age 29 years, range 22-45 years, 3/6 M/F, 8 T-ALL, 1 T-LBL) treated according to pediatric-like adapted schedule. Cytogentics data and molecular biologic features will be provided on site. Induction cycle included Vincristine, daunoblastine, L-asparaginase and Prednisone. After induction, all the patients received consolidation therapy with cyclophosphamyde, L-asparaginase, Cytarabine and 6-Mercaptopurine. Subsequently all the patients receveid Nelarabine 1500 mg/sqm (days 1-3-5 every 21) for two cycles. All the patients shared the same strategy for intensification, which consisted in allogenic stem cell transplantation, if available, or additional courses of consolidation chemotherapy. Durations of complete remission (CR) and overall survival (OS) were estimated according to the Kaplan-Meier method. The CR duration was dated from start of CR to first evidence of disease recurrence. Results. After a single induction course, 9/9 patients obtained a CR (100%). Eight patients underwent an allogenic bone marrow transplantation. After a median follow-up of 24 months, 7/9 patients (78%) are alive in CR. The median CR duration and OS were 13.4 and 24.4 months, respectively. Neurological toxicity of grade 3 has not been reported. We did not observe grade 3-4 haematological toxicity. Conclusions. Nelarabine is a promising drug, which induces a remarkable complete remission rate at the expense of a very low and manageable toxicity. Acknowledgments. European LeukemiaNet, AIL, AIRC, PRIN 2010-2011, Fondazione del Monte di Bologna e Ravenna.

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MULTICENTER SURVEY ON OUTCOME OF AML REFRACTORY TO FIRST INDUCTION CHEMOTHERAPY IN THE LAST 3 YEARS (2010-2012)

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Introduction. Despite the development of a variety of new investigational therapies, Acute Myeloid Leukemia (AML) refractory to first induction chemotherapy remains a very difficult clinical problem and there are few recent and controlled trials in this group of patients. Patients and Results. In this multicenter survey we evaluate the outcome of 68 patients (pts) with AML, diagnosed and treated in four Institutions (Udine, Verona, Treviso, Padova), between 2010 and 2012. All cases were refractory to first induction course of chemotherapy. Data were updated as of march 2013. There were 37 male and 31 females with a median age 62 years, (range: 19-79); 62% (42/68) of pts were younger than 65 yrs and 43% (29/68) had AML secondary to MDS. At diagnosis 51% of cases presented an adverse karyotype. According to the risk stratification based on cytogenetic/molecular profile (Dohner *et al*, Blood 2010), 16/68 (24%) of pts were classified as a Favorable/Intermediate-I risk group and 41/68 (60%) as Intermediate-II/Adverse risk group; in 11/68 (16%) cases cytogenetic/molecular profile were not evaluable. After a median follow up of 9 months (range 1-32), 47/68 (69%) of pts had died and 21/68 (31%) were alive; of these only 10/21 with a cytologic Complete Remission. Twenty-four pts (35%) underwent allogeneic hematopoietic stem cell transplantation (HSCT) and of these 44 % (11/24) were alive at last follow up (march 2013). The 12 and 20 months probability of Overall Survival for the whole population was 45% and 25%, respectively. The probability of Overall Survival was not affected by age (log-rank, p=ns) but it was significantly improved by HSCT procedure (35% *vs* 5% at 24 months; log-rank, p=0,0001) and with favourable/intermediate-I cytogenetic/molecular risk at diagnosis (63% *vs* 36% at 12 months; log-rank, p=0,024). Conclusions. Treatment options of refractory AML are still limited and the prognosis of these pts remains extremely poor. This survey shows that pts with refractory AML are rarely cured without undergoing allogeneic HSCT and confirms the importance of initiating an urgent unrelated donor search in patients with no matched sibling donor, who fail to respond to induction chemotherapy.

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AZACYTIDINE AS SALVAGE THERAPY IN OLDER PATIENTS WITH RELAPSED ACUTE MYELOID LEUKEMIA

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Recurrence of disease after complete remission (CR) achievement represents the most relevant adverse prognostic factor in acute myeloid leukemia (AML) and long-term survival is strictly related to the possibility of receiving allogeneic stem cell transplantation (allo-SCT), after second CR obtainment. In older AML patients salvage chemotherapy is associated with extremely poor results and allo-SCT can be offered to a small minority of them. Of interest, a considerable proportion of elderly patients relapse with limited bone marrow (BM) blast count, mimicking a clinical picture of advanced myelodysplastic syndrome. Here we report therapeutic results achieved with single agent azacytidine (AZA) in 3 patients aged over 60 years (61, 69 and 72 years old, respectively), in whom AML at relapse was characterized by less than 30 % BM blasts (12%, 21 % and 24 %, respectively) and absence of blast cells in peripheral blood. Of note, 2 patients had previously received autologous SCT (ASCT) and one conventional induction chemotherapy followed by consolidation with intermediate dose cytarabine. At the time of relapse. all patients presented with severe pancytopenia and needed blood and/or platelet transfusion. AZA was given to all patients at a schedule of 75 mg/mq subcutaneously for 7 days (75 mg/m^2 for 5 days on, 2 days off, 2 days on; total dose 525 mg/m^2) on an outpatient basis. Two patients achieved substantial hematologic improvement and durable transfusion independence (response duration 32+ and 14+ months, respectively). Of interest, in both cases a reduction of bone marrow blasts was observed (21 to 10 % and 12 to 7 %, respectively), without achieving criteria for definition of CR. The third patient showed a clear disease progression after the first AZA course (BM from 24 % to 90%) with onset of leukocytosis and worsening of anemia and thrombocytopenia and was switched to aggressive salvage chemotherapy. In the two responders, best response was observed after 4 and 3 courses, and treatment with AZA is still ongoing after 25 and 12 courses, respectively. Toxicity was exclusively hematologic and manageable on an outpatient basis. In particular, there were no grade 3-4 infectious episodes. We conclude that single agent AZA can be an effective treatment for selected older patients with relapsed AML (BM blast <30%, absence of leukocytosis). In addition, substantial and durable clinical benefit can be achieved, without necessarily obtaining second CR.

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PREDICTIVE VALUE OF POST-INDUCTION AND POST-CONSOLIDATION MRD: A COMPARISON OF MULTIPARAMETER FLOW CYTOMETRY AND WT1 RT-PCR TECHNIQUES IN 75 ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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We evaluated post-induction and post-consolidation bone marrow mimimal residual disease (MRD) in 75 AML adults, with median age of 57 years (range: 17-80) from two centers. MRD was measured both by multiparameter flow cytometry (MPFC) and by WT1 expression, by RT-PCR, by using the same techniques, as described by Buccisano *et al*. and Cilloni *et al*. Cytogenetics, NPM and FLT3 status were known in 73, 63 and 75 patients respectively, defining the molecular-cytogenetic risk in 62 patients. With a median follow-up of 16 months (range 2-55), CR rate was 85% and 3 years Relapse Free Survival (RFS) was 56%; according to cytogenetics and molecular markers, CR rate was 100% in favorable cytogenetic, 88% in NPM+FLT3-, 86% in triple negative, 80% in FLT3+, 79% in unfavorable cytogenetics. WT1 was +ve in 63/75 patients (85%) at diagnosis (median 1056; range:8,2-232.056), in 10/59 (16,9%)

post-induction (median 21; range:0,6-134.633) and in 8/52 (15,4%) post-consolidation (median 18,6; range:0,8-45.338). MPFC MRD was +ve in 28/58 (48%) patients after induction and in 17/46 (37%) after consolidation. WT1 and MPFC were concordant in 22/39 patients after induction and in 22/28 patients after consolidation. We analyzed the predictive value of MRD in terms of CR, RFS, OS, adjusted by: cytogenetics, NPM and FLT3 status, gender, secondary disease, hyperleukocytosis, age. Post induction WT1 was the only significant factor at multivariate analysis, with 67% of CR in WT1+ve patients *vs* 94,5% in WT1-ve patients (RR 5,9, 95%CI: 1,2-28, p=0,026). Both WT1 (post-induction and post-consolidation) and post-induction MPFC significantly predicted RFS at univariate analysis. However WT1 negativity post-induction was the only significant predictor of 2 yrs RFS at multivariate analysis, with 17% in WT1+ve *vs* 71% in WT1-ve post-induction patients (RR: 8,57; 95% CI: 2,57-28,57; p= 0,02). Post-consolidation WT1 remained the only significant factor at the multivariate analysis with 25% 2 yrs OS in WT1+ve patients *vs* 56% in WT1-ve patients (RR: 4; 95% CI: 1,5-10,5; p= 0,01). In conclusion our data show that, for MRD evaluation, a considerable degree of discordance exists between MPFC and WT1 expression; as regards the MRD status, both post-induction and post-consolidation, WT1 by RT-PCR predicts better than MPFC the outcome (in terms of RFS and OS respectively) and might influence post-consolidation therapy choice. These data should be confirmed in a prospective study.

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ACUTE LEUKEMIA AND HIGH-RISK MYELODYSPLASTIC SYNDROMES IN HIV-POSITIVE PATIENTS: A CLINICAL STUDY OF THE RETE EMATOLOGICA LOMBARDA (REL)

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Background. Acute leukemia (AL) and high-risk myelodysplastic syndromes (HR-MDS) are relatively uncommon among HIV-positive (HIV+) patients (pts) and optimal therapy has not been defined yet. Aims. To evaluate the feasibility of chemotherapy (CT) and autologous (autoSCT) or allogeneic (alloSCT) stem cell transplantation in HIV+ pts. Methoths: We retrospectively evaluated 13 HIV+ pts with acute myeloid (AML) or lymphoblastic (ALL) leukemia or HR-MDS treated in four Haematology centres of the REL. Results. From 1994 to 2011, 13 HIV+ pts were recorded (2 HR-MDS, 8 AML, 3 ALL). B-mature ALL was excluded. Median CD4+ T-cell count was 336/L (range 100-2048) and median time from HIV diagnosis to AL or HR-MDS was 9 years (range 2-24). Ten pts were in highly active antiretroviral therapy (HAART) before the diagnosis; all pts continued HAART during CT. Three pts received only palliative care because of poor performance status and died after a median of 51 days (range 16-54), whereas 8 were treated with standard CT, 1 with front-line alloSCT and 1 with azacytidine (HR-MDS). The patient treated with azacytidine showed progressive disease after 2 cycles and died after 4 months (mo). After induction CT or front-line alloSCT, 8 pts entered complete remission (CR) and 1 died of pneumonia in aplasia. All pts treated with standard CT and in CR received consolidation, 2 pts underwent autoSCT and 2 alloSCT. Neutrophil engraftment was achieved after a median of 10 days (range 10-11) after autoSCT and 19 days (range 11-30) after alloSCT. Table 1 shows pts characteristics and complications occurring during treatment. Two out of 8 pts (25%) relapsed after a median of 9 mo from first CR (range 4-14) and received standard CT or a 2nd alloSCT. One patient developed chronic myelomonocytic leukemia (CMML). Overall, 5 pts died: 2 of infection, 1 of cerebral hemorrhage after reinduction, 1 of CMML and 1 of acute graft versus host disease. Two pts are alive at 65 and 45 mo, 2 were lost to follow up at 2 and 9 mo from last CT. Median overall survival of CT-treated pts was 9 mo (range 1-73) and 3-y survival 20%. Conclusion: These data suggest that standard CT followed by autoSCT or alloSCT is feasible in a significant proportion of HIV+ pts with AL. CR can be successfully reached after induction CT and cure can be achieved in a sizeable fraction of them. We conclude that, with a careful monitoring of infectious complications, treatment should not be denied to HIV+ subjects with AL.

Table 1.

Characteristics	n. evaluable	n
Number of patients	13	13
Median age (range)		46 (28-59)
CD4 positive T cell count (median (range))	11	336/µl (100-2048)
Replicant HIV virus at diagnosis	10	3
HCV coinfection	11	2
Diagnosis	13	
AML		8
(with MDS related changes)		(3)
ALL		3
HR-MDS		2
(therapy related)		(1)
Cytogenetics	10	
complex karyotype		2
t(7;11)		1
inv(16)		1
normal karyotype		4
other abnormalities (not adverse risk)		2
Toxicities:		
induction	9	
infection (opportunistic infection)		3(1)
FUO		4
non hematological toxicities grade 3/4		2
therapy related mortality		1
consolidation	6 (9 cycles)	
infection (opportunistic infection)		3(0)
FUO		2
non hematological toxicities grade 3/4		0
therapy related mortality		0
autologous SCT	2	
infection (opportunistic infection)		0
FUO		0
non hematological toxicities grade 3/4		0
therapy related mortality		0
allogeneic SCT	3	
infection (opportunistic infection)		1(0)
FUO		1
non hematological toxicities grade 3/4		2
therapy related mortality		1

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REAL LIFE EXPERIENCE: HIGH DOSE CYTARABINE FEASIBILITY AND EFFICACY IN AN ELDERLY COHORT OF 149 AML PATIENTS EVALUATED WITH MULTIDIMENSIONAL GERIATRIC ASSESSMENT

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Acute Myeloid Leukemia has a dismal prognosis in elderly patients. Aim of this prospective phase II study was to evaluate the feasibility and efficacy of the so called Memorial including high dose Cytarabine (HD-ARAC) and Idarubicine plus Amifostine, a cytoprotective agent, in a subset of AML patients older than 60 years. Between 1999 and 2010 149 non M3 AML Patients with a median age of 68 yrs (range: 60-79) underwent multidimensional geriatric assessment as described by Balducci and Exrtermann: 58 were frail and received best supportive care, while 91 (61%) received a Memorial plus Amifostine induction course. Baseline characteristics are shown in Table 1. We observed 76% CR (67/88 evaluable patients), 5 toxic deaths. Grade III-IV extrahematological toxicity consisted of 67 febrile episodes, 13% mucositis, 9% hepatic toxicity. The median time for neutrophil recovery >1.500x10^6/L was 15 days (range 9-70) and 16 days for platelet recovery >20.000x10^6/L (range 3-47). The median duration of hospitalization was 30 days (range 15-69).

Sixty-one patients in CR received an HD-ARAC consolidation course and 49 CCR patients underwent PBSC mobilization, 4 received Allogeneic Transplant from HLA-identical sibling donors, 22 Autologous Transplant (ASCT) and 23 Gemtuzumab Ozogamycin (GO) (3 mg/sm for 6 courses) on an ITT basis. The Univariate and Multivariate analysis of factors influencing CR rate showed that patients with unfavorable karyotype achieved a 63.6% CR rate, significantly lower than the 85% observed in other patients (p= 0.03). Up to now 20 patients are alive, 5 after ASCT, 15 after GO, with a median follow up of 70 months (range: 24-124), 17 in 1st CCR and one in 2nd CR after GO retreatment. The 8 years Overall Survival (OS), Disease Free Survival (DFS) and Event Free Survival (EFS) are respectively 20,4%, 22,9%, 17,9%, with a median duration of 11,4 and 7,8 and 7 months respectively. Patients identified as unfit or frail had in comparison a significantly lower OS since all died within 18 months with a median OS of 1,5 months (p<0.001). In conclusion Memorial plus Amifostine resulted to be feasible in 61% of elderly AML patients with an acceptable toxicity, a 24% transplant feasibility, a superior outcome in comparison to best supportive care patients and some efficacy even in patients with unfavorable cytogenetic risk showing a 63.6% CR rate. This intensive regimen could be therefore explored in larger multicenter series.

Table 1. Patients Characteristics of the 91 Memorial and 58 BSC patients

	Memorial N (%)	BSC N (%)	p		Memorial N (%)	BSC N (%)	p
Gender:			0.87	PS:			0.003
Male	50 (55)	31 (53.4)		0-1	80 (87.9)	32 (55.6)	
Female	41 (45)	27 (46.6)		2	7 (7.7)	26 (44.4)	
				3	4 (4.4)	0	
Karyotype:			0.05	WBC count:			0.45
Favorable	5(6.2)	0		<50,000/mcl	80 (88)	48 (82.4)	
Intermediate	43(53.1)	11(35.5)		≥50,000/mcl	11 (12)	10 (17.6)	
Unfavorable	33(40.7)	20(64.5)					
			0.005	Age			<0.001
De novo AML	55 (60.4)	27 (46.5)		<70 yrs	52 (57.1)	10 (17.2)	
Secondary AML	36 (39.6)	31 (53.4)		>69 yrs	39 (42.9)	48 (82.7)	

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INTENSIVE CHEMOTHERAPY DOES NOT PROLONG SURVIVAL COMPARED TO AZACITIDINE IN PATIENTS AGED ≥ 70 YEARS WITH UNTREATED ACUTE MYELOID LEUKEMIA (AML)

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Recent findings suggest that hypomethylating agents have encouraging clinical and biological activity in AML with limited toxicity. The aims of our study were: 1) to retrospectively investigate the efficacy of azacitidine in a series of untreated AML elderly patients who were diagnosed according to World Health Organization (WHO) criteria; 2) to compare overall survival (OS) of patients treated with azacitidine with a historical group of patients who received intensive chemotherapy (IC). We evaluated 74 untreated AML patients, median age 76 years (range 63-88), 63 (85%) aged ≥ 70 years. Fifty-seven (77%) had a white blood cell count (WBCc)<10x10^{-9}/L, 22 (30%) AML secondary to myelodysplastic syndrome (MDS) and 36 (49%) a bone marrow (BM) blast count <30%. Karyotype was evaluable in 50 (67%) patients and according to European Leukemia Net criteria (ELN) 36 (48%) had intermediate- risk cytogenetics and 14 (19%) an adverse risk profile. Criteria to receive azacitidine were the absence of uncontrolled infections, adequate renal and hepatic function, life expectancy greater than 4 months. IC group consisted of 75 patients who received mitoxantrone, cytarabine, and etoposide (ICE) and 2 cycles of a consolidation (mini-ICE). Median age was 69 yrs (range 65-78), 34 (45%) patients were older than 70 years, 64 (85%) had BM blast count ≥30%. Karyotype was evaluable in 54 (72%) patients: 42 (56%) had intermediate, 1 (1%) good and 11 (15%) adverse risk profile. Fifty-six patients (76%) received azacitidine subcutaneously at the conventional dose of 75 mg/m^2, 24% at a flat dose of 100 mg daily. The median number of cycles delivered was 6 (range 1-39) and 72% of the patients received ≥4 cycles of therapy. Median follow-up was 266 days (range 30-1281). Overall response rate (ORR) was 46%, according to recommendation of the International Working Group in AML, including 16 (22%) complete remission (CR), 4 (5%) CR with incomplete blood count recovery and 14 (19%) partial remission. Response to azacitidine was significantly associated with a WBCc <10x10^{-9}/L (p=0.004). At 2 years, OS for patients treated with azacitidine *vs* IC was 15% and 34%, respectively (p=0.024). In 97 patients aged ≥70 years, 63 azacitidine and 34 IC, 2 years-OS was 14% *vs* 29%, respectively (p=0.13, Figure 1). In conclusion in patients with AML, aged ≥70 years and with WBCc <10 x 10^{-9}/L, azacitidine is as effective as IC. Larger prospective studies are warranted to confirm our conclusions.

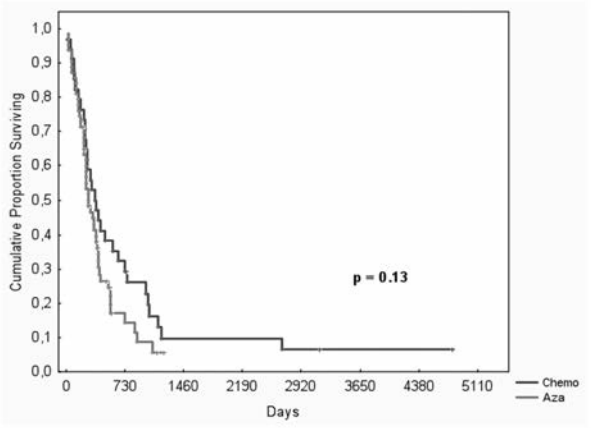

Figure 1.

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INDOLEAMINE 2,3-DIOXYGENASE (IDO) IS ASSOCIATED WITH HIGH INCIDENCE OF CHEMOREFRACTORY DISEASE IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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IDO is a heme-containing enzyme that catalyzes the rate-limiting step in tryptophan degradation; it's able to inhibit T-cell function by inducing the transformation of T-cells into regulatory T-cells. IDO is involved in immune tolerance induction during pregnancy, infection, transplantation, autoimmune diseases and neoplasias, including acute myeloid leukemia (AML), where it is expressed in a significant number of patients. Here, we addressed the correlation between IDO expression by AML cells, risk factors at diagnosis and patients' outcome. Adult AML patients from the Hematology Institute "L. and A. Seràgnoli" in Bologna were analyzed for risk characteristics at diagnosis and IDO expression by RT-PCR and Western- Blot analysis. Patients were stratified according to age at diagnosis, de novo or secondary disease, leucocytosis, cytogenetics, FLT3 and NPM mutational status. Fifty-two AML patients were analyzed for IDO expression both at gene and protein level. According to IDO transcript levels, patients were divided into IDO-negative (21%) and IDO positive (79%). Positive patients were

divided into three subgroups according to protein level: IDO-low (78%), IDO-intermediate (10%) and IDO-high expression (12%) patients. No statistically significant differences in the recurrence of prognostic characteristics at diagnosis between the four groups were observed, even though IDO-negative and IDO-low expression patients showed a higher median age at diagnosis than IDO-intermediate and IDO-high expression patients and an increased frequency of high-risk cytogenetics was found in IDO-high expression patients. Response to induction chemotherapy regimen was then analyzed: only patients who received cytotoxic chemotherapy were evaluated for response. Refractory patients were 60% among those who express IDO at high level and 27% among IDO- negative patients. A logistic regression analysis showed a significant difference between IDO-negative and IDO-high expression patients in terms of complete remission rate: IDO-high expressing patients showed an increased proportion of refractory disease than IDO negative patients. This finding may suggest a correlation between chemosensitivity and the induction of anti-leukemia immune response (immunogenic cell death). Since chemotherapy is known to kill tumor cells in an immunogenic manner, we hypothesize that IDO expression in AML cells may contrast such process, thus resulting in a decreased activation of leukemic cells.

P222

PI3K INHIBITION BY BKM120 ON ACUTE MYELOID LEUKEMIA: A PRECLINICAL STUDY

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Acute myeloid leukemia (AML) patients are often characterized by deregulation of various intracellular signaling pathways, including PI3K/Akt/mTOR, which concur to increased cell proliferation and abnormal survival. Stagnation of clinical results, especially in patients not eligible for aggressive treatment, have prompted the investigation on novel targeted therapies. Currently, several PI3K/Akt/mTOR inhibitors, especially those targeting downstream signals, have been investigated in AML. However, reactivation of critical upstream nodes have been associated with reduced activity of these molecules. BKM120 is a novel pan-class I PI3K inhibitor, characterized by a significant cell growth inhibition and apoptosis in a variety of solid tumors. Therefore, we investigated preclinically the activity of BKM120 on several acute leukemia (AL) cell lines and on primary AML samples. BKM120, kindly provide by Novartis, was tested on different leukemia cell models (U937, HL60, MOLM13) and primary AML samples at concentrations ranging from 0.5 to 5μM. Drug-induced cell growth inhibition was assessed using the MTT assay. Cell cycle changes and apoptosis induction were analyzed by the Acridine-Orange technique and Annexin V (AnnV) binding assay. The activation status of the molecular target and its modulation was evaluated by Western blot analysis. BKM120 exhibited dose- and time-dependent antiproliferative effects, with IC50s at 72h of 0.7, 1.1 and 1.8μM for the U937, HL60 and MOLM13 cell lines, respectively. On the U937 myeloid cell line growth inhibitory effects start to be seen at 24h (28.3% and 46.7% cell growth reduction with 1 and 2μM BKM120), reaching 80% of apoptosis induction after 48h of 5μM BKM120. These effects were associated with a complete abrogation of Akt phosphorylation. Results obtained on freshly isolated AML cells showed that BKM120 induces, at 24h, an increase of the subG1 peak from 19.9% (DMSO) to 33.7%, 40.7%, 47.6% and 62.8% at 0.5, 1, 2 and 5 M BKM120, respectively. In conclusion, our results demonstrate that BKM120 is a promising pan-class I PI3K inhibitor that impairs AML cells growth inducing pro-apoptotic activity and Akt dephosphorylation. Further studies are underway to define the potential antileukemic effect of this novel inhibitor.

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NOVEL HISTONE DEACETYLASE (HDAC) INHIBITORS: *IN VITRO* EFFECTS ON LEUKEMIC CELLS

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Histone deacetylase inhibitors (HDAC-I) are a group of small molecules that have been intensively investigated in a variety of malignancies because of their ability to induce a broad range of effects preferentially on cancer cells, including cell cycle arrest, apoptosis, cell differentiation, autophagy and anti-angiogenic effects. However, clinical responses have been obtained only in a proportion of patients, prompting further studies aimed at identifying more active compounds. Here we investigated the effects of two new HDAC-I, MS-275 and ST7612AA1 (doses ranging from 5 to 5000nM) on cell proliferation and apoptosis in acute leukemia cell line models (U937, MOLT4, Jurkat, Raji) and on freshly isolated AML samples. The cytotoxic effects were assessed by MTT assay. The drug concentration inducing 50% cell killing (IC-50) was calculated from the dose-response curve. Cell cycle inhibition and induction of apoptosis were analyzed by flow cytometry using the Acridine-Orange (AO) and Annexin V techniques. Both the two novel HDAC-I displayed a growth inhibitor activity on all but one, the Raji cell line. The IC50 ranged, in fact, from 86 nM to 278 nM and from 134.5 nM to 661 nM, in the ST7612AA1 and in the MS-275, respectively. Both HDAC-I resulted more active compared with the approved FDA compound, the SAHA (from 299 nM to 746 nM). The Raji cell line displayed resistance. The further analysis of freshly isolated AML samples confirmed the pro-apoptotic activity of both HDAC-I (ranging between 50 and 80% at 500nM), displaying however an heterogeneous sensitivity in individual cases. In conclusion, our results indicated that HDAC-I MS-275 and ST7612AA1 are two effective inhibitors of leukemic cell growth in leukemia models, resulting in AML primary cells in pro-apoptotic activity.

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FATTY ACID OXIDATION AND METABOLIC TARGETING OF LEUKEMIA CELLS BY THE CPT1 INHIBITOR ST1326

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The sustained rate of proliferation of cancer cells requires metabolic adaptation to satisfy the increased energetic demand and the need of biosynthetic precursors. Growing evidences show that tumor cells adopt enhanced rate of glycolysis and divert fluxes from alternative substrates, such as glutamine and fatty acid (FA), to sustain anabolic processes. Targeting the metabolic reprogramming of neoplastic cells may result in an effective novel approach for pharmacological intervention. In order to evaluate in acute leukemias this strategy, we preliminary measured cellular FA uptake differences between normal and leukemia cells, by flow cytometry using a fluorescent fatty acid analog (BODIPY-C12). Thereafter, taking advantage of ST1326 (Sigma Tau), a molecule capable of selectively and reversibly inhibiting Carnitine Palmitoyl-Transferase 1 (CPT1), a protein catalyzing the FA import into the mitochondria, we evaluated the effectiveness of FA oxidation targeting in acute leukemias. The cytotoxic drug effects on leukemia cell lines and on primary acute myeloid (AML) and lymphoid (ALL) cells were evaluated using the MTT test. The drug concentration capable of inducing a 50% cell killing (IC50) was calculated from the dose- response curve. The flow cytometry Acridine-Orange technique and Annexin V (AnnV) binding assay were used to examine cell cycle changes and apoptosis. As expected, a higher FA uptake was observed in leukemia cell lines compared to normal peripheral blood lymphocytes (NPBL). In fact, in the U937 cell line, the MFI fold increase was 58.1 compared to 32.5 in the NPBL. We further evaluated the activity of ST1326 on leukemia cells. In MOLT4 cells, an increase in the subG1 peak (72h) up to 95.8% was observed at 50 M (IC50=39.2 M). The myeloid cell lines HL60, HL60/MX2 and U937 were more sensitive

(IC50 <15). K562 and CEMR proved resistant (IC50=n.d.). *in vitro* exposure of primary cells from 12 AML patients to ST1326 confirmed a significant dose- and time-dependent pro-apoptotic activity with AnnV+ cells increasing from 23.27% ± 13.63 to 75.30%±11.52 (p=0.00018) at 72h up to 50 M. ALL primary samples (6/8) showed a significant (p<0.005) increase of apoptosis only at the highest concentration (50 M). In conclusion, ST1326 is capable of inducing cell growth inhibition and pro-apoptotic effects on acute leukemia cells, supporting this approach of targeting metabolic pathways in leukemia.

P225

AZACITIDINE IN THE TREATMENT OF OLDER PATIENTS AFFECTED BY ACUTE MYELOID LEUKEMIA: A REPORT BY THE RETE EMATOLOGICA PUGLIESE (REP)

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Background. The optimal treatment of older patients (>60 years) with Acute Myeloid Leukemia (AML) remains challenging in daily clinical practice; a choice has to be made between intensive chemotherapy and best supportive care. To guide physicians in their decisions, several prognostic factors have been identified and risk scores have been developed. Recently, the DNA methyltransferase inhibitor azacitidine has become available for MDS and AML patients with up to 30% bone marrow blasts. However, limited data are available on the setting of older unselected AML patients treated with azacitidine, regardless of their bone marrow blast count. Aim. To study the impact of azacitidine treatment in older patients with AML ineligible for intensive chemotherapy, we retrospectively analysed the outcome of 85 newly diagnosed AML older patients in 7 Institutions from the Apulia Region (REP). Patients and Methods. The study included 85 AML patients (44 males and 41 females, median age 75 years, range 61 to 90 yrs); FAB criteria subtypes were: 18 M0, 28 M1, 21 M2, 7 M4, 8 M5, 3 M6. M3 subtypes were excluded from the analysis. Cytogenetic evaluation was performed in 61 cases (72%): 40 (66%) patients were intermediate risk, 21 (34%) adverse, while no patients had a favorable prognosis. Median values of white blood cells and hemoglobin were $3.3x10^3$/mmc, 8.4 gr/dl, respectively. The median blast count in bone marrow and peripheral blood was 40 and 9, respectively. Results. After a median of 10 treatment cycles (range 1 – 23), median OS was 18 months. Azacitidine treatment failure was documented in 40 patients (47%), while the overall response rate after a median time of 4 months treatment was 53% (34% CR and CRi), 19% PR). Responder patients showed a better overall survival than non responders (23 *vs* 6 months, p<.001), although neither primary treatment failure nor bone marrow blast count were correlated with a worse overall survival (18 *vs* 22 months, p=.127; 16 *vs* 19 months, p=.390), respectively. Conclusions. The results of our retrospective analysis seem to confirm the benefit of treatment with azacitidine for older AML patients in terms of OS, regardless of the bone marrow blast count, while primary treatment failure should not affect the continuation of treatment.

P226

POSITIVE IMPACT OF CD200 EXPRESSION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Introduction. Overexpression of CD200, a trans-membrane protein structurally related to co-stimulatory molecules CD80 and CD86, has been associated with poor prognosis in many solid tumor, in CLL, multiple myeloma and in a small cohort of patients with acute myeloid leukemia (AML). We retrospectively evaluated the incidence of expression and the impact on response to therapy in a series of 114 adults with AML. Methods One hundred fourteen patients with non-promyelocytic AML treated at our Institutions between 2007 and 2011 were included in this analysis. Blast cells immunophenotype and CD200 expression were evaluated by multiparametric flow cytometry. Results CD200 was expressed in 45/114 (39.5%) cases, with a median MFI of 11.8 (range: 3.8-88). No differences in CD200 expression rate were observed according to type of leukemia, WBC count, or between cases with myeloid or monocytic morphology. However, higher incidence of CD200 expression was observed in CD34+ compared to CD34- patients (36/59, 61% *vs* 9/54, 17%, P<0.0001). CD200 expression was strongly associated also to favorable cytogenetics (8/9, 89%) compared to patients with intermediate/unfavorable karyotype (29/87, 33%) (p=0.002). Moreover, a lower incidence of FLT3-ITD mutations was detected in CD200+ (3/30, 10%) compared to CD200- cases (17/53, 32%) (P=0.032). One hundred and three patients received a three-drugs fludarabine-based induction course and were evaluable for response to therapy. Complete remission (CR) was achieved in 27/42 (64%) CD200+ and in 45/61 (74%) CD200- patients (P=0.38), but a higher relapse rate was observed in the CD200- group (21/45 *vs* 6/27, P=0.047). Consequently, 3-years disease-free survival was 37% in CD200- patients and 67% in CD200+ ones (P=0.03) [Figure 1]. Conclusions Our data suggest a positive impact of CD200 expression on blasts of AML patients, in term of a lower incidence of relapse. This finding is in apparent contrast with what has been previously reported (Tonks, Leukemia 2007). Reasons for this could be the different way in detection of CD200 expression (gene expression and flow cytometry) and the high prevalence, in our CD200+ patients, of favorable features such as good-risk cytogenetics and FLT3 negativity. Moreover, use of fludarabine, that is known to reduce CD4+ lymphocytes and possibly also CD4+/CD25+ regulatory T cells that suppress the anti-leukemia response, could neglect the adverse effect of CD200 expression on AML blasts.

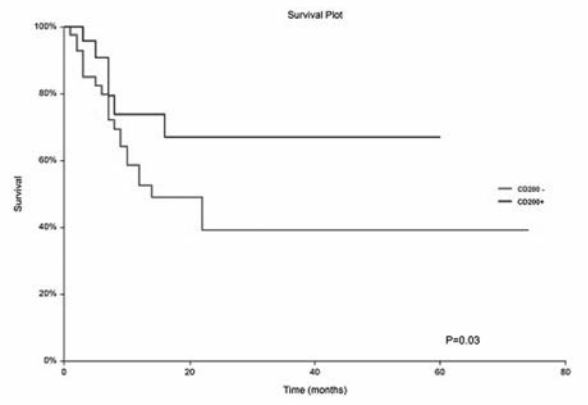

Figure 1.

P227

HEMATOLOGIC DIAGNOSTIC CENTRALIZED LABORATORY OF A BIG AREA ENABLES TO ACQUIRE ACTUAL EPIDEMIOLOGIC DATA OF INCIDENCE OF AML AND IMPROVES ACCURACY OF DIAGNOSIS: ANALYSIS OF TWO YEARS OF ACTIVITY FOCUSED ON ACUTE MYELOID LEUKEMIAS.

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Epidemiologic data on the real incidence of hematologic diseases, as for cancer in total, are still lacking in Italy, and also in Europe. Generally sources are represented by the Cancer Registries (CRs), that are locally distributed in the territory or are specifically dedicated to one disease. The recent publication (Blood, 2010) of the EUROCARE network reported data from most European CRs covering approximately 30% of the European population. The "Area Vasta Romagna" (AVR) laboratory was established from May 2009, it is a modern clinical laboratory created with the purpose of optimizing the use of public health resources without a negative impact on the quality of the service. It centralize samples from all the hospitals (excluding emergency service) and from all the blood drawing services of the Romagna area. We analyze the activity of the laboratory in diagnostic phase of acute myeloid leukemia (AML) from January 2011 to December 2012. In AVR during that period were made 130 new diagnosis of AML, with a crude incidence of 5,84 for year. The median age of the patients was 72 years (range: 2-88), with more than 75% of patient older than 60 years (101 patients). They were 55 females and 75 males. In 27 cases diagnosis was made exclusively on peripheral blood, confirmed by flow cytometry, and no further examination were required by clinicians. The median age of these patients was 81 years, and after diagnosis they had a median laboratory follow-up of 25 days (range 1-270). One hundred and three patients had confirmed diagnosis on bone marrow smears and in 96 cases karyotyping analysis was required. The rate of failure in kariotyping was about 5%. We performed analysis of risk for patients younger than 60, considering the cytogenetic classification reported by Grimwade in 2010 (Blood, 2010): high risk (presence of complex karyotype or adverse alterations) 29%, intermediate 50% and favorable 21%. In conclusion the AVR laboratory represents a real source of data on hematologic malignancies. We reported a higher incidence of AML in AVR compared to data of EUROCARE, probably due to the unselected patients referred to the laboratory, a population that generally do not have access to specialist Unit and to CRs. The second important point is represented by the good management of new diagnosis in patients candidate to specific therapies, with complete biologic characterization in selected cases, and the demonstration of growing expertise based on large numbers.

Myeloma and Monoclonal Gammopathies II

P228

HIGH-RESOLUTION GENOMIC ANALYSIS OF PLASMA CELL DYSCRASIAS IN DIFFERENT CLINICAL PHASES REVEALS CLONAL SELECTION ASSOCIATED WITH DISEASE PROGRESSION AND RELAPSE

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Multiple myeloma (MM) is a plasma cell (PC) malignancy characterized by a high genomic instability which may play a role in the mechanisms involved in disease progression and resistance to therapy. To provide a contribution to the profiling of the genomic complexity associated with myeloma progression, we investigated by genome-wide DNA analysis sequential samples of 7 MM and one primary PC leukemia (PCL) patients. Highly purified PC samples obtained at diagnosis and progression to symptomatic phase (2 MMs) or relapse after first line therapy (5 MMs and 1 PCL) were analyzed on Affymetrix®CytoScan™ HD Array. Copy number data were achieved using the Chromosome Analysis Suite software (Affymetrix). Single sample analysis was performed by setting the default parameters and a Reference Model provided by Affymetrix; a filter on the marker count (>30) and overlapping with Database of Genomic Variants were applied to define aberrant regions. Concerning regions of prognostic importance, 1p loss was identified as a new lesion or evolving from a sub-clone, in 2 relapsed and 1 progressed samples; two relapsed cases acquired 1q gain and 17p loss, respectively. Notably, some alterations were detected at diagnosis as sub-clones and then evidenced in the majority of neoplastic cells in at least one of 2 progressed and 3 relapsed MMs; these lesions involved single or combined gains or losses of whole chromosomes 3, 8, 9, 18, 20; aberrations of short/long arms of chromosomes 11, 13, 14, 15, 18, 21; or smaller altered regions in chromosomes 4, 10, 12, 16, 17, 19. Interestingly, deletions involving chromosome 5q (3/8, 1 progressed and 2 relapsed MMs) or 8q (2/8 relapsed MMs) were exclusively detected as novel lesions in relapsed/progressed samples. Our data reinforce the importance of using novel high-throughput approaches to provide insights into the characterization of genetic lesions which may be associated to the progression of the MM disease and/or the acquisition of mechanisms of drug resistance.

P229

ABSOLUTE LYMPHOCYTE AND MONOCYTE COUNT AT DIAGNOSIS PREDICTS OVERALL SURVIVAL IN PATIENTS WITH PLASMA CELL MYELOMA TREATED WITH BORTEZOMIB

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Baseline Absolute Lymphocyte to Monocyte Ratio (ALC/AMC) at diagnosis has been recently proposed as prognostic factor in lymphoproliferative diseases. Plasma Cell Myeloma (PCM) is a plasma cell neoplasm included in the Mature B-cell neoplasms in WHO classification. PCM is incurable and its Overall Survival (OS) varies from 3 to more than 10 years. Two scoring system has been proposed in the past: the Durie-Salmon (DS) and the International Staging System (ISS). We investigated the role of ALC/AMC in PCM in determining OS in PCM. Fifty consecutive patients diagnosed at our Institution, between 2005 and 2012, were collected; among them, we evaluated also other parameters with a prognostic role in PCM: hemoglobin, calcium, M-component, Ig level, b2-microglobulin, creatinine, albumin and k/ ratio. OS was used as clinical endpoint. Kaplan-Meier analysis was performed to compare survival curves. Mann-Whitney U test was used for testing differences in

the distribution of continuous variables. Median age of patients was 71.94 years (range 52-85), ISS stages were I, II and III in 18 (36%), 16 (32%) and 16 (32%) patients respectively. DS was 1A, 2A, 2B, 3A and 3B in 6 (12%), 6 (12%), 1 (2%), 23 (46%) and 14 (28%) patients respectively. Median follow up period was 16.6 months (range 0.57-78.17), mean OS was 58.83, median OS was not reached. Forty (80%) patients received bortezomib-containing regimens (BCR), 5 (10%) patients received thalidomide-containing regimens (TCR), and 5 (10%) patients received other treatments. Twenty-six (55.3%) patients achieved at least a very good partial response, while 21 patients (44.7) had a partial response or stable disease. We found a significant correlation between the ALC/AMC ratio (median value of 3.38 as cut-off) and both the hemoglobin and albumin level (p=0.01;p=0.03). Interestingly in patients treated with BCR we found a significant correlation between the AMC (median value of $0.39x10^3$/L as cut-off) and OS (p=0.03) (Figure 1). We demonstrated that the ALC/AMC ratio correlates with hemoglobin and albumin serum level in PCM. We found also that AMC is correlated with OS in patients treated with BCR. In conclusion, ALC/AMC ratio may represent a simple and reproducible prognostic factor in PCM Further investigations are needed to address the clinical advantage of such results.

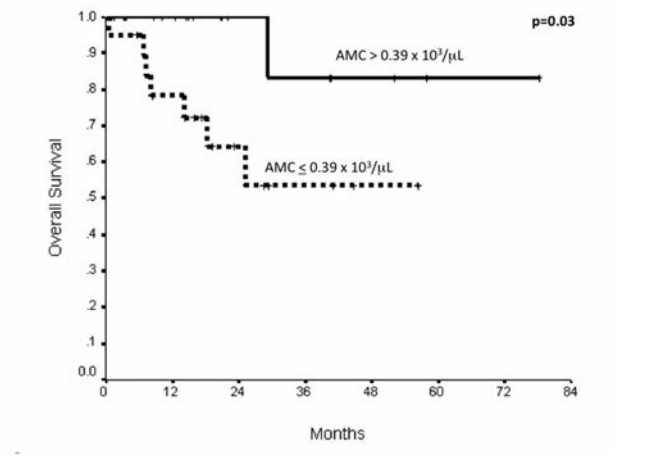

Figure 1.

P230

AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) FOR MULTIPLE MYELOMA (MM): A SINGLE-CENTER REAL LIFE EXPERIENCE

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Aims. to retrospectively review the entire experience of ASCT for MM at the Department of Hematology of Treviso. Methods. from November 2004 to December 2012, 120 consecutive patients (pts) underwent ASCT. Median age was 56.5 years (39-69), 13 pts (11%) were more than 65-yrs old. 59 pts were males, 61 females. Isotype was IgG in 55% of cases, IgA in 18%, Bence-Jones in 12%. At diagnosis, 58 pts (46%) were anemic, 25 (21%) had renal insufficiency, 89 (74%) had osteolytic lesions, documented by skeleton radiography and/or spine MRI. According to Durie&Salmon, stage was III in 78% of pts. Cytogenetic analysis (conventional and/or FISH) was performed in 75 pts (63%). Karyotype was normal in 37% of cases, del(13) was recorded in 28%, 15% of pts had high-risk karyotype. 1st-line therapy included novel agents (Thali-Dex, Vel-Dex, VTD, PAD) in 113 cases (94%); of them, 54 (48%) achieved a complete remission (CR), defined as a negative immunofixation and less than 5% of monoclonal plasma cells at bone marrow biopsy, and 34 (30%) achieved a very good partial remission (VGPR). Seventy-nine pts (66%) received a single ASCT, 41 (34%) underwent a double ASCT. Melphalan 200 mg/sm was the preparative regimen in 81% of the cases. Results No pts died from transplant-related toxicity. After 1st ASCT, 51 pts (64.4%) were in CR, 22 (28%) in VGPR; after 2nd ASCT, CR rate raised to 80.5%. After ASCT, 6 pts received consolidation with VTD, 35 pts were submitted to a maintenance treatment with Thalidomide. Fifty-five pts (45.8%) had a disease progression at a median of 43 months (1-92) after ASCT; 85.5% of them received various types of salvage therapy including also allogeneic stem cell transplant (3 pts, all dead, 2 from GVHD and 1 from invasive fungal infection). Time-to-progression (TTP) curve showed no evidence of plateau. At a median follow-up of 43 months, overall survival (OS) of the entire series is projected to 58% at 105 months, while the OS of the pts who progressed after ASCT is 32 months. The only parameter which retains a statistical significance in terms of TTP was maintenance with Thalidomide. Conclusions:1) at present transplant-related mortality (TRM) for ASCT in MM is close to zero; 2) 1st-line therapy including novel agents followed by one or two ASCTs can induce a CR in at least two thirds of cases; 3) the role of consolidation and maintenance after ASCT should be defined by prospective controlled clinical trials.

P231

THE NOTCH PATHWAY IS UPREGULATED DURING MULTIPLE MYELOMA PROGRESSION AND PLAYS A ROLE IN THE DEVELOPMENT OF CHEMO-RESISTANCE

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Multiple myeloma (MM) is a malignant plasma cell disorder accounting alone for approximately 10% of all hematologic cancers. Although the recent advances in treatment, myeloma remains incurable, with a median survival of 3-4 years after diagnosis. Once immortalized, myeloma cells are necessarily resident in the bone marrow (BM), as early tumor growth is entirely dependent on the BM microenvironment, especially on the paracrine support of IL-6 provided by BM stromal cells. During MM progression, at the stage of plasma cell leukaemia (PCL), the clone is proliferative and no longer confined to the BM and expands rapidly. Cells at this stage are substantially genetically altered and become independent of specific growth factors, particularly IL6. There are several intriguing evidences pointing to a possible role for Notch signaling in mediating these critical events. The Notch pathway is highly conserved and plays a critical role in cell-fate decision, tissue patterning and morphogenesis. Recently, the Notch receptor has been shown to be critical in MM which frequently displays deregulation of the Notch ligands, Jagged1 and Jagged2. Here we investigated a possible connection between Notch signaling and the development of IL6-independence by MM cells, and the role of these two pathways in MM chemo-resistance. The IL6-independent cell line CMA03/06 was derived from the IL-6 dependent cell line CMA03, and may represent a model of myeloma progression. Molecular characterization of CMA03/06 cells, compared with CMA03, evidentiated an upregulation of several members of the Notch pathway, namely Notch1, Notch2, Jagged1 and Jagged2, which results in the upregulation of Notch signaling. When assessing the response to Bortezomib, CMA03 growth impairement was more dramatic than that of CMA03/06. Sensitivity to Bortezomib was restored by Notch pathway blockade in accordance with the evidence that Notch signaling inhibition in MM cell lines down regulates the expression of drug-resistance genes. This is the first suggestion that Notch pathway activation may contribute to drug resistance in the transition from an IL6-dependent to IL6-independent growth. These preliminary data supports the rationale for a possible Notch-directed approach to overcome the drug resistance developed by MM cells during tumor progression.

P232

LONG TERM THERAPY WITH LENALIDOMIDE (LEN) SHOWS A LOW RATE OF SECONDARY PRIMARY MALIGNANCIES (SPMS) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS AND DOES NOT SIGNIFICANTLY AFFECT THE CELLULAR COMPOSITION OF THE BONE MARROW

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Long term therapy with Len has been associated with an increased risk of developing SPMs. Len has a known myelosuppressive effect, however its mechanism of action on the bone marrow (BM) still needs to be fully elucidated. In a large phase III study currently ongoing in the UK evaluating Len as induction and/or as maintenance therapy in newly diagnosed MM data on the occurrence of SPMs are being routinely collected. With a median follow up of 1.3 years (1 year from maintenance randomization) 10 SPMs have been reported (0.5% of the randomized population). The median time from trial entry to SPMs' development is 8 months (range 2.1-15.4) and 7/10 SPMs were developed during maintenance/follow up phase; 3/7 developed on Len maintenance, early in the course of treatment (2.0 to 5.9 months). Interestingly no hematologic SPM has been reported. To further evaluate the impact of Len on the BM we have performed a flow cytometry analysis of BM of Len treated patients. Three, 8 colour, panels (Pacific Blue, Pacific Orange, FITC, PE, PerCP-Cy 5.5, PE-Cy7, APC, APC-H7) were used to investigate relative percentages of the different BM populations (CD3, CD45, MPO7, CD79a, CD34, CD19, CD7, HLADR), B cells (CD20, CD45, TdT, CD10, CD34, CD19, CD123, CD38) and myeloid cells (CD16, CD45, HLADR, CD13, CD34, CD117, CD11b, CD10). Eighteen samples have been analyzed; 7 samples from patients never on Len were used as controls. There was no difference in terms of percentages of T cells and myeloid cells.

Table 1. BM populations in Len treated patients

	Time point	N pts			
Marrow subpopulations			Myeloid cells	T cells	B cells
	Never on Revlimid	7	53.4%	8.5%	6.2%
	Revlimid <12 months	11	51.6%	6.3%	3.6%
	Revlimid >12 months	8	53.7%	12.9%	2.1%
Tot		26			

	Time point	N pts						
B cell compartment			CD19+	Haematogones	Pre B	Naïve B	Mature B	CD19-
	Never on Revlimid	7	30.0%	1.1%	24.4%	48.9%	23.3%	63.0%
	Revlimid <12 months	10	9.7%	3.1%	37.2%	34.4%	10.5%	84.7%
	Revlimid >12 months	8	6.4%	2.6%	35.2%	29.0%	12.7%	93.5%
Tot		25						

	Time point	N pts					
Myeloid compartment			CD13+	Myeloid precursors	Myelo cytes	Metamyelo cytes	PMN
	Never on Revlimid	7	47.6%	0.6%	29.2%	35.2%	18.7%
	Revlimid <12 months	10	65.1%	4.4%	22.4%	26.4%	23.5%
	Revlimid >12 months	8	53.6%	0.8%	31.1%	27.5%	25.2%
Tot		25					

Len treated patients showed a decrease in the number of CD19+ B cells; this is not due to the reduction of specific subpopulations, but as a reduction of the CD19+ population as a whole. CD19+ decrease was proportional to the time on Len therapy. No significant difference was observed in the myeloid population either in the percentage of the CD13+ myeloid cells or in the relative percentages of the different fractions. We were unable to identify any cellular indication that Len treated patients are more likely to develop hematologic SPMs such as a significantly higher percentage of immature forms or the co-expression of aberrant markers. Our data clearly show a low incidence of SPMs in Len treated patients and do not confirm previous findings of an excess risk of SPM in association with the use of Len and melphalan. Longer follow up together with morphological, cytogenetic and molecular analysis is needed to elucidate the risk of Len associated SPMs.

P233

THE NOTCH PATHWAY CONTROLS MULTIPLE MYELOMA-INDUCED OSTEOCLASTOGENESIS

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Multiple myeloma (MM) is unique among hematological malignancies for its association to osteoclast-mediated bone destruction, and consequent osteoporosis, hypercalcemia, bone pain and fractures affecting more than 80% of patients. Bone marrow (BM)-infiltrating MM cells unbalance bone formation and bone resorption by inducing BM stromal cells to increase the production of osteogenic factors as the receptor activator of NF- B ligand (RANKL). MM cells can directly contribute to increase the pool of RANKL and of other pro-osteoclastogenic chemokines present in the bone microenvironment. These events alter the ratio between RANKL and its decoy, osteoprotegerin (OPG), thus increasing osteoclast (OCL) formation. Notch receptors are expressed by primary MM cells, bone marrow stromal cells (BMSCs) and OCLs. Of note MM cells can activate the Notch pathway because they overexpress the Jagged1 and Jagged2 ligands. Recently the Notch pathway was reported to play a key role in tissue remodeling and skeletal development by collaborating with the NF- B pathway and possibly in MM cells ability to increase mature OCLs activity. Here, we studied the role of Notch pathway in the MM-driven regulation of OCL development and activity. We demonstrate that Notch signaling is pivotal in OCL differentiation and activation induced by RANKL; most importantly, MM cells ability to induce effective osteoclastogenesis also requires active Notch signaling and does not necessarily involve a direct contact between MM cells and OCL progenitors. Specifically, the Jagged1/Jagged2-triggered Notch activation promotes RANKL secretion by MM cells, in turn Notch activation in pre-OCLs is necessary to respond to such osteoclastogenic stimulus. The evidence that Notch signaling inhibition blocks MM-driven osteoclastogenesis makes this pathway a promising therapeutic target to contrast the development of bone lytic lesions in MM patients and suggest to implement strategies to block the deregulated Notch ligands, with the final goal to reduce bone disease and improve the response to standard treatments, providing a valuable option for those subjects who suffer from advanced disease and have no effective alternative other than palliative cares.

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INTRA-CLONAL HETEROGENEITY IS A CRITICAL EARLY EVENT IN MULTIPLE MYELOMA DETECTABLE FROM DISEASE PRESENTATION

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Multiple Myeloma (MM) represents a good model in which to study the multistep acquisition of genetic events underlying cancer progression. The transition of normal plasma cells to MM is thought to be the result of the sequential acquisition of "genetic hits", and the existence and competition between different tumour clones, also termed intraclonal heterogeneity, has been recently revealed. In this work we have analysed the intra-clonal population of MM in order to better understand the most plausible disease evolution history at presentation that may impact on targeted therapies. Next generation sequencing (NGS) is an excellent tool which can identify complex intraclonal relationships in MM. Single cell analysis ensures the firm identification of the different clonal populations harbouring common and distinct mutations effectively validating the NGS data. We have used our sequence analysis of MM to identify mutations and translocation breakpoints, to be analysed at a single cell level. We aimed to define a phylogenetic tree of the disease on 7 MM samples at presentation. CD138-positive bone marrow plasma cells were selected to a purity >95%. For sequencing of MM samples, we used 50 ng of DNA and performed whole exome sequencing using 76 bp paired-end reads on a GAIIx (Illumina) to a median depth of 61x, with 99% at 1x and 85% at 20x exomic coverage. Data were aligned to the human genome (hg19) using Stampy and BWA and acquired SNVs and indels called using GATK. Single carnoy-fixed cells were sorted into lysis buffer on 96 well plates using a FACSAria cell sorter. Specific target amplification (STA) was performed before quantitative PCR using genotyping assays specific for the mutations of interest. The STA product was diluted before qPCR interrogation for mutations using the 96.96 dynamic microfluidic array and the BioMark HD (Fluidigm). The results show that the mutations are not all present in the same cells, but instead are acquired in different cells following a linear or branching evolution. A total of 5-6 clones per sample at presentation may be recognised with the tested mutations. This intra-clonal heterogeneity, formerly unveiled in several studies by our group, shows the emergence and disappearance of disease units of clonal selection. Different clones may become prevalent at distinct time points during disease evolution, increasing the complexity and effectiveness of targeted therapies.

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A PHASE II STUDY (PX-171-003-A1) OF SINGLE-AGENT CARFILZOMIB IN PATIENTS WITH ADVANCED RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background. Carfilzomib, a selective proteasome inhibitor, is approved in the US based on the single-arm, phase II study PX-171-003-A1 (NCT00511238) assessing single-agent use in patients with relapsed and refractory multiple myeloma (RRMM). Methods. Carfilzomib was administered intravenously over 2–10 minutes twice weekly for 3 weeks of a 28-day cycle at a dose of 20 mg/m^2 for Cycle 1 and 27 mg/m^2 thereafter. Results. The study population (N=266; intent to treat population) was heavily pretreated with a median of 5 prior lines of therapy (range, 1–20), including bortezomib (99.6%) and lenalidomide (94%). Overall response rate (ORR, primary endpoint) was 22.9% (95% CI, 18.0–28.5) with a median duration of response (mDOR) of 7.8 months (95% CI, 5.6–9.2). Median overall survival (mOS) was 15.4 months (95% CI, 12.5–19.0). In an exploratory analysis of subgroups defined as refractory to bortezomib (n=194) and refractory to bortezomib and lenalidomide/thalidomide (n=169), the ORR was 16.5% and 15.4%, respectively, and the mDOR was 7.8 months for each subgroup. In the safety population (N=266), the most common adverse events (AEs) of any grade included fatigue (49%), nausea (45%), and dyspnea (34%), and grade 3/4 AEs included thrombocytopenia (29%), anemia (24%), and lymphopenia (20%). The incidence of peripheral neuropathy was 12% overall and 1% for grade 3. Median treatment duration was 3.0 months (range, 0.03–16.9) with 18% of patients requiring at least 1 dose reduction. Reason for treatment discontinuation was primarily disease progression (59%) followed by AEs (12%). Five deaths (2%) on study or within 30 days posttreatment were deemed possibly related to carfilzomib. Conclusions: In this heavily pretreated population with advanced RRMM, single-agent carfilzomib provided clinically meaningful, durable responses and was generally tolerable with manageable AEs. The ongoing phase III FOCUS trial (NCT01302392), evaluating single-agent carfilzomib in RRMM patients versus best supportive care, will provide important information to facilitate regulatory approvals ex-US.

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SAFETY PROFILE OF SINGLE-AGENT CARFILZOMIB IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA FROM 4 PHASE II TRIALS

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Background. Carfilzomib is a selective proteasome inhibitor recently approved in the US for patients with relapsed and refractory multiple myeloma (RRMM). Results of a cross study analysis of 526 patients in 4 phase II trials including PX-171-003-A0, PX-171-03-A1 (both NCT00511238), PX-171-004 (NCT00530816), and PX-171-005 (NCT00721734) are presented herein. Methods. Carfilzomib was administered over 2–10 minutes and dosed at 20 27 mg/m^2 in a 28-day cycle for all studies except 005 (15 27 mg/m^2). Adverse events (AEs) were graded by NCI Common Terminology Criteria for Adverse Events (CTCAE) v3.0. Results. AEs ≥ Grade 3 in ≥15% of patients were thrombocytopenia (23.4%), anemia (22.4%), and lymphopenia (18.1%). Because of AEs, dose reductions and treatment discontinuations were required in 14.6% and 14.8% of patients, respectively. There were 37 deaths on study: 24 due to disease progression, 6 due to other reasons, and 7 due to an AE at least possibly related to carfilzomib (as determined by the investigator). While hematologic AEs were the most common ≥Grade 3 AE, they were transient. Worsening renal function was reported in 13% of patients, of which less than half were transient. Although 73.6% of patients had a past medical history of cardiovascular events, cardiac failure events were reported in 7% of patients, regardless of causality. Dyspnea was the most common AE in the pulmonary analysis (42.2%) and pneumonia was the most common respiratory infection (12.7%). While 71.9% of patients had active peripheral neuropathy at baseline, newly developed peripheral neuropathy was reported infrequently (13.9% overall). Conclusions: These data indicate that single-agent carfilzomib has an acceptable safety profile in heavily pretreated patients with RRMM. Few dose reductions and discontinuations due to AEs were needed. The safety profile of carfilzomib will be fur-

ther explored in the ongoing phase III FOCUS trial (NCT01302392) evaluating patients with RRMM (single-agent carfilzomib versus best supportive care), the results of which will be used to facilitate regulatory approvals ex-US.

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PROGNOSTIC SIGNIFICANCE OF SERUM FREE LIGHT CHAINS (SFLC) ASSAY IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) TREATED WITH BORTEZOMIB BASED REGIMENS

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Data on prognostic significance of sFLC assay in MM are still limited. We performed a retrospective analysis aimed to investigate the role of sFLC assay as predictor of outcomes in newly diagnosed MM patients (pts) treated with novel agents. We analyzed 90 pts who received first-line bortezomib-based regimens: 69% incorporated into autotransplantation and 31% combined with conventional chemoterapy. The involved light chain was kappa in 61% of pts, lambda in 37% and both in 2%. Overall, 83% of pts had an abnormal sFLC k/l ratio at baseline and 68% had an involved sFLC >=100 mg/L. Baseline sFLC >=100 mg/L correlated with higher frequency of Bence Jones isotype, higher PCR and lower Hb; no differences in high-risk cytogenetic abnormalities were observed. IMWG best response was: 49% stringent complete response (sCR), 12% CR, 22% VGPR and 10% PR. Overall, 67% of pts achieved a normalization of sFLC k/l ratio during treatment; of these, 78% were in sCR whereas 22% still had a detectable M protein. With a median follow-up of 28 months (mos), 33 pts progressed and 13 died. Thirty-seven % of relapsed pts showed a sFLC escape, defined as an increase of sFLC with no associated increase of intact M protein concentration. A baseline sFLC >=100 mg/L, compared to sFLC <100 mg/L, was associated with a reduced rate of sCR/CR (50% *vs* 81%, p=0.008), a lower probability to normalize sFLC k/l ratio (65% *vs* 92%, p=0.010) and a shorter TTP (35 *vs* 63 mos, p=0.019) and PFS (33 *vs* 46 mos, p=0.021). By the opposite, pts who achieved a normalization of sFLC k/l ratio during treatment, compared to those who did not, had an extended TTP (53 *vs* 18 mos, p=0.0001), PFS (53 *vs* 12 mos, p=0.0001) and OS (not reached *vs* 75 mos, p=0.024). No difference in post-relapse outcomes was observed in pts with or without sFLC escape, whereas sFLC >=100 mg/L at relapse was associated with earlier start of salvage therapy, compared with sFLC <100 mg/L (1 *vs* 4 mos, p=0.007). By multivariate analysis, the normalization of sFLC k/l ratio was as an independent factor predicting for extended TTP and PFS (p<0.001 and p=0.001, respectively). In conclusion, high baseline sFLC was associated with lower probability to achieve sCR/CR and shorter TTP and PFS. Moreover, high sFLC at relapse reflected on a short-lasting asymptomatic phase. By opposite the normalization of sFLC k/l ratio was a robust prognostic indicator of longer TTP and PFS.

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ROLE OF CHITOTRIOSIDASE AND CHITINASE-3–LIKE 1 ON OSTEOCLASTS ACTIVITY

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Pathological bone resorption is a cause of significant morbidity in diseases affecting the skeleton such as rheumatoid arthritis, osteoporosis and other cancer-associated osteolytic lesions such as in multiple myeloma. The biologic mechanism involved in bone resorption is still poorly understood. The family of chitinases (CHIA, CHIT-1, CHI3L1 and CHI3L2) exerts important biological roles in chronic inflammatory diseases. In this study, we compared the expression of CHIA, CHIT-1, CHI3L1 and CHI3L2 during the osteoclast differentiation. The expression levels of chitinases and chitinase-like proteins (C/CLPs) were investigated using quantitative real-time RT-PCR and western blotting assay. During the differentiation of monocytes into osteoclastic lineage the activity of CHIT1 was determined by a fluorimetric method using 22 M 4-methylumbelliferryl D-NNN-triacetylchitotriosidase. To determinate the resorpting activity the osteoclastic cells (5 104) were plated on dentine discs (BD BioCoatOsteologic Discs MultiTest Slides; BD Biosciences) and the resorption area was calculated after 10 days of culture by measuring the surface area of individual pits (150/treatment) using Image J software. The expression of CHIA and CHI3L2 resulted unchanged, whereas both RNA and protein expression of CHIT-1 and CHI3L1 increased significantly during osteoclast differentiation. We demonstrate, for the first time, that CHIT-1 and CHI3L1 are involved in osteoclast differentiation and function. In fact, CHIT-1 and CHI3L1 activity stimulated osteolysis, whereas siRNA of both CHIT1 and CHI3L1 resulted in a significant decrease in the bone resorpting activity. These effects occur, at least in part, as the result of a significant elevation in the expression and secretion of MMP9. Together these discoveries reveal a novel and key role for both CHIT-1 and CHI3L1 in promoting bone resoption and identify new potential candidate markers for skeletal disease progression and therapeutic targeting.

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ANGIOPOIETINS' LEVELS IN MULTIPLE MYELOMA CORRELATE WITH DISEASE STAGE, PROGNOSIS AND RESPONSE TO THERAPY

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Background. In most cases Multiple Myeloma (MM)derives from an asymptomatic premalignant stage(MGUS). Angiogenesis plays a relevant role into malignant evolution. "Angiogenic switch" is promoted by the over-production of angiogenic factors by myeloma cells and microenvironment. Recent evidence indicates that angiopoietins(Angs) and particularly Ang2 have a critical role in this process. Aims.To address the role of Angs in MM progression and prognosis and to assess the functional capabilities of Angs contained in sera in *in vitro* angiogenic assays. Methods.Ang-1 and-2 levels were determined by ELISA in 106 human samples (67/106 BM, 39/106 PB)obtained from patients(pts)affected by MM(n=59),Smouldering MM(SMM, n=16),MGUS(n=22)and healthy donors(HD, n=9). The correlation between Ang-2 and stages of the disease/clinical parameters was evaluated through Spearman correlation,t-test, Kruskal-Wallis test. Human umbilical vein endothelial cells (HUVEC)treated with Ang containing sera were tested in proliferation,permeability and morphogenesis assays on Matrigel; downstream signalling through the specific Tie-2 receptor was also investigated. Results.Pts with active MM have higher levels of Ang-2 versus SMM or MGUS pts(mean values were 5554pg/mL, 2317pg/mL, 3622 pg/mL,respectively) (p<0.05).Ang-2 significantly correlates with beta2microglobulin levels.High Ang-2 levels characterize pts belonging to ISS3 and DS III classes and predict no-response to therapy.Ang-2 levels significantly decrease in response to therapy.PB levels of Ang-2 correlate with BM levels. Sera containing high levels of Ang-1 trigger a quiescent profile in HUVEC resembling that obtained with HD sera,while those containing high levels of Ang-2 triggered an angiogenic response.Signalling through Tie-2 paralleled either condition. Conclusions.Our data suggest that the Angs play a clinical and pathogenic role in MM.In particular, Ang-2 correlates with a more severe disease and with B2microglobulin levels.Moreover, Ang-2 may have a prognostic impact because of its correlation with stadiation and poor response to therapy. Also, Ang-2 in sera is functional as demonstrated by its potential to promote angiogenesis *in vitro* possibly mirroring ongoing angiogenesis in patients. Finally,the statistical correlation between peripheral and marrow levels of Ang-2 suggests that this molecule might be easily monitored and should be tested as a biomarker in prospective studies.

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ROLE OF 18F-FDG-PET/CT, 99MTC-MIBI AND MRI IN THE PREDICTION OF OUTCOME OF MULTIPLE MYELOMA

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In the last years, newer imaging techniques are assuming a continuously growing role in the management of multiple myeloma (MM) including prediction of patient outcome. Our aim was to compare the relative contribution of 18F-FDG-PET/CT (PET/CT), 99mTc-MIBI (MIBI) and MRI in the prediction of progression-free (PFS) and overall survival (OS) in MM patients. In a previous study 33 newly diagnosed MM patients had been prospectively studied by whole-body PET/CT, whole-body MIBI and spine and pelvis MRI within 10 days. The number of eventual focal lesions and/or the presence or absence of diffuse bone marrow involvement assessed by each imaging methodology were recorded. Twenty-seven patients (7 females,20 males;mean age 62±11 y) were then subjected to a mean follow-up period of 60 months while 6 patients were lost. Univariate and Multivariate Analysis were performed including imaging findings and clinical prognostic parameters widely used in MM to test their capability in predicting PFS and OS. PET/CT,MIBI and MRI were positive in 26, 24 and 22 patients, respectively, showing diffuse bone marrow involvement in 4, 6 and 7 patients and a total of 185, 56 and 39 focal lesions, respectively. At follow-up, 18 patients were in complete or partial remission, while 9 patients developed progressive disease, 7 of which died of myeloma. Univariate analysis showed that PET/CT focal uptake (2=8.773 p=0.0031), MIBI focal uptake (2=4.633 p=0.0314), MIBI diffuse uptake (2=7.368 p=0.0066), MRI diffuse distribution (2=6.567 p=0.0104), haemoglobin (2=8.007 p=0.0047), 2-microglobulin (2=4.468 p=0.0345), stage (2=7.044 p=0.0080) and ISS (2=4.885 p=0.0271) were all predictive of PFS. When these variables were entered in the multiple regression model, only PET/CT focal uptake and MIBI focal and diffuse uptake were retained in the model (2=17.205 p=0.0006). Moreover, univariate analysis showed that PET/CT focal uptake(2=8.654 p=0.0033), MIBI focal uptake (2=7.596 p=0.0058), MIBI diffuse uptake (2=5,109 p=0.0238), MRI diffuse distribution(2=4.583 p=0.0323) and 2-microglobulin (2=3.772 p=0.0521) were predictive of OS. When these variables were entered in the multiple regression model, only PET/CT focal uptake and MIBI focal uptake were retained in the model (2=13.892 p=0.0010). In conclusion, PET/CT and MIBI may be used in the prediction of PFS and OS in myeloma patients, while the role of spine and pelvis MRI is more controversial probably due to its limits.

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OVEREXPRESSION OF SPHINGOSINE KINASE 2 IN MULTIPLE MYELOMA

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Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid generated upon phosphorylation of Sphingosine by two kinases, Sphingosine Kinase 1(SHPK1) and 2 (SHPK2). S1P was first characterized for its role in controlling apoptosis-survival balance, however in the last years many data showed that this molecule has a peculiar role in embryology and maturation of vascular compartment, trafficking and function of cell of the immune system, inflammation, cell transformation. SHPK1 is found in the cytosol while SHPK2 is found mainly in the nucleus. SHPK2 seems to be able to affect gene transcription. It is now accepted that these two kinases may have different role in cell functions. Recent findings suggest that these two kinases may play an important role in the growth and behaviour of cancer cells. There are no data concerning the expression of SHPK1 and SHPK2 in MM. The aims of this work was to assess the expression of SHPK1 and SHPK2 in bone marrow of Multiple Myeloma (MM). We analyzed the bone marrow of 14 individuals who underwent bone marrow biopsy for diagnostic purposes. 6 individuals had MM at the first diagnosis, 1 had MM and was at that moment in remission after chemotherapy, 2 were found to be normal, 5 had other hematological malignancies (ALL, AML, CML, CLL). cDNA was prepared acording to standard protocols and quantitative PCR was performed using validated specific primer for SHPK1 and SHPK2 using a 7500 Thermal Cycler. The results were analyzed using a dedicated software. We found that SHPK2 was overexpressed in marrow of MM ($p<0.001$) when compared to normal marrow and to other hematological malignancies ($p<0.05$). Intriguingly, the patients with MM in remission showed levels of SHPK2 comparable to normal marrow. Same levels of SHPK1 were measured in normal individuals and in patients regardless of the disease. These preliminary data indicate that SHPK2 is highly expressed in bone marrow of MM. SHPK1 was found to be expressed always at similar levels in normal individuals, individuals with MM or other hematological malignancies. Therefore, it is likely that the role of SHPK2 in MM is not, or not only, linked to phosphorylation of sphingosine. It is possible to speculate that here SPHK2 has a function due to its capacity to control gene transcription. More data are needed to confirm these results and to understand the meaning of the expression of this kinase in MM. However, these data may lead to new therapeutical approaches in MM through specific targeting of SPHK2.

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UNUSUAL LOCALIZZATION OF MULTIPLE MYELOMA: A CASE REPORT SERIES OF A SINGLE INSTITUTION

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Introduction. Extramedullary manifestations are relatively rare in clinical history of multiple myeloma (MM) patients. Pulmonary and pleural involvement has been described in case reports only. A stricking feature of myeloma plasma cells concern their tendency to reside in the bone marrow compartment during the main course of the disease evolution. Pleura effusion due to myelomatous involvement (MPE) is rare, occurring in<1% of cases. MPE is a manifestation of an aggressive course of MM. Discussion. Eight of 185 patients (4,3%) with MM, observed in our institution in the last seven years, had unusual presentation of MM. Median age was 67 years. Paraprotein isotype was Lambda light chain in 2 patients, IgG k in 3 patients; one IgGL and the last 2 patients with negative immunoelectrophoresis on serum and urine 24 hours collection. No IgA isotype was observed in our clinical records. 3 patients had MPE, diagnosed by thoracentesis, morphologic analysis with cytology and immunophenotyping of pleuric fluid. 2 patients with pulmonary localization diagnosed by lobectomy. One patient had sphenoidal localization as unique bone site of a Lambda chain MM and one patient with CNS localization in insula lobe diagnosed by cytology and immunophenotyping analysis of cerebrospinal fluid. Another patient with very rare gastric localization diagnosed by gastric biopsy.

Tabel 1.

ID	Age	gender	Ig class	Diagnosis	Extra site	Marrow plasma cells %	1st line Treatment	ISS	Gene mutation	Stage	Salvage	Survival Months
1	80	♀	L chain	Micromolecular L MM	MPE and ascites	40	TADD	II	Neg	IIIA	VMP+intracavitary Velcade	18
2	75	♂	-	Apical lung	Lung	2	Surgery + VMPx9	I	Neg	-	-	Alive
3	71	♂	IgG k	IgG k MM	MPE	100	VCDx3	III	Neg	IIIA	DTPACE	5
4	77	♂	-	Lower lung	Lung	1	Surgery + VMPx9	I	Neg	-	-	Alive
5	54	♂	IgG k	Gastric	Gastric	1	HSCTx2	III	Neg	IIIA	RDd-ASCT	6
6	63	♀	L chain	Micromolecular L MM	Sphenoidal bone	50	VCDx4	III	Del 13	IIIA	ID-EDX	10
7	76	♂	IgG k	IgG k MM	CNS	60	TADD	III	Neg	IIIA	DTPACE	9
8	50	♂	IgG L	IgG L MM	MPE	70	VCDx4	III	Complex karyotype	IIIA	HD/EDX-ASCT	Alive

Theese sites did not relate with medullary plasmocytosis or cytogenetics features (only two patients, one with chromosome 13 delection and one with complex karyotype). The patients with lung localization had low ISS and are still alive. One patient with MPE was refractory to the induction therapy (VCD) showing as massive MPE on the other side, and he was treated with DT-PACE schedule but the exitus occurred in five months. The patient with sphenoidal localization progressed on fourth cycle of VCD schedule induction, she had delection of chromosome 13 and died in ten months. The patient with CNS involvement of MM changed TADD chemotherapy with DTPACE but dead occured in 9 months. The patient

with MPE and complex karyotype performed ASCT and he is still alive. Conclusions. Although there is not strong correlation with genetic features, our series showed a very aggressive clinical course as high risk diseases. More aggressive therapeutic strategy may be indicated in this setting. More studies need to clarify the increasing incidence of unusual presentation of MM and the correlation to novel drugs use.

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INCREASED LEVELS OF INFLAMMATORY MONOCYTES IN MULTIPLE MYELOMA BUT NOT IN MGUS

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Background. In MM but not in MGUS, the immune function is impaired as consequence of an immunologically hostile microenvironment and cellular defects. The pro-inflammatory subset of monocytes (IM) defined as a subset of CD14+CD16+ monocytes is characterised by a peculiar release of TNFalpha in response to stress and inflammation, with immunosuppressive activity on CD8+ T cells and an enhancement of oxidative stress and endothelial dysfunction. In mouse model expansion of IM is induced by sIL-2R. Activin-A is potently up- regulated in monocytes as well as stromal fibroblasts by cognate interaction with activated T cells in the bone marrow milieu and plays a functional role in the suppression of inflammation. Aims. Quantify inflammatory monocytes and related cytokines in peripheral blood in MGUS/MM as surrogate of microenvironment defects typical of MM advanced disease. Methods. IM were quantified from peripheral blood of 25 MGUS and 35 MM using flow cytometry and their absolute number was correlated to serum level of circulating cytokines involved in inflammation (TNF alpha, s-IL- 2R, sCD44, IL-6, activin) detectable by commercially available ELISA kit in sera obtained from 10 MM, 10 MGUS and 10 healthy subjects matched for age and sex. Monocytes sorted from healthy donors using immune-beads at concentration $5x10^4$cells/mL were incubated with autologous serum or serum obtained from healthy subjects or MM patient. After 24 hours the percentage amount of CD14+CD16+ and the expression of TNFalpha in adherent fraction were detected by flow cytometry. Results Even though absolute count of monocytes was similar among healthy subjects, MGUS and MM, IM in MM were higher than in MGUS and healthy subjects (mean 79.1 ± 5.2/mmc *vs* 63.7 ± 5.4/mmc *vs* 52.7±7.2/mmc, p=0.015 respectively). Absolute count of MI was positively correlated with presence and extension of osteolytic MM-related bone disease and cytogenetics risk (according to Mayo criteria). In MM sCD44 and sIL-2R were increased when compared to healthy subjects (respectively mean 6.4±0.4 *vs* 3.4±0.5 ng/mL *vs* , p=0.02 and 2.1±0.2 *vs* 1.2±0.01 ng/mL *vs* , p=0.02). We observed a significant increase of circulating Activin A in sera of patients affected of MM compared to MGUS and healthy subjects (p<0.0001). Among MM patients, Activin A was significantly increased in presence of osteolytic lesions (p=0.014). Circulating levels of Activin A , Il-6 and s-IL-2-R were significantly positively correlated with the amount of circulating IM. Healthy monocytes sorted and cultured for 24 hours in presence of autologous or MM serum exhibited an increase in the percentage of CD14+CD16+ subset, intensity of mean fluorescence of CD16 and TNF-alpha expression. Finally, we tested if additional cellular subsets could be involved in this proinflammatory loop : we found positive correlation between the absolute count of circulating IM and Tregs (CD4+/CD25+/Foxp3+) and negative correlation with the expression of CD62L on CD8 cytotoxic cells, index of dysfunctional homing of T-cells. Conclusion: Taken together, our findings suggest a role for IM in sustaining inflammatory loop, contributing to the immunological impairment and driving the evolution from MGUS to MM.

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IN MULTIPLE MYELOMA NEUTROPHILS ARE DYSFUNCTIONAL AND IMMUNOSUPPRESSIVE

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Introduction. In Multiple Myeloma (MM) the immune function is impaired. The role of the effector function of neutrophils in MGUS and MM has been poorly investigated. Methods. In 60 consecutive newly diagnosed MM, 70 MGUS and 30 healthy subjects we evaluated the activation status of neutrophils (N) from peripheral blood using phagocytic activity and expression of surface markers including CD64, CD11b, CD62L,CD16 by flow cytometry. Moreover, we tested the immunosuppressive properties of N isolated from MGUS or MM patients, through functional assays, based on *in vitro* co-culture of N isolated from patients and T-lymphocytes from healthy subjects and we evaluated the expression of the immunosuppressive molecule arginase-1 (Arg-1) by RT-PCR. Results. Despite no differences in the absolute number of N between MM, MGUS and healthy donors, we found a functional impairment in MM not evident in MGUS patients. The capability of phagocytosis of MM-N was significantly reduced compared to healthy subjects (p<0.001) and MGUS (p<0.0001), and restored after induction chemotherapy. (p=0.02). Neutrophils expressions of CD64 were significantly elevated in MM patients compared to MGUS group or healthy controls (p=0.01 and p= 0.007 respectively); there was no significant difference between the group of MGUS and healthy individuals. No differences were observed among MGUS and MM for the other surface markers evaluated. MM-N exhibited an increased expression of ARG-1 compared to MGUS and healthy controls (25.5 *vs* 6.2 *vs* 1 fold changes in gene expression, p=0.003), confirmed by functional assay of enzymatic activity of ARG-1, positively correlated with advanced disease. After PHA-P stimulation, T-lymphocytes isolated from healthy donors missed the expression of activation markers such CD71, CD69, CD25, CD3 in presence of MM-N for 72 hours, and in a less extensive way in presence of MGUS-N. Conclusion. Compared to controls, neutrophils obtained from MM patients have a reduced phagocytic activity, a greater expression of Arg-1 and exhibit an immunosuppressive function on T lymphocytes. Taken together, these findings explain how neutrophils may contribute to impairment of immune function that characterizes MM p.

P245

BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE INCORPORATED INTO AUTOTRANSPLANTATION IS ASSOCIATED WITH MORE FAVORABLE OUTCOMES AFTER RELAPSE IN COMPARISON WITH THALIDOMIDE-DEXAMETHASONE PLUS AUTOTRANSPLANTATION IN MULTIPLE MYELOMA

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In the GIMEMA-MMY-3006 trial prospectively comparing VTD *vs* TD as induction therapy before, and consolidation after, double autologous stem-cell transplantation for newly diagnosed multiple myeloma (MM) VTD was superior to TD in terms of increased rate of CR/nCR and extended PFS. A detailed analysis of outcomes after relapse or progression (R/P) was performed among 182 out of a total of 226 pts with R/P. A lower percentage of pts in the VTD arm required salvage therapy due to symptomatic R/P (67% *vs* 83% in TD, p=0.016). Median time to salvage therapy (TtST, defined as the interval between start of induction and administration of the first dose of second-line therapy) was significantly longer for pts who experienced R/P in the VTD arm compared with those randomized to TD (35 *vs* 29 mos, p=0.018). Salvage therapy-free interval (STFI, defined as the interval between last admin istration of front-line therapy and start of second-line therapy) in the two arms was 22.5 and 15 mos, respectively (p=0.009). As expected, the majority of pts in the TD arm received a second-line therapy including bortezomib (68%), with lenalidomide being used in 12% of pts. By the opposite, in the VTD arm bortezomib- and lenalidomide-based salvage therapies were equally distributed (40% each). The probability to achieve at least a partial response to bortezomib as second-line therapy was 60% for pts with prior exposure to VTD *vs* 63% for those randomized to TD. No difference in post-R/P OS was seen between VTD- and TD-treated subgroups of pts who received bortezomib-based salvage therapies (2-

yr estimates: 48% *vs* 53%, p=0.59). In conclusions, VTD incorporated into front-line ASCT was superior in comparison with TD plus ASCT in terms of extended TtST e STFI. VTD-treated pts had a higher probability to experience long-lasting biochemical R/P not requiring salvage therapy than those in the TD arm. Similar rates of response and post-R/P OS were observed for VTD- and TD-treated pts who subsequently received bortezomib-based salvage therapies, suggesting that short-term exposure to VTD did not favor the selection of bortezomib-resistant clones at the time of relapse.

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PERSISTENT PFS BENEFIT AFTER A 5 YEARS' FOLLOW-UP WITH VTD VS TD INCORPORATED INTO DOUBLE ASCT FOR NEWLY DIAGNOSED MM PATIENTS

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In a randomized, phase 3 study, superior complete/near complete response (CR/nCR) rates and extended progression-free survival (PFS) after a median follow-up of 3 years were demonstrated with bortezomib-thalidomide-dexamethasone (VTD) *vs* thalidomide-dexamethasone (TD) as induction therapy before, and consolidation after, double autotransplantation (ASCT) for newly diagnosed myeloma (MM) patients (intention-to-treat analysis; VTD, n=236; TD, n=238) (Lancet 2010, 376(9758): 2075-85). An updated analysis of the study was performed after a median follow up of 5 years. On an intention-to-treat basis, median PFS was 62 months for patients randomized to VTD *vs* 48 months for those in the TD arm and 6-year PFS estimates were 48% and 36.5%, respectively (HR=0.65, p=0.001). No significant difference in 6-year estimates of OS was seen between patients randomized to VTD or TD arms of the study (75% *vs* 69%). PFS benefit with VTD incorporated into double ASCT was retained across prespecified subgroups of patients with low and high risk, including those aged ≤ 60 (p=0.031) and >60 years (p=0.025), 2-m ≤3.5 (p=0.043) and >3.5 mg/L (p=0.009), ISS 1 (p=0.022) and 2+3 (p=0.010), absence of t(4;14) and del(17p) (p=0.017) and presence of a single or both these abnormalities (p=0.001). In a multivariate analysis, randomization to the VTD arm was an independent factor predicting for prolonged PFS (HR 0.65, P=0.002), along with absence of t(4;14) and/or del(17p) (HR 0.48, P<0.001), 2-m ≤3.5 mg/L (HR 0.55, P<0.001) and achievement of CR after consolidation therapy (HR 0.60, P<0.001). An updated analysis of PFS from the landmark of starting consolidation therapy was also performed. With a median follow-up of 4 years, median PFS was 50 months for patients receiving VTD consolidation and 38 months for those treated with TD (HR 0.69, P=0.015). Superior PFS with VTD *vs* TD consolidation was seen in both low risk and high risk subgroups, including patients who failed at least nCR after the second ASCT (HR 0.48, P=0.003). In conclusion, in comparison with TD and tandem ASCT incorporation of VTD into double ASCT resulted in a significant reduction in the risk of death or progression that was maintained after 5 years' follow-up in the overall patient population and both low-risk and high-risk subgroups. Consolidation therapy significantly contributed to improved clinical outcomes seen on an intention-to-treat basis for patients randomly assigned to the VTD arm of the study.

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THE IMPACT OF DISEASE STATUS ON RESPONSE AND PROGRESSION FREE SURVIVAL IN LENALIDOMIDE TREATMENT FOR RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background. Survival of patients with relapsed refractory multiple myeloma (rrMM) has improved in the last decade with the introduction of immunomodulatory drug as lenalidomide in combination with dexamethasone (Len/Dex) which allows a long term treatment disease control with a well-tolerated continuous therapy. Objective. The aim of the study was to analyze the impact of disease status (relapse/refractory) and CRAB at relapse on response rate and progression free survival (PFS). Patients: 76 pts with rrMM treated with Len/Dex in the approved indication until progression or unacceptable toxicity from 2008 to 2012 were included in this retrospective, single-centre analysis. M/F 38/38, median age was 70 years (y) (range 40–83y), 17 pts were >75y; Isotype was IgG in 56 pts, IgA in 8 and BJ/FLC in 12; 45 (59%) pts were relapsed MM and 27 (35%) refractory MM. The relapsed ones were 26 CRAB positive and 16 CRAB negative. 38/76 (50%) pts received one prior treatment; 23 pts (30.2%) relapsed after autologous; 53 pts (69.7%) received bortezomib and 37 (48.6%) thalidomide-containing regimens. Four pts had renal failure at diagnosis. Median number of len/dex cycles was 8.5 (range 1-30), 10 (1-30) in relapsed and 6 (1-20) in refractory pts respectively. Results. Overall response rate (ORR) during len/dex was 77% comprising 28 pts (45,9%) with PR, 10 pts (16,4%) with VGPR and 9 pts (14,7%) with CR. Median time to best response was 8 mo (range 1-39). Median treatment duration with len/dex was 11.5 mo (range 0.3–51 mo). 39 pts (51.3%) were treated with len/dex >12 mo, 20 (26.3%) >24 mo, 7 (9.21%) >36 mo and 18 pts were still on treatment at the time of analysis. Among the 37 pts receiving len <12 mo, 11 pts discontinued treatment due to progression; in 16 pts treatment was stopped due to hematologic and/or extrahematologic toxicity; 4 pts proceeded to autologous transplantation and one to allogenic transplantation. No significant difference was found in ORR between relapsed *vs* refractory pts. While median PFS was significantly longer in asymptomatic relapsed pts versus CRAB positive at relapse (p<0.004), also in a multivariate analysis PFS was significantly affected by CRAB positivity and age (p<0,013), but not by disease status (relapsed/refractory pts) or pretreatments. Conclusion: Len/dex represents a well-tolerated long term treatment with a satisfactory ORR in rrMM, but with an advantage in term of PFS for patients with an asymptomatic disease.

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FLOW CYTOMETRIC ANALYSIS AND MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA: A COMPARISON BETWEEN FLOW CYTOMETRIC IMMUNOPHENOTYPING AND CYCLOSCOPE-MG

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Multiple myeloma (MM) is a plasma cell neoplasm which resides in the bone marrow (BM) and is critically dependent upon the BM microenvironment for its survival. Recent studies show that MM is consistently preceded by a precursor state,MGUS; however, we lack reliable markers to predict progression from MGUS to MM.In patients(pts) with MM, the depth of response is an important prognostic factor. The aim of this study is to analyze and compare the clinical utility of Minimal Residual Disease studies in MM with two differentes flow cytometric techniques: Flow Cytometric Immunophenotyping (FCI) and Cycloscope-MG. These studies included 45pts:15 with active MM,10 with non-active MM,10 with MGUS and 10 controls that were evaluated by FCI and Cycloscope-MG.The panel of monoclonal antibodies we used in our studies included the minimal test antigens for classifying plasma cells: CD38, CD138, CD45; CD19 and CD56 in order to distinguish myeloma plasma cells (CD19-,CD56+) from normal plasma cells (CD19+,CD56-), and CD117, CD40 and CD27 as recommended markers. Cycloscope-MG showed the aneuploidy of myeloma plasma cells (M-PCs) for the detection of minimal residual disease (MRD).
The results between morphological analysis, immunophenotypic analysis and Cycloscope analysis were compared in the different groups under study. Significant changes were found in the marrow infiltration of M-PCs, and,mainly, in the aberrant expression of phenotype and in the expression of aneuploid cells peak. Patients with active MM showed both the characteristic aberrant immunophenotype (CD38+CD138+ CD19-CD56+) and a visible peak in G0/G1 detectable in Cycloscope. The aberrant phenotype and the peak of aneuploid cells,were absent in patients used as control. The comparison between the three methods used for the evaluation of bone marrow infiltration in patients with MM (morfologia, FACS, aneuploidy) confirms the general overestimation of

infiltration if the only microscopy is performed. Our results show that FCI is directly compared with Cycloscope-MG so both methodologies may help to discriminate the risk categories of MM patients, based on the level of residual aberrant plasma cells. The Cycloscope-MG evaluation provided the possibility to establish a cut- off value that distinguishes patients with active disease (%M-PCs ≥2) from pts in post treatment remission,with non-active MM (%M-PCs ≤2) and MGUS (%M-PCs≤0.5).These studies have shown that residual disease above a level of 2% is clinically relevant.

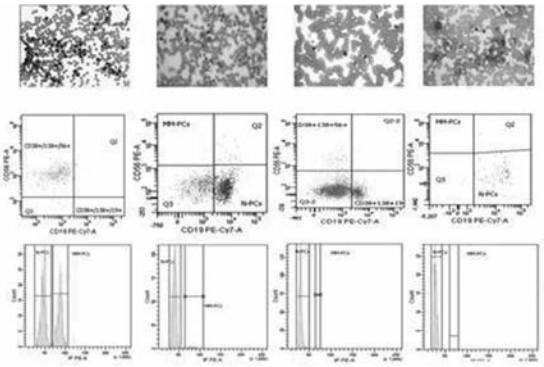

Figure 1.

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PROLONGED LOW-DOSE LENALIDOMIDE TREATMENT INCREASES THE NUMBER OF CIRCULATING NATURAL KILLER CELLS IN MULTIPLE MIELOMA PATIENTS

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High efficacy of lenalidomide in Multiple Myeloma (MM) patients is related to its direct anti-proliferative and anti-angiogenic properties inducing cell cycle arrest of myeloma cells, and downregulation of interleukin (IL)-6 and vascular endothelial growth factor levels in the tumor microenvironment, respectively. Furthermore, lenalidomide has been supposed to indirectly increase immunotherapy responses in MM patients, stimulating T-cell proliferation, enhancing IL-2, interferon-gamma and tumor necrosis factor-alpha secretion by T helper-1 cells, as well as downregulating regulatory T cells and upregulating cytotoxic effects of natural killer (NK) cells. To determine the effects of lenalidomide on NK cells, we evaluated the numbers of circulating NK cells in 15 MM patients (12 males and 3 females), with a median age of 75 years (range, 56-86), during treatment with continuous low-dose of lenalidomide (LD-R). Seven of these 15 MM patients were newly diagnosed MM patients receiving continuous alternate-day LD-R (10 mg on alternate days) in combination with low dose steroids (15 mg/day), the remaining were MM patients receiving continuous alternate-day LD-R maintenance therapy after autologous stem cell transplantation. Flow cytometry analysis was performed using the Cytomics FC500 (Coulter, Miami, FL) to determine the expression of T-cell antigens (CD3, CD4, CD8), NK-cell antigen (CD56) and NK-cell–activation antigens (CD2, HLA DR). All antibodies were obtained from Beckman Coulter. Phenotypic analysis of all these T- and NK-cell antigens were performed before and at month +1, +3, +6, +9 and +12 during R therapy. All MM patients showed a progressive increase in the percentage of CD3+ CD56+ NK cells during the first 6 months of R therapy reaching a plateau maintained until month +12 after initiation of therapy: the median percentage of NK cells was 4% before R treatment versus 10%, 13%, 30%, 31% and 27% at +1, +3, +6, +9 and +12 months, respectively. Mean fold increase of NK cells during R therapy was 1.5, 2.5 and 6.5 at +1, +3, and +6 months, respectively. Progressive increase of NK cells was concomitantly associated with reduction in tumor-linked monoclonal immunoglobulin in all patients. Our preliminary data document that prolonged low-dose R treatment in MM patients increases circulating NK cells further supporting that this drug may mediate its anti-MM effect, at least in part, by modulating NK cell number and function.

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EVALUATION OF MRD STATUS BY FLOW CYTOMETRY AND PCR IN MULTIPLE MYELOMA ELDERLY PATIENTS RECEIVING AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER BORTEZOMIB SUPPLEMENTED MOBILIZATION AND CONDITIONING

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Autologous Stem Cell Transplantation (ASCT) is still an option for eligible patients with Multiple Myeloma (MM). High-dose melphalan (HDM: 200mg/m^2) is the recommended conditioning before ASCT, but synergistic effects of Bortezomib (BOR) and HDM have been reported *in vitro* and *in vivo*. Patients and Methods. In a phase 2 prospective study we evaluated, in 56 fit elderly pts (median age 65 yrs) with MM, feasibility, efficacy and minimal residual disease (MRD) of a strategy based on BOR, Cyclophosfamide (CY) and dexamethasone (DEX): CY-BOR as induction, BOR-supplemented mobilization and HD-MEL. MRD status was evaluated with 4 colour flow cytometry (FC), and by ASO-PCR. Results. 44 pts were evaluable before ASCT: 32(73%) achieved ≥PR and 30(68%) were mobilized: 29(66%) collected PBSC and 25 underwent ASCT. Gr3 neuropathy in 3 pts, pneumonia in 2 pts and 1 toxic death were reported. At day +180 from ASCT 23 pts are evaluable for response and 21 for MRD status: 3 pts have progressive disease (PD), the remaining 20 are in PR(2),VGPR (4), nCR (10) and CR(4). Three pts(14%) are MRD neg by FC: 1/21 pts MRD neg at day +180 after ASCT, being positive after induction and at day +90 after ASCT; two pts MRD neg after induction (one became pos at day + 180 and relapsed at day +365 from ASCT; the other one became pos at day +90 after ASCT and is in CR 10 months after ASCT). MRD status has been also evaluated by ASO-PCR with patient-specific probes. Up to now 5 pts are evaluable: 1 achieved PCR negativity after induction and still maintains PCR negativity at 27 months from ASCT: this patient had positivity of MRD by flow cytometry after induction and at 90 days from ASCT, then became negative and is in CR. The second became PCR negative after ASCT: FC was negative too and the patient is in CR at 10 months from ASCT; the remaining three patients never achieved PCR negativity and were MRD positive at FC evaluation as well: two of them experienced progression of disease. Conclusions. ASCT with HDM and BOR is feasible in older patients, with very high RRs and without major toxicities. However only a minority of them was able to achieve the MRD negativity evaluated by FC, while at the moment, the low number of pts evaluable by PCR does not allow to compare the two methods (the evaluation by ASO-PCR of the remaining patients is ongoing); furthermore our preliminary data suggest that these two methods are not superimposable.

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COMORBIDITY IMPACT SURVIVAL IN MULTIPLE MYELOMA. A SINGLE INSTITUTION STUDY DEALING WITH VALIDATION OF ACE-27 SCORE

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Comorbidities increase in prevalence with age, but their impact on survival in patients with multiple myeloma (MM) is not known. The purpose of this study was to examine the impact of comorbidities on survival in multiple myeloma. Methods. All patients (pts) with MM diagnosed and treated at the Department of Hematology-Oncology of Catanzaro General Hospital (Italy) from 2000 to 2010 were included in an observational database. The study was evaluated and approved by the local ethical committee. Comorbidities were retrospectively graded as None (score=0), Mild (score=1), Moderate (score=2) or Severe (score=3) using the ACE-27 comorbidity index [Piccirillo *et al*. J Reg Mgmt 1999]. The primary endpoint was overall survival (OS), calculated from the

date of diagnosis and censored at the time of last follow-up. Results. 120 patients were identified in the database. Median age of patients was 69 years (range 41-86 years); 64 were male, 56 were female. Based on the ACE-27 comorbidity index, 24 pts (20%) had no comorbid medical conditions (score=0), 49 (40.8%) had mild comorbidities (score=1), 27 (22.5%) had moderate comorbidities (score=2), 20 (16.6%) had severe comorbidities (score=3). After a median follow-up time of 39 months (range, 2-160 months)53 patients died while median OS was 52 months. Survival curves by comorbidity category are presented in Figure 1. As shown, median survival of patients with ACE-27 comorbidity index 0-1 was 90 months therefore significantly longer than median survival of patients with ACE-27 comorbidity index >1 (39 monthts) (HR=0.421;95% CI, 0.175-0.625; P=0.0007). As expected patients with ACE-27 comorbidity index 0-1were significantly younger than patients with ACE-27 comorbidity index >1 (67 yrs *vs* 70 yrs; P=0.04) whereas no association could be found between the degree of comorbidity and international staging system (ISS)(P=0.130). Finally, ACE-27 comorbidity index impacted therapeutic decisions. As matter of fact, patients with score index >1 less frequently underwent autologous bone transplantation (P=0.02). Conclusion: The presence and severity of comorbidities confer a poorer prognosis to patients with MM. Further study is needed to determine to what extent comorbidities directly impact survival versus impacting therapeutic decision-making and tolerance of therapy.

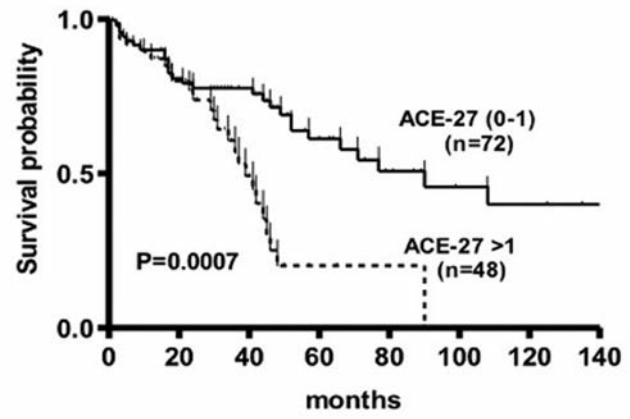

Figure 1.

Lymphomas II

P252

A GENETIC PROFILE WITH A PREVALENCE OF INHIBITORY KILLER IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) GENES MAY EXPLAIN ONE OF THE IMMUNE ESCAPE MECHANISMS OF HODGKIN AND REED-STEMBERG CELLS IN CLASSICAL HODGKIN'S LYMPHOMA

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Hodgkin and Reed-Stemberg (HRS) cells of classical Hodgkin's Lymphoma (cHL) are able to adopt multiple mechanisms to escape immune-surveillance. HRS cells shape the microenvironment by attracting specific T cell populations that provide growth-supporting factors and by suppressing antitumor response of the immune system. Moreover, it has been well established that a genetic predisposition to cHL is involved. An increased risk of developing the disease has been documented in monozygotic twins and in first-degree relatives or siblings. Genetic studies of the major histocompatibility complex (MHC) region have shown a strong association between genes codifying class I HLA-A molecules in Epstein-Barr virus (EBV)-positive cHL. Generally, down-regulation of the HLA class I molecules expressed on the surface of neoplastic cells induces activation of natural killer (NK) cells and exposes them to NK killing action. NK cell activity is modulated by killer immunoglobulin-like receptors (KIRs) with either inhibitory or activating functions. There is growing evidence that a reduced activity of NK cells may be related to the prevalence of inhibitory over activating KIR genes. In this study, we analyzed KIR gene profiles in cHL patients to investigate their possible involvement in immune escape mechanisms of HRS cells. To explore this hypothesis, we studied KIR genotypes, KIR gene frequencies and combinations of KIRs with their respective HLA Class I ligands in 109 consecutive cHL patients (60 males and 49 females, mean age 30 years) and a group of 121 healthy controls. Of the 109 patents examined, 76 had the nodular sclerosis subtype, 20 mixed-cellularity, 11 the lymphocyte-rich and 2 the lymphocyte depleted subtype. In our cohort of cHL patients, we found a significantly increased frequency of the KIR2DL2 (p=0.001) and KIR2DL5 (p=0.04) inhibitory KIR genes, besides an increased frequency of the KIR-ligand combination HLA-Bw4-absent/KIR3DS1present (p=0.0007). Moreover, patients had a significantly increased content of at least 8 inhibitory KIR genes (p=0.002) compared to the controls. These data show that cHL patients predominantly express inhibitory KIR genes, suggesting that the KIR gene profile in cHL patients may have a key role in one of the mechanisms by which HRS cells escape immune response.

P253

DOSE DENSE (DD)-ABVD FOR EARLY UNFAVOURABLE (EU) HODGKIN LYMPHOMA AND DOSE DENSE-DOSE INTENSE (DD-DI) ABVD FOR ADVANCED (A) HL: A MONOCENTRIC EXPERIENCE

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ABVD remains the standard of care for patients with early or advanced stage HL. According to EORTC criteria, early stage HL is divided into 2 groups:favourable and unfavourable.In (EU) HL is debated the role of standard ABVD+RT, in terms of effectiveness, as well as the role of BEACOPP, considered too toxic. In (A) HL, regimens such as esc.BEACOPP improved survival with increased acute and late toxicities.Hence, the need to test the efficacy of intensified chemotherapy (CT) such as ABVD, with no excessive toxicities. We analized 13 cases of HL: 7(EU) and 6 (A).PS:0-1.EU HL:6 males,1 female;19 to 39 years (median:28

years). VES>50 (43%), high LDH (57%) and B2-microglobulin(14%). B symptoms(43%). Bulky(43%).Nodal sites≥4(30%). Stage:IIA(57%), IIB (43%). (A) HL: 4 males, 2 females; 25 to 67 years(median:45 years). VES>50 (67%), high LDH (50%) and B2-microglobulin (67%). B symptoms (83%). Bulky (17%). Stage: IIIA (17%), IIIB (50%), IVB (33%). Extranodal sites (50%): liver,bone,lung,ovary. Splenomegaly (50%), BM (17%).(EU) HL were treated in accord to protocol dd-ABVD (d:1,8, q:21): Doxorubicin 25 mg/m^2,Bleomycin10U/m^2, Vinblastine 6mg/m2, Dacarbazine 375 mg/m^2, G-CSF from d+9 to d+16, for 4 cycles; followed by RT 20-30Gy on PET positive sites,at diagnosis. PET2 was positive in 71%.At the end of CT: ORR(100%), CR (86%), PR (14%). After RT:CR (100%). (A) HL were treated with 4 cycles of dd-di ABVD protocol (d:1,10,q:10) (Russo *et al*.,INT"Pascale"Naples): Doxorubicin 35mg/m^2,Bleomycin 10U/m^2, Vinblastine 6 mg/m^2, Dacarbazine 375mg/m^2, PEG-G-CSF (at d+12), followed by 2 cycles of dd-ABVD (standard dose of Doxorubicin 25mg/m^2, d1-10,q:10)+RT 20-30 Gy on persistent PET positive sites after CT.PET2 was positive in 50%. At the end of 6 cycles of CT,ORR was 100%:CR (67%), PR (33%). Only patients in PR (PET positive) after CT, underwent RT obtaining CR. No evidence of PD at PET-2.Treatments were well tolerated with similar toxicities: neutropenia grade III-WHO(46%),anemia grade II-WHO(38%), hypertransaminasemia grade II-WHO (46%), nausea and vomiting grade II-III-WHO(61%), skin toxicity (grade I-II-WHO) only in 33% of dd-di ABVD. After a median follow-up of 6 months (range:2-18 months),100% of patients are alive and in CR. In our experience, dd-ABVD and dd-di ABVD are highly effective and safety for treatment of HL.These regimens are attempting to test strategies to improve the effectiveness of standard ABVD with acceptable toxicity, in the subset of EU-HL,and to reduce toxicities maintaining the activity of CT,in the subset of (A) HL.

P254

THE "ELDERLY PLATFORM" BY THE FIL (FONDAZIONE ITALIANA LINFOMI: A PROJECT AIMED AT THE PROSPECTIVE VALIDATIONOF THE MULTIDIMENSIONAL ASSESSMENT FOR ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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The initial approach to the elderly patient affected by B-cell lymphoma (DLBCL) is, in most cases, based on the subjective judgment of the physician on the individual patient's ability to tolerate treatment with curative intent. The concept of the "frail" patient was introduced in the late 90s to identify patient whose clinical conditions represent a limit to the possibility of administering chemotherapy at full doses. In the past the FIL has used in its clinical trials a model of "Comprehensive Geriatric Assessment" (CGA) based on the use of the ADL (Activity of Daily Living), IADL (Instrumental ADL) and CIRS-G (Comorbidity Index Rating Scale for Geriatrics) scales. According to the CGA scale only FIT patients with the highest ADL and IADL scores and without severe comorbidities are eligible for curative treatments. Subsequently, the FIL conducted a prospective pilot study on elderly patients with DLBCL by using CGA evaluation. In addition to the categories of FIT and FRAIL patients, in this project, a class of patients with intermediate fragility, defined as "UNFIT", was introduced and identified by intermediate values of ADL, IADL and the absence of serious comorbidities. The project by evaluating 172 subjects demonstrated that the FIT patients (approximately 50%) treated with curative intent reach results similar to young patients, while non-FIT patients perform significantly worse, regardless of the therapy. Based on the preliminary results of the pilot study, FIL has decided to start a prospective study extended to all the FIL Italian's centers with the aim of collecting data by CGA in a large series of elderly patients (>65aa) with DLBCL. For the management of the study by an innovative modality, an easy computerized platform has been predisposed accessible from the private website of FIL, allowing a fast and objective evaluation of the single patient, restituting in real time the results of the CGA and directing the clinicians towards the choice of treatment. In conclusion the use of CGA gives the possibility of an objective and reproducible assessment of the elderly patient with DLBCL. With the "elderly platform" FIL has the intent of extending and simplifying the use of CGA, in further validating it and identifying eventual new criteria for improving the selection of patients.

P255

CHANGES IN ANGIOGENESIS AND HYPOXIA-INDUCIBLE FACTOR-1ALFA (HIF-1ALFA)PROTEIN EXPRESSION IN RELAPSED-REFRACTORY INDOLENT NON-HODGKIN LYMPHOMAS

Minoia C,[1] Quero C,[2] Asselti M,[2] Galise I,[3] Marzano AL,[2] Iacobazzi A,[1] Rana A,[1] Merchionne F,[1] Serratì S,[1] De Tullio G,[1] De Fazio V,[1] Maiorano E,[4] Simone G,[2] Zito FA,[5] Iacopino P,[1] Guarini A[1]

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Angiogenesis increases with disease progression in solid tumors as well as in a variety of haematological malignancies involving the bone marrow. Similarly, some studies have suggested the role of angiogenesis in the clinical progression of non-Hodgkin Lymphomas (NHL), but so far no direct evidence supports this hypothesis. This study evaluated specific immunohistochemical parameters that characterize angiogenesis in whole-tissue sections of matched lymph nodal biopsies at diagnosis and recurrence of eleven relapsed-refractory patients (7 Follicular Lymphoma, 3 Small Lymphocytic Lymphoma and 1 Nodal Marginal Zone Lymphoma). Immunohistochemical analysis was carried out for CD34 and HIF-1. For morphometric analysis, quantification of acquired digital images was performed. In the second biopsy, corresponding to relapse/progression, we observed an increment of area (P=0.009), Feret diameter (P=0.009), major axis length (P=0.032), minor axis length (P=0.009), perimeter (P=0.016), number of ramifications (P=0.065, borderline significance) and microvessel density (MVD) (P=0.006). The analysis performed only on Follicular Lymphoma confirmed a difference in area (P=0.028), Feret diameter (P=0.028), minor axis length (P=0.028) and MVD (P=0.047). Nuclear expression of HIF-1alfa was increased in lymphoma cells after disease recurrence (P=0.009) and this finding remained significant also in the subgroup of FL patients (P=0.028). This is the first direct demonstration of the formation at relapse/progression of a wider, more branched, and more complex vascular network in comparison with diagnosis. The observed variation of HIF-1alfa protein expression leads us to hypothesize a role of HIF-1alfa in the modifications of the angiogenic pattern during lymphoma progression. This evidence may constitute a further biological contribution to the use of complex anti-neoplastic agents, such as lenalidomide or drugs interfering with pathways downstream of HIF-1alfa, to treat relapsed/refractory disease.

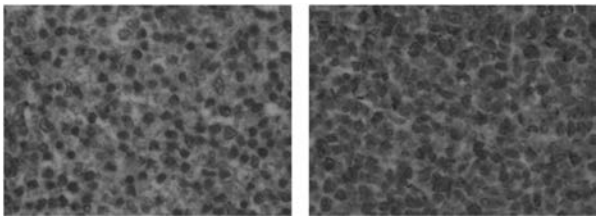

Figure 1. HIF-1alfa expression in whole lymph node sections (high magnification) analyzed comparatively at diagnosis and recurrence in a Follicular lymphoma grade 1.

P256

COMPARISON BETWEEN WHOLE BODY-MRI DWIBS AND 18FDG-PET CT IN THE STAGING OF LYMPHOMAS

Minoia C,[1] Ficco M,[2] Maggialetti N,[3] Ferrari C,[4] Iuele F,[4] Buquicchio C,[5] Tarantini G,[5] Iacobazzi A,[1] De Fazio V,[1] Merchionne F,[1] Florio CM,[2] Rubini G,[4] Guarini A[1]

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FDG PET-CT is currently regarded as the reference standard in the staging of HL and high-grade NHL. Its role is confirmed also in the staging of Follicular Lymphoma. A recent development of MRI is whole-body diffusion-weighted imaging (DWI), whose potential advantage over conventional MRI sequences in the evaluation of lymphoma is the high lesion-to-background contrast possible because of the relatively low diffusivity of lymphomatous tissue. MRI does not entail ionizing radiation and may be complementary to FDG PET CT in the staging of lymphoma. The aim of this prospective study was to assess the role of whole body-MRI/DWIBS in the staging of newly-diagnosed lymphomas (HL and NHL) in comparison to 18F FDGPET-CT. 25 consecutive pts were enrolled and underwent WB-MRI (coronal T1-weighted, coronal STIR and axial DWIBS sequences) and 18F FDG PET-CT. Axial DWIBS sequences included 3 acquisitions of b factor (0, 500, 1000). Lymph nodes larger than 10 mm in short-axis diameter, in the coronal plane on T1-weighted and STIR images, were considered positive. The agreement between WB-MRI-DWIBS and 18F-FDG-PET CT for each of the nodal and extranodal sites was evaluated. A statistical evaluation with the Cohen k was performed. 18F FDG PET-CT showed 75 involved nodal and 10 extranodal lesions. WB-MRI-DWIBS showed 80 nodal and 12 extranodal lesions. The concordance between the two techniques was excellent for neck (k 0.754), axilla (k 0.918), mediastinum (k 0.838), pelvis (k 0.763) and femoral stations (k 0.851) and good for abdomen (k 0.679). According to Ann Arbor criteria, the agreement was good. 2 of 25 (8%, 1 DLBCL and 1 SLL-CLL) patients showed a different stage with the 2 techniques, with an overstaging by WB-MRI/DWIBS. Our initial results show a good agreement between whole-body MRI-DWI and 18F FDG-PET-CT in lymphoma staging, both in the evaluation of nodal and extranodal involvement. Although 18F FDG PET-CT remains the gold standard for lymphoma staging, WB-MRI-DWIBS may be considered an emerging functional whole-body imaging modality. This new technique may provide complementary information and, due to the low toxicity profile, become a useful tool also in the follow-up.

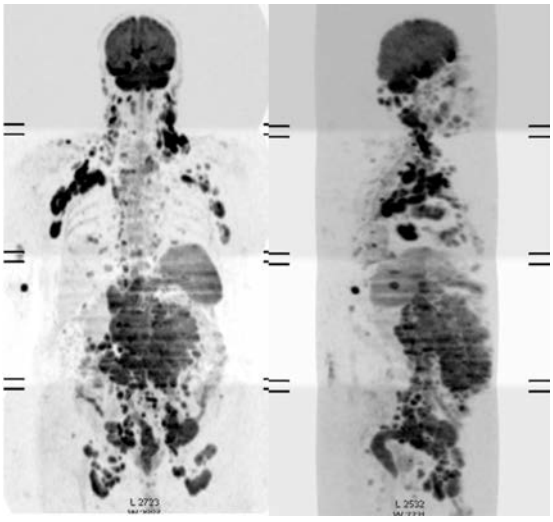

Figure 1. Volumetric Whole Body-DWIBS post-processing of the same patient, confirms MR findings.

P257

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

De Renzo A,[1] Masarone M,[2] Della Pepa R,[1] La Mura V,[3] Signoriello G,[4] Pugliese N,[1] Beneduce G,[1] Rosato V,[5] Migliaccio I,[1] Cimmino C,[1] Luponio S,[1] Perna F,[1] Pane F,[1] Persico M[2]

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Background. Occult HBV infection (OBI) is defined by the persistence of HBV in the liver without serum HBsAg and HBVDNA. It represents a life threatening risk if the carrier experiences immunosuppression. An OBI can be present in about 18% of HBcAb+ patients. International guidelines suggest a strict surveillance for ALT and HBV markers in patients undergoing immunosuppressive therapies, in particular monoclonal antibodies. In Non-Hodgkin Lymphoma (NHL), OBI reactivation can occur in 3 to 25%. The real prevalence remains to be established. Aims. To determine the prevalence of occult HBV reactivation in a large cohort of patients undergone immunosuppressive treatments for NHL and to confirm the association with monoclonal antibodies. Methods. We analysed 498 NHL patients in a single centre of Southern Italy from 2005 to 2011. We evaluated HBV markers, type and NHL localization, treatment type and HBV reactivation. Results. Fourty percent was treated with monoclonal antibodies and 60.3% without. Ninety six patients were HBcAb+ and HBsAg-. HBV reactivation occurred exclusively in ten subjects of this subgroup, 5 treated with Rituximab and 5 without. Every patient was treated with Lamivudine. No one experienced liver-related death. Summary / Conclusion: Our data report a prevalence of OBI reactivation of 10.42% in HBcAb positive patients. This event occurred in 50% of cases in patients treated with no monoclonal antibodies. Each reactivation was treated with Lamivudine. This report enlightens the importance and the cost- effectiveness of a strict surveillance in HBcAb+ HBsAgpatients, in order to detect an occult HBV reactivation, also in NHL patients treated with monoclonal antibodies-free protocols.

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LENALIDOMIDE WITH/WITHOUT RITUXIMAB OR STEROIDS IS ACTIVE AND SAFE IN RELAPSED/REFRACTORY AGGRESSIVE NON-HODGKIN'S B-LYMPHOMAS (NHL): RESULTS OF A MONOCENTRIC RETROSPECTIVE STUDY

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Relapsed or refractory aggressive NHL not eligible to high dose chemotherapy had a poor prognosis, however standard treatment for these patients is not clearly defined. On this basis, we conducted a monocentric retrospective study to investigate efficacy and safety of lenalidomide monotherapy or in association to rituximab (R) or steroids in patients with heavily pretreated NHL. End points of the study were overall response (ORR: complete response, CR, + partial response, PR, + standard disease, SD) and duration of response (DOR), feasibility and safety of treatment. Inclusion criteria were: relapsed/refractory NHL patients treated between 2007 to 2012 with lenalidomide 25 mg daily for 21 days every 28 as monotherapy or in association to weekly dexamethasone (20 mg bolus) or lenalidomide 20 mg daily for 21 days in combination with R (375 mg/sqm) every 28 days. Patients were treated until progression or unacceptable toxicities. Results. 53 patients were analyzed. NHL histotype were: 34 diffuse large B-cell (DLBCL),11 mantle cell, 5 follicular, 2 primitive mediastinum B-cell and one Burkitt. Clinical features at relapse before lenalidomide therapy were: stage 3-4 40 (75%), intermediate high/high risk International Prognostic Index 23 (43%), bone marrow involvement 20 (38%), bulky disease 20(38%). Eight (15%) patients received lenalidomide at first relapse while 24 (45%) underwent more than 3 previous lines of therapy, including autol-

ogous or allotransplant in 19 (36%). Median time from last previous therapy was 3.2 months (0.4- 38). At the time of the analysis, response assessment was done in 51 patients: ORR 18 (35%, CR 15%) and no response in 33 (65%). Concerning different schemes of therapy: among 29 patients treated with lenalidomide monotherapy ORR was 24%, in 11 receiving lenalidomide plus R was 55% and in 11 underwent lenalidomide plus steroid 45%. Among 34 DLBCL,ORR was 41%. Median DOR for all 51 patients was 12 months (0.2-24). At a median follow-up of 20 months, 5 patients were in stable CR, 7 continued lenalidomide, 11 relapsed and 28 died. Globally 91% of the expected dose of lenalidomide was underwent. One patient died due to heart failure during treatment. Grade 3-4 toxicity was mild: neutropenia 19%, anemia and thrombocytopenia 17%; 5 patients had grade 3-4 infections and one grade 3 thromboembolic event. In conclusion, lenalidomide monotherapy mainly in association with R or steroids is active and safe in patients with heavily pretreated NHL.

P259

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

De Renzo A,[1] Luponio S,[1] Della Pepa R,[1] Migliaccio I,[1] Pugliese N,[1] Beneduce G,[1] Salvatore D,[1] Marotta S,[1] Cimmino C,[1] Picardi M,[1] Masarone M,[2] Persico M,[2] Pane F[1]

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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, 88 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 February 2013. Patients were treated with different chemotherapy regimens: 40% (35/88) with R-CHOP; 52% (46/88) with R-FN; 3% (3/88) with R-F; 5% (4/88) with R-Leukeran. 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. 20% of the patients (18/88) were HBcAb positive. 64% of the patients (56/88) 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). 36% of the patients (32/88) are still in therapy with Rituximab and 9% of them are HBcAb positive (3/32): all these patients are at risk for HBV reactivation too. 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 (1/18). More ambitious prospective studies are required to establish the clinical utility of prophylactic therapy with lamivudine during the maintenance therapy with Rituximab.

P260

FAVORABLE OUTCOME IN PRIMARY HEPATIC LYMPHOMA WITH OR WITHOUT HCV INFECTION

De Renzo A,[1] Della Pepa R,[1] Perna F,[1] Masarone M,[2] Luponio S,[1] Pugliese N,[1] Migliaccio I,[1] Beneduce G,[1] Salvatore D,[1] Gravetti A,[1] Cimmino C,[1] De Sio I,[2] Ciancia G,[3] Persico M,[2] Picardi M,[1] Pane F[1]

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Background. Primary hepatic lymphoma (PHL) is an uncommon lymphoid tumor frequently associated with a poor prognosis. PHL was first described in 1965 by Ata *el al* and in 1986 Caccamo *et al* defined PHL as a lymphoma localized and limited to the liver without extrahepatic involvement. To date, less than 150 cases have been published. We report 11 patients with PHL diagnosed from 1995 to 2012 in 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). Tumor 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. Eigth patients received chemotherapy with CHOP regimen (6CHOP, 2 R-CHOP), two patients received R-FN, while a patient with a single focal lesion received surgical treatment. The complete remission rate was 100% (11/11); one of these patients, who had HCV- related cirrhosis, died because of hepato-renal syndrome, and another one died because 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.

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THE PROGNOSITC ROLE OF EBV IN PERIPHERAL BLOOD OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Approximately 9-15% of Diffuse large B cell lymphomas (DLBCL) are EBV-positive. Monitoring of EBV-DNA in peripheral blood is emerging as a biomarker in EBV-related lymphomas. We studied the role of EBV-DNA in whole blood (WB), mononuclear cell fraction (MNC) and plasma of patients with DLBCL at diagnosis treated with immunochemotherapy (R-CHOP). Our analysis included 136 patients with DLBCL (median age 62 years, range 15-92 years). EBV was detected using a commercial real-time PCR kit (BioQuant EBV, Biodiversity, Brescia, Italy) in peripheral blood (PB) compartments (WB n=133, plasma n=55, and MNC n=52). Lymph node samples from 61 DLBCL patients were analyzed for EBV infection through in situ hybridization for EBV-encoded small RNAs (EBER). EBV was detected in 35 of 133 WB samples (26%). The copy number varied between 200 and 196000 copies/ml. Presence and copy number of EBV in WB and MNC correlated ($p<0.01$, respectively), while there was no correlation between EBV-load in plasma and the other compartments. Presence or viral load of EBV-DNA in any blood compartment was not an indicator for the presence of EBV in the lymphoma cells in 61 patients studied with EBER–ISH (11 patients EBER positive). The presence and viral load of EBV in PB was not related to age or gender, or other disease characteristics as LDH level, stage, and IPI. In univariate analysis including 133 patients treated with R-CHOP, the presence of EBV-DNA in WB was associated with a significantly shorter event-free survival (EFS): 60% versus 79% at 2 years ($p<0.04$). As well, the EBV copy number was correlated with a worse outcome (HR of 1.86 for each logarithmic increase; 95% C.I., 1.17-2.97; $p<0.009$). Correcting for IPI in a multivariate Cox analysis, the presence of EBV-DNA in PB retained its prognostic significance (HR 2.02; 95%

C.I., 1.03-3.95; p<0.04). Our findings suggest that the presence and load of EBV-DNA in peripheral blood is not a surrogate marker for EBV-status in DLBCL, but EBV-DNA in PB associates with a worse outcome. Further studies are needed to explore the mechanisms of expansion of EBV-positive cells in patients with DLBCL.

P262

A RETROSPECTIVE ANALYSIS OF 54 PATIENTS WITH PERIPHERAL T-CELL LYMPHOMAS TREATED AT A SINGLE INSTITUTION

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Background. Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of rare diseases, usually manifesting clinical aggressiveness and a poor outcome. High-dose therapy and autologous stem cell transplantation (HDT/ASCT) have been used in up-front and salvage settings, with different success rates. However, no standard therapy has been established, due to the lack of randomized studies. Design and Methods. We retrospectively analyzed 54 patients (pts) treated in our institution between 2001 and 2011. Median age at diagnosis was 56 years. The histological subtypes were: 37 (68%) of peripheral T-cell NHL unspecified (PTCL-U), 13 (24%) anaplastic large cell lymphoma (4 pts ALK+), 2 (4%) of angio-immunoblastic lymphoma (AITL) and 2 (4%) of enteropathy-associated T-cell lymphoma (EATL). Forty-eight percent had B Symptoms, 33% serum elevated LDH levels and 24% bone marrow involvement. ECOG was 2-3 in 28%; 76% of pts had stage III-IV. According to the International Prognostic Index (IPI) and to the prognostic index for T-cell lymphoma (PIT) 11% and 22% were classified as low risk, 30% and 33% as low-intermediate risk, 12% and 33% as high-intermediate risk, 29% and 12% as high risk respectively. CHOP-like regimen was given in 32 (59%) pts of which 14 received a CHOEP regimen. The remain 22 (41%) were treated with more intensive third generation regimens (MACOP-B like). HDT/ASCT was planned in 16/54 (30%) as first line consolidation therapy. Results. Complete response (CR) was obtained in 30/54 (55%), partial response in 7 (13%) and 17 (31%) pts presented a progressive disease. No difference in terms of CR rate was observed between CHOP-like and MACOP-B like regimens (27% *vs* 27%). Five-year overall survival (OS) and progression-free survival (PFS) was 31.9% (95% CI 25.3-38.5) and 27.3% (95% CI 20.2-34.5), respectively. At the univariate analysis bone marrow involvement (p=0.003), PIT high risk group (p<0.001), lymphocytopenia (p= 0.06) predicted a shorter PFS. Five of 16(31%) pts had not the planned HDT/ASCT consolidation due to early progression. Patients who received HDT/ASCT as consolidation therapy presented a slightly better 5-years PFS than pts with chemotherapy alone (95% CI 37.7% *vs* 24.6%; p=0.008). Conclusions. The prognosis of PTCLs remains poor despite the use of intensive chemotherapy regimen including HDT/ASCT. More active induction chemotherapy regimens, including novel agents, are needed to improve the outcome for these patients.

P263

EFFICACY AND SAFETY OF BENDAMUSTINE-BASED REGIMEN IN THE TREATMENT OF REFRACTORY AND RELAPSED HODGKIN LYMPHOMA

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Background. The management of patients with refractory or relapsed Hodgkin lymphoma (HL), especially after stem cell transplantation (SCT) remains controversial. Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the dosage and combinations in HL. Aim. This phase II study evaluated the efficacy and safety of two different schedules of bendamustine-based regimens (high dose *vs* standard dose) in refractory/relapsed HL patients. Methods. From May 2011 to December 2012, 9 patients (6M/3F) with a median age of 25,5 years (range 18-34) received bendamustine as salvage treatment. Patients were divided by chance into 2 groups of treatment: 5 patients received standard intensity treatment (standard-B; bendamustine 90 mg/mq days 1-2 and Ara-C 0,5-0,75 g/mq day 1) and 4 patients received high intensity treatment (high-B; bendamustine 150 mg/mq days 1-2 combined with Ara-C 1-2 g/mq day 1 or modified BEACOPPesc regimen without adriamycin). Each cycle was repeated every 28 days. The treatment efficacy in both groups was evaluated according to Revised Response Criteria for Malignant Lymphoma. Any adverse event occurred was recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0). Results. The median number of previous chemotherapy lines was 3 for both groups. Three patients had failed prior autologous-SCT in the high-B group and 2 in the standard-B group, 2 patients in each group, were primary refractory to ABVD. A total of 26 cycles was administered (median 3,5; range 1-6). In the high-B group, 3 (75%) patients achieved complete remission (CR) and then underwent to SCT (two autologous and one haploidentical-SCT) and are in complete remission while one patient died for progressive disease (PD). By contrast, among the 5 patients who received standard-B, 3 (60%) were in stable disease (SD), 1 was in PD and 1 patient obtained partial remission (20%) and underwent to autologous-SCT. When bendamustine was used at standard doses, it didn't exert grade 3-4 side effects. Grade 3-4 treatment related adverse events reported in the high-B group were thrombocytopenia (75%) and anemia (50%). Conclusion Bendamustine used at high dose and in combination with Ara-C or a modified BEACOPPesc, seems to be effective in heavily pretreated patients with HL, suggesting a possible non cross-resistance with other agents. Its safety profile is acceptable and adverse events manageable also at this high dosage.

P264

ROLE OF CIRCULATING -DOUBLE NEGATIVE T CELLS (DNT) IN LYMPHOMA PATIENTS: PRELIMINARY RESULTS OF A PROSPECTIVE STUDY

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Numerous aspects of lymphoma pathophysiology indicate mutual interactions between the host immune system and lymphoma cells. An unconventional subset of CD4 CD8 double-negative Tcells (DNTs) has been recently described to specifically contribute to anti-tumor immunity,acting as both regulatory T cells and/or cytotoxic T cells.DNTs are T lymphocytes expressing either or T-cell receptor (TCR) and lacking of CD4,CD8 and CD56.In healthy human donors they constitute about the 1-5% of lymphocytes in the peripheral blood and in lymphoid organs.DNTs also demonstrated to have a direct *in vitro* anti-tumor activity against lymphoma but few data are available on their prognostic significance in lymphomas, their interaction with other immune cells and on their functional attitude.

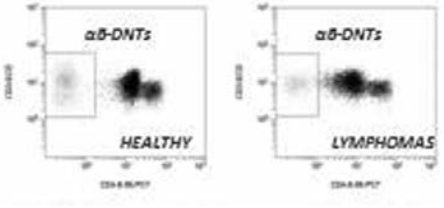

Figure 1. Circulating αβ–DNTs in gated CD3+T cells. Representative staining of two different subjects are shown

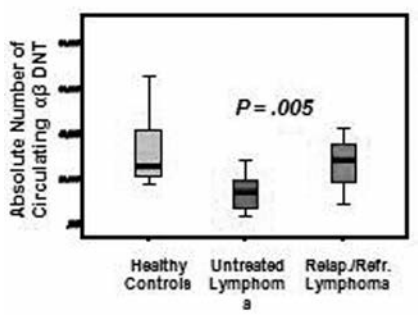

Figure 2. Absolute number of circulating (TC Rαβ) DNTs : DNTs are reduced in Lymphoma patients compared to healthy controls (p=0.005) and they correlate with disease relapse/progression

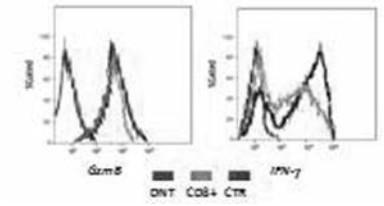

Figure 3. Expression of GzmB and IFN-γ in DNTs as compared with autologous CD3+Tcells

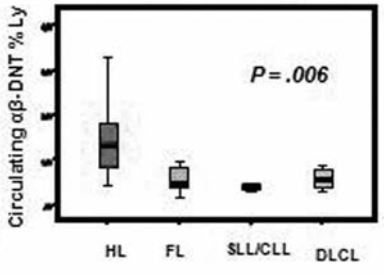

Figure 4. Percent of circulating DNTs as %Ly: Hodgkin?s Lymphoma showed higher number of αβ–DNT cells as compared with other histotypes (p=0.006)

The aim of this study is to assess the frequency and the functional attitude of circulating DNTs in Lymphoma patients pts and healthy donors as controls, in order to evaluate the role of DNTs on clinical outcome. Peripheral blood of 30 Lymphoma (pts) and 16 healthy donors as controls were prospectively collected to perform phenotypic and functional characterization of DNTs using the following monoclonal antibodies (MoAbs): CD3, CD4, CD8, CD56, CD45, TCR , CD45Ra, CD45Ro, CCR7, CD27, CD28, CD30, CD69, GITR, CD95, CD178, CD152, IFN- , TNF-, granzymeB,perforin. For functional studies DNTs were purified from PBMCs by immuneselection. Data was acquired using a 8-colour flow cytometer and compared among the groups using the Mann-Whitney non parametric test or Kruskal–Wallis one-way analysis of variance. We observed a significant decrease (p = 0.006) of -DNTs in the PB of pts with untreated lymphoma (20.5±4.8 SE,) (Mean + SE) as compared with healthy controls (31.3±3.4),and their number correlated with disease relapse/progression (fig.1-2).In Hodgkin's Lymphoma pts the -DNTs frequencies were significantly increased as compared with other histotypes (fig. 4). Interestingly, after ex vivo expansion, DNTs, acquired a immunomodulatory cytokine profile, characterized by the secretion of IFN- and granzyme B which are known as central components of antitumor immune responses (fig.3). Our study has demonstrated for the first time that -DNTs may play an important role in both the development and the progression of lymphomas. In addition,it is likely that ex-vivo expanded DNTs exert an anti-tumor activity,thus suggesting their possible use as new strategy for adoptive immune-therapy.

P265

RITUXIMAB INDUCES HYPOGAMMAGLOBULINEMIA IN PATIENTS WITH NON HODGKIN LYMPHOMA

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Background. Rituximab (R) is a monoclonal antibody that binds the CD20 antigen on all peripheral B cells. Its favorable toxicity profile and effectiveness have led to its wide use in induction and maintenance regimens for Non Hodgkin Lymphoma (NHL). Aim. This retrospective single center study aimed to evaluate the hypogammaglobulinemia (hypoIg) associated with R use. Patients and Methods. We performed serial quantitative serum immunoglobulin (SIg) concentration at the baseline, after chemotherapy, during and after R maintenance therapy. IgG, IgA and IgM deficit were respectively defined by level below 700 mg/dL, 70 mg/dL and 40 mg/dL. Symptomatic patients were defined as having 2 or more non-neutropenic infections in a 6-month period after or during R. Results. 88 patients with NHL and SIgG studies were analyzed, 24% were relapsed or refractory. The median age of patients was 61 years (range: 28-80). The histologies included follicular lymphoma (FL) (n=53), small lymphocytic lymphoma (SLL) (n=9), marginal zone lymphoma (ML) (n=11), mantle cell lymphoma (MCL) (n=9), diffuse large B-cell lymphoma (n=6). Patients received a median of 11 doses of R (range: 6-27). The median follow-up of surviving patients was 3,6 years. Before treatment with R, 9/88 (10.2%) had low SIgG levels (6 FL, 1 MCL, 1 SLL, 1ML) and 4/9 (44.4%) required, during R maintenance treatment, IVIG administration. After R-chemotherapy, IgG deficiency appeared in 20/88 (22.7%), no one needed IVIG, despite 7/20 (35%) were symptomatic. After or during R maintenance 22/88 (25%) showed IgG deficiency after a median of 10 R cumulative doses; the deficit occurred in the 77% (17/22) within the fourth R maintenance dose and in no one after the sixth R administration. In this category, 12/22 (54.5%) were symptomatic and 4/22 (18.2%) required IVIG. All 8 patients who needed IVIG showed at least two Ig isotypes deficiency. Conclusions. We observed that R administration was associated with a high risk of hypoIg. In addition, we found that the number of R doses correlated to the development of symptomatic hypoIg. Finally we observed that the risk of hypoIg increased in patients who received maintenance R. The decision to introduce therapy with IVIG in non-neutropenic patients was related to repeated episodes of infection. HypoIg often is underestimated also for the presence of confounding symptoms. Our study suggests that the baseline and periodic Ig monitoring should be considered in these patients subset.

P266

PET IN THERAPY RESPONSE ASSESSMENT FOR PATIENTS (PTS) WITH FOLLICULAR LYMPHOMA (FL) AND BULKY DISEASE: A SINGLE CENTRE EXPERIENCE

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Background. According to the International Harmonization Project (IHP), response assessment after treatment by PET is not recommended for FL. Persistence of residual masses after chemotherapy does not necessarily indicate residual disease and CT has a limited ability to distinguish between scar tissue and viable tumours in residual masses. Previous studies showed that PET is an accurate imaging modality for staging and assessment of treatment response in FL with positive and negative predictive values. Aim The aim of our study was to retrospectively evaluate PET's role in response assessment after treatment in FL with bulky disease at diagnosis and with positive CT after treatment. Patients and Methods. We examined 24 patients with grade 2-3A FL and bulky disease, observed at our Institution between 2005 and 2012. All were evaluated by CT and PET at diagnosis and at the end of treatment. R CHOP chemotherapy was administered (6 cycles). All pts had evidence

of residual masses at CT after treatment. PET was positive in 10 pts (41%) and negative in 14 (59%). Because of the size of the residual masses, all pts underwent: R DHAP therapy, peripheral blood stem cells collection and autologous stem cells transplantation (ASCT). After ASCT only 3(12%) pts had positive PET results, while in all pts CT showed residual masses. Results Six (60%) pts with positive PET after first line treatment and 4 (14%) with negative PET underwent progression after second line treatment. Mean Progression Free Survival (PFS) was 39 months for PET positive and 68 months for PET negative pts. The difference in progression rates and PFS between PET-positive and PET-negative pts is statistically significant. Conclusion. In our experience FDG PET is a useful tool for response assessment after treatment in pts with FL and bulky disease. It is able to distinguish between scar tissue and active residual disease, and is strongly predictive of outcome. More clinical trials are needed to assess the utility of FDG PET in the management of pts with FL.

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BENDAMUSTINA INCLUDING DHAP REGIMEN IN HIGH RISK AND RELAPSED/REFRACTORY LYMPHOMA PATIENTS: A SINGLE-CENTER STUDY

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Background. Many studies have demonstrated the efficacy and safety of Bendamustine combinations in heavily pretreated Lymphoma patients (pts). Aims. Our study was designed to assess the safety of Bendamustine including DHAP regimen in pts with high risk or relapsed/refractory lymphomas. Moreover we evaluated the mobilizing potential of this Bendamustine combination. Methods. Twelve patients were treated at 4-weekly intervals with Bendamustine 90 mg/mq on days 1,2; Cisplatin 100 mg/mq over 24 hours on day 2; Cytarabine 2000 mg/mq (two doses) on day 3; Dexamethasone 40 mg on days 1–4 with or without Rituximab 375 mg/mq on day +4. Palonosetron was given as antiemetic prophylaxis on days 1,3. A total of 28 courses were administered and each patient received at least 2 cycles of therapy. Six patients received a mobilizing chemotherapy plus G-CSF at a dosage of 10 mcg/Kg starting from the day +6. Patient's characteristics are shown in tab 1. Results. Grade 3 and 4 haematological toxicity consisted of anemia (8 pts), neutropenia (12 pts) and thrombocytopenia (9 pts). The median nadir of neutropenia was observed at day +12 (range 11-14) with an haematological recovery after a median of 4 days (range 3-6 days) from the nadir. Four patients experienced a febrile neutropenia with hospitalization in three cases. Two patients died because of K. Pneumoniae sepsis. No grade 3-4 extra-haematological toxicity was observed except for grade 1-2 gastrointestinal toxicity. Five of the six patients undergoing a mobilization course of chemotherapy have reached the target cell dose (tab 2).

Table 1. Patient's Characteristics

PARAMETERS		N°
Histology	HL	4
	DLBCL	2
	PTCL	1
	SMZL	2
	RICHTER	1
	FL	2
Primary Refractory Disease		7
Relapse After ASCT		3
Previous Lines of Chemotherapy≥2		4
Disease Status at Enrollment	Relapsed/Refractory Disease	9
	Partial Response	1
	Complite Response	2
Age Median (range)	53	(26-77)

Table 2. Patients Receiving a Mobilization course of Chemotherapy

Pt	Histology	Age	Failed previous mobilization	Previous extensive radiotherapy	Previous Fludа/Lena/Mel	Previous Lines of CHT≥2	Refractory Disease	BOM+ /BM cellularity≤30%	Harvest	Day of Apheresis	N° Apheresis	CD34/Kg	Mozobil
1	HL SN	29	N	N	N	N	Y	N	Y	+18	1	4,8 X10⁶/Kg	Y
2	LNH FL	67	N	N	Y	N	N	N	Y	+15	1	6,5 X10⁶/Kg	N
3	SMZL	45	N	N	N	N	Y	N	N	-	-	-	Y
4	RICHTER	58	N	N	N	N	N	N	Y	+15+16	2	5,12 X106/Kg	Y
5	LH SN	53	N	N	N	N	Y	N	Y	+18+19	2	5,4 X10⁶/Kg	N
6	DLBCL	52	N	N	N	N	N	N	Y	+14	1	5,0 X10⁶/Kg	N

Y: yes; N: no; Pz: patient LH: Hodgkin Lymphoma; LNH: non Hodgkin Lymphoma; SN: nodular sclerosis; CM: mixed cellularity; SMZL: Splenic Marginal Zone Lymphoma; PTCL: Pheripheral T Cell Lymphoma; DLBCL: Diffuse Large B Cell Lymphoma

Conclusion. In our experience the addition of Bendamustine to DHAP regimen seems safe with an acceptable toxicity profile if compared with an historical control group of pts treated at our Institute with DHAP alone. The two pts died of sepsis had a primary refractory disease after ASCT. Survival and response rate analysis cannot be evaluated because of the small number of pts and the short follow-up. Only one patient failed mobilization. Although none of the patients undergoing a collection attempt met the criteria to be defined as poor mobilizer, half of them have not reached the circulating CD34+ cells target peak requiring intervention with new mobilization agents. Many studies on larger series are needed to explore these preliminary results.

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TELOMERE LOSS IS AN EARLY AND PERSISTENT ABNORMALITY IN LEUKOCYTES FROM PATIENTS TREATED WITH CONVENTIONAL CHEMOTHERAPY BUT NOT FROM THOSE EXPOSED TO RITUXIMAB ALONE

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Introduction. Patients with lymphoma often require treatments with chemotherapy. Several recent reports suggest that the exposure of leukocytes to chemotherapeutic drugs may induce telomere shortening. However, the cell type involved and the time course for the onset of chemotherapy-induced telomere shortening remain to be elucidated. In the present study changes in telomere length (TL) before and after cytotoxic drug exposure were evaluated. Main aims were: i. to verify whether TL shortening is a phenomenon induced by extensive chemotherapy treatments or it may occur even after minimal drug exposures; ii. to identify possible cell populations that are particularly susceptible to drug-induced telomere; iii. to investigate the different effect of chemoimmuno- or immuno-therapy alone. Methods. Peripheral blood (PB) cells were obtained from 34 lymphoma patients undergoing chemoimmunotherapy (22 R- CHOP, 5 ABVD, 1 BEACOPP, 4 R-Bendamustine, 1 R-MINE, 1 R-OxDHA) and 6 patients with primary immune- thrombocytopenia (PTI) treated with Rituximab (R). Median age of patients was 60 years. All but four lymphoma patients were at their first treatment line. TL was assessed on granulocyte (GN), mononuclear cell (MNC) and on total leucocytes (total PB) before and after each chemotherapy course. In 25 lymphoma patients and in all PTI, TL was assessed also at long term since last therapy. TL was evaluated by southern-blot analysis. Results. A marked reduction in TL was detected in 28/34 (82%) patients undergoing conventional chemotherapy in all PB cells investigated. In particular a marked TL loss following chemotherapy compared to pre-treatment values was observed in GN (p=0.001), in MNC (p=0.034) and total PB (p=0.002). In most patients TL shortening was detectable already after the first (21 pts) or the second (8 pts) chemotherapy course. In addition, TL shortening remained virtually unchanged up to 6 months since the last therapy in 25 patients evaluated at long-term. As shown in Figure 1, no difference in TL was detected before and after drug exposure in 6 patients receiving R monotherapy, even in patients followed up to 10 months since last R infusion.

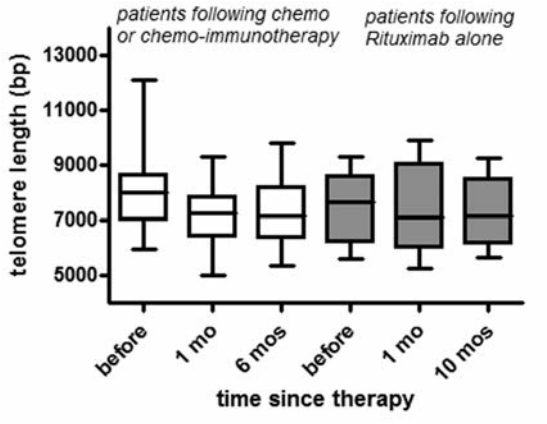

Figure 1. Changes of telomere in patients following chemo or chemo-immunotherapy and in patients treated with Rituximab alone

Conclusions. Results indicate that telomere shortening: i. can be detected early following chemotherapy exposure and is persistently detectable for several months since chemotherapy; ii. can be most easily detectable in granulocytes; iii. is not observed in patients affected by PTI treated with Rituximab.

Allogeneic and Autologous Transplantation

P269

CYTOMEGALOVIRUS REPLICATION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS ASSOCIATED WITH A DECREASED RELAPSE RISK IN PATIENTS WITH LYMPHOMA

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Recent studies found an association between post-transplant CMV replication and a reduced risk of relapse for patients with acute myeloid leukemia. This prompted us to evaluate the impact of CMV replication on the outcome of a retrospective cohort of 92 patients with Hodgkin (HL) or Non-Hodgkin Lymphoma (NHL) undergoing allogeneic HSCT from HLA identical siblings and homogeneously treated with a conditioning regimen including thiotepa, fludarabine and cyclophosphamide. Post-HSCT CMV replication was detected by pp65 antigenemia. Patients characteristics are summarized in Table 1. CMV replication occurred in 45 patients (49%) at a median of 40 days (range 9-530) and was independent from disease histology (p=0.09), pre-transplant disease status (p=0.5), sensitivity or refractory to previous chemotherapies (=0.9), number of lines of treatment (p=1), conditioning regimen (p=0.08), acute (p=0.2) and chronic (p=0.2) GVHD (Table I).

Table 1.Patients Characteristics according to post-transplant pp65 antigenemia (CMV replication)

Characteristics	All Patients	Patients with pp65-antigenemia	Patients without pp65-antigenemia	P value
Patients	92	45 (49%)	47(51%)	
Histologic Subtype				
Follicular non-Hodgkin Lymphoma	17	8 (47%)	9 (53%)	0.09
Aggressive non-Hodgkin Lymphoma	28	9 (32%)	19 (68%)	
T cell non-Hodgkin Lymphoma	10	8 (80%)	2 (20%)	
Chronic Lymphocytic Leukemia	6	4 (66%)	2 (34%)	
Hodgkin Lymphoma	31	16 (52%)	15 (48%)	
Patient Age Median (range)	49(20-67)			
<40	24	11 (46%)	13 (54%)	0.8
≥ 40	68	34 (50%)	34 (50%)	
Donor/Patient CMV Serostatus				
Negative/Negative	5	0 (0%)	5 (100%)	0.05
Others	87	45 (52%)	42 (48%)	
Chemosensitive	56	29 (52%)	27 (48%)	0.5
Refractory	36	16 (44%)	20 (56%)	
Disease Status at HSCT				
CR	40	20 (51%)	20 (50%)	0.9
PR	36	18 (50%)	18 (50%)	
SD/PD	16	7 (44%)	9 (56%)	
Number of lines of CT				
≤2	33	16 (49%)	17 (51%)	1
>2	59	29 (49%)	30 (51%)	
Preparative Regimen				
MAC	34	21 (61%)	13 (39%)	0.08
RIC	58	24 (41%)	34 (59%)	
aGVHD				
Yes	40	23 (57%)	17 (43%)	0.2
No	52	22 (42%)	30 (58%)	
cGVHD				
Yes	42	24 (57%)	18 (43%)	0.2
No	50	21 (42%)	29(58%)	
Post-HSCT Relapse				
Yes	44	13 (29%)	31 (71%)	0.003
No	48	29 (60%)	19 (40%)	

P value with Fisher's test. Abbreviations: CR, complete remission; PR, partial remission; SD/PD, stable disease/progressive disease; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; aGVHD, acute graft versus host disease; cGVHD, chronic graft versus host disease; HSCT, hematopoietic stem cell transplant; CT, chemotherapy

Forty-four patients experienced relapse at a median of 186 days (range 34-1022): 13 had pre-relapse CMV replication and 31 did not, compared with 29 and 19 in patients who did not relapse, respectively (p=0.003). With a median follow-up of 1068 days (range 8-4705 days), in univariate analysis (Kaplan-Meier) CMV replication was associated with longer relapse free survival (3yy-RFS; 62% *vs* 39%, p=0.02) and no difference in terms of non-relapse mortality (NRM), progression-free survival (PFS) and overall survival (OS). In particular, we observed that the benefit of CMV replication was more evident in patients with HL (p=0.03) and with disease in complete remission pre- transplant (p=0.04). In multivariate analysis including disease type, pre-transplant disease status, type of conditioning and pp65 antigenemia, the last one was confirmed as a strong independent predictor for RFS (HR: 0.3, 95% CI 0.1-0.7; p=0.01),

together with pre- transplant disease status and disease type (HL, CLL and aggressive NHL compared to follicular lymphomas). In multivariate analysis, pre- transplant disease status was an independent factor for PFS, OS and NRM. CMV replication was an independent variable negatively affecting NRM (HR: 4.3, 95% CI 1.05-17.9; p=0.04). In conclusion CMV replication after allogeneic HSCT for lymphoma represents a multi-faceted event influencing patients outcome, both by reducing relapse risk, especially for patients with HL, and increasing the chance of NRM.

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SECOND ALLOGENEIC HSCT IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES RELAPSED AFTER A FIRST ALLOGENEIC TRANSPLANTATION: A RETROSPECTIVE STUDY

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Background. Leukemia relapse after allogeneic hematopoietic stem cell transplantation (HSCT) remains a significant problem and has been unchanged over the past three decades. Aim of the study. We retrospective analyzed the outcome of 60 patients with hematological malignancies , who relapsed after allogeneic HSCT , and received a second HSCT and evaluated overall survival (OS) and NRM (non relapse mortality). Patients and Methods From January 2008 to December 2012 60 patients with hematological malignancies underwent to a second allogeneic HSCT. Patients characteristics are listed in Table 1. In univariate and multivariate analysis for OS and NRM we analyzed: 1) interval time from 1st transplant to relapse (<> 6 months), 2)diagnosis: acute leukemia (AL), chronic leukemia (CL) and severe aplastic anemia (SAA), 3) presence of active disease at second transplant, 4) second donor type: HLA identical sibling (SIB.), cord blood (CB),unrelated mismatched (UD), haploidentical related (Haplo), 5)patients age, 6)diagnosis of Invasive Aspergillosis(IA) (probable or proven) after second transplant. Results With a median of follow up of 636 days (range: 54-3472 days) post second transplant actuarial OS and cumulative incidence of NRM are 25% and 40% respectively. In univariate analysis negative predictors of OS are: active disease at second transplant (p=0.016), second donor type (p=0.0021), patients age (p=0.023) and diagnosis of IA (p=0.008). In multivariate analysis on OS significant negative predictors are : active disease at second transplant (p=0.003), unrelated or CB donors, as compared to HLA identical siblings and Haplo (p=0.004) and a diagnosis of invasive aspergillosis after second transplant (HR 2.0, p=0.04). Negative predictors of NRM in univariate analysis are: interval time from 1st transplant to relapse <6 months (p=0.05), donor type (unrelated and CB) (p=0.0012), diagnosis of IA (p=0.012). In multivariate analysis only donor type remains significant (p=0.02)

Table 1. Characteristics of patients and multivariate analysis for OS and TRM.

	Total=60	Alive, n= 17	Dead, n=43	OS Multivariate analysis HR(95%CI) P-value	TRM Multivariate analysis HR(95%CI) P-value
Median age, years (range)	45 (18–64)			-	-
Underlying disease				-	-
Acute leukemia	38	12(32%)	26(68%)		
Severe aplastic anemia	4	0	4(100%)		
Chronic leukemia	18	5(28%)	13(72%)		
Donor				**0.0041**	**0.021**
Cord Blood	14	1(7%)	13(93%)	1.00	1.00
Unrelated Donor	12	1(8%)	11(92%)	0.64(0.28-1.48)	1.50(0.48-4.68)
Identical Sibling	19	8(42%)	11(38%)	0.24(0.10-0.72)	0.28(0.08-1.01)
Haploidentical	15	7(47%)	8(53%)	0.28(0.11-0.72)	0.19(0.04-1.03)
Time from first transplant to relapse .					
< 6mo	21	5(23%)	15(77%)	-	-
>6mo	39	12(30%)	27(70%)	-	-
Active disease at second transplant				**3.06 (1.47-6.37) 0.003**	-
RC	31	10(32%)	21(68%)		
Active disease	39	7(17%)	32(83%)		
Aspergillosis post second transplant				**2.06 (1.00-4.25) 0.049**	
Yes	15	1(6%)	14(94%)		
No	45	16(36%)	29(64%)		

Conclusions This retrospective analysis shows an acceptable OS for very advanced patients undergoing a second allogeneic transplant. Second transplant is associated with lower NRM and improved survival if the disease is in complete remission at time of second transplant, and if relapse has occurred beyond 6 months from the first transplant. This is a group of patients in which primary antifungal prophylaxis , with mould active agents is warranted.

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A POSSIBLE CORRELATION BETWEEN DIFFERENT TOLL-LIKE RECEPTORS, MONOCYTES, LYMPHOCYTE SUBSETS, TYPE OF INFECTIONS AND OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Bacterial, fungal and viral infections are important complications after allogeneic stem cell transplantation (SCT), whose outcome can be influenced by effective and balanced immune responses against infections. Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs), such as protein, carbohydrate or DNA/RNA pattern motifs. Extracellular PAMPs are recognized by surface TLRs (TLR-1,TLR-2,TLR-4,TLR-5, and TLR-6). Intracellular TLRs (TLR-3,TLR-7,TLR-8 and TLR-9) bind mainly to foreign nucleic acids. TLRs are also receptors for endogenous ligands and damaged tissue, suggesting that both pathogen-derived molecules and products of damaged tissue can modulate innate and adaptive immune responses. We assessed the expression of TLRs in 35 patients by flow cytometry as mean fluorescence intensity at day +30. Functional data were obtained by ELISA assay after TLRs activation. The cell supernatants were collected and assayed for TNF-alpha, IL-4, IFN-gamma, and MCP-1. Monocytes values, lymphocyte subsets and TLR expression and function were analysed in relation to early infections (day +100) and outcome of SCT. Bacterial infections were associated with lower values of monocytes, CD4+lymphocytes and NK cells, decreased expression of TLR-7,9 on T-lymphocytes and monocytes, and increased expression of TLR-4 on monocytes (p<0,04). Patients with fungal infections showed lower values of CD4+,CD8+ lymphocytes, B cells, NK cells and a decreased expression of TLR-7 on T-lymphocytes (p<0,04). T-lymphocytes and monocytes of patients with CMV reactivation had an increased expression of TLR-5 and lower values of IFN-gamma induction upon TLR-3,-4,and -9 activation (p<0,05). CMV reactivation was also associated with lower values of monocytes and NK cells (p<0,04). Relapse rate correlated with increased expression of TLR-6 on T-lymphocytes. Transplant related mortality was associated with decreased expression of TLR-1 on T-lymphocytes and lower values of monocytes and IFN-gamma after TLR-3 activation (p<0,05). Bacterial, fungal, and CMV infections correlated with specific combinations of lymphocyte/monocyte values, TLR expression and function. An atypical involvement of some TLRs in the interaction between these pathogens and the immune system was also observed. A particular pattern of lymphocytes and TLRs could be associated with the outcome of SCT as well.

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IMPACT OF HLA-DPB1 DISPARITIES IN UNRELATED DONOR (UD) HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Background. High-resolution HLA matching for HSCT recipients and UDs is associated with the best clinical outcomes. The impact of polymorphic HLA locus DPB1 remains controversial. We retrospectively analyzed the impact of HLA-DPB1 disparities on the outcome of a single center series of 71 consecutive patients who underwent 10/10 HLA allelic-matched unrelated HSCT at our institution between 1994 and 2013. Overall survival, relapse and acute and chronic GvHD incidence were

the main clinical endpoints analyzed. Materials and Methods. median age was 37 (range, 1-65) years; the underlying disease was a malignant disorder in 93% of cases (n = 66). A reduced-intensity conditioning regimen was used in 25% of cases (n =18). The conditioning regimen included antithymocyte globulin in 65 patients (91%) and alemtuzumab in 2 patients. For GvHD prophylaxis all patients received Cyclosporine and short-course methotrexate. Stem cell source was either PBSC in 28 patients (39%) or BM in 43 (61%). All grafts were T-cell repleted. HLA-DPB1 mismatches were evaluated for each D/R pair. Among the 71 D/R pairs, HLA-DPB1 allele mismatches were found in 56 cases (1 mismatch, n = 28; 2 mismatches, n = 28). As defined by the presence of T-cell-epitope mismatching, 25 (35%) pairs had non-permissive HLA-DPB1disparities. Results. the incidence of aGvHD II-IV was 13.3+ 0.8% and 35.8+ 0.4%, (p=0,26) in matches and mismatches pairs, respectively. The incidence of cGvHD was 6.6 + 0.4% and 24.2+ 0.4%, (p=0.47) in matches and mismatches pairs, respectively. The relapse incidence was significantly reduced in HLA-DP1 mismatches pairs as compared to matches pairs, 39.1+1.1% *vs* 61.1+3.5% respectively (p=0.046). No difference in aGVHD and cGVHD and relapse incidence was observed in permissive versus non permissive and in 1 allele mismatch versus 2 alleles mismatches HLA-DPB1 disparities. After a median follow-up of 11 years, DFS was 29+13.5% and 33+7.4% in matches and mismatches pairs, respectively (p=0.184). No difference was observed in OS between the two groups (47+17% and 44+8%, p=0.115). Conclusion. This study does not confirm the adverse prognosis of HLA-DPB1 non permissive disparities after 10/10 HLA matched UD HSCT. In HLA-DPB1 mismatches, independently of permissive or non permissive disparities, we observed a significantly reduced relapse incidence, although there was no impact on DFS and OS. In order to have a definitive conclusion a largest homogeneous patient population is required.

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LUNG TRANSPLANTATION FOR THE TREATMENT OF CHRONIC GRAFT VERSUS HOST DISEASE OF THE LUNG

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Introduction and objectives. Chronic graft versus host disease (GVHD) of the lung, also known as bronchiolitis obliterans syndrome (BOS), is a serious complication after hematopoietic stem cell transplantation (HSCT). Therapy of BOS includes topical treatment and systemic treatment with corticosteroids and immunosuppressive therapy. However, when medical treatment fails, lung transplantation (LTX) may be indicated as the ultimate treatment option. Methods. We searched our institutional data base for lung and bone marrow transplantation and three patients were found with an history of both procedure performed. We collected demographic and outcome data about transplant procedures. Results. Between 1998 and 2005, 3 patients (2 females and 1 male), with a median age of 24 years, (range 21-49) underwent alloHSCT because of myelodisplastic syndrome, B-precursor acute lymphoblastic leukemia and multiple myeloma. In all cases, donors were HLA identical siblings while the cell source was the bone marrow for one patient and the peripheral blood for the other two. No patient presented acute GVHD and in all cases a de novo chronic GVHD developed and eventually, respiratory failure. LTX was needed after a median time of 4 years (range 3-9) from HSCT. Bilateral LTX was performed in 2 out of 3 patients, cardiopulmonary bypass in 2 and only one of them required tracheostomy, but before LTX. Of the three patients, one died in hematological complete remission 6 years after LTX (10 years after HSCT). The clinical course of this patient was soon complicated by acute and then chronic lung rejection and by severe infections, with development of progressive respiratory failure. This patient was taken into account for a second LTX but unfortunately he died for multi organ failure after a surgical procedure. The other two patients are alive and in hematological complete remission after 4 and 5 years from LTX, without other signs of chronic GVDH. They are leading an active life, currently maintained with three immunosuppressive agents and their pulmonary function tests show normal or middle reduction of the diffusing capacity of the lung for carbon monoxide (DLCO) and of the lung volumes, with stable values over time. Conclusion. Our data indicate that lung transplantation may represent an effective treatment option of BOS with remarkable improvement of the quality of life. In selected, good prognosis HSCT patients, lung transplantation should not be unnecessarily delayed or refused.

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TOWARDS CLINICAL APPLICATION OF TCR GENE EDITING FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION TO TREAT ACUTE LEUKEMIA AND MULTIPLE MYELOMA

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TCR gene transfer has yielded promising clinical results in cancer patients. To completely tailor T cell specificity, we developed a TCR gene editing approach, based on the combination of: i. Somatic knockout of the endogenous TCR genes (by transient exposure to and chain specific Zinc Finger Nucleases), and ii. Introduction of tumor-specific TCR genes by lentiviral vectors. With this approach, TCR gene transfer can follow the knockout of either a single endogenous TCR chain, with the generation of "single-edited" T cells (SE), or both and chains, yielding "complete-edited" (CE) lymphocytes. By avoiding competition for surface expression between exogenous and endogenous TCR and chains, and by abrogating the risk of inappropriate TCR pairing, the TCR editing approach permanently overcomes several major limitations of TCR gene transfer. TCR edited T cells targeting WT1 recognized leukemic targets without mediating Graft versus Host Disease (GvHD) (Provasi *et al*., Nat. Med. 2012). To simplify its clinical application, we established a scalable protocol that generates edited T cells in a single round of T cell stimulation by targeting a single endogenous TCR chain. This approach fully abrogates expression of the endogenous TCR repertoire, responsible of GvHD in allogeneic transplantation. To generate not only potent anti-tumor effectors, but also memory T cells able to continuously patrol against tumor cells, we applied the editing approach to a recently developed protocol, designed to enrich genetically modified cells in the newly described memory T cell subset (TSCM), characterized by self-renewing, stem cell-like abilities (Cieri, Blood 2012). In addition, we validated the TCR gene editing approach by targeting NY-ESO-1, expressed by solid tumors and hematological malignancies. We observed higher levels of NY-ESO-1 specific TCR expression in edited versus transferred T cells (Relative fluorescence intensity to untransduced cells: CE: 30; SE: 25; unedited-TCR transferred: 14). Edited T cells were more efficient than unedited-TCR transferred T cells in killing NY-ESO-1-pulsed cell lines and NY-ESO-1+ myeloma cell lines, while displaying no activity against NY-ESO-1- targets. These results suggest that the TCR gene editing approach can support a more potent anti-tumor effect while potentially eliminating the risk of GvHD associated with the infusion of TCR engineered autologous or allogeneic lymphocytes to patients with hematological malignancies.

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THE ROLE OF PET AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN PERIPHERAL T-CELL LYMPHOMA: HIGH RATE OF FALSE POSITIVE RESULTS

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Several studies have shown that positron emission tomography (PET) plays an important role in staging and response assessment after therapy in many lymphomas. Dodero *et al* (Cancer 2010) and Lambert *et al* (Blood 2010) reported the use of PET scan to detect persistent residual tumor mass or early relapse of B-cell lymphoma after allogeneic stem cell transplantation (AlloSCT). To date the role of PET in peripheral T-cell lymphoma (PTCL) is not fully elucidated neither before nor after alloSCT. In this study we therefore investigate the impact of PET after alloSCT in patients who underwent alloSCT for relapsed PTCL. We retrospectively analyzed 23 PTCL patients (PTCL-NOS n=12, ALCL Alk neg n=6, ALCL Alk pos n=1, Extranodal NK/T-cell lymphomas n=1, Enteropathy T-cell lymphomas n=1, Hepatosplenic n=2) receiving alloSCT in our centre from June 1999 to March 2013. Patients received a conditioning regimen including thiotepa, fludarabine and cyclophosphamide [using matched sibling (n=9), matched unrelated (n=12) and haploidentical donors (n=6)]. All but one patients were investigated by PET before alloSCT and only 2 were PET positive. PET was performed regularly after alloSCT in 16 patients until a median of 2 years. Seven patients were never evaluated by PET due to early disease progression (n=3) or non-relapse mortality (n=4). Five of 16 patients (31%) were characterized by persistent PET negativity after alloSCT. Conversely pathological FDG uptake was documented in the remaining 11 patients (69%) (multiple sites n=8). In all these patients concomitant or subsequent CT evaluation was performed in order to confirm disease progression. CT and clinical assessment excluded or confirmed the lymphoma relapse in 3 and 4 patients, respectively. In 4 patients PET, CT and clinical evaluation were not definitively diagnostic and for this reason they underwent a biopsy that was negative in all cases. In one patient, the biopsy was diagnostic for sarcoidosis; conversely in the others an inflammatory reaction with polyclonal lymphoid hyperplasia was documented. Despite the limited number of patients, this study suggests that PET evaluation after alloSCT may be characterized by a high rate of false positives (7/16; 43%) that may represent a significant limitation for its use in the follow up of allografted PTCL patients.

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IN VITRO STUDY OF THE MECHANISMS INVOLVED IN THE BONE MARROW MESENCHYMAL STROMAL CELL MODULATORY EFFECT ON B CELL FUNCTIONS

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Human bone marrow Mesenchymal Stromal Cells (MSC) are potent modulators of T cell activation and proliferation, mainly through the production of partially defined soluble factors, including the IFN- -induced tryptophan-degrading enzyme IDO, a key immunosuppressive effector pathway. Actually, MSC may affect the functions of virtually all immune effector cells, including B cells. However, current literature concerning MSC immunomodulatory activity on B cells is still controversial, due to both biological peculiarities of B cells, which do not produce IFN- , a key MSC-triggering cytokine, and to different and poorly comparable experimental approaches. Human purified B cells, either resting or activated for 4 days with a stimulation cocktail (CD40 ligand + enhancer polyhistidine mAb MAB050+rhIL-2+mouse F(ab')2 anti-human IgM/IgA/IgG+CpG oligodeoxynucleotide 2006) were co-cultured with MSC, either at resting conditions or following inflammatory priming (MSC pre-incubation with IFN- +TNF- for 48 hours), or with MSC supernatants. CD27-positive (memory) and CD27-negative (naïve) B cell survival, proliferation, and intracellular activation status (through signaling network analysis by Phosphoflow) were assessed. Our results showed that MSC are normally supportive cells, not intrinsically capable of suppressing B cell proliferation, and require inflammatory priming to acquire B cell inhibitory potential. Inflammatory-primed MSC impair significantly activated B cell growth in a cell contact-independent manner, as supernatant is sufficient to provide maximal inhibition of B cell proliferation. B cell inhibition by MSC is not related to either induction of B cell apoptosis or early signaling events necessary for B cell activation. In addition, IDO pathway triggered in IFN- -primed MSC seems to have a role also in B cell inhibition by interfering with the tryptophan metabolism. Overall, B cell behavior following the interaction with MSC depends on the functional state of both B cells and MSC. The role of IDO in B cell regulation needs further investigation, as it may be relevant to develop new therapeutic approaches in pathological conditions related to B cell hyper-activation.

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HAPLOIDENTICAL UNMANIPULATED BONE MARROW TRANSPLANTATION FOLLOWING THIOTEPA-BUSILVEX-FLUDARABINE CONDITIONING REGIMEN FOR PATIENTS WITH HIGH-RISK ACUTE MYELOID LEUKEMIA

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Introduction. The outcome of high-risk AML patients not undergoing an allogeneic transplant is extremely poor. We report results of unmanipulated, G-CSF primed, haploidentical BMT in high-risk AML patients lacking an HLA identical sibling. Materials and Methods. Between January 2006 and March 2013, 50 patients (median age: 44 yrs, range 5-66) with high-risk AML (CR1=29; CR2=12; advanced stage=9) underwent BMT from haploidentical donor in 2 transplant Centers (Rome n=32; Pescara n=18). For patients grafted in CR1, AML was refractory (n=8), secondary (n=9), with cytogenetic-molecular high-risk characteristics (n=10) or MRD+ after consolidation (n=2). All patients were prepared with a myeloablative (MAC) or a reduced intensity (RIC) conditioning regimen consisting of Thiotepa at days -7 and -6, i.v. Busulfan combined with Fludarabine at days -5, -4 and -3 (TBF-MAC n=35). In the TBF-RIC (n=15), one dose of Thiotepa and one dose of Busulfan were omitted. All patients received an identical GvHD prophylaxis. Donors were primed with G-CSF at 4 mcg/Kg/day for 7 days. BM was infused unmanipulated. Results. the median number of CD34+ and CD3+ cells infused was 2 (0.7-11) and 29 (9-170)x10^6/Kg, respectively. Two patients died early after transplant and engraftment was reached at a median of 20 days (13-35) with full donor chimerism, with a 30-day CI of 96+/-%. The CI of III-IV grade A-GVHD was 14.6+/-0.3%. The CI of extensive C-GVHD for 39 evaluable patients was 16.4+/-0.5%. At a median follow-up of 34 mon (3-63 mon), the CI of TRM and relapse was 28+/-0.4% and 41+/-1%, respectively. For all 50 patients, the 1- and 5-yr probability of OS was 59+/-7% and 49+/-9%, respectively and that of DFS was 55+/-7% and 39+/-7%. For the 41 patients transplanted in CR1 and CR2, these probabilities were, respectively, 70+/-7% and 57+/-8% for OS, 65+/-8% and 49+/-9% for DFS. Thirty patients in remission were conditioned with TBF-MAC regimen and the 5-yr probability of OS and DFS was 65+/-9% and 52+/-10%, respectively. The 3-yr OS and DFS of 11 patients in remission, 9 of whom aged >55 yrs (55-66) and prepared with the TBF-RIC regimen, were 45+/-15% and 45+/-15%, respectively. Conclusions. This transplant procedure not requiring expensive laboratory tools and advanced expertise in cell manipulation can be widespread extended to all transplant Centers and could be offered as alternative option to high-risk AML patients lacking an HLA identical sibling and on urgency to be transplanted.

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HAPLOIDENTICAL STEM CELL TRANSPLANTATION AFTER NEGATIVE DEPLETION OF T CELLS EXPRESSING THE ALFA-BETA+ CHAIN OF THE T-CELL RECEPTOR (TCR) FOR ADULTS WITH HEMATOLOGICAL MALIGNANCIES

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Premise. For many years,T cell depletion (TCD) of hematopoietic stem cells (HSCs) has been based on either positive or negative selection of mobilised peripheral blood cells (PBPCs). Selective elimination of + T cells has been recently performed to achieve a 4,5–5 log TCD and to retain in the graft NK, dendritic cells, monocytes and T lymphocytes.A rapid immunological reconstitution and very promising outcome were observed in pediatric patients.With the aims of confirming these results even in adults, we have recently launched this programme and here we report our preliminar clinical data. Methods. Eight patients, median age 40 yrs (range19-65), with AML(n=6), ALL(n=1)or HL(n=1) entered the study.Conditioning consisted of Treosulfan 12gr/sqm x3 days,Fludarabine 30mg/sqm x5 days,Thiotepa 5mg/Kgx2 days and ATG 1,5mg/kg x4 days. G-CSF was used to mobilize PBPCs from one-haplotype mismatched donors (3 mothers,4 brothers,1 cousin). Mobilized mononuclear cells were incubated with a biotinylated anti-TcR antibody and subsequently with an antibiotin antibody conjugated to magnetic microbeads(Miltenyi Biotec,Germany).Under a strong magnetic field,TcR T lymphocytes were retained,whereas all nonmagnetized cells were recovered. Short sirolimus(1mg/day x3 weeks)was used as GVHD prophylaxis in 3 cases whose grafts contained >$2x10^5$/kg +Tcells. Results.Grafts contained a median of $14,4x10^6$/kg (range7- 15,7)CD34+ cells, $6,6x10^6$ CD3+Tcells/kg (range 2,14-13) with $19x10^4$/kg (SD 21,24) +T cells and $7,78x10^6$ Tcells/kg (range2,1-12,6), $0,08x10^6$ B cells/kg (range 0,003–0,32) and $24x10^8$ CD56+NKcells/kg (SD 1,04). All but one patient,who required a second graft from the same donor to boost hematopoietic reconstitution,achieved a full donor sustained engraftment. Median time to reach 500 neutrophils and 50,000 platelets was 13 (range 9-18) and 11 days(range 9-13),respectively. Two patients had skin grade I/II aGVHD. No patients has so far developed cGvHD. 100 CD4+cell/mcL were reached in a median of 60 days.Only 1 CMV reactivation occurred. Overall,3 patients have so far died (2 non-hematologic causes and 1 relapse).All were in relapse at transplant. Five survive event-free at a median follow-up of 92 days (range 30-163). Conclusions.The infusion of /CD19-depleted grafts was safe and effective also in adult setting,resulting into rapid donor engraftment and early expansion of donor-derived T lymphocytes,without life-threatening infectious complication.

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EARLY RECONSTITUTION OF CMV IMMUNITY AFTER HLA-HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION IS A STRONG SURROGATE BIOMARKER FOR A LOWER INFECTIOUS MORTALITY

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Introduction. HLA-haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is a readily available option for patients with high-risk hematological malignancies who lack an HLA-compatible donor. Unfortunately, the profound state of immune incompetence associates with a high infectious mortality. Different strategies have been developed to speed-up immune reconstitution after haplo- HSCT. The development of these strategies is however limited by the current lack of validated T-cell biomarkers predictive of clinical events. Aim. To analyze the immune reconstitution following haplo-HSCT in order to find early surrogate biomarkers of infectious mortality. Results. We prospectively studied T-cell immune reconstitution in 89 pts treated with haplo-HSCT from day 30 until day 360 post transplantation. Eighteen patients (20%) were given suicide gene-modified DLI, while 71 patients (80%) received an unmanipulated graft followed by rapamycin. The incidence of grade III-IV acute GVHD and chronic extensive GVHD were 12% and 28% respectively. T-cell counts recovery was accelerated: at day 90, median CD3+ cells were 378 per microL (0-2817), CD4+ 127 (0-804), CD8+ 173 (0-1922). There was a progressive normalization of both memory differentiation phenotype and TCR spectratyping complexity score. Nevertheless, none of these biomarkers performed enough to be considered for surrogating a lower infectious mortality. In this series at high risk for CMV reactivation (CMV serostatus: H+/D+ 68%, H+/D- 27%), the event was observed in 46 pts (52%) and CMV disease in 8 pts (9%), all treated according to guidelines. By using Receiver Operating Characteristics (ROC) curve analysis of CMV-specific IFN-gamma ELISPOT results, we found that cut-off values of 1000 spots/mL allowed to discriminate with high specificity (>95%) pts that did not reactivate the virus. Strikingly, while in pts with <1000 spots/mL, the 2-yrs infectious mortality was 32%, in those with >1000 spots/mL, this was 0% (P<0.05). Interestingly, the infectious mortality of pts that achieved or not a CD4+ cell value of 200 per mL were not significantly different (21% *vs* 30%, P=0.8). Conclusions. These indicate that the early reconstitution of T-cell immunity to CMV after haplo-HSCT is a strong surrogate biomarker for a lower infectious mortality. Moreover, they warrant the investigation of a CMV-specific IFN-gamma ELISPOT cut-off value of 1000 spots/mL as a predictive biomarker in larger, multicenter study.

P280

PURE RED CELL APLASIA IN ABO-MISMATCHED HAEMATOPOIETIC CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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ABO mismatching between donor and recipient in allogeneic hematopoietic stem cell transplant (HSCT) setting is common, occurring in up to 40% of all HSCTs. ABO mismatch is major when the recipient had naturally occurring antibody against the red cell antigen of the donor's red blood cells (donor A,B, AB; recipient O); it is minor when the donor had naturally occurring antibody against the red cell antigen of the recipient's red blood cells (donor O; recipient A,B, AB). Even if it is not considered a contraindication, ABO mismatch is a risk factor for delayed red cell engraftment and acute/delayed hemolytic reactions including the particular setting of pure red cell aplasia (PRCA); it is a serious, life threatening rare condition characterized by severe anemia with absence of erythroid precursors in the bone marrow. The incidence of post-HSCT PRCA may vary according to the conditioning regimen and is reported to vary between 6 and 30%. Modern reduced-intensity and reduced-toxicity regimens cause much less myeloablation than conventional myeloablative regimens. Therefore, recipient cells producing donor red cell-specific antibodies are more likely to survive after a major ABO-mismatched transplant and cause pure red cell aplasia (PRCA). The incidence of PRCA with reduced-toxicity regimens is not established. We therefore collect data about PRCA and ABO mismatch in patients submitted to allogeneic HSCT from 2007 to march 2013 at our institution with suitable bone marrow aspirate on day +30. They were 85 patients (51 male, 34 female) with a median age of 49 years (range 16-65 years) submitted to allogeneic HSCT for hematologic malignancies. PRCA was diagnosed when the bone marrow biopsy on post transplant day 30 demonstrated adequate myeloid, lymphoid and megakaryocyte populations in the setting of absent or nearly absent erythroid precursors. PRCA was thus recognized in 7 patients (4 male, 3 female) with a median age of 57 years (range 24-63 years). Analyzing data according to ABO mismatch, we found a statistically significant difference in major ABO mismatch incidence between patients showing PRCA (5 out of 7 patients) and patients without PRCA (18 out of 78): 71.5% *vs* 23.1%, p=0.006 (chi2 test). Two groups were comparable in terms of age, sex, CD34+ cell dose and time to neutrophil and platelet engraftment. These preliminary data confirm the impact of major ABO mismatch on PRCA incidence and underline the required attention for this subset of patients.

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IN VIVO RAPAMYCIN-INDUCED TREGS DO NOT TRAFFIC TO THE BONE MARROW: PRESERVATION OF THE GVL EFFECT AFTER T-CELL REPLETE HLA-HAPLOIDENTICAL HSCT

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Introduction. We have recently completed a Phase I/II study of T-cell replete hematopoietc stem cell transplantation (HSCT) from an HLA-haploidentical donor in 113 pts with hematological malignancies followed by GVHD prophylaxis with rapamycin. Incidences of grade III-IV acute GVHD and chronic extensive GVHD were 21% and 35%, respectively. There was expansion of circulating CD4+/CD25+/FoxP3+/IL-7R - Tregs (median 6.5% range 0.2-37.2, P<0.01 compared with controls), which were suppressive ex vivo. Tregs frequencies correlated with GVHD severity. One-year PFS was 44% in pts with early disease (n=18) and 30% in pts with advanced disease (n=95). Aim. A long-standing question is whether Tregs-based strategies may interfere with the GVL effect. We thus aimed at verifying if rapamycin-induced Tregs traffic to the bone marrow (BM) suppressing effector T cells locally. Results. The BM of rapamycin-treated pts was depleted of Tregs (at day 30, BM Tregs frequencies: median 0.3% range 0.0-2.2, P<0.01 compared with PB Tregs). High-level CXCR4 expression on circulating Tregs suggested a specific antimigratory effect of rapamycin. On the contrary, the BM was heavily infiltrated by CD45RA-/CD62L- effector memory CD8+ Tcells that expressed VEGFR-2. Circulating Tcells co-cultured with VEGF-producing BM-resident stromal cells promptly up-regulated VEGFR-2, resulting in a dose-dependent suppression of proliferation. Interestingly, VEGFR antagonists, such as sorafenib and sunitinib, but not dasatinib, partially reverted the antiproliferative effects of stromal cells. Conclusions: While protecting from GVHD, rapamycin-induced Tregs do not traffic to the BM, thus leaving the GVL effect unharmed. The potential immunosuppressive effect of BM-resident stromal cells merits further investigation.

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DECIPHERING T-CELL DYNAMICS IN THE FIRST MONTH AFTER T-CELL REPLETED HAPLOIDENTICAL HSCT

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Several biological events that play a critical role for transplant outcome occur within the first month after allogeneic hematopoietic stem cell transplantation (HSCT). While stem cell engraftment and hematological reconstitution are carefully monitored, the shape of T-cell dynamics within this timeframe remains largely unknown. We are currently studying patients with high-risk acute leukemia, undergoing T-cell repleted haploidentical HSCT within a rapamycin-MMF immunosuppressive regimen and post-transplant cyclophosphamide (PT-Cy) to tame alloreactivity. This platform, devoid of anti-tymocyte globulins, allows a thorough analysis of circulating cells in this delicate early post- transplant phase. Strikingly, results from the first 10 analyzed patients show a predominance of CD4 T-cells that are significantly more represented than CD8 cells from day 1 to day 15. This picture is gradually reversed in the following days, and by day 30 CD8 T cells become the most represented T-cell subset. Given the initial unexpectedly high CD4:CD8 ratio, we investigated the relative contribution of different T-cell subsets and correlated their distribution with that of the infused graft. No early expansion of circulating regulatory T cells was observed. On the contrary, we observed an expansion of antigen-experienced CD8 and CD4 T-cells including central memory, effector memory, effector cells and the recently described stem memory T-cells (TSCM). Similarly to naïve cells (TN), TSCM are characterized by the co-expression of CD45RA and CD62L but differently from TN, TSCM express CD95, a marker of memory T cells. We found that by day 5 after HSCT, TN cells disappear by the peripheral blood, while TSCM readily accumulate and reach a peak by day 15. Of note, PT-Cy efficiently abates T cell proliferation (assessed by Ki-67 by staining). Unexpectedly, T-cell numbers progressively increase (median CD3 count at day 5: 19 cells/μL; day 15: 65 cell/μL), suggesting residual T-cell proliferation in extravascular sites. Importantly PT- Cy does not eliminate viral-specific T cells, as CMV-pp65 specific T cells were detectable as early as at day 15. Of note, we were able to detect also tumor-specific T-cells against PRAME and WT-1 in HLA-A02 or A01 patients and such antitumor responses correlated with complete remission (up to 120 days post-HSCT). Analysis of T-cell dynamics in a larger cohort of patients will prompt the identification of immunological correlates with clinical outcome.

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IMPACT OF CD3/T REGS RATIO IN DONOR GRAFT ON SURVIVAL RATES IN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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The therapeutic efficacy of allogeneic stem cell transplantation (alloSCT) for hematological malignancies relies largely on the graft versus leukemia (GvL) effect exerted by the donor CD3 cells, but an uncontrolled graft-versus-host-disease (GvHD) bears a risk of complications. On the other hand, T regs cells (CD4+CD25high Foxp3+) are believed to maintain tolerance and to inhibit GvHD after alloSCT; also, the Foxp3 gene encodes a transcription factor that is a key for thymic development, so T regs cells could preserve an optimal microenviroment for the reconstitution of functional immunity after alloSCT. Moreover, when looking at post allotransplant patients' outcomes, there is no evidence that donor graft CD3/T regs ratio may determine an effect in terms of OS, NRM and relapse free survival rates so far. In this study we analyzed the graft CD3+/Tregs ratio (gCD3/Tregs R) and determined its impact on acute GVHD (aGVHD), immunological recovery and survival rates (OS, NRM and Relapse) after myeloablative alloPBSCT. We analyzed 90 consecutive patients transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor (n=59) or an HLA identical unrelated donor (n=31); diagnoses were acute myeloid leukaemia (n=75), acute lymphoblastic leukaemia (n=15). The median CD3+ and Tregs dose administered was 238 (range (r): 67-550) and $12{,}5x10^6$/Kg (r: 2-21), respectively; the median gCD3/Tregs R was 19 (r: 8-250). Patients were subdivided into a high gCD3/Tregs R(>36)group(n=36) and a low gCD3/Tregs R(<36) group (n=54). The incidence of aGVHD (grade II-IV) in the low gCD3/Tregs R (LR) group was lower than in the high gCD3/Tregs R (HR) group (8/54 or 14% *vs* 3/36 or 83%, p<.001). The OS, NRM and relapse rate at 3 years was 54, 29 and 34%, respectively. Comparing LR with HR group a statistically significant difference is demonstrated for OS and NRM rates (65 *vs* 31%,p<.004; 3 *vs* 71%, p<.001), respectively, but not for the R one (35 *vs* 30%, p=ns). Comparing aGVHD+ with aGVHD- group OS, NRM and relapse were always statistically significant different (39 *vs* 65%,p<.005; 61 *vs* 7%,p<.001; 9 *vs* 53%,p<.002). Taken together, our data may suggest that Tregs content is able to mediate protective effects against aGVHD, while preserving GvL effects as demonstrated by relapse rate comparison between H and LR groups. However, larger studies are needed to understand the real contribution of gCD3/Tregs R on survival rates.

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TELOMERE ANALYSIS SHOWS THAT HEMATOPOIESIS IN RECIPIENTS OF UMBILICAL CORD BLOOD IS BIOLOGICALLY YOUNGER COMPARED TO THE AGE-MATCHED HEALTHY POPULATION

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Background. Several studies indicate that high proliferative stresses, including bone marrow (BM) regeneration after transplantation, produce a non-physiological telomere length (TL) shortening. However, recent reports suggest that post-autograft TL correlates with that of grafted cells (Ruella *et al*, 2010). In the present study, TL has been evaluated in adult subjects engrafted with umbilical cord blood (UCB), a graft source characterized by cells harboring very long TL. Aim of the study was to verify whether despite the high replicative stress during engraftment, UCB cells may renew the recipient BM, maintaining the biologic characteristics of the graft. Patients and Methods. TL was evaluated on peripheral blood (PB) cells from 43 adults who received UCB transplant (UCBT) for high-risk hematologic malignancy. TL analysis was performed at a median of 15 mos. (range 0-156) since UCBT, in subjects in continuous remission of their previous disease and with complete and stable hematological recovery. TL was evaluated by Southern blot analysis as terminal-restriction fragment (TL-TRF) length, on both whole and separated PB cells, *i.e.* mononuclear cells (MNC) and granulocytes. Results. Overall, TL in PB cells following UCBT showed a rapid fall compared to TL of CB cells. When values were distributed according to the age, TL was markedly higher in UCBT recipients than in age-matched normal subjects, with median TL-TRF on MNC of 9,118 bp (range 6,573-13,695) and 7,179 bp (range 4,375-10,467), in UCBT recipients and healthy controls, respectively (p=0.0003), as shown in Figure 1. Similar results were obtained when TL was assessed on whole PB cells and separated granulocytes. In fact, the most marked difference in TL between UCBT recipients and controls was observed on granulocytes. In particular, among subjects aged 40-65 yrs., the median TL-TRF of granulocytes was 8,111 bp (range 6,260-11,138) in UCBT recipients compared to 6,864 bp (range 5,773-10,946) in age-matched controls. Lastly, no difference in TL was observed according to time elapsed since transplant, with analogous TL values at less or more than 12 months since UCBT. Conclusions. Despite the high replicative stress during BM engraftment, UCBT regenerate a hematopoietic system characterized by long TL. This suggests that in the transplant setting the use of young donor cells such as UCB may replace patient BM with not only a healthy but also a biologically young hematopoietic system.

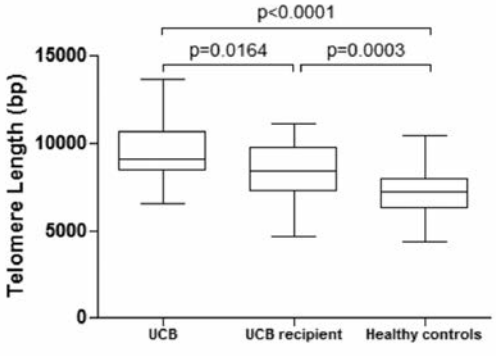

Figure 1. Comparison of TL of mononuclear cells from Umbilical Cord Blood (UCB), recipients of UCB transplant and age matched (17-65 yrs) healthy controls

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HAPLOIDENTICAL IS NON-INFERIOR TO MATCHED RELATED AND UNRELATED DONOR TRANSPLANTATION: AN INTENTION-TO-TREAT ANALYSIS OF 241 PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Understanding of leukemia biology and advances in the transplant field have improved safety and access to allogeneic hematopoietic cell transplantation (HCT) for patients (pts) affected by acute myeloid leukemia (AML). The trend of growth of HCTs can be expected to continue based on acceptance and availability of alternative donor. Our policy is to offer a haploidentical HCT to pts lacking a matched donor in the appropriate time according to clinical indications (www.leukemianet.org, www.ebmt.org). Methods. Here we are reporting the intention-to-treat (ITT) analysis of HCT in all consecutive AML pts referred to our Institution between Jan'04 and Apr'12. Results. Indication to HCT was given to 241 pts (tab1), of note pts in 1st complete remission (CR1) are evenly distributed according to donor source (HLA-matched sibling donor - MSD, unrelated donor - URD, haploidentical related donor -haplo-HCT). HCT was performed in 201 pts (median time from diagnosis to HCT and from HCT-indication to HCT are 222 and 80 days). In ITT analysis, 83% of candidate pts received an HCT. The 3y overall survival (OS) analysis for pts transplanted in CR is 56%, for pts in morphologic leukemia free state (MLFS) 21%, for pts in persistence of disease (PD) 11% (p<0.0001). The relapse incidence (RI) for pts in CR at 3y is 21%, for pts in MLFS 43%, for pts in PD 36% (p0.002). The 3y transplant related mortality (TRM) is 28% for CR pts, 44% for MLFS pts, 53% for PD pts (p<0.0001). For pts transplanted in CR, the 3yOS according to donor source (MSD, URD, haplo-HCT) is 57% *vs* 59% *vs* 53% (p ns); the 3y TRM is 37% *vs* 18% *vs* 28% (p ns); the 3y RI is 14% *vs* 23% *vs* 22% (p ns). For pts in CR1, the 3yOS according to donor source (MSD, URD, haplo-HCT) is 53% *vs* 73% *vs* 67% (p ns). TRM and RI are superimposable. For pts transplanted in PD, the 1y OS/TRM/RI according to donor source are superimposable, long term survivors are present only in haplo-setting (5yOS 10%). Comparison between relapsed *vs* refractory pts didn't show any difference. Conclusions. In high risk AML, HCT from a MSD provides the best chance for long-term survival. In our experience, the option of HCT for adults with AML is not limited to those pts with MSD thanks to the exploitation of an high-committed strategy of donor search and of haplo-HCT as alternative donor. So far haplo-HCT, in comparison with MSD and URD, offers equal outcome in any setting, noteworthy in CR1 and regardless of disease characteristics.

Table 1. Patients, disease and transplant characteristics

Patient-related	n = 201
Median Age	51-y (r 17-72)
Over 60-y	53 (26%)
Male sex	108 (54%)

Disease-related	n = 201
Primary AML	129 (64%)
Therapy related AML	15 (8%)
AML with myelodysplasia-related changes	57 (28%)

Transplant-related	n = 225			
Donor	MSD	URD	UCB	haplo-HCT
n	45 (20%)	42 (19%)	7 (3%)	131 (58%)
Status at transplant				
- CR1	26 (58%)	24 (57%)	0	26 (20%)
- CR2/>2	4 (9%)	12 (29%)	2 (28%)	24 (18%)
- MLFS	1 (2%)	3 (7%)	2 (28%)	7 (5%)
- relapse	14 (31%)	3 (7%)	3 (43%)	74 (57%)

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HAPLOIDENTICAL CELLULAR THERAPY IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: DESCRIPTION OF ITS USE IN HIGH RISK PATIENTS

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Guo *et al*. reported an innovative approach for the treatment of Acute Myeloid Leukemia (AML) in a 2011 issue of Blood. The report showed that the infusion after chemotherapy of peripheral blood stem cells (PBSC) from haploidentical donors significantly improved the outcome in elderly AML patients, without major toxicities. So far, these data have not been confirmed by other groups worldwide. We treated with this protocol 3 elderly patients with high-risk AML. Pt. #1 had treatment-related AML following 6 lines of chemotherapy for ovarian cancer and her blasts had a mixed phenotype with complex karyotype. Pt. #2 and #3 had AML with myelodysplasia-related changes, one of them (#2) had received 4 azacitidine courses without response. Institutional ethics approval was obtained for this protocol. All three patients received a 3+7 induction regimen followed by the infusion of G-CSF mobilized PBSC from haploidentical familial donors, according to the original protocol. The potential reactivity of patient T- cells against their haploidentical counterparts was proved *in vitro* by mixed lymphocyte reaction. This *in vitro* reactivity resulted *in vivo* in graft-rejection, with detectable microchimerism in only 1 patient and absence of GVHD in all patients. Pt. #1 had a partial response, with BM blast reduction from 90% to 45%. She then received an intensified salvage regimen followed by a second infusion of haploidentical PBSC, resulting in complete hematological recovery and CR. Six weeks later, AML relapsed, the patient was not further treated and died shortly thereafter. Pt. #2 and #3 received 1 infusion of haploidentical PBSC after induction chemotherapy. Pt #2 was refractory, he refused further treatment and died few weeks later. Pt #3 achieved CR, however she was unable to receive consolidation therapy due to the development of endophtalmitis and chronic sinusitis by Fusarium. She completely recovered within 3 months, with a mild residual chronic renal insufficiency. Despite lack of consolidation therapy, she is still in CR at 11 months of follow up. Our observation confirms that this approach is feasible and safe. Compared to Guo *et al*, we treated AML patients with very unfavorable prognosis. The achievement of CR in 2/3 patients was encouraging and somehow unexpected, especially for the patient in long lasting CR after a single treatment. Therefore, this "nonengraftment haploidentical cell-therapy" approach should be further investigated in a prospecitve trial.

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CARDIOVASCULAR COMPLICATIONS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Nowadays allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is the choice treatment for several hematological and non hematological malignancies. This treatment has greatly improved the prognosis of these patients creating many long-term survivors. However this is not a completely risk-free therapy, in fact Cardiovascular diseases (CVD) are emerging late effects of this treatment. Materials and Methods. We have retrospectively examined 149 patients undergoing allo-HSCT from January 2008 to December 2009. We have recorded clinical data relative to diagnosis, age, comorbidities, pre-existing risk factors, exposure to anthracyclines chemotherapy, exposure to total body irradiation (TBI), development of acute or chronic graft versus host disease (GvHD). We have observed the incidence of cardiovascular complications: onset of new cardiovascular events and cardiovascular risk factors not present at baseline. Results. Our data show that new cardiovascular risk factors have emerged as effect of this treatment as hypertension, diabetes and dyslipidemia: 27 patients (18,12%) developed hypertension 38 patients (25,5%) dyslipidemia and 9 patients (6,04%) diabetes. We observed the incidence of new cardiovascular events: 6 patients (4%) developed heart failure and 19 (12,8%) patients showed lower limbs edemas. Moreover 5 patients (3,35%) developed Acute Coronary Syndrome, 25 patients (16,8%) suffered from chest pain (defined as "aspecific"), 3 patients (2%) developed acute pulmonary edema (cardiogenic or not cardiogenic), 14 patients (9,4%) pericardial effusion and 14 patients (9,4%) hypotension. 9 patients (6%) developed atrial arrhythmias (as atrial fibrillation and/or atrial flutter), 14 patients (9,4%)conduction disturbances (as branch block, atrioventricular block, long QT, 12 patients (8%)alteration of ventricular repolarization. As expected, an age >45 years is associated with a higher risk of heart failure (p<0,03) and atrial arrhythmias (p<0,05). Conclusions. These results leads our attention to the onset of cardiovascular complications following allo-HSCT. From our experience we can deduce that a high level of attention is required towards potential cardiovascular disease in these patients for the future. In our opinion a baseline and a follow-up clinical and cardiological evaluation is mandatory in these patients, especially in patients with pre-existing risk factors.

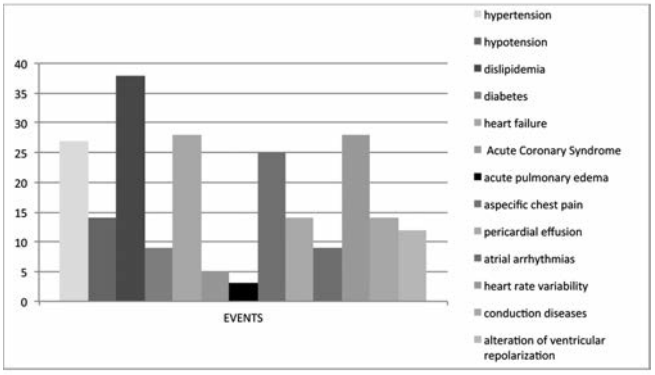

Figure 1.

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CHRONIC GRAFT *VS* HOST DISEASE OF THE GENITAL TRACT: DIAGNOSIS, CLINICAL IMPACT AND MANAGEMENT FROM A SURVEILLANCE PROGRAM IN A PRELIMINARY COHORT

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Chronic graft-versus-host disease(GvHD) of the genital tract(gcGvHD) is an under-recognized complication impacting on quality of life. Female gcGvHD(FgcGvHD) was reported in a quarter of long-term survivors, while only limited data with regard to male gcGvHD(MgcGvHD) are available. FgcGvHD have clinical features ranging from vulval or vaginal irritation, discharge and ulceration to vaginal stenosis. Our surveillance programme for FgcGvHD includes gynecological and colposcopic examination at 6 months after transplantation for all patients and regular follow-up. The main symptoms were vaginal dryness, burning and soreness, which corresponded at the signs of vaginal dryness, reduction elasticity and pain on touching the labia. In the past 2 years, we systematically evaluated 10 female underwent an allogeneic stem cell transplantation. We classified the gcGvHD based on the NIH criteria; 3 cases of gcGvHD were identified. One received a diagnosis of severe gcGvHD for the presence of concentric fibrous banding of the vagina with vaginal stenosis and reduction capacity; she also had a severe cGvHD involving the liver and the skin. The second, with moderate cGvHD, had mild gcGvHD with genital tract discomfort and inflammatory redness of vaginal mucosa. These 2 patients were subjected to systemic and local immunosuppressive therapy(IST). The local terapy was made with steroid vaginal application, with oestrogen and progesterone replacement as appropriate for age, gonadotrophin levels

and co-morbidities. The first died for the underlying hematological disease; the second got a good response with reduction of global grading of GvHD. The third patient had mild cGvHD and showed moderate gcGvHD with reduction in vaginal elasticity and dyskeratosis vulvar; she also received systemic and local IST, with large benefit. MgcGvHD was not systematically studied. On 12 patients, only one complained symptoms or signs suggestive for cgGvHD. He had leucoplasiform and acetowhite areas involving about 50% of glans with mild burning; he had no other localization of cGvHD. Not responding to steroid, he was treated with a carbon dioxide laser to excide the premalignant lesion. Even with the caveat of the cohort size, our data confirm that cGvHD may affect the genital tract both in females and males. A systematic evaluation by specialized teams should be encouraged to prevent long-term complication which may severely affect sexuality and thus overall self-being and quality of life.

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TREOSULFAN-FLUDARABINE-THIOTEPA AS PREPARATIVE REGIMEN FOR ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION IN HIGH RISK PATIENTS WITH ADVANCED LYMPHOPROLIFERATIVE DISEASES

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Background. Reduced-intensity conditioning regimens for allogeneic HSCT are accepted treatment for high risk hematologic patients non fitting criteria for high-intensity approach. The association Treosulfan-Fludarabine has demonstrated to be tolerated and myeloablative. To increase anti-tumor activity we added Thiotepa to this combination in a consecutive prospective series of patients with advanced lympho-proliferative disease. Primary objectives of this prospective phase II study were feasibility and safety of this regimen; secondary objectives were OS, DFS, TRM and relapse rate. Methods. Since November 2006 to November 2012, 25 consecutive patients (15 males,10 females) entered this study. Median age was 43 years (range 19-60). Underlying diseases were: non Hodgkin lymphoma (n. 13), Hodgkin lymphoma (n. 7), acute lymphoid leukemia (n. 2), chronic lymphoid leukemia (n. 2) and Burkitt lymphoma (n. 1). All patients were heavily pretreated (16 had received previous autologous transplant. Only 1 patient was in first complete remission; 13 were in second or subsequent CR, 2 in PR, and 9 in resistant or progressive disease. Mean HCT-CI was 1. Conditioning consisted of Treosulfan 14 gr/m^2 for 3 days, Fludarabine 30 mg/m^2 for 5 days and Thiotepa 10 mg/kg single day. CSA+short MTX was used as GVHD prophylaxis. Anti-lymphocyte globulin was added in case of MUD transplants. Thirteen patients received HSC from HLA identical siblings and 12 from match unrelated donors. Results. Twenty-three patients (92%) regularly engrafted. Two patients were not evaluable for engraftment because of early death. Five patients experienced severe GI toxicity (4 grade III mucositis, 1 GI bleeding), 1 patient had grade I renal toxicity, 1 patient presented CNS hemorrhage, 2 patients had mild skin toxicity. Twelve patients presented grade I-II acute GVHD, 1 grade IV. CrGVHD occurred in 8/19 evaluable patients (extended in 1). With a median follow up of 24 months (range 3-67) 15 patients are alive (60%), 14 in complete remission. Ten patients died: 4 by recurrent disease and 6 by TRM. Conclusions. The association Treosulfan-Fludarabine-Thiotepa can be safely and effectively used for allogeneic conditioning regimen in very heavily pre-treated patients with advanced lymphoproliferative disease. The role of Thiotepa in increasing anti tumor effect should be confirmed in a randomized trial.

Myeloproliferative Disorders II

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A DIFFERENTIAL CENTROSOME LOCALIZZATION OF WILD TYPE (WT) AND JAK2V617F PROTEIN IN HUMAN LEUKEMIA CELL LINES

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The strong activation of JAK2V617F stimulates homologous recombination, centrosome and ploidy abnormalities. The centrosome ensures symmetry and bipolarity of the cell division process, which is essential for accurate chromosome segregation and cell-cycle progression into mitosis. The centrosome amplification occurs frequently in both solid tumors and hematological malignancies and it is thought to contribute to the development of chromosomal abnormalities in these disorders. The main aim of our research is to probe the role of Jak2 in the centrosome genomic stability and cell polarity. Then, we evaluate if JAK2 status is related to centrosome malfunction and malignant transformation. To determine if JAK2 is a centrosomal partner, we performed co-immunoprecipitation and co-immunofluorescence (CIF) assays between JAK2 and the centrosome marker gamma-tubulin in several cancer cell lines, expressing JAK2WT (K562 and BV173, derived from CML patients; and human bone marrow stromal cell line HS5) or carrying JAK2V617F (SET-2 and HEL cell lines). Furthermore, we also evaluated the JAK2 centrosomal localization in CD34+ cells isolated from MPN patients. CIF assay shows a neat co-localization of JAK2 and gamma-tubulin in K562, BV173 and HS5 cell lines as well as in CD34+ cells isolated from MPN patients with JAK2WT (in more the 90%±5% of cells) in a cell cycle independent manner. Moreover, we observed that JAK2 centrosomal interaction is strictly dependent on the intact microtubule network, since nocodazole treatment, inducing depolymerization of the microtubule network, was able to reverse JAK2 centrosomal localization. By contrast, in SET or HEL cell lines, carrying JAK2V617F, we observe colocalization of JAK2 and gamma-tubulin in only 40%±5% and 10±3% of the cells, respectively. In BM CD34+ cells isolated from MPN patients with JAK2V617F mutation, we also observe a partial JAK2 co-localization on centrosome. Notably, in 90% of HEL cells, lacking of JAK2-gamma-tubulin co-localization, we identified high percentage (61%) structural and/or numeric centrosome abnormalities. Interestingly, we observed that the exogenous expression of JAK2V617F in K562 cell line increased significantly the numbers of centrosome abnormalities. Our preliminary data strongly suggests that JAK2 protein interacts with centrosome structure and that JAK2V617F is associated with centrosome malfunction and transformation.

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FACTORS CORRELATED TO MYELOFIBROTIC EVOLUTION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA: A SINGLE CENTER EXPERIENCE

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Introduction. Evolution to myelofibrosis (MF) represents a relatively rare but always severe event in patients with essential thrombocythemia (ET) and polycythemia vera (PV). Few reports have focused on the clinical and biological features at diagnosis of ET and PV that correlate with progression to MF. We studied a series of patients with post-ET and post-PV MF and compared with a group of ET and PV patients with a long follow-up without myelofibrotic evolution, with the aim to identify prognostic factors for MF. Methods. Thirty-three patients with post-ET (n=22) and post-PV (n=11) MF followed at our institution were compared with 29 ET and 27 PV patients with at least 10 years of follow-up without evolution. Diagnosis of ET and PV was confirmed according to WHO criteria (including JAK2 analysis), evolution to MF was defined according to IWG-MRT proposed criteria. Results. Median time from diagnosis of ET/PV and progression to MF was 96 months (range: 16-

161). Comparing baseline characteristics of patients who evolved to MF and those who did not, we found a trend for higher risk of progression for older age (53 *vs* 47 years, p=0.07) lower Hb level (15.0 *vs* 16.1 g/dL, p=0.06), higher platelets (706 *vs* 610x10^9/L, p=0.10) and higher WBC (10.8 *vs* 9.1x10^9/L, p=0.08) counts. Considering only the 22 post-ET MF and the 29 ET patients, a higher platelet count (806 *vs* 676x10^9/L, p=0.02) was associated with myelofibrotic progression, with a trend for lower Hb (14.0 *vs* 14.8 g/dl, p=0.11) and age (52 *vs* 44 years, p=0.06). In the 11 post-PV MF and 27 PV patients, progression to MF was predicted by lower platelets (334 *vs* 526x10^9/L, p=0.03) and higher WBC (17.5 *vs* 9.8x10^9/L, p=0.001) counts. Conclusions. Our data suggest a possible role of age, anemia, platelet count and leukocytosis as adverse risk factors for progression to MF in myeloproliferative neoplasms. In particular, high platelet counts in ET and leukocytosis in PV are associated with higher risk of fibrotic evolution.

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RUXOLITINIB SYNERGIZES WITH PANOBINOSTAT TO OVERCOME DRUG RESISTANCE RELATED TO BONE MARROW STROMA MICROENVIRONMENT IN PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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JAK2V617F constitutively deregulates signaling of the JAK/STAT pathway conferring proliferative and survival advantages in the chronic Ph-negative Myeloproliferative Neoplasms (MPNs). Many drugs target different pathways critical for MPN development, like JAK inhibitor ruxolitinib, known to decrease spleen size and alleviate constitutional symptoms in myelofibrosis (MF). Other drugs work through remodeling of chromatin with a key role in epigenetics, like the pan-histone deacetylase inhibitor panobinostat. This drug in a phase I study for patients with MF, showed to be clinically active, regardless of JAK2 V617F status. JAK2V617F tumor cell lines HEL and SET2 were treated with ruxolitinib and panobinostat In RPMI medium, defined as regular media (RM), or on monolayers of stroma cell line HS-5 or bone marrow stroma secreted cytokines, defined as HS5/Stroma Conditioned Media (HS5/SCM). In RM condition, panobinostat or ruxolitinib induced a significant apoptosis in SET2 and HEL cells in a dose-dependent manner. Indeed, when SET2 cells were treated with 30nM panobinostat or 300nM ruxolitinib in the presence of HS-5/SCM, the drug-related apoptosis is significantly reduced (40% ±18% and 30%±8%, respectively) respect to cell line treated in RM (79% ±15% and 58%±12%, respectively; p<0.05). Similar results have been achieved for HEL cell line but only when cells were treated with Panobinostat (22% ±4% in HS-5/SCM *vs* 46%±6% in RM; p<0.05), since ruxolitinib exerts no effect on HEL viability. The IC50 of SET2 cells treated with panobinostat or ruxolitinib is significantly increased in the presence of HS-5/SCM (32nM and 1261nM, respectively) versus the IC50 in RM (15nM and 281nM, respectively). Co-treatment of panobinostat and ruxolitinib strongly synergizes, increasing SET2 (96%±1%) and HEL (73%±5%) apoptosis, regardless HS5/SCM exposition. Finally co-treatment of MPN-CD34+ cells with panobinostat and ruxolitinib strongly decrease CFU counting in a stroma-independent manner, respect to the use of the single agent. Disrupting the cross-talk between the malignant clone and its BM milieu is an attractive therapeutic strategy in MPNs. Here, we present evidence that, combination therapy with HDAC and JAK inhibitors has a potential value to overcome the "protective effect" of the stroma BM on the JAK2V617F cells.

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THE PI3K INHIBITOR BKM120 ALONE AND IN MULTIPLE COMBINATIONS INHIBITS GROWTH OF JAK2V617F CELLS

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Introduction. In addition to the dysregulated JAK/STAT signaling, activation of other downstream pathways such as ERK and PI3K/mTOR pathways has been documented in myeloproliferative neoplasms (MPN). We explored the efficacy of targeting PI3K/mTOR pathway with the selective PI3K inhibitor BKM120 alone or in combination with other inhibitors. Methods. BKM120, RAD001 and Ruxolitinib were provided by Novartis. Inhibition of cell growth effects was evaluated with WST1 assay. Quantification of apoptosis and cell cycle were done by flow cytometry. Protein analysis was assessed by SDS-PAGE Western Blotting assay. CD34+ cells from MPN patients were plated in semisolid medium. In-vivo studies were conducted on SCID mice injected with Luc+BaF3 JAK2V617F cells. Results. We found that both human SET2 and murine BaF3 JAK2-mutated cell lines resulted more sensitive to PI3K inhibitors (respectively 1±0.3μM and 1.1±0.2μM) compared to K562 and BaF3 JAK2-WT (respectively 4.5±0.8μM and 3.1±0.9μM). G2/M phase of cell cycle was increased with 1μM BKM120 compared to untreated cells and apoptosis was induced at >3 μM dose. Clonogenic growth of MPN erythroid, myeloid and megakaryocytic progenitors was inhibited by BKM120 at doses significantly lower (from 5 to 10-fold) than normal cells and EPO-independent colony formation was inhibited at nM doses (9±10nM). Western Blot analysis showed drug-induced inhibition of PI3K downstream effectors mTOR, 4eBP1 and p70 but also of JAK2 and STAT5 phosphorylation. We also explored double and triple drugs combinations with BKM120 and other key pathways inhibitors. A synergic effect (Combination Indexes <1) was observed for the JAK1/2 inhibitor Ruxolitinib (C.I.:0.4), the PIM1/2/3 inhibitor SGI1776, (C.I.:0.7), the MEK1/2 inhibitor AS703026 (C.I.:0.5), and the PI3K/mTOR inhibitors RAD001 and PP242, (C.I.:0.2 and 0.8). In in-vivo studies, mice receiving 60mpk and 45mpk had statistically significantly increased overall survival (27.8±4.9 and 27.3±4.2 days post-injection respectively) compared to controls (20.2±5.6 days). Conclusions. These in-vitro and in-vivo data prefigure a role of BKM120 in MPN treatment strategies; studies in JAK2V617F KI murine model are ongoing to confirm and expand these results. Thus, concurrent targeting of different pathways might optimize efficacy and reduce toxicity in patients with MPN. At this regard, a phase Ib clinical trial of BKM120 associated with Ruxolitinib in MF patients is ongoing.

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RET: "RETE EMATOLOGICA TOSCANA". A REPRESENTATIVE NETWORK "PATIENT CENTRICITY": A REAL-LIFE OF CHRONIC MYELOID LEUKAEMIA MONITORING AND TREATMENT IN TUSCANY

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CML incidence is relatively consistent in all countries where adequate statistics are available. Occurring at about 1 to 2 per 100,000 population, CML is a rare disease in children, where it makes up no more than 5% of the leukaemias. The median age of onset is 45 to 55 years. Half of CML patients are older than 60 years. As in Italy, CML incidence in Tuscany is 2 per 100,000 population, mortality is around 400 per year, so CML prevalence is almost 1 per 18,000 population. The hematologic network started on October 29, 2009. Then, the Tuscan centers are connected to ensure a correct and uniform management of patients with chronic myeloid leukemia. From 2009, 162 patients have been enrolled. To be sure to have enough available data, we utilized patients who received CML diagnosis from 2002. The distribution of patients is among 9 Tuscan centers, and Firenze (coordination), Pisa and Siena are referred ones. All enrolled patients are CML in chronic phase. The therapy is so distributed: 72% treated with Imatinib standard dose, 8% with Nilotinib 800 mg/day, 6% with Nilotinib 600 mg/day and 14% treated with Dasa-

tinib at different doses (50-140 mg/day). Tuscan Network offers the availability to perform molecular analysis with RT-PCR International Scale (IS) to monitor patients, and the availability to detect ABL mutations by Denaturing-HPLC-Based assay and genomic sequencing. Twenty five percent of patients effectuate 3 molecular determinations per year and 48% at least 2 per year. In 40 months 66 samples have been studied to detect ABL mutations and on 9 patients (5%) ABL mutation has been identified. Twenty nine patients (18%) resulted non-mutated and for 28 patients the analysis was not performed because not sufficient amount of transcript was revealed. Regards the rate of response, 21% of observed patients is in MR 4-MR 4,5, consistently to literature data. For 11 patients (7%) the follow up is not still available, but this rate is continuosly decreasing thanks to centers cooperation. All Tuscan centers are now linked to be closer to patients and to ensure the correct monitoring for them and the right treatment, too. This network could offer opportunities to clinician to share several decision in second generation TKis and early switch era.

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IS THE MARROW FIBROSIS EVALUATION WORTHWHILE IN THE PROGNOSTICATION OF NEWLY DIAGNOSED POLYCYTHEMIA VERA PATIENTS?

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Based on WHO revised classification, bone marrow biopsy is no longer needed in the diagnosis of Policythaemia Vera (PV). However, the prognostic value of marrow fibrosis at baseline has been recently raised by Barbui *et al*, and this point still deserves further confirmations. To address this issue, we revised retrospectively the histological features at diagnosis of 195 patients with PV [M/F 100/95, median age 58.4 yrs, Interquartile Range (IQR) 48.1 – 67.2, median Hb 18.4 g/dl (IQR 17.1 – 20.1), median Ht 56.6% (IQR 53.0 – 61.8), median WBC 10.4x10^9/L (IQR 8.4 – 14.0), median PLTs 505x10^9/L (IQR 346 – 717)] observed at our Institution from 1/1982 to 12/2010 and with an available bone marrow analysis. One hundred twenty-eight patients (65.6%) had a bone marrow fibrosis grade 0 while 67 patients (34.4%) a bone marrow fibrosis grade >0 (grade 0 -1 in 15 cases, grade 1 in 36 cases and grade 1 – 2 in 16 cases, respectively): the different features at diagnosis of the 2 groups are compared in the Table 1.

Table 1. Features at diagnosis of PV patients with and without fibrosis

	Fibrosis grade 0	Fibrosis grade >0	p
Gender, M/F (%)	61/67 (47.6/52.4)	39/28 (58.2/41.8)	0.161
Median age, years	58.9	57.9	0.896
(IQR)	(48.0 – 67.0)	(48.9 – 67.2)	
Median Hb, g/dl	18.5	18.1	0.213
(IQR)	(17.1 – 20.4)	(17.2 – 19.5)	
Median WBC, x10^9/L	10.3	11.1	0.299
(IQR)	(8.3 – 13.7)	(8.4 – 14.6)	
Median PLTS, x10^9/L	497	562	0.296
(IQR)	(324 – 738)	(357 – 711)	
Median JAK2-V617F,%	56.2	62.5	0.998
(IQR)	(15.8 – 93.0)	(29.5 – 81.8)	
Spleen, enlarged/normal	52/73	28/38	0.913

After a median period of observation of 131.6 months (IQR 52.3 – 204.1), the 2 groups were also compared as concern thrombotic episodes during follow-up [reported in 45/195 patients (23.1%)], evolution in myelofibrotic phase (MP) [21/195 patients (10.8%)], evolution in blastic phase (BP)[15/195 patients (7.7%)] and overall survival (OS): the presence of marrow fibrosis > grade 0 was associated with a reduced occurrence of thrombotic episodes during follow-up [9/67 (13.4%) *vs* 36/128 (28.1%), p=0.021] and with a decreased rate of evolution in MP [3/67 (4.5%) *vs* 18/128 (14.0%), p=0.040], while no difference was observed as to evolution in BP and OS. In conclusion, while the histological marrow evaluation is not required for the diagnosis of PV, the recognition of fibrosis seems to have a role in the prognostication of adverse events during follow-up and should be evaluated at baseline.

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IN PRIMARY MYELOFIBROSIS (PMF) A NEW SIMPLE SCORE BASED ON THE PRESENCE/ABSENCE OF ANEMIA AND/OR BLASTS IS A POWERFUL PREDICTOR OF LEUKEMIC EVOLUTION: A COMPARISON WITH THE IPSS MODEL

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To correlate the clinico-biologic features at presentation with the risk of blast phase evolution in PMF patients, we retrospectively collected the clinical records of 362 patients followed in 12 hematologic units of Latium, between 1981 and 2010. Criteria for PMF diagnosis were those accepted at the time of first observation. After a median follow-up of 89 months (1-252) the actuarial overall survival rates at 5, 10 and 20 years were 70%, 35% and 22%, respectively. Forty-two (11.6%) of 362 patient progressed to a blast phase. The overall leukemia-free survival (LFS) rates at 5, 10 e 20 years were 86%, 72% and 66%, respectively. At the univariate analysis, Hb (≤10 gr/dL), blasts (>1%), symptoms, hepatomegaly and any cito-reductive therapy were the only variables significantly correlated with a worse LFS. At the multivariate analysis, Hb and Blasts maintained their independent prognostic value. Therefore, 250 pts with all these two parameters available, were scored as follows: score 0 = none of these two factor (137 pts); score 1 = 1 of the 2 factors (82 pts) and score 2 = both factors (31 pts). The 6 years LFS rates of these groups were 89%, 80% and 37%, respectively (p<.0001). The Present score system was compared with IPSS scores in 216 patients. The resulting 6 years LFS rates were 30%, 80% and 89% (p<.0001) for,respectively, the classes 2, 1 and 0 of the present score, and 48%, 80%, 88% and 90% (p<.0001) for the IPSS 3, 2, 1 and 0 groups. Moreover, the present score allowed a clear distinction of the 3 curves starting from 25 months, whereas the the IPSS score 0 and 1 curves are overlapping and the trend of the score 2 and 3 curves started to diverge at 100 months. Thus, the present score, despite the degree of freedom of only 2, showed a log rank value higher than that of the IPSS (59.93 *vs* 31.24). In conclusion, even in the absence of cytogenetic data, difficult to achieve in this setting for the frequent presence of a dry-tap aspiration, the present score is a powerful predictor of progression to blast phase in PMF. It is simpler than the IPSS being based on two easily and worldwide detectable parameters. It would be extremely relevant to confirm these finding in other larger series of PMF patients, given the forthcoming availability of new targeted drugs that require an ever more precise and simple prognostic score of patients.

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FRONT-LINE TREATMENT WITH TKIS SECOND GENERATION IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA HIGH RISK EARLY IDENTIFY CANDIDATES FOR STEM CELL TRANSPLANT

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Nilotinib and Dasatinib have shown greater efficacy than Imatinib in patients with newly diagnosed Philadelphia-positive chronic myeloid leukemia (CML)in chronic phase (CP) after a minimum follow-up of 12 months. Results from ENESTnd and ENESTst studies support Nilotinib as a first-line treatment option for patients with newly diagnosed disease. Nilotinib is a BCR-ABL inhibitor rationally designed to be more

potent and selective than Imatinib. Here we report how the huge selectivity of this inhibitor can early identifies patients who will never respond to therapy. They could be candidates for stem cell transplant (SCT). Four CML-CP patients, 3 females and 1 male (median age 44 yo) received Nilotinib treatment 300 mg BID as first line therapy. Sokal Risk was Int for 2 patients and High for the other two, same distribution for Hasford Score. Eutos risk was Low for 3 patients and High for the last one. The cytogenetic frame was typical Ph+ without added chromosomal anomalies.All patients early documented severe haematological toxicity and interruption of treatment occurred. The median duration of administration therapy before adverse event was 35 days (47-22).The median dosage of Nilotinib was 541 mg/day. All patients developed grade 4 thrombocytopenia and for 2 of them no recovery of platelets occurred, even though the suspension of treatment for 180 days. Other two patients, after a respectively 30 days and 14 days of temporary suspension of the drug, restarted Nilotinib treatment at 200 mg BID and 150 mg BID.After 27 days and 14 days of treatment respectively, grade 4 thrombocytopenia occurred again. For one of them also grade 4 of neutropenia occurred and hospitalization was requested for persistent high fever. For 2 patients bcr/abl ratio was more than 50% at 3 mths,for other 2 patients the transcript ratio was 10% and 12% respectively at 3 mths.For one of them,trisomie 8 occurred in 5 metaphases analysed (10 metaphases totally analyzed),even though complete cytogenetic response was reached (only FISH analysis available).For 2 patients, sibling donor was available and SCT was performed after 9 months from diagnosis and after 8 mths respectively. Median of follow up after SCT is 11 mths. Both patients are alive in Molecular Response (MR)4,5.One patient is still in aplasia in trasfusional support, with bcr/abl ratio 8%.The other patient is in Imatinib 400 mg/day treatment with recurrent grade 2 thrombocytopenia and with bcr/abl>10%.

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SERUM TRYPTASE LEVELS DIFFERENTLY CHANGE OVER TIME IN INDOLENT SYSTEMIC MASTOCYTOSIS WITH OR WITHOUT SKIN LESIONS

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Background. Serum tryptase levels (sTL) are thought to reflect the individual burden of mast cells (MC). In patients with normal sTL, there is evidence for a decline over time of tryptase concentration during long-term immunotherapy (IT) for Hymenoptera venom allergy (HVA). Little is known about sTL changes over time in patients with mastocytosis. Aims and Methods. To analyze the sTL individual variations in patients with Indolent Systemic Mastocyosis (ISM) we retrospectively evaluated the registry of our Multidisciplinary Mastocytosis Outpatient Clinic. Patients with at least three consecutive measurement of sTL and a minimum follow-up of 24 months were included in the analysis. Changes of sTL over time were calculated as a linear regression analysis and the individual slopes were determined. Results. Ninety-two patients were studied. They were equally distributed by gender (M/F=52/40), median age at first observation was 50 years (range 19-80), median follow-up was 45 months (range 24-80). Half of the patients (n=46) had a diagnosis of ISM without skin lesions (ISMs-) and the other half (n=46) had ISM with skin lesions (ISMs+).ISMs- were referred for severe HVA (n=44), anaphylaxis after food (n=1) or severe osteoporosis (n=1). ISMs+ were referred for skin lesions only (n=28) or skin lesions associated with HVA (n=16), anaphylaxis after drug (n=1) or severe osteoporosis (n=1). sTL at first observation were 27.5±19.6 and 59.6±56.0 for ISMs- and ISMs+ patients, respectively. Overall, mean sTL tended to decline over time in ISMs-, while in ISMs+ there was a progressive increase (Figure 1). Means of individual slopes were significantly different in ISMs- (-0.51±4.71) *vs* ISMs+ (+2.91±10.2, p=.043). After diagnosis of ISM, 52 patients with severe HVA underwent long-term IT (39 ISMs- and 13 ISMs+). sTL values during IT tended to decline (mean of individual slopes was -0.35±4.32) but the difference was not significant *vs* patients not undergoing long-term IT (+2.76±10.77, p=.092). In 2 patients a rapid increase of sTL over 100 ng/mL during the follow-up accompanied the diagnosis of other non-MC hematologic malignancies.

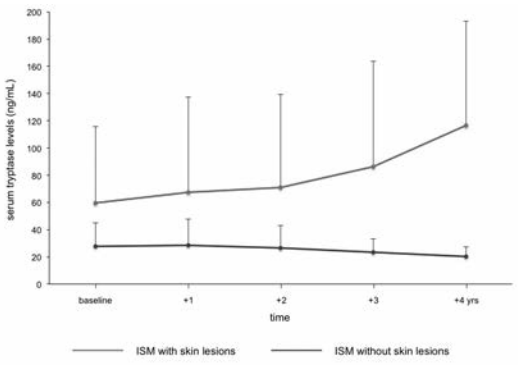

Figure 1.

Conclusions. sTL changes over time are significantly different in ISMs- and ISMs+ patients. This could in part reflect the reduction of MC burden during long-term IT for HVA. However, it is likely that, as reported by others, ISMs- and ISMs+ represent separate biologic entities, arising from neoplastic MC with a different potential of evolution.

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A SIMPLE SCORE PREDICTS CLONAL MAST CELL DISORDERS IN PATIENTS WITH SYSTEMIC REACTIONS TO HYMENOPTERA VENOM WITHOUT SKIN LESIONS

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Background. Systemic reactions after Hymenoptera sting are a common initial manifestation of clonal mast cell disorders (CMD). Since the majority of these patients lack classical skin lesions, sensitive and expensive diagnostic techniques are needed to make the diagnosis. The Red Espanola de Mastocytosis (REMA) had proposed a score to identify among patients with Hymenoptera venom allergy (HVA) those with high likelihood of having mastocytosis, in order to specifically address the diagnostic work-up. Aims and Methods. To validate the REMA score in an independent large series, we evaluated 98 consecutive patients referred to our Multidisciplinary Mastocytosis Outpatient Clinic for systemic reactions to Hymenoptera and suspect for CMD in the absence of skin lesions. Patients performed bone marrow smears and biopsy, flow cytometry analysis and D816V KIT mutation detection by allele-specific RT-PCR. The diagnosis of mastocytosis was made according the World Health Organization criteria.Results. CMD were diagnosed in 75/98 patients (78.8%), 53 males and 22 females, median age at first observation was 51 years. Indolent Systemic Mastocytosis without skin lesions was diagnosed in 72 patients, the remaining 3 had monoclonal MC activation syndrome, lacking coexpression of CD25 on MC but showing KIT D816V mutation. Notably, 5/8 patients with normal serum basal tryptase (<11.4 ng/mL) had a CMD. As in REMA score, a tryptase level >25 ng/mL, the presence of syncope/presyncope and absence of erythema or angioedema confirmed their predictive power of CMD at univariate analysis. In our series, male gender and tryptase level <15 ng/mL had not independent predictive value. Based on multivariate analysis, we propose a simplified score, considering only 3 variables (Table 1). Our score showed the same specificity as the original REMA score and the sensitivity was improved from 0.90 to 0.93, thus allowing to foresee the diagnosis of CMD in other 2 patients. Conclusions. A simple clinical score applied to patients with systemic reactions to Hymenoptera venom without skin lesions reliably predicts the presence

of underlying CMD and identifies patients for which a complete diagnostic work-up is needed.

Table 1.

Variable		Rema score	Our score
Gender	Male	+1	0
	Female	-1	0
Clinical symptoms	Absence of (urticaria and) angioedema	+1	0
	(Urticaria and/or) angioedema	-2	-1
	Presyncope and/or syncope	+3	+2
Basal tryptase level	<15 ng/mL	-1	0
	≥25 ng/mL	+2	+1
Score value with high probability of CMD		≥2	≥2
AUC ROC		0.864 (0.764-0.965)	0,877 (0.781-0.973)
Significance AUC		<0,001	<0,001
Sensitivity		0.90	0.93
Specificity		0.74	0.74
PPV		0.92	0.92
NPV		0.71	0.77

*AUC=Area Under Curve; ROC=Receiver Operating Characteristics; PPV=Positive Predictive Value; NPV=Negative Predictive Value

P300

USE OF ANAGRELIDE IN A COHORT OF PATIENTS FROM THE LAZIO REGION: COMPARISON WITH RECOMMENDATIONS FROM THE ITALIAN GUIDELINES

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Background. The aim of treatment of Essential Thrombocythemia (ET) is to prevent thrombotic/hemorrhagic complications, or leukemic transformation, due either to the natural history of the disease or possibly to the use of chemotherapeutic agents. The Italian guidelines for ET therapy were developed from an Expert Panel and an Advisory Committee, and published in 2004. Anagrelide (ANA) was recommended as 1st line therapy in patients with no child-bearing potential, either <40 years or ≥40<60 years but without a history of major thrombotic events. Aims. To describe the use of ANA in a population of ET patients from the Lazio region and to evaluate the adherence between the Italian Guidelines recommendations on the use of this drug in the clinical practice. Patients. Between 1981-2012, 148 ET patients (99F;49M) were treated with ANA for a median period of 4.4 years (0.1-22.3). Median age at diagnosis: 42 years (20.9-87.7); median age at the start of ANA: 45.4 years (22.7-87.8); median follow-up: 10.5 years (0.6-31.8). Results. ANA was used as 1st line treatment in 52/148 patients (35%), as 2nd line treatment in 78/148 (52.7%) and as 3rd line therapy in 18/148 (12.3%). Responses (plts <600x10^9/L) were obtained in 121/148 patients (81.7%). While on ANA, 11 thrombotic (3 venous, 8 arterial) and 43 hemorrhagic events (7 major, 36 minor) were observed. One hundred and two patients (72 F; 30 M) started ANA after the publication of the Italian guidelines: in 26/102 (25.5%) ANA was used as 1st line treatment, in 65/102 (63.7%) as 2nd line and in 11/102 (10.8%) as 3rd line therapy. In the Table 1, age, platelet number at the start of therapy and previous thrombosis are described for patients undergoing ANA as 1st line medication. In 76/102 (74.5%) patients treated with ANA as 2nd/3rd therapy line, 16 were <40 years and 29 between 40-60 years (45/72, 62.5%). Conclusions. These data show that although ANA was indicated as 1st line treatment in patients <40 and ≥40<60 years with no thrombotic events, it has been more widely used as 2nd/3rd line therapy. In our series, when prescribed as 1st line, in 5/26 cases the recommendations were not followed: 3 patients were ≥40<60 years with previous thrombotic events and 2 patients were >60 years. We conclude that the use of ANA has not been very adherent to the Italian recommendations, but it depended more on the experience of the single center.

Table 1.

Patients age (years)	N° of patients with plts >450x 10^9/L + previous thrombosis	N° of patients with plts >450 ≤1000x10^9/L No thrombosis	N° of patients with plts >1000 ≤1500x 10^9/L No thrombosis	N° of patients with plts >1500x 10^9/L No thrombosis	Total
<40	1	3	4	2	10
≥40 <60	3	3	7	1	14
≥60 <70		1			1
≥70		1			1
Total	4	8	11	3	26

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PREVALENCE OF MPL MUTATIONS AND ASSESSMENT OF THE ASSOCIATED THROMBOTIC RISK IN PATIENTS WITH JAK2 V617F-NEGATIVE ESSENTIAL THROMBOCYTHEMIA

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Background. Mutations in the exon 10 of the thrombopoietin receptor gene MPL have been reported in a small minority (3-4%) of patients with essential thrombocythemia (ET), exceptionally associated with JAK2 V617F. Among the JAK2 wild-type patients, those with MPL W515L/K mutations have been reported to be slightly more prone either to arterial (Vannucchi AM *et al*, Blood 200) or venous thrombosis (Beer PA *et al*, Blood 2008). Aim of the study. To assess the prevalence of MPL exon 10 mutations in a cohort of patients with ET, and to investigate the associated thrombotic risk. Patients and methods. The initial cohort comprised 315 patients with ET referred to our Center and diagnosed according to the WHO 2008 criteria. Of them, 98 patients (31.1% of the cohort, M/F 26/72) were JAK2 V617F-negative. Mutational analysis of MPL exon 10 was performed by high resolution melting (HRM) in all the patients without JAK2 V617F and positive cases were then characterized by direct sequencing analysis (Pietra D *et al*, Haematologica 2011). Results. MPL mutations were detected in 12 patients (12.2% of the patients JAK2 V617F-negative, 3.8% of the overall cohort). Six patients had the W515L mutation, 3 the W515L mutation, and 3 the S505N mutation. Among the patients JAK2 V617F-negative and MPL mutation-negative (n=86, M/F 22/64, median age at diagnosis 41 years, range 16-86), 8 had a thrombotic event (9.3%) since 2 years before diagnosis of ET: 1 had acute myocardial infarction (AMI), 1 ischemic stroke, 2 deep vein thrombosis of the legs, 2 cerebral vein thrombosis, 1 superficial vein thrombosis of the legs, 1 retinal vein thrombosis. The median age at the time of thrombosis was 45 years (range 30-78). Among the patients JAK2 V617F-negative and MPL mutation-positive (n=12, M/F 4/8, median age at diagnosis 51 years, range 31-83), 1 with S505N mutation had AMI at 31 years of age 8.3%). The risk for thrombosis was not different in the carriers of MPL mutations in comparison with the non-carriers (odds ratio 0.88, 95%CI 0.10-7.78, p=1.00). Conclusions. In this cohort the prevalence of the MPL mutations is 12% among the JAK2 V617F-negative patients, not different from previous observations. However, the risk of thrombosis among patients harboring MPL mutations is not different from that of the non-carriers.

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IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA, TICLOPIDINE IS NOT PROTECTIVE AGAINST THROMBOTIC EVENTS

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Low dose aspirin (ASA) is widely used in the clinical practice both in primary and secondary prevention of cardiovascular complications. Since in Polycythemia Vera the efficacy of ASA in preventing thrombosis has been proven by the ECLAP study, the same aspirin dose is commonly used also in patients with Essential Thrombocythemia (ET) because ASA reduce microvascular disturbances and re-thrombosis, even if no randomized trials have assessed the efficacy of ASA in ET. Ticlopidine is commonly used in patients with contraindication to aspirin therapy because its efficacy in the prevention of recurrent stroke, myocardial infarction and vascular death. Even if ticlopidine efficacy in ET has not yet been demonstrated, it is often used as an alternative to ASA. The aim of our study was to compare ASA and ticlopidine efficacy in the prevention of thrombotic complications in ET. We retrospectively evaluated 173 patients with ET (50 M, 123 F; mean age at diagnosis 55 ± 17 y) diagnosed in agreement with WHO criteria, all under antiplatelet treatment. Eighty patients were low risk and 93 high risk (older than 60 years and/or with a previous thrombosis). The latest were all under cytoreductive-treatment. Anti-aggregating treatment was established by the presence of cardiovascular risk factor or previous arterial thrombosis: aspirin 100 mg/die and ticlopidine 250 mg BID. The Pearson's 2 test with Yates correction was used to compare categorical variables. Long term ASA therapy (median follow-up 8.13 y) was administered in 156 (90.2%) and ticlopidine (median follow up 8.62 y) in 17 patients (9,8%). Nine patients treated with ASA (5.8%, 1 low and 8 high risk) after a median treatment of 7 y and 7 patients treated with ticlopidine (41.2%; 2 low and 5 high risk) after a median treatment of 6.8 y developed thrombosis ($p<0.0001$). Ticlopidine seems to be less protective than aspirin in ET patients, in the prevention of thrombotic complications both high and low risk patients. In this study, less than 10% of ET patients under long term ASA therapy seem to be "ASA-non-responders". While recently it has been proposed to use 200 mg of aspirin daily in patients with high platelet count to obtain adequate inhibition of thromboxane A2, this seems to be not necessary from a clinical point of view.

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MUCOCUTANEUS TOXICITY INDUCED BY HYDROXYUREA IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Introduction.Essential thrombocythemia (ET) and polycythemia vera (PV) are clonal stem-cell disorders of the group of Philadelphia-chromosome negative chronic myeloproliferative neoplasms (MPNs). ET and PV patients with risk factors for thrombotic complications have been shown to benefit from cytoreductive therapy, the most common agent used being Hydroxyurea (HU). This drug is usually well-tolerated although systemic and/or localized toxicities have been reported and in particular mucocutaneus toxicity. AIM This single center retrospective study aimed to evaluate dermatological toxicity in MPNs patients treated with HU. Materials and Methods. From 1981 until march 2013, 256 patients received diagnosis of ET (n=212) and PV (44) (according PVSG and after WHO 2008 criteria) and were treated with HU as the first line treatment at a dose ranged from 0,5 to 2 g, with a median of 1 g. The median observation time was 75 months. The median age at diagnosis was 51 years (range 18–85 years). The population included 59% females and 41% males, 64% of patients with ET were JAK-2 positive versus 96% JAK-2 positive patients among PV. Results. The 89% of patients treated with HU had problem of dry skin and skin spots also if they were advised to take the drug away from sun exposure. Forty-six percentage of patients showed nail and skin discoloration. Moreover, 24 patients (9,4%) developed ulcers while on treatment with HU. The grade of ulcers was inversely proportional to the compliance with clinical controls because the progression of the ulcer is not very fast and a timeline of 6 months seems to be adequate to prevent more severe complications. The patients with ulcer were prevalent females (58%). Negative predisposing factors were hypertension (83%), diabetes mellitus (46%) and venous insufficiency (42%). The median time from HU initiation to the development of ulcers was 27 months (range 2-92 months). Since both HU therapy and the underlying MPNs can predispose to other skin disorders like squamous and basal cell carcinoma (4 patients), epidermal atrophy and dermal fibrosis. Conclusion. It is very important to monitor appropriately, with clinical observation and specialist consulting, patients treated with HU and especially patients with microvascular disease and problems of the peripheral circulation. The timely suspension and the use of appropriate therapies are essential factors for a faster and adequate resolution.

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SINERGISTIC INTERACTION BETWEEN ALL-TRANS RETINOIC ACID AND INTERFERON ALPHA *IN VITRO* MODEL OF CHRONIC MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background. Although IFN has proven efficacy in Polycythemia Vera (PV) and Essential Thrombocythemia (ET), many patients experience unacceptable side effects. ATRA synergizes with the growth inhibitory effect of IFN. Their pathways converge on the promoter of STAT1 that induces IRF-1 transcription; this tumor suppressor exerts proapoptotic effect trough TRAIL and caspase activation. These drugs cooperate in blocking cell cycle by inducing p21WAF1/CIP1. Aims We investigated *in vitro* the synergistic activity of ATRA and IFN in MPN, in order to reduce IFN doses. Methods. HEL and SET2 cell lines were treated with a scalar dose of IFN (1-10000U/mL), ATRA (0,1nM-1x10^6 nM) and their combination. Proliferation rate, apoptosis and cell cycle were evaluated after 24-48-72-96-120-168 hrs. CD34+ cells purified from BM of four MPN patients (PV=1, ET=3) at the time of diagnosis were plated (2x103 cells/mL) in semi-solid methylcellulose medium and were incubated with IFN (10000U/mL), ATRA (0,01mM) or their association. Colony forming cell assay (CFC) was performed at day 14. RAR, and RXR, gene expression was evaluated. Results. IFN (10000U/mL) and ATRA (0,01mM) induced SET2 growth inhibition (p=0,0006; p=0,0008) and apoptosis (p=0,001; p=0,04) compared to untreated cell line. Moreover, their combination synergistically increased growth suppression (IFN *vs* ATRA+IFN p=0,0001 and ATRA *vs* ATRA+IFN p=0,0006) and apoptosis (IFN *vs* ATRA+IFN p=0,036 and ATRA *vs* ATRA+IFN p=0,00001). IFN (6,2%) and IFN+ATRA treatment (3,9%) reduced SET2 cells in S phase of cell cycle. Apoptosis assay showed that HEL cell line is less sensitive to IFN (p=0,0002), ATRA (p=0,0026) and their combination (p=0,00001) than SET2. RT-qPCR showed a significant down-regulation of RAR and upregulation of RAR in HEL respect to SET2. This differential expression of RAR subunit may explain the different responsiveness to ATRA. CFC showed that IFN reduced CFU-GM (54+34; p=0,04) without affecting BFU-E and CFU-GEMM formation, while ATRA alone inhibited BFU-E (34+10 p=0,01) growth. Although the addition of ATRA seems to neutralize the IFN inhibitory effect on CFU-GM (127+24 p=0,25), their combination suppresses BFU-E (3+2 p=0,003) and CFU-GEMM (23+7 p=0,004). Conclusion. These results indicate that, the *in vitro* combination of IFN and ATRA acts synergistically in inhibiting SET2 cell line and in suppressing BFU-E and CFU-GEMM growth and suggests that it has a potential interest for the treatment of PV and ET.

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INTERFERON ALPHA IS EFFECTIVE AND HAS A FAVOURABLE TOXICITY PROFILE IN THE TREATMENT OF ESSENTIAL THROMBOCYTHEMIA

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Introduction. Interferon-alfa (IFN) has shown significant activity in ET, furthermore it is the only therapy able to induce complete molecu-

lar remission with the reduction or the suppression of tumor burden. Although IFN effectiveness, many patients discontinue therapy due its side effects. In contrast, IFN hasn't leukemogenicity effects and it's safety in pregnants. Here we reported our monocentric experience. Methods. From 1992 until today, 60 newly diagnosed ET patients were treated with IFN -2b. All patients received subcutaneously administration at induction dose of 3 MU 5 times a week, reduced on the basis of clinical-hematological response. Results. After a median follow up of 68 months, 73,3% of patients achieved complete hematological response (CHR) according to the European Leukemia Net criteria, of these 13% were taken off therapy and 39% are receiving very low doses of IFN (3 MU every 7-15 days). 20% experienced partial (PHR) or absence of hematological response. The median time to response was 3 months. Response was not influenced by gender (p=0,8), baseline values of hemoglobin (p=0,12), platelet (p=0,1) and WBC count (p=0,28). Furthermore JAK2V617F mutational status and allele burden didn't influence the response (p=0,8). Under IFN therapy no patients showed thrombo-embolic or emorragic event, independently on the basis of cardiovascular risk. As many as 20 patients experienced flu-like symptoms, 5 myalgias, 6 dermatitis, 2 alopecia, 5 mood disorders, 7 autoimmune thyroditis, of them only 2 patients had hypothyroidism and had to suspend IFN treatment. Spleen volume average at the baseline was 495 mL, after a median follow-up of 36 months, spleen volume was 472 mL, no differences in spleen enlargement was observed in CHR/PHR compared with no responsive patients. Bone marrow fibrosis grade didn't worsened after IFN treatment both in patients who achieved CHR and PHR. We can't evaluated data about no responsive patients, because this parameter could be influenced by their second line treatment. Conclusion. IFN was safe and effective therapy for patients with ET. IFN ability in controlling disease pathology and modifying the clinical course of the disease is confirmed by our data. This response is sustained for prolonged periods in some patients, also, after therapy discontinuation. IFN should be considered as election treatment, furthermore in young patients, for its no leukemogenic and teratogenic merits.

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A PERSISTENT COMPLETE REMISSION AND A SIGNIFICANT DECREASE OF THE JAK2V617F TRANSCRIPTION LEVELS IN A PATIENT WITH ESSENTIAL THROMBOCYTEMIA TREATED WITH THE HDAC INHIBITOR (HDACI) VALPROIC ACID

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Current therapies of ph-negative myeloproliferative neoplasms (Ph-MPNs) are unlikely to cure or offer remission to patients. New treatments are needed and HDAC inhibitors are a new class of drugs with a potential activity in this group of disease. Among these, valproic acid (VPA) is a well tolerated and long used drug for the treatment of epilepsy and Bipolar Disorder. At concentrations of 50-100 ug/ml, VPA acts as a powerful HDACi, that has been shown to be promising in the treatment of both solid and hematological tumors. Therefore herein we present a 52 years-old woman with JAK2V617F essential thrombocytemia (ET) who received VPA for a bipolar disorder. Diagnosis of ET was done 2 years before the patient came to our observation, at the time of an episode of angina pectoris treated with 3 coronary by-passes. Patient started treatment with antiplatelet drugs and hydroxyurea (HU). However, during therapy she developed a severe intolerance to HU, without reaching disease control. In the same period, the patient developed heightened mood, decreased need for sleep and hyperactivity. A psychiatric evaluation led to the diagnosis of bipolar disorder II (BD II). Thus the patient completely stopped HU and started VPA at increasing doses, reaching a therapeutic serum level of 76 mcg/mL after four months with a dosage of 1.5g/die and achieving the complete remission of manic phase of BD II. As for the clinical course of ET her hemochromocitometric values before VPA were: Hb 14.3 g/dL, WBC count $11.3x10^9$/L and platelets $793x10^9$/L. After reaching the VPA therapeutic level we observed a significant decrease in the hemochrocitometric parameters. Complete remission of disease was obtained 12 months after the start of VPA therapy (Hb= 14 g/dL, WBC and Platelet that resulted, $5.6x10^9$/Land $420x10^9$/L, respectively). After 2 years of VPA therapy the patient is in complete remission. The JAK2V617F DNA mutational burden ratio at diagnosis and at 12 and 16 months after starting VPA therapy were: 15%, 10% and 9%, respectively. The JAK2V617F expression levels were assayed by q-RT-PCR method in which the B2M was used as housekeeping gene. The JAK2V617F/B2M ratios were 2.56±0.1; 1.5 ±, 0.7± and 0.09±0.01 % at diagnosis and at 12, 16 and 24 months of therapy. Herein, for the first time, we provide evidence of the potential efficacy of VPA, a safe and cheap inhibitor of HDACs. These data should provide the rational to test VPA in larger controlled clinical trials.

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SEQUENTIAL EVALUATION OF JAK-2 V617F ALLELE BURDEN IN HETEROZYGOUS PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET)

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JAK-2 V617F mutation is a cornerstone in the diagnosis of Essential Thrombocythemia (ET); however, it is still unclear how the JAK-2 V617F allele burden can be modified by the natural evolution of disease or by the cytoreductive treatment. To address this issue, 86 patients with ET and a 1st evidence of JAK-2 V617F mutation in a heterozygous state (allele burden <50%) were prospectively studied for allele burden variations. There were 30 males and 56 females, with a median age at diagnosis of 56.6 years [interquartile range (IR) 45.9 – 68.7]. Median time from diagnosis to 1st JAK-2 allele burden evaluation was 4.2 years (IR 0.5 – 9.6); in particular, 54 patients received 1st allele burden evaluation <6 years since diagnosis and 32 patients ≥6 years since diagnosis. Median interval between 1st and 2nd JAK-2 allele burden evaluation was 12.1 months (IR 11.1 – 14.1). As to treatment, 29 patients (33.7%) were not receiving any treatment while 57 patients (66.3%) were receiving Hydroxyurea (HU) when both JAK-2 allele burden analyses were done. JAK-2 V617F allele burden median values at 1st and 2nd analysis in the whole population were 17.2% (IR 6.1 – 32.7) and 22.3% (IR 8.9 – 35.3)(p=0.003). In the 29 patients without treatment, 1st and 2nd analysis were 22.7% (IR 14.2 – 31.6) and 25.9% (IR 14.7 – 34.7) (p=0.013); in the 57 patients receiving HU, 1st and 2nd analysis were 15.2% (IR 4.2 – 34.5) and 20.0% (IR 6.8 – 36.3) (p=0.038). Patients with a shorter disease duration (<6 years since diagnosis) showed a V617F allele burden increase from the 1st median value of 14.3% (IR 3.5 – 28.0) to the 2nd median value of 15.0% (IR 6.7 – 31.8) (p=0.028); patients with a longer previous disease duration (≥6 years since diagnosis) had an allele burden increase from the 1st median value of 29.0% (IR 10.4 – 35.4) to the 2nd of 31.0% (IR 17.9 – 40.0) (p=0.027). Moreover, 64/86 patients had a 3rd JAK-2 V617F allele burden analysis with a median interval of 11.9 months (IR 11.2 – 13.4) from the 2nd sample: in these 64 patients, JAK-2 V617F allele burden median values at 1st, 2nd and 3rd analysis were 16.9% (IR 4.7 – 32.7), 21.6 (IR 10.1 – 36.4) and 28.2% (IR 11.6 – 41.7)(p<0.001). In conclusion, JAK-2 V617F allele burden seems to increase over the time in patients with ET; this increase seems to be significant during all disease course. In addition, HU does not seem to reduce the allele burden rise. However, a larger cohort of patients is warranted to confirm these data.

Chronic Myeloid Leukemia II

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MODERATE/ SEVERE PLEURAL EFFUSION IN VERY OLD CHRONIC MYELOID LEUKEMIA (CML) PATIENTS DURING IMATINIB TREATMENT

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The advent of Imatinib Mesylate (IM) has provided an effective therapeutic choice for elderly Chronic Myeloid Leukemia (CML) patients that, just like their younger counterpart, can achieve a complete cytogenetic remission and a major molecular response. However, fluid retention, a common side effect of IM therapy, seems to occur more frequently in elderly individuals. Indeed, peri-orbital and ankle oedema are common patients complaints, whereas pleural effusions have not been usually observed in IM-treated individuals. We conducted a retrospective survey on 211 Italian CML patients above the age of 75 receiving IM for chronic phase CML. Among the 73 patients aged >80 years, we observed 5 cases (6.8%) with severe (grade 3: 4 patients) or moderate (grade 2: 1 patient) pleural effusions. Conversely, such a side effect was not observed in any of the remaining 138 subjects aged <80 years. The 5 patients displaying a pleural effusion were all males, age range 80.3 - 88.7 at the time of IM start. One patient was in late chronic phase, and had received hydroxyurea for more than 2 years before commencing IM, whereas the other 4 started IM within 2 months from diagnosis. Four of 5 patients presented at least one cardio-vascular co-morbidity. Pleural effusions developed after 3-8 months of treatment. Two patients also displayed pericardial effusions while one developed peripheral oedema. IM was definitively discontinued in 2 patients (one of them had cytogenetically resistant disease) whereas the drug was resumed, after a few months of interruption, in the other 3 subjects. Three patients died: two of myocardial infarction and one for CML blast crisis, 45, 31 and 54 months from diagnosis. Two patients are alive (one in major cytogenetic response after resuming IM twice) 12 and 30 months after diagnosis. Our survey found that >5% of very elderly CML patients develop pleural effusions during IM treatment. Compared to the more common dasatinib-induced effusions, IM-dependent effusions were more severe sometimes leading to pericardial involvement. Moreover, they were restricted to very elderly (>80 years of age) patients and possibly correlated to cardio-vascular co-morbidities. In conclusion, although IM still represents the best CML treatment for most elderly patients, individuals >80 years old receiving this drug should be strictly monitored for fluid retention and for the possible appearance of a pleural and or pericardial effusions.

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A RARE CHRONIC MYELOID LEUKEMIA CASE WITH PHILADELPHIA CHROMOSOME BCR-ABL E13A3 TRANSCRIPT. MONITORING OF MRD BY QUANTITATIVE RT-PCR WITH A PATIENT SPECIFIC PROBE

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More than 90% of patients with CML express BCR-ABL transcripts with a e13a2 or e14a2 fusion, translated into the p210 BCR-ABL. CML with p190 BCR-ABL or p230 BCR-ABL are less frequent. Atypical BCR breakpoints outside these cluster regions have also been described.CML cases with fusion transcript e13a3 in which ABL exon 3 rather than exon 2 has fused to BCR, are extremely rare. We describe a patient with CML, with the e13a3 transcript and the use of patient-specific TaqMan probe for monitoring of MRD. A 51 -year-old man patient was referred to our hospital in July 2010 with a clinical picture consistent with CML. Cytogenetic and FISH analysis documented t(9:22);no additional chromosomal abnormalities was found. Real-time quantitative PCR performed (according to EAC Protocols) on cDNA obtained from bone marrow at diagnosis did not detect MBCR-ABL, m-BCR-ABL nor -BCR-ABL transcripts. Because of the discrepancy of cytogenetic findings and molecular results, PCR amplification of the BCR-ABL gene were performed using Biomed-1 protocol. An unusual size of BCR/ABL amplicon was detected. The PCR product was sequenced (ABI 3130 sequencer) and the exact DNA breakpoint was identified. The breakpoint was between the end of exon 13 in BCR(NM021574.2) and exon 3 of ABL gene (NM005157.4).For MRD study we developed a detection method based on quantitative real-time PCR by TaqMan technology. We designed (Primer express software)a patient-specific TaqMan assay probes: the forward primer was designed at the position of the junctional regions, between the end of exon 13 and the first nucleotide of exon Abl a3. Reverse primer and probe were designed on Abl a3.The system did not amplify other related heterogeneous c- DNA sample and the assay achieved sensitivity of 5 log. The amount of e13a3 transcript was evaluated by 2E- Ct method. For the housekeeping gene we used ABL gene. The patient starts with Imatinib therapy (400mg) in November 2010 and responded well to therapy. At 3 months patient achieved hematological response and PCgR, at 6 months CCgR ,at 12 months he achieved a reduction of transcript of 4 log (2E - Ct =0.0002) and the same value was reported at 18 and 24 months. In this case, in agreement with the few cases described in the literature, seems that CML with the BCR-a3 fusion product is associated with slow progressiveness and good response. Patient- specific probe is a new, good assay to monitoring MRD in CML patient with rare fusion transcript.

P310

CLINICAL OUTCOME OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS WITH DELETION AND INSERTION EVENTS (DI) IN THE TYROSINE KINASE DOMAIN OF BCR-ABL

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TKI clinical management has shown the clinical importance of acquired resistance to therapy, mostly due to point mutations (PM) occurrence in the Bcr-Abl kinase domain (KD). Lately, isolated Bcr-Abl transcript variants characterized by DI in Abl KD have been described in Imatinib-resistant patients. However, mostly due to the rarity of these variants, their significance for clinical setting is not yet defined. We evaluated the DI frequency in early chronic phase CML patients treated in front line with Imatinib and progressively enrolled from 2003 until 2010 in our institution. A total of 376 patients were monitored by RTQ-PCR for 9 years, with a median follow-up time of 58 months (6-115), according to ELN recommendations. Bcr-Abl KD mutations were detected using Sanger sequencing and DI were confirmed by re-sequencing of DHPLC recovered fractions. Overall survival (OS), failure free survival (FFS), event free survival (EFS), progression free survival (PFS) and TKI best molecular response were defined according to ENL recommendations. Sokal risk index was available for only 128 patients: 62 high, 42 intermediate and 24 low risk. In our cohort, 91% of OS, 53% of EFS and 5% of PFS were observed. In particular, 66% of 165 tested patients reduced ≤10% at 3 months, 62% of 326 tested patients ≤1% at 6 months and 60% of 359 ≤0.1% at 18 months. However, only 196 out of 376 patients were optimal responders to TKI, while 141 suboptimal and 42 failure responders, for which we performed mutation analysis. In particular, 98 (54%) were no mutated (WT), 68 (37%) showed PM, whereas 16 showed DI events. In particular, 5 DI patients showed an open reading frame of Bcr-Abl proteins. The majority of DI had high (36%) and intermediate (36%) Sokal risk, respect to PM (24% high and 27% intermediate) and WT (12% high and 34% intermediate). Moreover, the DI showed reduced OS (p=0.0444) and FFS (p=0.0342) respect to PM and WT (p<0,0001). Over 9 years of follow-up, complete molecular response was achieved in 31% of DI, in contrast to 41% of PM and 61% of WT. Nevertheless, none of DI and PM patients was optimal responder, while 56% of DI and 51% of PM were failure. In conclusion, since the OS and FFS were significantly decreased in patients with Abl DI versus patients with Abl PM or Abl WT, the presence and the type of Abl mutations may be relevant to be integrated in the clinical decision algorithm.

P311

HETEROGENEITY OF MOLECULAR OUTCOME IN CHRONIC MYELOID LEUKEMIA AFTER IMATINIB (IM) DISCONTINUATION: LONG-TERM FOLLOW-UP IN CLINICAL PRACTICE

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Recent studies suggest that IM may be safely discontinued in some pts with long-lasting complete molecular respone (CMR: undetectabe BCR-ABL trascript on QR-PCR). However, published data, are for short-term follow-up. Herein we aim to update data we reported in 2005 and add our experience on IM cessation outside clinical trials. Four previously reported pts (late chronic phase, prior IFN): a pt who discontinued IM in Oct 2003 (CMR duration before IM withdrawal: 14m) is still off IM (113m) with transcript level fluctuating between undetectable and 0.004%IS. A pt who resumed IM 10m after discontinuation (CMR duration: 17m) because of molecular recurrence, rapidly regained a sustained CMR; 44m later she explored a second discontinuation attempt and is treatment-free (60m) with fluctuating transcript levels (from undetectable to 0.009% IS). A pt who resumed IM 8m after discontinuation (CMR duration: 19m) reachieved a second CMR and remained negative for 21m; a second discontinuation attempt was followed by an increase in transcript level >0.1%IS (MR3) requiring therapy. The last pt discontinued IM after 13m in CMR, but lost MR3 18m later and resumed IM. Five additional pts (low Sokal risk; IM first-line): one pt (CMR at 6m) discontinued IM after 55m of therapy because of a severe rheumatologic syndrome and is still in CMR 65m from discontinuation. A young male pt decided to stop IM after 82m of therapy (BCR-ABL trascript fluctuating around negativity) whising to conceive, as he was oligoasthenoteratospermic. He conceived, but refused treatment resumption and is still treatment-free (42m) in stable MR3. In 2012, three female pts (diagnosis in 2005; CMR at 6 m; stable CMR) discontinued IM, as they desired to become pregnant. One of them (37 yrs) was not able to conceive, lost MR3 in 6m and resumed IM. One pt decided to resume IM after delivery, although CMR was sustained during pregancy. The last pt became BCR-ABL positive 2m after IM cessation; now she is pregnant in MR3. None of the pts lost complete cytogenetic response. The outcome of pts who discontinue IM is puzzling: 1) long-term IM therapy, early CMR and sustained molecular negativity do not always predict maintenance of response; ii) a sustained CMR is foreseeable in a very few cases; iii) in some pts with fluctuating detectable BCR-ABL below the MR3 threshold the disease might remain stable for a long time off therapy, due to biologic and immunologic mechanisms still unclear.

P312

MYELOID DERIVED SUPPRESSOR CELLS (MDSC) ARE INCREASED AND EXERT IMMUNOSUPPRESSIVE ACTIVITY IN CML PATIENTS AT DIAGNOSIS

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Background. In some solid tumors it has been demonstrated that a subpopulation of myeloid cells, defined as "myeloid-derived suppressor cells" (MDSCs), plays an important role in inducing T cell tolerance by production of arginase 1 (arg1) that depletes microenvironment of arginine, an essential aminoacid for T cell function. Since chronic myeloid leukemia (CML) patients have high levels of immature myeloid cells it is of interest to investigate if these cells have MDSCs phenotype and activity. Aim The aim of this study was to analyze MDSCs and investigate their activity in CML patients. Methods. MDSCs were analyzed in peripheral blood (PB) of healthy donors (HD; n=20), 30 CML patients at diagnosis, 21 of which followed during treatment with imatinib (IM; n=8), nilotinib (NI; n=2) and dasatinib (DA; n=2) as first line therapy. Granulocytic MDSCs (G-MDSCs) were identified as CD11b+CD33+CD14-HLADR- cells, while the monocytic MDSCs (Mo-MDSCs) as CD14+HLADR by cytofluorimetric analysis. Arg1 expression was assessed using real time PCR. Arg activity was measured in granulocyte lysates using a colorimetric test after enzymatic activation and arginine hydrolysis. Microvesicles (MV) were isolated from CML serum at diagnosis (n=5) by sequential ultracentrifugation. Results. CML patients showed high levels of Mo- and G-MDSCs at diagnosis in comparison to HD (63±8 and 83±12,2% respectively in CML *vs* 4,9±2,1 and 55,8±5,3% in HD; p<0.001) while after TKIs therapy MDSC levels returned to normal values. Also T-reg was significantly increased at diagnosis in respect to HD (from 5,9±0,8% to 9±2%; p<0.001) with a significant correlation with the percentages of Gr-MDSCs (r=0,6996; p<0.001). Either in PB and purified granulocytes, arg1 expression showed a 30 fold increase in CML at diagnosis (p<0.001) and decreased after therapy. Arg enzymatic activity in granulocytes resulted also increased in CML (n=10) compared to HD (n=10) (p<0.001). The suppressive function of CML granulocytes was demonstrated by their ability to inhibit the proliferation of CFSE+ HD T cells (p<0.001). In addition, an increase of Mo-MDSCs *in vitro* was observed after incubation of HD Mo with CML sera (29±13%; p<0.0001) or MV (8±2,8%; p<0.05). Conclusions MDSCs are increased in CML patients at diagnosis and decrease during TKIs treatment. CML granulocytes have high arg1 activity and immunosuppressive activity. Moreover, CML serum as well as CML microvesicles increase the percentage of HD Mo-MDSCs.

P313

DISCONTINUATION OF ALPHA-INTERFERON TREATMENT IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN LONG-LASTING COMPLETE MOLECULAR REMISSION

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Background. Treatment with alpha-interferon (IFN) was widely employed before the advent of Tyrosine-kinase inhibitors in patients (pts) with Chronic Myelogenous Leukemia (CML), with the achievement in few cases of a prolonged Complete Molecular Response (CMolR) possibly leading to drug discontinuation. Aim. To evaluate the clinical follow-up after IFN discontinuation, 23 pts (M/F 11/12) who achieved CMolR at RT-nested PCR with IFN at our Institution have been

revised. Patients and Methods. Median age at diagnosis was 43,3 years [Interquartile Range (IR) 35.8 – 51.3], Sokal score was low in 18 pts, Intermediate in 3 and not evaluable in 2, median WBC at diagnosis was 39.9 x 109/l (IR 24.4 – 67.6). IFN was given alone in 16 pts or in association with autologous bone marrow transplantation (ABMT) in 7 pts (ABMT at onset followed by IFN in 4 pts, IFN followed by ABMT in 3 pts). Median time to Complete Cytogenetic Response was 21.4 months (IR 14.4 – 37.4), median time to CMolR was 63.7 months (IR 30.3 – 106.0). Results. After a median period of IFN treatment of 105.8 months (IR 56.1 – 127.3), all pts discontinued treatment due to prolonged CMolR (12 pts), intolerance (8 pts) or planned ABMT (3 pts). After 12.5 months from IFN discontinuation, 1 pt developed an extramedullar lymphoid blast crisis and died from disease progression. Four pts needed to start imatinib (2 for cytogenetic relapse after 24,8 and 44,0 months, 1 for molecular relapse after 39,8 months and 1 for progressive rise in molecular transcript after 39,7 months), all achieving a new molecular response which was complete in 3 out 4. The remaining 18 pts are still off-therapy after a median time from IFN discontinuation of 125.5 months (IR 86.9 – 205.3); among them, 5 pts resulted always negative at the molecular follow-up, 6 pts presented a sporadic positivity (bcr-abl ratio ≤ .0.01) and 7 had a mild rise of transcript levels with a long-lasting stable positivity (bcr-abl ratio <.0,5 without further increments). At the last molecular evaluation, 11/18 pts were in CMolR, 4/18 in MMolR and 3 had bcr-abl ratio between 0.5 and 0.1. Conclusions. Our data show that CML pts who achieved a prolonged CMolR with IFN and discontinued the treatment had a very low risk of relapse; it is worth of note that in many cases the reappearance of a bcr-abl positivity < .0.5 did not precede a disease relapse but was sporadic or stable over long time, suggesting a possible role for immunological mechanisms induced by IFN.

P314

CO-EXPRESSION OF P190 AND P210 BCR-ABL FUSION TRANSCRIPTS AND RESPONSE TO TKI THERAPY IN CHRONIC MYELOID LEUKEMIA: THE RET (RETE EMATOLOGICA TOSCANA) EXPERIENCE

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The hallmark of chronic myeloid leukemia is the presence of the Philadelphia chromosome and its resultant fusion gene BCR-ABL, and fusion protein, p210. Occasionally patients with CML can have a smaller fusion transcript, resulting in a p190 protein. A co-expression of both proteins, p190 and p210 has been described in CML; p190 mRNA usually is expressed at low level and its presence seemed to have no impact on prognosis in the pre-TKI era. 82 patients with new diagnosis of CML have been referred to our institution between 2008 and 2013, within the RET (Rete Ematologica Toscana) project. 13 cases (16%) expressed both transcripts. Median age was 55 years (range 31–83 years). At diagnosis 11 patients were in chronic phase (CP), 2 in accelerated phase (AP), none was in blastic phase (BP). Sokal risk was low in 2 patients, intermediate in 6 patients, and high in 3 patients. As frontline therapy, 8 patients received Imatinib 400 mg/day, 3 Nilotinib 600 mg/day, two patients in AP Dasatinib 100 mg/day and Imatinib 600 mg/day, respectively. The median follow-up was 22 months (range 3–57 months). Amount of p190 transcript was always low at the diagnosis (ratio BCR-ABL/ABL <1% in IS), becoming undetectable within 12 months in all patients; occasional, sporadic positivity at very low levels was documented during the follow up, with no correlation with raise in p210 amount. The patient in AP treated with Dasatinib obtained a CCyR (complete cytogenetic response) and a MMR (major molecular response) within 6 months, the other one in AP was resistant to Imatinib and underwent allogeneic bone marrow transplant from an unrelated donor; 3 patients in CP treated with Imatinib obtained a MMR at 12 months, 3 were resistant and switched to second generation tyrosine kinase inhibitors, a case under Imatinib at 18 months showed still a ratio of 0,21, while BCR-ABL/ABL ratio of the last patient with only three months of follow up is not available. Nilotinib as first line therapy induced a MMR at six months in 3 patients. Co-expression of p210 and p190 transcripts is not a common event in CML, especially in chronic phase. Presence of low levels of p190 transcripts at the diagnosis appears to be related to an increased Sokal risk and/or advanced phases, and to an apparent reduced probability of response to Imatinib. The majority of patients showed fluctuating, very low levels of p190 transcripts over the time, with no correlation with changes in p210 transcript.

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REDUCED EXPRESSION OF CHIBBY IS A COMPONENT OF BETA-CATENIN ACTIVATION IN CHRONIC MYELOID LEUKEMIA STEM CELLS

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Bcr-Abl fusion gene is the causative genetic lesion of chronic myeloid leukemia (CML). However, it has a marginal role in leukemic stem cell (LSC) proliferation and survival, mostly contingent upon -catenin signalling. Chibby (Cby) is a -catenin antagonist encoded by C22orf2 on chromosome 22q13.1, in close proximity to the Bcr breakpoint (22q11). Cby hinders -catenin binding with Tcf/Lef transcription factors and drives -catenin nuclear export and cytoplasmatic relocation in a stable tripartite complex encompassing 14-3-3 scaffolding proteins. Here we proved that Cby is a component of -catenin activation associated with Bcr-Abl, in particular, in the LSC compartment. FISH analysis established that one C22orf2 allele was invariably translocated to the derivative 9 chromosome in CML patients with the typical t(9;22)(q34;q11) translocation or to the second fusion gene in patients with variant translocations, and fused to upstream Bcr sequences. C22orf2 translocation was associated with a significant reduction of its Cby protein in the majority of CML patients compared to healthy donors. The analysis of gene expression in CML patients at diagnosis and at the moment of major molecular response (MMR) established that Cby reduction was restricted to the Bcr-Abl+ leukemic clone. However, it was not contingent upon transcriptional events, as proved by the marginal reduction of C22orf2 transcripts associated with Bcr-Abl expression in mononuclear cell fractions (MCF) from bone marrow of CML patients at diagnosis. Notably, the stem cell compartment identified by a CD34+ phenotype, where -catenin has a prominent impact, exhibited lower levels of C22orf2 transcripts associated with an even greater decrease of Cby protein compared with the MCF. Chibby transcriptional downregulation in Bcr- Abl+ LSC is driven by epigenetic modifications encompassing the gene promoter hypermethylation. In conclusion, our study let identify Cby downregulation as a component of -catenin activation associated with Bcr-Abl in a LSC compartment, which is resistant to imatinib and second generation tyrosine kinase inhibitors. C22orf2 promoter hypermethylation is one cause of Cby downregulation, supporting de-methylating agents as a complementary therapeutic option in CML.

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COMPARISON BETWEEN BCR-ABL TRANSCRIPT LEVELS AND COMPLETE CYTOGENETIC RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS: RESULTS FROM THE SCREEN STUDY

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Targeted therapy with tyrosine kinase inhibitors (TKIs) has changed

the clinical course, long-term outcome and treatment goals of chronic myeloid leukemia (CML). Historically, the achievement of a complete cytogenetic remission (CCyR) represented the first surrogate marker associated with therapeutic benefit and increased survival. More recently, early and deep molecular responses measured by Real-Time Quantitative RT-PCR (RQ-PCR) have also been associated with superior responses to TKI treatment and improved survival. However, unlike RQ-PCR, cytogenetic analyses are commonly associated with 10-15% failure rates that can deprive both patients and physicians of critical information concerning treatment efficacy. We therefore set to establish if BCR-ABL transcripts could be employed to identify a molecular threshold associated with the attainment of a CCyR. We investigated a cohort of 230 consecutive chronic-phase CML patients recruited in the SCREEN (Sicily and Calabria CML REgional ENterprise) study between January 2005 and December 2011. All subjects received Imatinib Mesylate 400 mg/daily and were analyzed for clinical, cytogenetic and molecular responses. Median follow-up of the patient cohort was 42 months. Clinical responses were defined according to the 2009 ELN criteria. Bone marrow cytogenetics were assessed at diagnosis, at 3 and 6 months and then every 6 months by evaluating no less than 20 metaphases. BCR-ABL transcripts were measured from peripheral blood samples drawn at diagnosis and every three months thereafter, using RQ-PCR. BCR-ABL values were expressed on the international standardized scale (IS) employing ABL as a reference gene. We analyzed 321 samples collected simultaneously from bone marrow aspirates and peripheral blood specimens of CML patients in confirmed CCyR (*i.e.* absence of leukemic metaphases in two consecutive cytogenetic analyses). We found that patients achieving a CCyR displayed median BCR-ABLIS transcripts of 0.97% (range 0.09% - 1.88%). Our findings suggest that BCR-ABLIS values of 0.97% are associated with the achievement of a CCyR and that, CML patients displaying BCR-ABLIS transcripts >1.88% are not likely to be in CCyR. In the molecular era of TKI therapy, these BCR-ABLIS transcript values may represent an additional tool in the CML monitoring armamentarium, allowing the identification of patients in CCyR when conventional cytogenetics fail.

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HIGH BCR-ABL LEVELS AT DIAGNOSIS ARE ASSOCIATED WITH UNFAVORABLE RESPONSES TO IMATINIB

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The approval of second-generation tyrosine kinase inhibitors (TKIs) for the first line treatment of Chronic Myeloid Leukemia (CML) has generated a need for early molecular parameters associated with inadequate responses to Imatinib (IM). We correlated quantitative determination of BCR-ABL transcripts at diagnosis with the outcome of 230 newly diagnosed CML patients receiving IM 400 mg/die. BCR-ABL transcripts were measured from peripheral blood samples drawn at diagnosis using Real-Time Quantitative PCR (RQ-PCR). All molecular determinations were performed twice (in triplicates) on the same sample using either ABL or glucuronidase-beta (GUS) as reference genes. BCR-ABL values were then reported on the international scale (IS). High BCR-ABL/ABLIS levels at diagnosis were associated with inferior IM efficacy (p=0.007). Correlations between high BCR-ABL transcripts at diagnosis and unsatisfactory IM responses were strengthened by the use of GUS. Indeed, high BCR-ABL/GUSIS at diagnosis was associated with lower rates of complete cytogenetic response (CCyR) after 12 months of treatment (p<0.0001), and with inferior probabilities of failure-free survival (p<0.0001), event-free survival (p<0.0001) and optimal response as defined by the 2009 European Leukemia Net recommendations (p<0.0001). Elevated BCR-ABL/GUSIS levels were also associated with suboptimal responses and IM failure (p<0.0001) and with inferior rates of disease transformation to the accelerated phase or blast crisis (p=0.012). Using receiver operating characteristic curves and the achievement of an optimal response as a specific endpoint, we found that 15.96% BCR-ABL/GUSIS at diagnosis defined a threshold distinguishing low risk from high risk patients. High BCR-ABL transcripts at diagnosis measured by RQ-PCR employing GUS as a reference gene allow the identification of CML patients unlikely to benefit from IM that should receive alternative forms of treatment.

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RETROSPECTIVE ANALYSIS OF SAFETY AND EFFICACY PROFILES OF SECOND GENERATION TKIS IN CML

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Second generation tyrosine kinase inhibitors (TKIs) in Chronic Myeloid Leukemia (CML) have shown more rapid and deeper responses than imatinib, so their use in first line has been approved. As we know little about their safety and tolerability profile, we explorated it both in their first and second line use. Thirty-four patients were diagnosed with CML in our institution from 2003; mean age was 43 years, 47% male and 53% female patients; 11% received second generation TKIs as first line treatment, while 89% switched after imatinib. BCR-ABL transcript was b2/a2 in 31% and b3/a2 in 68%; one patient showed both. Sokal risk was low in 48%, intermediate in 27% and high in 25% of patients. Mean duration of imatinib treatment was 54 months; switch to second line TKI was for resistance in 77% and for intolerance in 23% of the patients. During Imatinib treatment all patients showed muscle cramps , fatigue and periorbital edema, with 23% grade 3-4 events. Other side effects were anemia (23%), conjunctival injection (13%) and diarrhea (13%). Five patients received nilotinib as first line treatment with a mean duration of 18 months; 12 patients switched to Nilotinib in 50% of cases for intolerance while in the other 50% the main reason was resistance to imatinib. Median duration of second-line treatment was 21 months. Seventy-five percent of resistant patients achieved molecular response while 25% had only hematological and cytogenetic response. Biochemical abnormalities (46%) included increase of serum lipase (15%) and transaminase(31%); other side effects were muscle cramps (31%) and nausea (10%). Fourteen patients switched to dasatinib in 10% of cases for intolerance and in 90% for resistance to first/second TKIs therapy. This group included patients with more aggressive disease course. Median duration of treatment was 35 months. Molecular response ($MR^{4.5}$) was achieved in 60% of resistant pts, while 40% had only hematological and cytogenetic response. As to side effects, 20% of pts had pleural effusion, 10% of pts had muscle pain and rarely headache. Grade 2/3 events were thrombocytopenia (10%)and neutropenia in one patient. Second generation TKIs show a good efficacy profile in the treatment of CML not only in first line but also in second line, recovering a substantial number of patients failing first line with Imatinib. Moreover, in our hands, these TKIs have a good safety profile, showing manageable side effects in the majority of patients.

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PROTEOMIC SIGNATURE OF CD34+ PROGENITORS FROM CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA

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Tyrosine kinase inhibitors (TKIs) represent the successful molecular therapy for patients with chronic myeloid leukemia (CML), nevertheless several mechanisms have been associated with resistance to TKIs, including the presence of rare quiescent leukemic stem cells, less susceptible to TKIs. Moreover, Bcr-Abl activates additional signal transduction pathways (STP), such as RAS/MEK/ERK, PI3K/Akt, Wnt and STAT5 pathways, potentially contributing to CML TKIs drug resistance. Therefore, in this study we aimed to investigate, at the protein level, proliferative and apoptotic STP in CML CD34+ cells as compared to normal CD34+ cells in order to identify additional aberrant signals potentially therapeutic targetable. CD34+ cells were purified from peripheral blood (PB) of seven newly diagnosed chronic phase (CP) CML patients and one normal bone marrow (nBM). The phosphorylation status of 46 proteins from various STP and the expression of 32 proteins of the apoptotic machinery were assessed by using a customized direct phase proteome profiler antibody array. The resulting dots were visualised using ECL and quantified by densitometric analysis. CML samples had a WBC count ranging between 41900-421400 per microliter. The Sokal risk category was low (1/7) and intermediate (6/7). CML CD34+ cells were characterized, as compared to nBM, by lower phosphorylation (>1.2 fold decrease) of three members of the protein-tyrosine kinase oncogene family (Fyn, Scr, Yes), of the serin-threonine kinase Chk-2, and of STAT2. In contrast, higher phosphorylation was found for the cell cycle inhibitor p27 and for the PI3K pathway component p70s6K (fold increase 1.4 and 1.6, respectively). Protein overexpression, ranging between 1.4-2.1, was further noticed in members of the HSP family (HSP70, HSP60, HSP27), in the IAP family members (survivin, XIAP, cIAP-1, cIAP-2), and for Bcl-2. In conclusion, in this study we have found that CD34+ from CP CML are characterized by a proteomic and phospho-proteomic profile that promotes cell survival in a quiescence-like state through inhibition of both proliferation and apoptosis. The understanding of the critical balance between quiescence and proliferation in the proteomic profile would be useful to predict CML progression.

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THE CHRONIC MYELOID LEUKEMIA ITALIAN REGISTRY OF NILOTINIB: A GIMEMA CML WP ANALYSIS OF THE OUTCOME OF PATIENTS TREATED FRONTLINE WITH NILOTINIB-BASED REGIMENS

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Background. Nilotinib is a potent and selective BCR-ABL inhibitor approved for the frontline treatment of CML. The latest update (4-yr follow-up) of the ENESTnd study demonstrated sustained superiority of nilotinib *vs* imatinib (Kantarjian H *et al*, ASH 2012, abstract 1676). The CML Italian Registry of Nilotinib is the largest series of pts treated frontline with nilotinib-based regimens, outside of Company- initiated trials. Therefore, it represents an important resource for an independent evaluation of the outcome of such pts. Aims: to analyze the response rates and outcome in an independent cohort of pts treated frontline with nilotinib-based regimens in Italy. Methods. The CML Italian Registry of Nilotinib includes 215 pts, enrolled in 2 multicenter phase II studies conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00481052 and NCT00769327) or treated at the Bologna University Hospital, with nilotinib 300 mg or 400 mg BID as initial treatment; 123 pts received a sequential treatment with nilotinib and imatinib, with a 3-mo rotation period. The median age was 53 yrs (range 18–86). Ten out of 215 pts (5%) had a high EUTOS score. The median follow-up was 33 mos (range 21–51 mos). We analyzed the rates of Complete Cytogenetic Response (CCyR) and Major Molecular Response (MMR); the failure-free survival (FFS, according to ELN 2009 definitions), progression-free survival (PFS), and overall survival (OS, any death included). Results. Rates of CCyR were 72% and 92% by 3 mos and 12 mos, respectively. Rates of MMR were 56% and 84% by 3 mos and 12 mos, respectively. The cumulative rates of CCyR and MMR were 93% and 88%, respectively. Overall, 9 (4.2%) pts progressed to accelerated-blast phase (AP/BP), 6 of whom subsequently died. Eight progressions occurred during the 1st year of therapy. Nilotinib-resistant mutations were identified in 5 pts (4 T315I; 1 Y253H). No difference in the rate of progression to AP/BP was observed between pts receiving nilotinib alone or nilotinib and imatinib in sequential schedule. The 3-yr OS, PFS, FFS, and EFS were 94%, 93%, 90%, and 83%, respectively. Conclusion. Our National experience confirmed that pts treated frontline with nilotinib-based regimens obtain fast and high rates of complete cytogenetic and major molecular response. Progression to AP/BP occurred in most cases within the 1st year. These results translate in high 3-yr survival measures. Acknowledgements: European LeukemiaNet, COFIN, Bologna University.

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MEGAKARYOCYTIC BLAST CRISIS IN A CHRONIC MYELOID LEUKEMIA PATIENT

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Chronic myelogenous leukemia (CML) is a chronic myeloproliferative neoplasm with a triphasic clinical course: chronic, accelerated and blastic phase. Myeloid blastic transformation of CML is more common than lymphoid; megakaryocytic blast crisis is very rare. A 46 year-old caucasian woman with elevated leukocytosis, mild peripheral blastosis and splenomegaly was diagnosed with CML in accelerated phase, intermediate and high risk according respectively to Sokal and Euro score. In addition to the Ph chromosome, she showed an additional cytogenetic abnormality, *i.e.* an isochromosome i(17)q. After a short course of Hydroxyurea, Nilotinib 600 mg daily was started. The patient subsequently had progressive severe pancytopenia and transfusion support rapidly occurred; the bone marrow biopsy showed a block maturation of three hematopoietic series. At third months, the patient had a high platelet count (817.000/microL) and the bone marrow biopsy showed a megakaryocytic blast crisis with fibrosis: CD34 positive, MPO, CD68PGM1 and LAT negative blasts, reticulin fibrosis grade I. Oddly enough but not too much RQ-PCR demonstrated 0.39% BCR-ABL/ABL transcript, while mutational screening analysis of BCR-ABL kinase domain did not revealed mutations. After a bankruptcy therapy lasted barely a month with Dasatinib (BCR-ABL/ABL% transcript raised to 89) we started Ponatinib, a third-generation tyrosine kinase inhibitor, at doses of 45 mg/daily that has demonstrated significant activity against the BCR-ABL fusion oncogene. The evaluation at 2 months showed a significant decrease of the transcript and above all an important reduction in the bone marrow blastosis (<10%). The patient is obviously a candidate for an early allogeneic transplantation from HLA-matched family. Megakaryocytic blast crisis occurs extremely rarely, accounting for <3% of cases of CML in blastic transformation; the prognosis is poor. Obviously in such blast crisis the molecular mechanism of the

BCR-ABL rearrangement is accompanied and well exceeded by many other factors; consequently the amount of BCR-ABL transcript is not necessarily in relation to the size of the disease, like in this patient. The allograft is essential, but it must be preceded by a debulking therapy. In this case it seems to have achieved partial remission of disease; obviously allogeneic transplantation remains the only chance of survival and perhaps cure for this patient.

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CONCOMITANT OCCURRENCE OF BCR-ABL REARRANGEMENT AND MPL W515L MUTATION IN A SINGLE PATIENT

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Few isolated cases of patients with simultaneous occurrence of typical chronic myeloid leukemia (CML) and classical Ph-negative myeloproliferative neoplasm (MPN), with BCR-ABL rearrangement and JAK2 V617F mutation have been described so far. The physiopathologic mechanisms underlying the development of this co-mutated phenotype and its correct management are not yet defined. Till now, no MPN cases with mutations involving genes other than JAK2 and concomitant CML have been reported. Here we report a 58-years-old man with BCR-ABL rearranged CML and concomitant MPL-positive primary myelofibrosis(PMF). At diagnosis he presented with leucocytosis (WBC 15.9x10^9/L), anemia (Hb 10.6 g/dl) and thrombocytosis (PLT 921x10^9/L); a e13a2 BCR-ABL rearrangement was documented, t(9;22)(q34;q11) being the sole cytogenetic abnormality (14/20 metaphases). He started Imatinib 400 mg/day; after 3 months, complete hematologic response wasn't obtained, as thrombocytosis and anemia worsened (WBC 7.41x10^9/L, Hb 8,17 g/dl, PLT 909x10^9/L),despite of complete cytogenetic response and major molecular response (MMR, IS 0.01%). A bone marrow biopsy was performed and a diagnosis of PMF was done in accordance to WHO criteria. Thus the MPL screening was retrospectively conducted on DNA stored at diagnosis and the W515L mutation was detected (allele burden 31%). To assess the clonal evolution of these 2 lesions, 41 hematopoietic colonies cloned in semisolid medium after 3 months of therapy were individually picked and genotyped for both BCR-ABL and MPL: 3 colonies were normal, 37 were MPL-heterozygous and BCR-ABL negative and 1 harboured both lesions, consistent with the achievement of MMR (IS 0.01%) and the stable MPL mutation burden. On the basis of these results, we can hypothesize that in the clonal evolution of the disease the MPL mutation arose first. Then, as second hit, the BCR-ABL rearrangement occurred in a MPL-positive cell and the double-mutant clone progressively expanded. Imatinib selectively targeted the cells carrying both mutations but allowed the expansion of the residual clone carrying only the MPL mutation. After 12 months of follow-up, the patient is still under Imatinib; he is in MR4, whereas severe thrombocytosis and anemia persist, due to PMF (MPL mutant burden unchanged, 29%). This is the first case of concomitant BCR-ABL rearrangement and MPL mutation; further studies are requested to clarify the molecular pathogenesis and clonal predisposition to develop MPN.

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REDUCED EXPRESSION OF CHIBBY IN CHRONIC MYELOID LEUKEMIA ARISES FROM THE BCR-ABL EFFECTS ON ITS STABILITY

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BCR-ABL1 tyrosine kinase (TK) is the cause of chronic myeloid leukemia (CML). However, it is not the critical signal for leukemic stem cells (LSC), whose self-renewal is under the control of -catenin signaling. Accordingly, imatinib (IM) has revolutionized the disease prognosis, but is unable to eradicate the BCR-ABL1+ LSC compartment. We have identified the transcriptional down-modulation of Chibby (Cby) as a component of -catenin activation in the BCR-ABL1+ LSC compartment. Cby is a -catenin antagonist encoded by C22orf2 on chromosome 22q13.1. The BCR-ABL1 TK impact on CBY stability was first prompted by Cby protein reduction in the mononuclear cell fraction (MCF) from bone marrow samples of the great majority of CML patients at diagnosis, not contingent upon transcriptional events. Cby reduction in such a cell context was associated with catenin increment and nuclear translocation. Those results suggest that BCR-ABL1-associated downregulation of Cby is mostly contingent upon post-transcriptional mechanisms. Further investigation was therefore carried in K562 cell line (which exhibits low levels of C22orf2 transcript and no Cby protein) and C22orf2-transfected K562 cells, where the enforced expression of Cby drove -catenin cytoplasmic accumulation and functional inactivation. In parental K562, Cby induction in response to IM was followed by its nuclear export in complex with 14-3-3 and -catenin. Thereafter, the complex dissipation within the cytoplasmic compartment drove the increment of Cby and the decline of -catenin, addressed towards the proteasome degradation, while 14-3-3 levels remained steady. Further experiments carried in presence of inhibitors of the 14-3-3 binding domain BV001 or of the JNK (whose activation elicits 14-3-3 phosphorylation at a critical residues for binding with partners proteins) inhibitor let establish the critical role of 14-3-3 in Cby subcellular partitioning and stability. Accordingly, the enforced expression of a C22orf2 mutated construct coding for a protein S20A unable to bind 14-3-3 abrogated both Cby and -catenin nuclear export in response to IM, in spite of the drug-induced gene transcription. Further experiments are currently in progress to elucidate the participation of post-transcriptional mechanisms in Cby enhanced stability driving -catenin functional inactivation in BCR-ABL1+ cells and, in particular, in LSC.

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REDUCTION IN IL-33 PLASMA LEVELS MIGHT BE INVOLVED IN T-CELL DYSREGULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is a proliferative disorder that requires the help of its microenvironment to be maintained and to progress. In CLL neoplastic B cells inhibit normal T lymphocytes, and the alteration of several cytokines may also contribute to this T-cell dysregulation. Particular relevance may have some recently discovered cytokines, such as interleukin (IL)-33 and IL-31. IL-33 can activate dendritic cells directly driving polarization of naïve T cells towards a Th2 phenotype. IL-31 is capable of inducing chemokines in several diseases. We analyzed the plasma levels of IL- 33, and IL-31, in 77 patients with B-CLL. In the same subjects we also evaluated the lymphocyte immunophenotypical pattern, and we performed IgVH gene analysis, CD38 positivity and ZAP-70 expression to evaluate a possible correlation between interleukin concentrations and biological risk. In a small group of patients the dosage of the cytokines was performed before and after different treatment protocols. Plasma from 63 normal subjects were also included as controls. IL-31 and IL-33 protein levels were measured using the commercially available ELISA kits. Data were presented as interquartile range IQR and range except age presented as mean ± Standard deviation. There was a significant difference ($p<0.0001$) between the levels of IL-33 in patients affected by CLL (411.5 and 617.2 pg/mL) and those measured in controls (1,375.3 and 2,035.4 pg/mL). There was not a significant difference between the levels of IL-31 in patients affected by CLL (1,783.5 and 10,692.8 pg/mL) and those measured in controls (3,278.4 and 10,067.1 pg/mL). There was a significant difference, although not in a statistically way (P=0.072), between the IL-33 levels in CLL patients before and after therapy (83.67 and 1,421 *vs* 837.22 and 1,790.38 pg/mL). We found a negative correlation in patients between IL-31 levels and IL-33 and CD20 expression (respectively rho = -0.6, P = 0.014 and rho= -0.43, P=0.031). There was a positive correlation in patients between levels of IL-33 and CD3 expression (rho = 0.81, P = 0.027). To the best of our knowledge, ours is the first study that demonstrated decreased concentrations of IL-33 in patients with CLL, and this reduction might justify the reduction of the Th2 response observed in these patients.

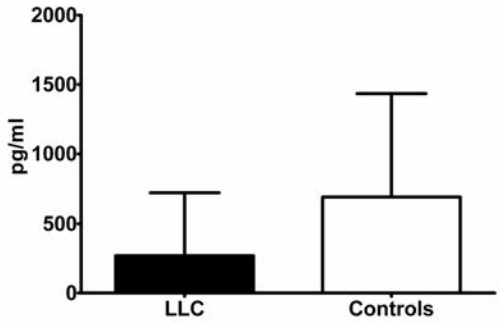

Figure 1.

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UNEXPLAINED SEVERE COOMBS-NEGATIVE HEMOLYTIC ANEMIA ASSOCIATED WITH THE EMERGENCE OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PHENOTYPE ON RED BLOOD CELLS (RBC) IN A CHRONIC LYMPHOCYTYC LEUKEMIA (CLL) PATIENT RECEIVING ALENTUZUMAB: A CARE REPORT

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Coombs-negative haemolytic anaemia (HA) may represent a difficult diagnostic challenge in CLL patients. Alentuzumab is a monoclonal antibody directed to CD52, a glycosylphosphatidylinositol (GPI) anchor protein, which has a conventional structure similar to CD55 and CD59. The potential role of alentuzumab in inducing PNH-like acute HA, as observed by us in a CLL patient, has been rarely reported. A 70 years old CLL woman was urgently admitted for a severe acute haemolysis, initially considered to be of autoimmune origin. Four years before she was diagnosed as having CLL which immunophenotype was CD19+, CD5+, CD23+, CD38+, CD11c+, Kappa+, CD79b+, ZAP-70+; FISH analysis revealed trisomy 12 in 50% of B-cells. Somatic mutations of VH 4-39, D 6-13, and JH 5 sequences was detected by molecular analysis; NOTCH1, TP53, SF3B1 e BIRC3 were unmutated. She received six monthly standard courses of fludarabine and then rituximab at weekly dose of 375 mg/m^2 for 4 weeks. A complete response (CR) was achieved. Therefore, with the aim to delay the disease recurrence, rituximab 150 mg/m^2 was monthly given for 9 months. The patient maintained the CR until about a year later, when a disease relapse occurred. The combination fludarabine-cyclofosphamyde was given for six cycles achieved a second CR. As consolidation, alentuzumab (10 mg/m^2 three times a week) was started. After two months of treatment, a severe Coombs-negative HA occurred. On clinical basis, she received prednisone and rapidly recovered. A PNH-like syndrome was suspected and a flow cytometry evaluation demonstrated a reduction of CD55 and CD59 expressions, revealing alentuzumab-induced GPI anchors protein changes on red blood cells. After cessation of alentuzumab, haemoglobin levels stabilized. The patient died because of disease complications three years later. However, no recurrence of HA was recorded. Alentuzumab has been associated with some rare cases of haemolytic anaemia which the underlying pathogenic mechanism has been referred to the loss of anchors protein structurally related to CD52, such as CD 55 and CD 59, thus resembling PNH. Although in our case this correlation has not been definitely proven, we conclude that the regular monitoring of peripheral CD55/CD59 levels could be justified in patients receiving alentuzumab given that the increased number of RBC lacking of their expression may predispose to severe haemolytic complications.

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TWO NOVEL MUTATIONS OF NOTCH1 PEST DOMAIN IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Chronic Lymphocytic Leukemia (CLL) prognosis and evolution can be predicted using different biological parameters. Among them, unmutated immunoglobulin heavy-chain variable (IgHV) genes, cytogenetics alterations as 11q deletion, NOTCH1 mutations and TP53 disruptions are recognized markers of adverse outcome. NOTCH1 is a transmembrane receptor that acts as an intracellular mediator to regulate different physiological phenomena such as cellular differentiation, proliferation and apoptosis. Recently, it has been observed that NOTCH1 mutations affect more frequently CLL patients harbouring trisomy 12, and are almost exclusively localized at the proline, glutamic acid, serine, threonine-rich (PEST) domain. As reported in T-cell acute lymphoblastic leukemia patients, mutations at the PEST domain alter NOTCH1 protein degradation resulting in intracellular protein accumulation accompanied by increased transcription of NOTCH1 target genes. With this study we report two previously unrecognized mutations in the C-terminal NOTCH1 PEST domain, one of whom leading to disruption of the

protein functionality. The first mutation was characterized by the substitution of a cytosine with a thymine (g.7459C>T), which generated a stop codon (p.Q2488*) leading to the disruption of the regulatory domain of NOTCH1, with consequent intracellular protein accumulation. This alteration was identified in a 62 years old patient harbouring deletion in 13q14 and 17p13 and showing a rapid disease progression complicated by the onset of immune thrombocytopenia within a year from diagnosis. The second was characterized by the substitution of a valine with an isoleucine (p.V2537I) without affection of the structure and function of the protein. This patient had a complex cytogenetic profile (+12, del13p14, del 17p13 and dup 8q) and rapidly transformed to Richter's syndrome. Our data suggest that previously unrecognized NOTCH1 mutations involving the PEST domain are likely to be associated with an aggressive clinical behaviour. Our study strengthens the importance of NOTCH1 mutational screening to predict CLL prognosis since it is known that the intracellular NOTCH1 protein accumulation increases B-cell clone proliferation and evasion from apoptosis.

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RECURRENT MOLECULAR ABNORMALITIES ASSOCIATED WITH B-CELL RECEPTOR CONFIGURATION AND IGVH MUTATIONAL STATUS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND TRISOMY 12

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Trisomy 12 (+12) is the third most frequent cytogenetic aberration in chronic lymphocytic leukemia (CLL) retrievable both as the sole chromosomal abnormality or in association with additional alterations. NOTCH1 mutations are known to prevail among +12 patients, while mutations of FBXW7, a gene involved in NOTCH1 degradation, leading to the constitutional activation of NOTCH1, have not been investigated in this setting. In this study, a unicentric cohort of 44 CLL patients harboring trisomy 12 was screened for mutations of TP53, NOTCH1 and FBXW7 genes and correlated with B-cell receptor (BCR) configurations. FBXW7, TP53 and NOTCH1 mutations were identified in 7%, 13.6% and 18.2% of patients, respectively. FBXW7 and NOTCH1 mutations appeared in a mutually exclusive fashion, suggesting that both aberrations might affect the same biological pathway. We found that 44.1% of +12 CLL patients had stereotyped BCRs, which is significantly more prevalent than what observed in CLL patients without +12 (27%, p=0.01). Subsets #1, #8, #10, #28 and #59 were the most represented stereotyped patterns and IGHV4-39*01 was the gene configuration most commonly used. There was a significantly higher risk for Richter Syndrome (RS) transformation in patients with NOTCH1 or FBXW7 mutations: 4 of the 7 (57%) patients developing RS were characterized at least by one of the two abnormalities. These observations suggest that, similarly to the aberrations of NOTCH1, FBXW7 gene mutations may also result in cell proliferation and evasion from apoptosis in patients with +12 CLL. Together with the extremely high frequency of stereotyped BCRs and RS transformation, these abnormalities appear to cluster in +12 CLL patients, suggesting a connection with the prognosis of the disease.

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THE TNF-FAMILY CYTOKINE TL1A/DEATH RECEPTOR 3 FUNCTIONAL AXIS MODULATES B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA PROLIFERATION

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Compelling evidence suggests that survival and proliferation of B-cell chronic lymphocytic leukemia (B-CLL) cells depend on a crosstalk with microenvironmental bystander cells and that the dynamic and interactive interplaying between tumor necrosis factor (TNF) superfamily members and their cognate receptors has a major role in this crosstalk. Death receptor (DR3) 3 is a member of the TNFR superfamily. Its ligand is TNF-like ligand 1A (TL1A), a member of the TNF superfamily. TL1A/DR3 interactions have been reported to modulate the functions of T cells, NK, and NKT cells and play a crucial role in driving inflammatory processes in several T-cell-dependent autoimmune diseases. Recently, we showed that the TL1A/DR3 axis reduces B-cell proliferation induced by anti-IgM and IL-2. The objective of this study was to analyze DR3expression and its possible effects on B-CLL proliferation and apoptosis. To this aim, first peripheral blood B cells and lymph node specimens from 35 B-CLL patients were analyzed for the expression of DR3 by flow cytometry, western blotting and immunofluorescence. Flow cytometry analysis showed that, similarly to healthy B cells, B-CLL did not express DR3 in basal conditions, whereas stimulation of B cell receptor (BCR) with anti-IgM antibodies induced significant de novo expression of DR3 in B-CLL ($p<0.001$). These data were confirmed by western blotting analysis. The relevance of these results were further validated by immunofluorescence analysis in lymph node specimens that showed the in situ expression of DR3 in antigen-stimulated B-CLL cells *in vivo*. Then, we analyzed proliferation and apoptosis in B-CLL cells stimulated with anti-IgM, IL-2, CpG, or CD40L (or a combination of them), in presence or absence of TL1A. Among these stimuli, only the combination of CpG with suboptimal dose of anti-IgM is able to induce proliferation of B-CLL. Remarkably, TL1A reduces B-CLL proliferation induced by anti-IgM antibodies and CpG., whereas no effects of TL1A were detected in the presence of anti-IgM in combination with IL-2, or CD40L. This study describes for the first time the expression of DR3 molecules in B-CLL cells and reveals a novel role of the TL1A/DR3 functional axis in modulating leukemic proliferation *in vitro*. These data suggests that TL1A/DR3 may modulate cell crosstalk in the B-CLL microenvironment *in vivo*, therefore contributing to B-CLL pathogenesis and progression.

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CLL PATIENTS WITH LOW-RISK CYTOGENETIC ABNORMALITIES SHOW CLINICAL HETEROGENEITY ACCORDING TO IGVH MUTATIONAL STATUS AND CD38 EXPRESSION

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Chronic Lymphocytic Leukemia (CLL) is one of the most common hematological malignancies characterized by heterogeneous clinical course and outcome. Several prognostic factors have been validated for this disorder, the most important including cytogenetic abnormalities, IgVH mutational status and CD38 expression. The aim of this study was to correlate the clinical features of patients with 13q- or with normal FISH to the above quoted CLL prognostic factors, in an effort to define a scoring system useful for the risk stratification of patients. We retrospectively analyzed 212 CLL patients, with a mean follow-up of 70±64 months (range from 7 to 297 months), referred to the Hematol-

ogy Unit of Padua University Hospital from 1989 to 2011. 54% of subjects (n=114) were 13q- while 32% of patients (n=67) did not show any chromosome abnormality (N). Both IgVH mutational status (mutated, MUT or unmutated, UNMUT) and CD38 expression (CD38+ or CD38-) were available before starting treatment. Considering these two parameters, we developed a scoring system assigning score 0 to MUT&CD38- patients, score 1 to UNMUT&CD38- or MUT&CD38+, and score 2 to UNMUT&CD38+ patients (Fig. 1A). On the basis of the above-mentioned scoring system, 13q-&N patients were stratified in 3 different groups: 59% (n=107) score 0; 29% (n=57) score 1, and 9% (n=17) score 2 (Fig. 1A). These groups were different for time to first treatment (TTT) and overall survival (OS) (Log-rank; p<0.005) (Fig. 1B and 1C). In particular, all score 0 patients were alive at 5 years from diagnosis and only 20% of them needed treatment; conversely, score 2 patients showed an aggressive disease with TTT and OS at 5 years of 80% and 60%, respectively. Cox regression model showed that treatment and death related hazard ratio of score 0 *vs* score 1 were 3.0 and 6.2 (p<0.005), while score 0 *vs* score 2 were 5.2 and 13.6 respectively (p<0.005). 13q- and normal FISH have been usually expected to assign a favorable outcome to CLL patients. Despite this, some patients are treated or die few years from the diagnosis, indicating that CLL patients included in this group do not present the same clinical behavior. We disclosed this clinical diversity demonstrating that del13q&N patients UNMUT&CD38+ (score 2) have a shorter TTT and OS than score 0 MUT&CD38- patients. The need to develop a prognostic system that allows risk stratification and helps in the follow-up is emphasized.

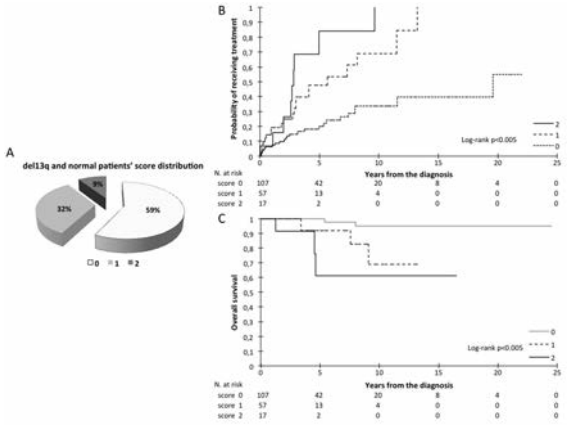

Figure 1.

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

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Background. Chronic lymphocytic leukemia (CLL) is characterized by a highly variable clinical course. Among the biological features underlying this heterogeneity, genetic lesions and the mutational status of the immunoglobulin heavy chain variable genes (IGHV) are of importance. Therapeutic options in CLL have been considerably expanded during recent years. The combination of fludarabine, cyclophosphamide and rituximab (FCR) has become gold standard in the first-line treatment of physically fit patients. Bendamustine demonstrated clinical activity in pre-treated hematological malignancies due to its unique mechanism of action distinct from standard alkylating agents. Aim. We have assessed the efficacy and safety of bendamustine in elderly patients with pre-treated chronic lymphocytic leukaemia. Methods. In the last 30 months we treated 20 elderly patients (8 F and 12 M, median age: 78 years, r.: 75-88 years) with relapsed/refractory CLL who had been heavily pre-treated (more than 4 lines of treatment). Patients received bendamustine 90 mg/m^2 on days 1 and 2 of a 4-week cycle. 18/20 patients received six cycles, while the remaining two received four cycles. Results. Overall response rates were 80% (16/20 patients), with clinical complete response rates of 50%. At present (+18 months after the end of treatment), 19/20 patients are alive and show no disease progression. One patient died due to respiratory infection six months after the end of therapy. Thrombocytopenia and gastrointestinal toxicities were more frequent adverse events. Grade ≥3 adverse events were infrequent and most commonly included thrombocytopenia (20%), anemia (10%), and infection (10%). Conclusions. Our results suggest that the bendamustine monotherapy is effective and well tolerated in the treatment of relapse/refractory CLL in elderly patients. Although further data are required to fully establish the efficacy of intravenous bendamustine in the management of this subset of patients, it appears to be a useful addition to the armamentarium of currently available therapies for this haematological malignancy.

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ROLE OF STROMAL CELL-MEDIATED NOTCH SIGNALING IN B-CELL ACUTE AND CHRONIC LYMPHOPROLIFERATIVE DISEASES

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Stromal cells are essential components of the bone marrow (BM) microenvironment regulating and supporting the survival of different tumors, including B-cell acute and chronic lymphocytic leukemia (B-ALL and CLL). In this study, we investigated the role of Notch signalling in human BM-mesenchymal stromal cell (hBM-MSC)-promoted ALL and CLL survival and chemoresistance. The block of Notch signalling through -secretase inhibitor (GSI) XII reverted the protective effect mediated by co-culture with BM-MSC. The treatment with combinations of anti-Notch neutralizing Abs resulted in the decrease of B-ALL cell survival, either cultured alone or cocultured in presence of BM-MSC from normal donors and B-ALL patients. The inhibition of Notch-3 and -4 or Jagged-1/-2 and DLL-1 resulted in a dramatic increase of apoptotic B-ALL cells by 3 days, similar to what is obtained by blocking all Notch signaling with the GSI XII. The same Notch receptors are involved in CLL survival except for Notch-1 that, in CLL, mediates a synergistic effect with other Notch receptors in inducing the anti- apoptotic phenotype. Overall, our findings show that stromal cell-mediated Notch signaling has a role in promoting ALL and CLL survival and resistance to chemotherapy. Therefore, the target of Notch pathway activation may represent an useful strategy to overcome drug resistance and improve the efficacy of conventional treatments.

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CLINICAL SIGNIFICANCE OF SF3B1 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Del Poeta G,[1] Del Principe MI,[1] Dal Bo M,[2] Rossi D,[3] Ragusa D,[1] Rossi FM,[2] Rasi S,[3] Perrotti AP,[1] Zucchetto A,[2] Buccisano F,[1] Niscola P,[1] Bulian P,[2] Maurillo L,[1] Bomben R,[2] Venditti A,[1] de Fabritiis P,[1] Amadori S,[1] Gaidano G,[3] Gattei V[2]

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Among the novel genetic alterations disclosed by whole genome/exome sequencing, NOTCH1, SF3B1 and BIRC3 lesions are more represented in refractory and advanced CLL clinical phases as well as TP53 and ATM. SF3B1 mutations deregulate normal and alternative mRNA splicing, have been identified in hematopoietic tumors of the myeloid compartment and their prevalence rises from 7% at diagnosis to 17% in relapsed and fludarabine refractory CLL patients (Rossi *et al*, 2011). The primary endpoints of our study were: 1) to determine time to first treatment (TTFT) and overall survival (OS) on the basis of SF3B1

mutations; 2) to correlate SF3B1 mutations with other biological and clinical markers, and finally 3) to assess the independency of SF3B1 mutations as prognostic factor. We investigated 340 patients, with a median age of 65 years whose 112 had a low Rai stage, 219 an intermediate and 9 a high stage. SF3B1 mutations were found in 24 out of 340 (7%) of CLL patients at diagnosis. SF3B1 mutations correlated with multiple thoracic/abdominal lymphadenopathies >3 cm in diameter and/or splenomegaly (14/24; P=0.005) and soluble CD23 >70 U/ml (13/22; P=0.01). On the other hand, no signicant correlation was found between SF3B1 mutations and FISH cytogenetic abnormalities, while SF3B1 mutated patients presented frequently unmutated IGHV status (16/24, P=0.0001). Noteworthy, SF3B1 mutations were mutually exclusive with TP53 disruption, NOTCH1 mutations and BIRC3 disruption (only 3 and 2 SF3B1 mutated patients were positive both for NOTCH1/BIRC3 and TP53, respectively). With regard to clinical outcome, 20/24 (83%) SF3B1 mutated patients received chemotherapy (P=0.0004) with 13/24 cases (54%) undergoing two or more lines of treatment (P<0.0001). Moreover, both significant shorter TTFT and OS were observed in SF3B1 mutated patients (6% *vs* 35% at 12 years, P=0.003 and 51% *vs* 74% at 12 years, P=0.02; Figure 1). In multivariate analysis of TTFT, SF3B1 mutations showed only a trend for significance (P=0.08), probably due to the low number of SF3B1 mutated cases, while IGHV status (P<0.001), NOTCH1 mutations (P=0.0009), TP53 disruption (P=0.003) and BIRC3 disruption (P=0.0001) were confirmed to be independent prognostic factors. Therefore, SF3B1 mutations represent a molecular marker more useful for the late identification of high risk CLL patients who are wild type for TP53, NOTCH1 and BIRC3 and that is consistent with their accumulation in the more advanced phases of the disease.

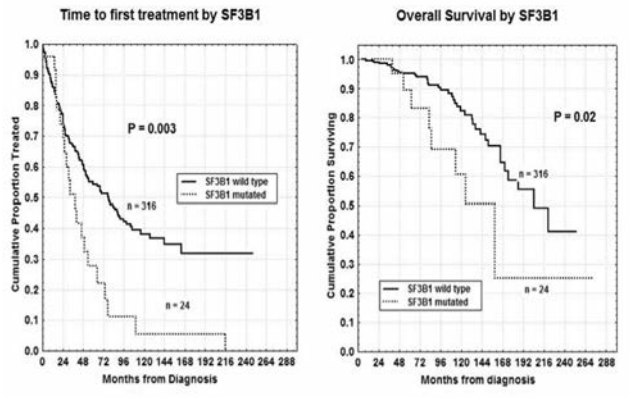

Figure 1.

with a median age of 65 years whose 112 had a low Rai stage, 219 an intermediate and 9 a high stage. BIRC3 disruption was found in 36 out of 340 (11%) of CLL patients (14 mutated, 18 deleted and 4 mutated and deleted) at diagnosis. BIRC3 lesions correlated with multiple thoracic/abdominal lymphadenopathies and/or splenomegaly (21/36; P=0.006) and soluble CD23 >70 U/ml (20/33; P=0.001). Significant correlations were also found between 11q22-q23 deletions (16/33, P<0.0001) or unmutated IGHV status (19/35, P=0.002)and BIRC3 disruption. Noteworthy, BIRC3 disruption was mutually exclusive with TP53 disruption and NOTCH1 mutations (only 2 and 4 BIRC3 disrupted patients were positive for TP53 and NOTCH1, respectively). With regard to clinical outcome, 29/36 (80%) BIRC3 disrupted patients received chemotherapy (P=0.00006) with 9/36 cases (25%) undergoing two or more lines of treatment (P=0.006). Moreover, both significant shorter TTFT and OS were observed in BIRC3 disrupted patients (18% *vs* 31% at 14 years, P=0.00007 and 39% *vs* 65% at 14 years, P=0.01). Interestingly, BIRC3 mutated cases showed an OS significantly shorter than BIRC3 deleted and BIRC3 wild type patients (43% *vs* 77% *vs* 86% at 10 years, P=0.02, Figure 1). In multivariate analysis of OS, BIRC3 disruption was confirmed to be an independent prognostic factor (P=0.003) together with age (P=0.005), TP53 disruption (P=0.003), NOTCH1 mutations (P=0.00001) and IGHV status (P=0.0001). Therefore, BIRC3 disruption may represent a molecular marker useful for the early identification of high risk among CLL patients who are wild type both for TP53 and NOTCH1 and provides the rationale for targeted anti-NF-kB therapy in this unfavorable clinical setting.

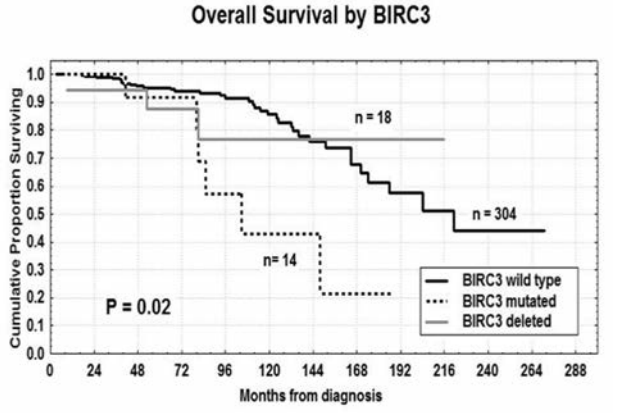

Figure 1.

P333

CLINICAL SIGNIFICANCE OF BIRC3 DISRUPTION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Del Principe MI,[1] Dal Bo M,[2] Niscola P,[1] Rossi D,[3] Ragusa D,[1] Rossi FM,[2] Rasi S,[3] Perrotti AP,[1] Zucchetto A,[2] Buccisano F,[1] Bulian P,[2] Maurillo L,[1] Bomben R,[2] Venditti A,[1] de Fabritiis P,[1] Amadori S,[1] Gattei V,[2] Gaidano G,[3] Del Poeta G[1]

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Recently, next generation sequencing has revealed new recurrent alterations in CLL, targeting the NOTCH1, SF3B1, and BIRC3 genes which provide new biomarkers for the identification of poor risk patients and new therapeutic targets mainly within the high risk disease. BIRC3 inactivating lesions (mutations and deletions) drive NF-kB activation which contributes to chemorefractory CLL and correlates with poor outcome (Rossi *et al*, 2012). The primary endpoints of our study were: 1) to determine time to first treatment (TTFT) and overall survival (OS) on the basis of BIRC3 disruption; 2) to correlate BIRC3 disruption with some biological and clinical markers, and finally 3) to confirm BIRC3 disruption as an independent prognostic factor. We investigated 340 patients,

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CHLORAMBUCIL PLUS RITUXIMAB AS FRONT-LINE THERAPY IN ELDERLY/UNFIT PATIENTS AFFECTED BY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS OF A SINGLE-CENTRE EXPERIENCE

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At present the standard first line therapy for fit patients affected by B-CLL/SLL are fludarabine-analogue based regimens. Elderly patients or patients with comorbidities poorly tolerate purine analogue-based chemotherapy and they are often treated with Chlorambucil (Chl). However, complete response (CR) rates are relatively low (up to 7%) as are overall responses with Chl. On the basis of promising results about the addiction of Rituximab (RTX) to Chl (Chl-R), we investigated this combination in treatment-naïve elderly/unfit patients with B-CLL. All patients were treated with Chl (1 mg/Kg monthly for 8 cycles) plus Rituximab (375 mg/m^2 for the first course and 500 mg/m^2 for subsequent cycles until the 6th cycle). The primary endpoints were efficacy and safety of the Chl-R. We included in the study 27 patients with a medi-

an age of 72 years (range 58 to 85 years). Nine patients (33%) were unfit (CIRS>6) with a median age of 72 years (range 58 to 79 years). On an intention to treat basis the OR rate was 74%. Seven pts (26%) obtained a complete response, 13 pts (48%) obtained a partial response. PFS was reached at a median time of 29 months. Among fourteen pts who experienced progression after treatment, the median time was 19.5 months (range 2-36). TTR was reached at a median time of 32 months. At present, six patients died, the median follow-up is 30 months (range, 11-51) with an OS rate of 78%. Twenty-four patients completed the planned treatment. The safety analysis demonstrated a rate of grade 3- 4 neutropenia of 18.5%. Extra-hematological grade 3-4 side effects including infections were not recorded. None of the patients required reduction of dose, delayed therapy or needed hospitalization. Our study shows that the Chl-R combination is a safe and effective therapeutic option for untreated B-CLL pts unfit for fludarabine-based regimens. Further studies are required to confirm these data on a larger series. Furthermore, is also needed to compare the CHL-R combination with Bendamustine-Rituximab which seems to play an emerging role in the same setting with the aim to determine which is the most appropriate currently available treatment for this large subset of patients with CLL.

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IMPACT OF THE BIOLOGICAL AND CLINICAL FEATURES OF THE DISEASE ON THE REAL LIFE OF THE CLL PATIENTS. A RETROSPECTIVE SINGLE CENTRE STUDY

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Chronic Lymphocytic Leukemia (CLL) is the most common lymphoproliferative disorder in Western countries. No comprehensive data on epidemiological, clinical and biological characteristics and evolution of the B-CLL population are available in a single centre analysis. This retrospective study has the purpose to analyse demographic, clinical and biological characteristics of our B-CLL population. From our database consisting of 507 diagnoses during the last 15 years, we excluded 105 patients (pts) referred to our centre for consultancy. Thus we selected 402 pts affected by B-CLL diagnosed from January 1997 to December 2011 and followed until December 2012. We recorded a mean of 27 B-CLL diagnosis per year. The median age at diagnosis was 66 years with a ratio M:F of 1.68:1. The Binet stage was A in 286 patients (71.1%), B in 82 pts (20.4%) and C in 34 pts (8.5%). Positivity for ZAP-70 was detected in 133 pts (36.0%), for CD38 in 94 pts (24.9%), for CD49d in 71 pts (40.6%). FISH analysis was performed in 364 pts: del(13q14) resulted in 97 pts (26.6%), +12 in 52 pts (14.3%), del(11q22) in 30 pts (8.2%) and del(17p13) in 23 pts (6.3%). The analysis of the IgVH mutational status showed 200 pts (60.1%) mutated and 133 pts (39.9%) unmutated. Analysing the time dependent outcomes, the median time to progression (TTP) was 4 years: 220 pts (63%) showed progressions, the projected rate of progressions at 5-years and 10-years was 57% and 76% respectively. The median time to treatment was 5 years: 193 pts (55%) were treated; 51% of all pts were untreated at 5 years and 32% after 10 years. Ninety-six pts (28%) died: 56% of the deaths were due to B-CLL, 31% to extra-haematological diseases, 8% to infections and 4% undetermined. The 5 and 10-year overall survival (OS) was 86% and 68% respectively. Most of the deaths seem to be related to CLL: among the extra-haematological deaths only 13% did not experience progression. ZAP-70, del(17), unmutated-IgVH, beta-2-microglobulin and CD49d identified a group of pts with significantly shorter TTP and OS, while CD38 and del(11) only for TTP. By comparison with the data reported in the literature, our cohort is representative of the B-CLL population. A comprehensive characterization of the clinical features and these standard biological prognostic parameters of the disease is essential to allow to the clinicians the best management of the pts.

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ANGIOPOIETIN-2 PROMOTER METHYLATION DEGREE IS STRONGLY ASSOCIATED WITH GENE EXPRESSION LEVELS AND PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Increasing evidence suggests a key role for Angiopoietin-2 (ANGPT2) in influencing the aggressiveness of chronic lymphocytic leukemia (CLL). In the presence of vascular endothelial growth factor (VEGF), ANGPT2 causes vessel destabilization leading to neoangiogenesis. Accordingly, high ANGPT2 expression levels and high angiogenesis degrees have consistently been associated with poor prognosis in CLL but the molecular mechanisms behind the variability of ANGPT2 expression are still to be discovered. Here we analyzed the DNA methylation status of a CpG island located inside ANGPT2 promoter in a large CLL cohort (n=88) using pyrosequencing and correlated data with ANGPT2 expression, prognostic factors and outcome. The methylation percentages of the CpG island were very variable among CLL cases however showed significant inverse correlations with ANGPT2 mRNA expression ($p<0.001$). Dividing our CLL patients in two subsets according to 73% cut-off value for ANGPT2 methylation, we found significant associations between high methylation degree and low ANGPT2 expression ($p<0.001$), IGHV mutated status ($p<0.001$) and low FISH risk ($p=0.039$). Moreover, low ANGPT2 methylation status was found to predict a shorter time to first treatment (HR 2.407; 95%CI 1.098-5.275, $p=0.028$) and overall survival (HR 2.435; 95%CI 1.137-5.215, $p=0.022$), confirming its association with CLL poor prognosis. Finally, we examined the effects of DNA methyl transferase inhibitor 5-aza-2'-deoxycytidine (DAC), followed by histone deacetylase inhibitor Trichostatin A (TSA) treatment, on ANGPT2 gene expression and promoter methylation in MEC1 and RAMOS cell lines, to understand if DNA methylation can regulate transcriptional silencing of ANGPT2 gene. An increased activation of ANGPT2 gene was observed in both cell lines after 72 hours of incubation with increasing amounts of DAC, however the highest ANGPT2 induction was observed with DAC treatment followed by overnight TSA incubation. Concordantly, ANGPT2 methylation degree was found to proportionally decrease in both cell lines with increasing DAC concentrations, suggesting that ANGPT2 transcription is inhibited by DNA methylation in an inversely proportional manner. In conclusion, we demonstrated that ANGPT2 mRNA expression is strongly regulated by DNA methylation and that a lower ANGPT2 methylation is associated with poor outcome in CLL, pointing to an important target in this yet incurable disease.

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CHANGES IN CD4+ CD25HIGH FOXP3+ REGULATORY T CELLS IN PATIENTS WITH HAIRY CELL LEUKEMIA TREATED WITH CLADRIBINE

Ricci F,[1] Tedeschi A,[1] Vismara E,[1] Tessarolo S,[3] Incontri A,[3] Perseghin P,[3] Farioli L,[2] Frustaci A,[1] Pecoraro V,[2] Morra E,[1] Montillo M[1]

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Introduction. Regulatory T cells (Tregs) have a critical role in preserving immunologic homeostasis. In the last few years some studies have suggested a possible implication of this T cells subset in the pathophysiology of the malignant process. The aim of our study was to evaluate the quantitative alterations of systemic Tregs in hairy cell leukaemia (HCL) pts after treatment with 2CdA Methods. Sequential immunophenotyping was performed on the peripheral blood samples of 15 pts with diagnosis of HCL. After gating the lymphocyte population, the CD4+ CD25high FoxP3+ population were selected and analyzed. Collection of blood samples was performed at the time of disease progression before treatment (baseline) and subsequently after 2CdA treatment at month 1, 3, 6, 9 and 12. The number of circulating Tregs at baseline was correlated with Jansen staging and with treatment response assessed by bone

marrow biopsy (BM) performed at 6 months. Results. The median number of FOXP3+ circulating cells was significantly lower (median 2,44 cells/mmcL) in HCL pts with progressive disease compared with the number in a control group of 5 healthy individuals (median 11,9 cells/mmcL; p= .0046). A significant increase in the number of FOXP3+ cells was observed in pts at month 1 after therapy (p= .047) and thereafter a stabilization of the FOXP3+ levels was reached at the subsequent samples collection. The level of Tregs at baseline was greater in pts with Jansen stage=1 (median 2,9 cell/mmcL) compared to pts with Jansen stage >1 (median 0,6 cells/mmcL), and in pts with BM infiltration <5% (2,6 *vs* 0,9 cells/mmcL). However in both cases the difference was not statistically significant, being p= .1172 and p= .4179 respectively. Discussion: Available data of literature suggest that the role of Tregs is context-dependent, as higher density of Tregs may be associated with either a positive or negative clinical outcome. In particular a favourable prognostic impact of higher Tregs density has been reported lymphomas of germinal center (GC) B cell derivation like MALT and Follicular Lymphoma. No data are available in the literature in HCL. Although the results of this study do not allow a definitive conclusion, our findings confirm the concept of lymphoma-associated immune disfunction and support the hypothesis of a close relationship between the hairy cells and the GC B-cells. However, the role of Tregs disregulation in the lymphomagenesis of HCL needs to be finalized.

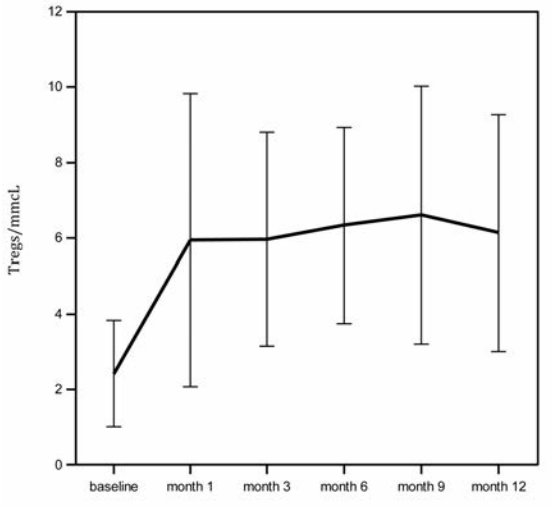

Figure 1.

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BENDAMUSTINE PLUS RITUXIMAB (B-R) IN ELDERLY PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND WALDENSTROM MACROGLOBULINEMIA: PRELIMINARY DATA OF A SINGLE CENTRE STUDY

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Background. Bendamustine (B), a cytotoxic alkylating drug, has shown considerable activity as single agent in the treatment of lymphoid malignancies. B has recently become available for clinical use, as a first-line treatment for chronic lymphocytic leukaemia (CLL) and as salvage therapy after Rituximab or Rituximab-based regimen, for early relapsed or refractory indolent B-cell lymphoma (NHL). Aims. to assess the efficacy and toxicities of B in combination with Rituximab (BR) in elderly previously untreated patients with CLL and Waldenström macroglobulinemia (WM). Methods. From November 2007 to June 2011, 30 patients (M/F=18/12) were enrolled in the study. The median age was 74 yo (range: 65-85); twenty-seven (86%) patients were more than 70 yo; 21 (70%) and 9 (30%) patients had B-CLL and WM, respectively. Eighteen (60%) patients present an ECOG PS >1. Twenty-four (80%) patients had co-morbidity with more than 2 diseases in 44% of cases. R-B regimen consisted of Rituximab 375 mg/mq iv on day 1 for the first course and 500 mg/mq on day 1 for all subsequent courses combined with Bendamustine 80 mg/mq iv on days 1, 2; all patients received four-six cycles delivered every 21 days. Median number of cycles delivered was 5 (range 3-8); 14 (46%) patients completed the planned treatment; G-CSF and ESA support were needed in 9 (30%) and 4 (13%) patients, respectively. Results. CR was achieved in 19 (64%) and PR in 11 (36%) of patients with an ORR of 100%. After a median follow up of 28 months (range 0-46), 6 (20%) of 30 responsive patients had relapsed. Median Duration of Response was 23 months (range 2-57). Ten out of 11 (91%) patients with B-CLL achieved CR, while all patients with WM obtained a stable RP. The regimen was safe and well tolerated with dose reduction occurring only in 4 (13%) patients. Mainly adverse event was neutropenia occurring in 13 (43%) patients; severe neutropenia (WHO grade 3-4) was recorded only in 6 (20%) patients. No extra-haematological toxicity was observed. With a median follow-up of 28 months OS and PFS were 90% and 80%, respectively. Conclusions: B-R may be a good option for elderly patients who need treatment for CLL and WM with major but tolerable toxicity consisting in myelosuppression. A larger study-population, including a carefully evaluation of comorbidities is needed to better define response duration and long-term safety.

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PROGNOSTIC ROLE OF PERIPHERAL BENZODIAZEPINE RECEPTOR IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. The peripheral benzodiazepine receptor (PBR) is a critical component of the mitochondrial permeability transition pore (MPTP). PBR is a multiprotein complex located at the contact site between inner and outer mitochondrial membranes, and is intimately involved in the initiation and regulation of apoptosis. PBR is a small evolutionary conserved protein, associated with the voltage-dependent anion channel (VDAC) and adenosine nucleotide translocase (ANT) that form the backbone of MPTP. Consistent with its localization in the MPTP, PBR has been involved in the regulation of apoptosis, but also in the regulation of cell proliferation, stimulation of steroidogenesis, immunomodulation, porphyrin transport, heme biosynthesis, anion transport and regulation of mitochondrial functions. Transfection-enforced overexpression of PBR attenuates apoptosis induced by oxygen radicals or ultraviolet light. Chronic lymphocytic leukemia (CLL) cells have increased levels of radical oxygen species (ROS). Aims: In the present study, we have investigated PBR function by this receptor in leukocyte subsets from healthy donors and CLL patients before and after chemo-immunotherapy. Moreover, we evaluated in lymphocytes of healthy donors and CLL patients the levels of toxic aldehydes (MDA) by thiobarbituric acid (TBA) assay as expression of oxidative stress. Results. Preliminary data showed that CLL cells from 10 different patients had increased PBR receptors normalized for mitochondria expression if compared to normal lymphocytes. In leukemic cells we evidenced an increased DNA repair activity detected by plasmid-based assay than normal lymphocytes. After 6 months from the beginning of chemotherapy and biological therapy PBR/mitochondria ratio resembles that one of healthy controls. In addition, we evidenced an increased activity of caspase-3 in all responders patients. These effects were correlated to increased lipid peroxidation and nitric oxide levels in CLL cells that were enhanced by the treatment. Interestingly, the 2 patients who resulted resistant to treatment displayed also higher PBR levels and lower caspase 3 activation and TBA levels. Conclusions: These data suggest that PBR expression could be a molecular prognostic factor predictive of response in CLL patients. Moreover, PBR could also represent a useful therapeutic target in order to increase treatment activity in CLL patients.

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FACTORS PREDICTING RESPONSE TO LENALIDOMIDE IN CHRONIC LYMPHOCYTIC LEUKEMIA BELONG TO ANGIOGENESIS, WNT AND INTERFERON SIGNALING PATHWAYS

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Lenalidomide is an IMID® immunomodulatory agent clinically active in chronic lymphocytic leukemia (CLL) patients. In this study, we analyzed samples collected from 27 relapsed/refractory CLL patients being treated with lenalidomide within a multicenter Phase II trial with a protocol approved by the Institutional Review Board. Patients received a median lenalidomide dose of 10 mg ranging from 2.5 mg to 25 mg. After the 4th course, 16 patients remained valuable for response: 10 partial responses (PR), 2 stable diseases (SD) and 4 progressive diseases (PD). We evaluated whether CLL patients who respond to therapy with lenalidomide show different modulation of angiogenesis-related parameters. Plasma basic fibroblast growth factor (bFGF) levels diminished in responders from 168±105 pg/mL at baseline to 41±11 and 19±8 pg/mL after one week and 4 months of treatment, whereas increased levels were measured in nonresponders from 120±58 pg/mL at baseline to 426±195 and 234±147 pg/mL ($p<0.05$ in all instances). Moreover, we found that vascular endothelial growth factor (VEGF) plasma levels significantly decreased during treatment exclusively in responders (83±26 pg/mL at baseline to 26±6 pg/mL after 4 months, $p=0.007$). Lastly, treatment with lenalidomide reduced circulating endothelial cells (CEC) in CLL patients. Higher CEC number during treatment was detected in nonresponders compared to responders ($p=0.013$). Then, we evaluated gene expression profiles of purified CLL samples collected before lenalidomide treatment from 16 patients (10 responders and 6 nonresponders). Supervised analysis identified 78 genes up-regulated and 119 genes down-regulated in responders compared with nonresponders (Fold change [FC]≥2, $p<0.01$). The down-modulation of genes involved in interferon (INF) and Wnt signaling pathways characterized patients who respond to lenalidomide. In particular, responders showed a 23-fold increase in Wnt inhibitor SHISA3, whereas a decrease of WISP3, CDH4, HOXB7 and WNT10A. Moreover, leukemic cells collected from responders down-regulated interferon-induced proteins (IFI44, IFI44L, IFIT2) and STAT1. Nonresponders up-regulated lipoprotein lipase (LPL, FC=+7.5). In conclusion, our study indicates that factors associated with angiogenesis and genes involved in Wnt and INF signaling pathways may represent potential biomarkers of lenalidomide response in relapsed/refractory CLL patients.

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PREVALENCE OF HEPATITIS B VIRUS INFECTION IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS. MONOCENTRIC EXPERIENCE

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The prevalence of chronic hepatitis B virus (HBV) infection in oncohematological patients is higher than in general population. Recent data showed that Occult hepatitis B virus infection (OBI) is significantly more prevalent among patients with B-cell chronic lymphocytic leukemia (CLL) than in age and sex-matched controls. The aim of the study was to determine the prevalence of HBV infection in the CLL population observed at our Hematology Department during the last 15 years and its correlation with clinical and biological parameters of the disease. Moreover, the effect of the OBI on survival and management of the disease in CLL pts undergone or not to chemo-immunotherapy was evaluated. We analysed 402 patients. HBV infection was detected in 7.7% of the pts (31/402): 28 were defined as OBI (HBsAg negative, HBcAb and/or HBsAb positive, HBV-DNA negative) and 3 pts as HBV carriers, 2 of them HBsAg positive without detectable serum HBV-DNA and 1 with both HBsAg and HBV-DNA positivity. In all pts the HBV infection was concomitant or preceded the CLL diagnosis. Only the pt with HBsAg and HBV-DNA positivity had received therapy for HBV infection at CLL diagnosis. Twenty-three/31 pts were male, 8 female. The median age of the HBV/CLL population was 66 years (range, 48-83). The median lymphocyte count at CLL diagnosis was 7500/mmc (range, 2000-67000): 20 pts were in Binet stage A, 10 in stage B and 1 pts in stage C. Twenty-three pts were studied for IGVH mutational status (74.2%): 13 had unmutated, 10 had mutated IGVH genes. FISH analysis was done in 30/31 patients: 16 showed normal karyotype, 6 pts had had del(13q14), 5 trisomy 12, 1 trisomy 12 and del(13q14). Two patients showed a high risk karyotype: 1 pt had del(17p) and 1 had del(11q). Eighteen pts experienced disease progression. Sixteen/18 pts were treated at a median time of 47 months (range, 1-189). The HBV infection did not influence the treatment choice. At a median time of 76 months (range, 27-189) 5 pts are dead (16%), 3 of them for disease progression and 2 for infectious disease not HBV-related. Our preliminary analysis showed no significant differences in terms of clinical-biological characteristics, disease progression and overall survival between HBV positive B-CLL pts and the entire B-CLL cohort. In the future the pathogenetic role and the management of HBV positive B-CLL patients will be explored.

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COEXISTENCE OF LYMPHOPROLIFERATIVE DISORDERS AND ESSENTIAL THROMBOCYTEMIA: THREE CASE REPORTS AND LITERATURE REVIEW

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Introduction. We report three cases of lymphoproliferative disorders (LD) associated to Essential Thrombocytemia (ET): Chronic Lymphocytic Leukemia (CLL), Hodgkin Lymphoma (HL) and non Hodgkin Lymphoma (nHL). Case Reports. From 2006 to 2012, we observed in our center three patients with the coexistence of a LD and ET. All the patients were female with an age of: 73 yrs (CLL), 60yrs (nHL) and 27 yrs (HL). In the first case we diagnosed CLL and ET at the same time. In the other two cases the diagnosis of ET was made later. These patients had previously received chemotherapy for the nHL (R-CHOP, six courses) and for the HL (ABVD, 6 courses). Diagnosis and staging were performed according to guidelines: imaging techniques (PET, CT scan, ultrasound scan) and laboratory tests (full blood count, bone marrow smears and trephine, flow cytometry immunophenotyping, molecular biology). In all the patients full blood count at onset showed a platelet count>500x10^3/mcl. Molecular biology (performed in bone marrow or peripheral blood samples) showed V617F mutation of JAK2 gene in all the cases. The patient with CLL does not receive any treatment for CLL and ET (neither chemotherapy nor antiplatelets drugs). The patients with nHL and HL are in Complete Remission for lymphoma: the first one is on hydroxyurea treatment, the second one is on acetylsalycilic acid. Conclusions. Coexistence of LD and Chronic Myeloproliferative Diseases (CMD) is a rare event. Etiopathogenesis is unknown. CMD following LD could be caused by a defective host immunesurveillance due to the disease and/or chemotherapy. Molecular biology allows to diagnose new cases of CMD not only during the follow up but also at onset of LD, when thrombocytosis could be misdiagnosed as reactive.

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TREATMENT WITH PENTOSTATIN PLUS SEQUENTIAL RITUXIMAB IN PATIENTS WITH HAIRY CELL LEUKEMIA.

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Rituximab offers a vantage in all B cell lymphomas, but little is known in a specific subset as hairy cell leukemia. Here we discuss about eight cases of hairy cell leukemia who gained response after pen-tostatin and subsequently were treated with rituximab every one or two months respec-tively for one or two year. At diagnosis the characteristics of the patients were: median age 50 (range 41-66); male/female ratio 6/2; diagnosis was confirmed by bone marrow aspiration or biopsy in all patients; splenomegaly in all cases but two, mean longitudinal spleen diameter 167 mm (range 139-200); peripheral pancytopenia was 3/3 and 1/3 respectively in two cases, 2/3 in six 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 three patients. Actually we will not evalue one patient, because rituximab therapy is ongoing. After pentostatin all patients obtained complete haematological responses with pancyto-penia 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 reduced. After rituximab therapy, the bone marrow involvement and the splenomegaly disappeared in all patients. All the patients have been maintaining these responses for 5 (two),34,35 (two),37 and 108 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 improved. Ac-cording this follow up time long enough in all patients but in two patients, 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.

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CLINICAL MONOCLONAL B LYMPHOCYTOSIS VERSUS RAI 0 CHRONIC LYMPHOCYTIC LEUKEMIA: A COMPARISON OF CELLULAR, CYTOGENETIC, MOLECULAR, AND CLINICAL FEATURES

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Flow cytometry techniques for chronic lymphocytic leukemia (CLL) diagnosis has resulted in an enhanced specificity and sensitivity, allowing the detection of small levels of circulating B-cell populations (monoclonal B-cell lymphocytosis, MBL) in otherwise normal subjects. A clonal B-cells value <5x10^9/L and the absence of palpable lymphadenopathy and/or organomegaly has been proposed to differentiate clinical MBL (cMBL) from Rai stage 0 CLL (Rai0-CLL) patients. However, the clinical and biological differences between Rai0-CLL and cMBL remain to be clarified. We investigated prospectively a group of 136 cMBL and 216 Rai0-CLL cases belonging to a panel of 462 newly diagnosed CLL patients enrolled in a multicenter Italian study (O-CLL1 protocol clinicaltrial.gov identifier NCT00917540). We compared the incidence and clinical relevance of classic and new prognostic markers, IGHV gene mutational status and chromosomal abnormalities. IGHV-mutated cases were more frequent among cMBL (P=0.005), while the distribution of CD38 and ZAP-70 positive cases, NOTCH1 and SF3B1 mutated cases, as well as the major cytogenetic abnormalities were similar in the two groups. Moreover, no significant differences were found in IGHV/IGHD/IGHJ gene usage as well as in the overall prevalence of stereotyped IGHV gene sequences between cMBL and Rai0-CLL patients. Gene and miRNA expression profiling analyses (GeneChip® Gene 1.0 ST Array (Affymetrix) and Human miRNA Microarray V2 platform (Agilent Technologies)) were performed in a subset of cases in a further attempt to identify transcriptional differences distinguishing the two groups. Notably, no specific signatures were identified in either case at a relatively high stringency level (q-value=0); in fact, only a single transcript (LOC400986) and miRNA (miR-130a) were found to be differentially expressed in cMBL versus Rai0-CLL (down- and upregulated in cMBL, respectively). cMBL diagnosis was predictive of longer progression free survival. Overall, our study based on a prospective series of patients indicates that no major differences in biomarkers exist between the circulating cells from cMBL and Rai0-CLL, suggesting a difference only in the initial size of the monoclonal cell population which may influence the subsequent timing of clonal expansion and clinical manifestations.

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THE PRO-INFLAMMATORY IL23/IL23R AXIS IS ACTIVE IN EARLY STAGE CLL PATIENTS WITH POOR PROGNOSIS

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Introduction. IL23 is a pro-inflammatory cytokine involved in T-cell responses. Its receptor (IL23R) is a heterodimer consisting of the IL12R 1 chain and a unique IL23R chain. We evaluated the potential role of IL23/IL23R-axis expression on CLL pathogenesis and patient PFS. Methods. Newly diagnosed Binet-A CLL cases (N=233, clinicaltrials.gov NCT00917540) were studied for IL23R expression by flow-cytometry (FC) (median %IL23R expression=22.7, range:1.2-91.1). MicroRNA expression profiling was performed on the Human miRNA Microarray V2 platform (Agilent Technologies). For *in vitro* activation, purified CLL cells were co-cultured with stable CD40L-expressing NIH-3T3 cells. Results. The median percentage of IL23R subunit expression was 23% (range 1.2-91.2),while the IL12 1 subunit, not expressed in CLL samples, was significantly up-regulated after CD40/CD40L cross-linking. In situ immunohistochemistry showed IL23R variably expressed in 9/16 CLL biopsies, with significant expression in neoplastic cells effacing lymphnodes or populating bone marrow infiltrates. Double-marker analysis confirmed co-localization of IL23 and IL23R in CLL infiltrates. MiRNA pro-

filing of 93 CLL cases (n=40 IL23R–, n=53 IL23R+) revealed 15 miRNA statistically anti-correlated with IL23R expression. Of these, Web-based algorithms predicted 5 miRNAs target IL23R, 2 miRNA target IL12R 1 and one IL23A. Upon CD40L engagement, expression of miRNAs targeting the IL23R/IL12R 1 heterodimer (miR-532-3p, -324-5p and -500) were down-regulated, suggesting that the microenvironment may control IL23R expression through miRNA regulation. Precursors of potential miRNA identified above, were screened by 3' UTR reporter gene assay in HEK-293 cells confirming the direct effect of miR-146b-5p on IL12R 1 expression. Gain and loss of function assays using miRNA mimics and inhibitors in BJAB cells confirmed the down- or up-regulation, respectively of IL2R 1 at the protein level. PFS evaluation showed 8/102 IL23R–(≤20%) and 23/101 IL23R+ CLL cases required therapy. The 2-year PFS probability of IL23R– patients was 89.7% compared to 80.7% of IL23R+ cases [2 7.7, P=.006; HR=3.0, 95%CI (1.3-6.6)]. Interestingly, miR-146b-5p, -155, -324-5p, -500 and -532-3p showed a significant predictive power on PFS. Conclusions: The microenvironment plays a precise role in regulating expression of IL23R subunits and of potential miRNAs controlling IL23/IL23R turnover, prompting further investigation into the specific function of this axis and its targets in the context of CLL.

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ANALYSIS OF THE PROGNOSTIC VALUE OF CD 20 ANTIGEN EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. Chronic lymphocytic leukemia (CLL) is an heterogeneous disease with variable outcome. The identification of factors that could predict the clinical course of CLL is a crucial objective. Flow cytometry makes it possible to evaluate malignant B cell immunophenotypic characteristics and study important prognostic factors, such as either CD38 or ZAP 70 expression. Aims. We investigated the relationship between immunophenotypic variables such as the CD20 intensity of expression and clinical outcome in CLL. Correlation between time to progression, measured as the time elapsed between diagnosis and first treatment, was addressed. Methods. We retrospectively evaluated patients (pts) with de novo CLL treated at the University Hospital of Bari (Italy) between April 2006 and February 2011. At diagnosis pts were studied for clinical characteristics and peripheral blood multicolor flow cytometric analysis. The intensity of CD20 Antigen expression was analyzed by means of flow cytometry. A cut-off of 70% was considered to define weak (<70%) and strong (>70%) expression of the antigen. Median time to progression was calculated for all pts and related to CD20. Patients were divided in 2 groups according to time to first treatment. The first group included 45 pts treated within 3 months from diagnosis; the second group included 13 pts treated after 5 years of observation. Univariate analyses of each group were performed to evaluate the correlation between CD20 antigen expression and time to treatment. Results. In the group of pts treated within 3 months from diagnosis, 12 % pts presented weak expression (<70%) of CD20 and 88 % pts strong expression (>70%); in the group of pts treated after 5 years of observation 69% presented weak CD20 expression and 31% strong; the difference was statistically significant (P: 0.005). Conclusions. In this study a lower CD20 expression was associated to a shorter time to first treatment. The immunophenotypic intensity of CD20 expression could have a prognostic role and help to recognize at diagnosis subset of patients that may show rapid evolution to progressive disease. Multicentric studies and more consistent cohorts of patients are warranted to confirm these preliminary data.

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IDENTIFICATION OF THYMOSIN BETA 4 AS A DIFFERENTIALLY REGULATED PROTEIN IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. TB4 is a ubiquitously expressed G-actin sequestering protein engaged in tissue repair processes and differentiation of stem/progenitor cells, cell migration and angiogenesis. MALDI-TOF MS profiling identified TB4 as differentially expressed in CLL *vs* normal B-cells. We describe the regulation of TB4 expression by miRNA and indicate a potential functional role in lymphoid neoplasms. Methods. GEP (N=217) and miRNA (N=221) profiling were obtained using Affymetrix HG-U133A GeneChip Arrays (Affymetrix). GEP and miRNA profiling was performed on purified primary CLL, BJAB cells alone or in co-culture with CD40L. *in vitro* and *in vivo* functional studies (cell proliferation, migration and F/G-actin Ratio assays) were performed on miRNA precursor-transfected and treated cells with increasing doses of TB4 (1-100nM). Results. GEP analysis comparing CLLs and the different normal B-cell subpopulations also confirmed TB4 mRNA down-regulation in CLL (3604±1244 *vs* 5715±1004, respectively; mean±SD; p<0.001). miRNA microarray profiling identified 19 miRNA significantly anticorrelating with TB4 gene expression which were then crossed with databases for predicted miRNAs targets of TB4. Six potential miRNA were identified and their precursors were screened by 3'-UTR reporter gene assay in HEK-293 cells, confirming the direct effect of miR-128, -210 and -532-3p on TB4. Gain or loss of function with miRNA mimics/inhibitors confirmed down- or up- regulation of TB4 by miR-532-3p and -210 in BJAB cells at mRNA and protein levels. Time-course and dose-response assays showed both TB4 and miR-532-3p inhibitors alter the expression of pro-angiogenetic genes (PECAM1,COL15A1, ANG, MMP2, FBLN5, VEGFA) in CLL samples and BJAB cells. Treatment of CLL cells with TB4 caused an increase in cell migration when combined with SDF1. Given the actin sequestering properties of TB4, we evaluated changes in the F/G-actin ratio by WB. Treatment with TB4 induced a decrease of F-actin *vs* the G-actin in BJAB cells and showed a rapid alteration of the F/G-actin pool in CLL cells. Co-culture of CLL cells in presence of CD40L induced a down-regulation of TB4 expression by RT-PCR. Conclusion:TB4 mRNA and protein are both downregulated in CLL compared with normal B cells. We show that inversely correlated miR-532-3p, -128 expressed in CLLs and CD40L control TB4 expression. Functional studies indicate that TB4 alters cell migration and regulate angiogenetic genes. Our results show that TB4 may play an important role in maintaining CLL adhesion and migration.

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LOW-DOSE SUBCUTANEOUS ALEMTUZUMAB THERAPY IN PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: LONG TERM OUTCOME FROM A SINGLE INSTITUTION EXPERIENCE

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Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with an extremely variable clinical course. Despite the use of highly effective

chemo-immunotherapy, CLL remains incurable with conventional therapy. Patients (pts) with relapsed and/or fludarabine-refractory CLL still represent a challenge, because they have poor outcomes with current salvage treatments, with a median overall survival (OS) of 10 months. Low-dose alemtuzumab has shown to be effective and safe in relapsed/refractory CLL. We retrospectively evaluated the efficacy and tolerability of subcutaneous (sc) low-dose alemtuzumab (defined as a total weekly dose </=45 mg and a cumulative dose </=600 mg given for up to 18 weeks) in 60 pts with relapsed/refractory CLL treated at our institution from January 2003 to October 2009. Median age was 68 years (range 41-83), male to female ratio was 2.1. The fludarabine-resistant pts were 53,4% of our cohort, while del(17p) was detected by FISH analysis in 21,2% of pts. According to the revised IWCLL 2008 criteria, the overall response rate (ORR) was 63,4%, including 26,7% of complete responses (CR). Overall response rate was 45,8% in the fludarabine-resistant pts and 70% in pts with del(17p). Thirty-one pts (51,6%) were treated with maintenance therapy (subcutaneous alemtuzumab 10 mg weekly for up to 12 weeks). After a median follow-up of 39,5 months (range 2-118), 17 pts (28,3%) are alive and 7 (12%) are still in remission (5 in CR and 2 in PR).We observed 42 deaths, 31 of which related to CLL (disease progression, associated with lethal infections in most cases). Causes of death unrelated to CLL included one acute myeloid leukemia and 2 myelodysplastic syndromes-therapy related, 3 Richter's syndromes and 5 second solid tumours. During the follow-up, 1 patient was lost and 41 pts (68,4 %) progressed. The median overall time to disease progression (TTP) was 12 months. Thirty-nine pts (65%) received alternative treatment. The median time to alternative treatment (TTT) was 13 months. Therapy with low-dose alemtuzumab was well tolerated: grade 3-4 neutropenia was observed in 22%, and cytomegalovirus (CMV) reactivation in 31,6% of the pts, without CMV disease. We observed 8 severe (grade 3–4) infective complications. In conclusion, our results confirm that low-dose subcutaneous alemtuzumab is effective and safe in poor prognosis relapsed/refractory CLL. Noteworthy, 28,3% of the pts are long term survivors and 12% maintain the response obtained.

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TARGETING THE BRAF-MEK-ERK PATHWAY IN PRIMARY HAIRY CELL LEUKEMIA CELLS *IN VITRO*

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Background. Hairy cell leukemia (HCL) is a mature B-cell lymphoma characterized by indolent clinical course and distinct morphology and phenotype. We recently identified the BRAF-V600E kinase mutation as the genetic lesion distinguishing HCL from other leukemias and lymphomas, including HCL-like neoplasms such as splenic marginal zone lymphoma and HCL-variant. The BRAF-V600E mutation is a known oncogenic driver in solid tumors thorough constitutive phosphorylation of the MEK and, in turn, the ERK kinases. Thus, the BRAF-MEK-ERK pathway appears a rational therapeutic target in HCL to be attacked by available small-molecule kinase inhibitors, including the BRAF inhibitors PLX4720, GDC-0879, Vemurafenib and Dabrafenib (the latter two already successfully used in phase 3 clinical trials of BRAF-V600E-positive metastatic melanoma patients) and the MEK inhibitor Trametinib. Aims. To study the *in vitro* effects of BRAF and MEK inhibitors in terms of reversion of the distinct biochemical, morphological and anti-apoptotic features of primary HCL cells. Methods. Primary leukemic cells purified through CD19-MACS from 13 HCL and 7 HCL-like patients were exposed to low escalating concentrations (up to 1µm) of BRAF or MEK inhibitors for different time periods (up to 24 hours), and monitored for the activation status of MEK and ERK by Western blotting. Primary HCL cells were also monitored (up to 5 days) for the potential loss of the F-actin-rich hairy projections by confocal microscopy after phalloidin staining (in 6 HCL and 5 HCL-like patients), and for apoptosis induction by AnnexinV/Propidium Iodide flow cytometry staining (in 7 HCL and 4 HCL-like patients). Results. Upon treatment with BRAF inhibitors, HCL cells showed, in a time frame of minutes/hours, a consistent and sustained reduction of phospho-ERK and phospho-MEK levels as opposed to vehicle-treated HCL cells and to inhibitor-treated HCL-like cells. Also Trametinib rapidly induced marked ERK dephosphorylation in HCL cells. These biochemical events were followed, in a time frame of 2-3 days, by loss of the hairy projections in still-living (AnnexinV-negative) cells and, after another 1-2 days, by apoptosis induction (from 20% to 59% increase in apoptotic cells relative to the drug vehicle) specifically in HCL cells but not in HCL-like cells. Conclusion. These data form preclinical evidence of the anti-leukemic activity of BRAF-MEK-ERK pathway inhibition in HCL.

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BENDAMUSTINE WITH OR WITHOUT RITUXIMAB IN CHRONIC LYMPHOCYTIC LEUKEMIA. SINGLE CENTER EXPERIENCE IN UNTREATED AND PRETREATED PATIENTS

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Bendamustine is a novel agent with structural similarities to both alkylating agents and purine analogues active for lymphoid malignancies. The combination of bendamustine and rituximab (BR) has been demonstrated to be highly active with low toxicity in patients (pts) with CLL, NHL and MM. We analysed 26 pts treated with BR or bendamustine single agent affected by refractory or relapsed CLL, 9 untreated (aged >65) and 17 pretreated (aged <65 when fludarabine containing regimens were not indicated). Among pretreated: 11 were treated with fludarabine containing regimens, 2 with alemtuzumab and 2 with both. 4 pts received >1 line of fludarabine containings regimens. The majority of pts had nodal involvement and/or organomegaly (18 deep abdominal nodes; 2 splenomegaly; 12 both). 12/26 pts had impaired renal function (GFR < 60). HBV and HCV status were evaluated in all pts: 14/26 were HBV carriers and received lamivudine or tenofovir. 4/9 untreated pts recevied at least 4 courses of bendamustine. Rituximab was added in 8/9 untreated pts and in 11/16 pretreated ones. 6 did not receive Rituximab for severe infusion reactions (3) or for presence of Rituximab in two or more previous lines of therapy (3).

Table 1.

tients characteristics at first course of bendamustine	Untreated patients	Pretreated patients
ale/Female	3/6	11/6
ge (mean - range)	72 (62 - 82)	68,2 (52 - 83)
ean follow-up (months from diagnosis)	36.5 (1-70)	91 (48-190)
bsolute lymphocyte count (mean - range)	126,6 (8 - 413)	52.7(3.1 - 120)
emoglobin (mean - range)	11,2 (6.8 - 14.6)	12,4 (9.5 - 14)
atelets (mean - range)	140 (22 - 248)	120 (3 - 198)
net stage		
	1	3
	3	6
	5	8
evious lines of therapy (median - range)	##	3 (2-6)
onths from diagnosis to first course of BR (mean - range)	32.6 (1-69)	66.9 (19-160)
onths from last therapy to first course of BR (mean - range)	##	8,45 (1-25)
elapsed/Refractory	##	12/5
tuximab Yes/No	7/2	11/5
BV and HCV status		
BV occult carriers	2	5
active HBV	2	2
tive HBV infection	1	2
CV infection	0	1
itial bendamustine dose (mg/sqm)		
	9	6
	0	8
wer than 70	0	3
ompleted	6	15
umber of courses		
	3	5
	2	4
	0	4
	1	2
verall response rate	75%	79%
R	25%	29%
CR	0%	21%
R	50%	29%
ean duration of response -months (range)	##	15 (2-29)

Table 1 shows pts characteristics and overall response rates (ORR). Responses were defined as International Workshop on Chronic Lymphocytic Leukemia (Ann Intern Med 1989): unconfirmed CR if pts meet all the criteria for CR, but restaging bone marrow biopsy was not performed. We observed an ORR of 79% in pretreated and 75% in untreated. Mean duration or response was 15 months. One patient (pt) died during therapy due to disease progression; 2 pts died at a mean of 14 months after the end of BR with progressive disease. Grade 3 or 4 hematologic

toxicities developed in 4/9 untreated and 10/17 pretreated. One pt developed severe neutropenia (grade 4) five months after the end of BR. Two pretreated pts were hospitalized for severe infection. No pt developed immune cytopenia and pts with hemolytic anemia at the beginning of BR (2) controlled the complication. No pt had complications related to HBV or HCV. In conclusion we assess the bendamustine (BR or alone) safety and efficacy both in untreated and pretreated pts. This regimen is feasible even in pts with multiple comorbidities. Bendamustine can be a valuable choice also in heavily pretreated pts who received different lines of therapy containing purine analogues and/or alemtuzumab.

Myelodysplastic Syndromes

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COMPLETE REMISSION OF SWEET'S SYNDROME AFTER AZACYTIDINE TREATMENT FOR CONCOMITANT MYELODYSPLASTIC SYNDROME

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Sweet's syndrome (SS) is characterized by painful erythematous papules associated with systemic inflammatory symptoms. It is due to a dermal neutrophilic infiltrate the pathogenesis of which is unclear. A significant proportion of cases is malignancy-associated, with acute myeloid leukemia and myelodysplastic syndromes (MDS) being most commonly involved. Steroid treatment is effective, though possible refractoriness to available therapeutic options may have disabling implications. We report the first case of clinical remission of refractory SS after hypomethylating therapy with azacytidine (5-Aza) in a patient with MDS who concurrently experienced an excellent hematologic response. A 66-year-old man was diagnosed with Sweet's syndrome on a skin biopsy of recurrent painful, papular skin lesions at the upper limbs, trunk, neck and face (Figure 1a, 1b). Symptoms proved refractory to oral and intravenous steroids, colchicine, indomethacin, dapsone, minocycline and methotrexate. Finally, low dose thalidomide and oral methylprednisolone obtained moderate improvement of symptoms, with recurrence at any attempt to reduce steroid dosage below 0,25 mg/Kg/day. Two years after onset of SS, a diagnosis of refractory cytopenia with multilineage displasia was made, with rapid progression to refractory anemia with excess of blasts-2, normal karyotype, IPSS intermediate-2.

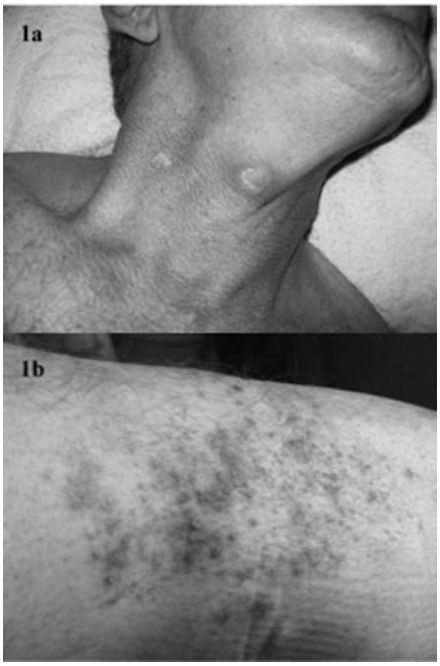

Figure 1. Nodular skin lesions (1a), with occasional vesicular or pustular appearance (1b).

Treatment with 5-Aza was started, thalidomide stopped and methylprednisolone tapered until discontinuation over 20 weeks. No relapse of SS was documented from the fifth 5-Aza cycle onwards and hematologic complete remission was obtained. The patient is currently receiving the 13th 5-Aza cycle. Several indirect lines of evidence support a causal relationship between azacytidine treatment and clinical response in our patient, particularly i) the temporal association between 5-Aza administration and reduction of skin lesions, which were refractory to multiple prior lines of treatment and ii) the association between hematologic and cutaneous response. This suggests that modification of methylation pattern of bone marrow cells might have provided hematologic improvement and reduced propensity to neutrophilic dermal infiltration. The absence of cytogenetic abnormalities precluded the demonstration of a possible clonal relationship between dermal neutrophils and myelodysplastic progeny. It is worth noting, however, that no dysplastic changes of dermal granulocytes were documented in our case.

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AZACITIDINE IN THE TREATMENT OF HIGH-RISK MYELODYSPLASTIC SYNDROMES (MDS)

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Background. The management of myelodysplastic syndrome (MDS) remains challenging. The goals of treating older patients with myelodysplastic syndrome (MDS) are different than for younger patients. Few elderly patients are able to pursue an allogeneic stem cell transplant for potential cure of the disease. The focus for the treatment of older patients with MDS is therefore not curative, but rather alleviation of symptoms, improvement in quality of life, maintenance or improvement of functional status, and continued independent living. Azacitidine (AZA) improves long-term outcomes of higher-risk myelodysplastic syndrome (MDS) and is now the reference frontline therapy of higher-risk MDS not eligible for allogeneic stem cell transplant. Aim. We report our experience on using the azacitidine in patients with high-risk MDS, evaluating the efficacy and safety. Methods. In our Institution from October 2009 to March 2013 we have treated 23 elderly patients (13 male and 10 female, median age 75 years, r. 72-86) affected by HIGH-RISK MDS (IPSS INT-2/HIGH). Patients received subcutaneous azacitidine at 75mg/m^2 daily for 7 days every 4 weeks. Five patients received from the third cycle, a dose 25% reduction due to excessive hematologic toxicity. All patients completed at least 6 cycles of therapy. 9/23 (39%) patients underwent more than 8 cycles of therapy. Results. Complete response, partial response, and hematologic improvement were observed in 4 (17%), 6 (26%), and 7 (30%) patients, respectively. Complete cytogenetic response was observed in 25% of evaluable 20 patients. The median number of cycles to clinical response was 4 (range 4-8), and duration of remission was 410+ days (range 250-512+). The 2-year rate of acute myeloid leukemia-free survival was 48%. Five serious adverse events occurred in five patients with one fatal outcome. Conclusions. Our results confirm the effectiveness of the therapy with azacitidine in HIGH-RISK MDS elderly patients with acceptable toxicity profile. Peripheral cytopenias were the most commonly occurring adverse event, with gastrointestinal adverse events (*e.g.* nausea, vomiting and diarrhoea) and injection- site reactions among the most commonly occurring non-haematological adverse events. In conclusion, azacitidine is an important agent for use in the treatment of elderly patients with MDS. The agent resulted in clinical and hematological improvement in these patients with acceptable side effects.

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RISK FACTORS IN MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic Syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by peripheral-blood cytopenias and risk of progression to acute myeloid leukemia. The clinical, morphological and prognostic heterogeneity is not sufficiently addressed even in current classification systems. Aim. We investigated the role of WT1 gene expression and its association with the expression of the chemokine receptor CXCR4 on bone marrow CD34+ cells of MDS patients. Methods. From January 2007 to January 2013 BM samples from 71 MDS patients (according to WHO classification: 20 RA, 12 RCMD, 10 RAEB I, 8 RAEB II, 9 RARS, 10 deletion of 5q, 2 MDS unclass) were tested for WT1 expression at diagnosis and every 6 months. WT1 gene expression was evaluated by methods of real-time quantitative PCR (RQ-PCR). Surface CXCR4 expression were measured flow cytometrically. Results. At diagnosis, 29 BM samples (10 RA, 7 RCMD, 6 RAEB I, 4 RAEB II, 1 RARS, 1 MDS unclass) expressed WT1 transcript amounts greater than the ranges level. The degree of WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. A significant correlation was found between WT1 expression levels, blast cell percentage and CXCR4 over-expression on blast cells (as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5). After 6 months, 9 patients (2 RA, 5 RAEB I, 2 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 and CXCR4 expression and a further elevation of WT1 expression level after 6 months. Conclusions. WT1 expression has been previously reported to be increased also in myelodysplastic syndromes. In this study, the data obtained show that in most MDS, including a large percentage of RA and almost the total number of RAEB I and II, WT1 is expressed above the range observed in normal controls in BM and that its expression is directly correlated with the type of MDS. A strong association is present between the level of WT1 expression and the blast percentage and the CXCR4 over-expression. The identification of a molecular marker so able to establish the tendency of MDS to progression can be of great help in decision making for MDS patients. Our results justify further investigation into the role of CXCR4 in MDS and suggest that WT1 and CXCR4 should be incorporated into the risk assessment of MDS patients.

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OBSTACLES TO ADHERENCE TO AZACITIDINE ADMINISTRATION SCHEDULE IN OUTPATIENT MYELODYSPLASTIC SYNDROME AND RELATED DISORDERS: A SINGLE CENTER EXPERIENCE

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Introduction. Azacitidine (aza) is a hypometylating agents with high therapeutic profile in myelodysplastic syndrome (MDS) and related disorders [acute myeloid leukemia (AML) and chronic myelomonocytic leukemia (CMML)]. Recently, the original seven days every four weeks subcoutaneous administration shedule was flanked and overtaked by one more feasible, such as 5+2+2 every four weeks. However, in the daily practice, several problems may limit the full adherence to the planned schedule. Data on adherence to planned schedule in MDS/AML/CMML patients (pts) undergoing treatment with aza are scarce. Aim. To evaluate the adherence to 5+2+2 subcoutaneous aza administration schedule during outpatient management. Method. By reviewing medical records of pts from May 2011 and April 2013, intervals between date of administration of consecutive aza cycles [defined as: (day 1 of cycle n) - (day 1 of cycle n-1)] were extracted; in case of delay (defined as interval duration >28 day), the causing reasons were identified. Results. During the 24 months period, we administered 199 cycles in 21 pts; median age 68 years (37-85); gender: 15 male, 6 female; diagnosis: MDS 12 (57%), AML 5 (24%), CMML 4 (19%). Median cycles / pt were 9 (range 1-21). Evaluable intervals were 178. Median interval duration was 28 (27-217) days; interval was >28 days in 56 (31%) cycles; 29-35 days in 26 cycles (15%),

36-42 days in 15 cycles (8%), >42 days in 15 cycles (8%). Reasons for delay were: i. clinical issues in 33 cycles (59%): a. hematological toxicity 11 (19,7%), b. temporary discontinuation during disease evaluation 7 (12,5%), c. complications 15 (26,8%); ii. organizative obstacles in 19 cycles (33,9%): center-related organizative obstacles (public holidays) 15 (26,8%), patient-related organizative obstacles 4 (7,1%); iii. unknown reasons 4 (7,1%)). Delay causing complications were: infections in 12 (21,4%) and other in 3 events (5,4%). On univariate analysis age, gender and diagnosis were not statistically related with delay risk. Conclusions. Delay on aza administration is frequent due both to clinical and organizative obstacles; delay effect on dose intensity and therapy efficacy is unknown but, probably, effective. Clinical issues could be addressed by ameliorating complications prophylaxis. Organizative obstacles could be addressed as following: center opening extension to non-working days, external pharmacy drug dispensation or, in the future, oral hypometylating agents use.

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AZACITIDINE IN 'REAL LIFE' PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML): A SINGLE CENTRE EXPERIENCE

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CMML is a clinically heterogeneous disease for which, according to recently reported experiences, hypomethylating therapies have provided significant clinical benefits. Herein, we present the outcome of 10 patients (pts) who fulfilled the WHO 2008 criteria for CMML (CMML-2 in 6, CMML-1 in 2) or CMML-related acute myeloid leukemia (AML) with <30% bone marrow blast (2 pts) and have been treated with azacitidine (aza) at our institution between 2010 and 2012 after informed consent has been obtained. Median age at diagnosis was 75 years (range 62–86). Four pts had proliferative CMML. Three pts were transfusion dependent at some time point of disease course. Two out of 10 pts had abnormal kariotype (46,XY,Inv12 and 45,Y,-X, respectively). Two pts had secondary CMML; one to a 7-years lasting myelodysplastic syndrome (refractory anemia), whereas the other, who has undergone radiochemotherapy for a solid tumor 3 years before, presented a likely therapy-related CMML. Prior therapies included cytoreductive therapy and erythroid stimulating agents. The MDAPS was low, Int-1, Int-2 and high in 1,2,4 and 1 CMML pts, respectively. Pts were treated with azacitidine, 75 mg/m^2 x 7 days, 5+2+2 schedule, every four weeks, subcoutaneously). Supportive care was given as required. Bone marrow (BM) response was assessed in 9 pts (following the sixth cycle in 8 pts and the fourth in 1); response was not assessed in 1 pt only, due to death (multiorgan failure) occurrence after second cycle. Responses were classified according to the modified IWG criteria; 4/9 evaluable pts achieved complete remission (CR) and 3 partial remissions (PR) with an overall response rate (CR+PR) of 70%; 2 pts maintained stable disease. No progressing pts continued the treatment. Two pts progressed to AML following the sixth and the fourteenth cycle respectively, after having obtained CR. With a median follow-up of 12 (2 – 23) months, 6 pts are alive and 4 of them continue to receive the treatment; four pts have died, 2 of AML, 1 of multiorgan failure (before response assessment), 1 of sudden cardiac death (with stable CMML); median survival from therapy start was 12,9 months. Treatment was well-tolerated and no remarkable side effects were recorded. In conclusion, our experience was encouraging; the use of aza in our hands achieved good responses in 70% of the treated pts, despite their high risk of disease and unfavorable prognostic profile.

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RESPONSE TO ERYTHROPOIETIN IN A MULTICENTRIC REAL-LIFE COHORT OF MYELODYSPLASTIC PATIENTS: THE GROM EXPERIENCE

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Erythropoietin (EPO) have been widely employed in the treatment of patients with low-risk Myelodysplastic Syndromes (MDS) and anemia, with response rates ranging from 30 to 60%. These data, however, have been derived only from controlled clinical trials or unicentric single-arm studies. A wider survey evaluating the use of EPO in the real-life clinical practice is still lacking. To address this issue, the Gruppo Romano Mielodisplasie (GROM) retrospectively revised 394 MDS patients (M/F 225/169, median age at diagnosis 73.9 yrs, IR 67.0 – 79.3) treated with EPO from 1/2002 to 12/2010 at 11 Hematological Centers in the metropolitan area of Rome. According to the WHO classification, there were 81 (20.6%) patients with RA, 7 (1.8 %) with SA, 160 (40.7%) with RCMD, 17 (4.3%) with RCMD-S, 75(19.0%) with RAEB-1, 27 (6.8%) with RAEB-2 and 27 (6.8%) with isolated del5q. The IPSS score was calculated in 307 patients with an available karyotype: 145 (47.2%) patients were low-risk, 135 (44.0%) int-1, 24 (7.8%) int-2 and 3 (1.0%) high-risk. Median interval from diagnosis to EPO start was 3.7 months (IR 0.9 – 12.1). At EPO start, median age was 74.5 yrs (IR 68.3 – 79.9) with a median haemoglobin level of 8.9 g/dl (IR 8.2 – 9.6): 138 patients (35.3%) had a previous transfusion requirements. Median serum EPO level was 50.0 mU/L (IR 26.2 – 110.0). The initial doses of EPO were ≤ 40.000 UI/week in 259 patients (65.7%) (standard doses, -EPO in 104 patients, -EPO in 143 patients, darbepoietin in 12 patients) and 80000 UI/week in 135 patients (34.3%). Erythroid response was observed in 228 (57.9%) patients, with Hb increase >1.5 g/dl in 210 patients (53.3%) and transfusion independence in 18 (4.6%). Patients starting with high EPO doses had higher response rates compared to those receiving standard doses [94/135 (69.6%) *vs* 134/259 (51.7%), p=0.002]. Significant factors predicting for erythroid response were hemoglobin level at baseline > 8 g/dl (p=0.017), no previous transfusion requirement (p<0.001), serum EPO <50 mU/l at baseline (p<0.001), normal creatinine levels (<0.001) and ferritin levels <250 ng/ml (p=0.009). Median overall survival from EPO start was 70.7 months (CI 95% 52.5 - 88.8) in responders versus 41.7 months (CI 95% 27,6 - 55,7) in resistant patients (p= 0.018). Our real-life data from a single homogeneous geographic area outline that EPO treatment is safe and effective also in the current clinical practice, beyond controlled clinical trials.

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ENDOTHELIAL-HEMATOPOIETIC STEM CELL INTERPLAY DISTURBANCE IN MYELODYSPLASTIC SYNDROMES

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Endothelial cells are relevant to the normal hematopoiesis. Vascular niches within the bone marrow allow hematopoietic stem cell proliferation and differentiation, to the point that the contemporary administration of endothelial progenitor cell facilitates the homing of transplanted hematopoietic cells (Salter et al. Blood 2009; 113:2104). We explored the hypothesis that in MDS patients the vascular niche might be damaged and unable to adequately sustain the hematopoietic differentiation. To this purpose, we differentiated normal CD34+ cells on confluent layers of endothelial colony forming cells (ECFCs, Ingram et al. Blood. 2004;104: 2752) obtained from patients with MDS or from

healthy blood donors. Normally, the presence in culture of ECFCs amplifies the expression of lineage-specific genes of differentiating CD34+ cells; this effect is highly dependent from the physical contact between hematopoietic and endothelial cells. Our preliminary results suggest that an altered crosstalk between endothelial and hematopoietic cells exists in MDS. Actually, the expression of lineage-specific genes in normal CD34+ cells co-cultured on MDS endothelial layers was perturbed, with higher expression of genes involved in the early differentiation (such as PU.1 and RUNX1) and lower expression of genes usually up-regulated during the late differentiation (such as MPO and GP1b) (Teofili et al, Blood , ASH Annual Meeting, 2012;120:1718). In this study, we compared through PCR Arrays the gene expression profiles of normal and MDS ECFCs, focusing our attention on genes involved in Endothelial Cell Biology (PAHS-015Z, QIAGEN, Milan, Italy). In addition, we analyzed the level of cytokines and growth factors in surnatants of cultures of MDS ECFC in comparison with normal ECFCs, using the Bio-Plex pro human cytokine 27-plex assay (Bio-Rad, Milan, Italy). Basically, we found that in MDS ECFCs several adhesive molecules such as ICAM-1, L-selectin and V-CAM were significantly overexpressed. Moreover, the cytokine milieu in the surnatants of ECFCs significantly differed between normal and MDS samples. In particular, the MDS cultures contained significantly higher amounts of IL13, IL1b, PDGFb and VEGF. Conversely, the levels of IL6, IL7, IL10, G-CSF, CXCL-10, CCL-2 and CCL-3 were significantly lower in MDS than in normal ECFC cultures. Overall, these data highlight a possible dysfunction in the vascular niche in MDS, contributing to the pathogenesis of myelodysplasya.

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T-CELL RECEPTOR REPERTOIRE KINETIC DURING AZACITIDINE TREATMENT

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Patients with myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML) with multilineage dysplasia display several immunological abnormalities. Azacitidine, beside the well known effects on bone marrow precursors, has been demonstrated to influence T-cell polarization. The aim of this study is to monitor the kinetic of the T-cell receptor (TCR) repertoire during Azacitidine treatment in order to explore its potential ability to reverse the immune derangement typical of these patients. Our study consists in a flow cytometric and CDR3 spectratyping analysis performed at baseline and then every 3 cycles on the peripheral blood of 9 patients (4 with MDS and 5 with AML with multilineage dysplasia) and 30 normal controls.

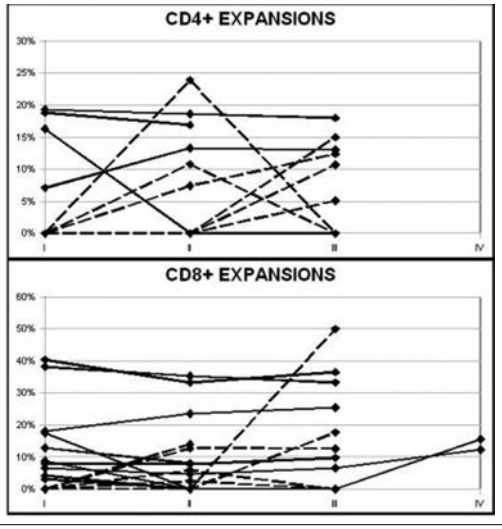

Figure 1. The figure shows the kinetic of expanded T-cell subpopulations in MDS patients during Azacitidine treatment at different time points (every 3 months). Expansions already present at baseline or emerged during therapy are represented as continuous or dashed lines respectively. By convention values below the mean +3 standard deviations are represented with a value of 0,0%

At baseline, in CD4+ cells only 4 patients showed a single BV expansion, one of which disappeared after 3 cycles while 3 were stable during treatment. When reassessed, 2 of the patients showed each the appearance of 3 new BV expansions. Overall CD4+ expansions were 10 and their size ranged from 7 to 24%. Within the CD8+ subset, at baseline 6 out of 9 patients showed at least one T-cell expansion. In details 2 patients showed a single expansion while 4 of them displayed 2 different BV expansions. Of these 10 baseline expansions 6 were stable during treatment, while 4 of them quickly disappeared.

Noteworthy, one of these expansions which had disappeared in a patient in remission reappeared at disease relapse. Six patients showed the appearance of a single BV expansion during treatment. Overall CD8+ expansions were 16 and their size ranged from 2 to 50%. Preliminary data on spectratyping analysis showed a gradual improvement in the TCR repertoire diversity, due to the progressive reduction in the frequency of non Gaussian CDR3 profiles, especially remarkable as regards the number of missing BVs. Our findings firstly confirmed in our patients an overall derangement of the TCR repertoire which however seems to gradually improve during Azacitidine treatment, as witnessed by the disappearance of some BV expansions observed on flow cytometry - particularly within the CD8+ subset-, as well as by the progressive restoration of the CDR3 diversity detected by spectratyping. These preliminary data seem to suggest that Azacitidine could be potentially able, not only to restore the hematopoietic function, but also to reverse the immune derangement typical of patients with MDS and AML with multilineage dysplasia.

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R-IPSS, RPS14 AND WT1 LEVELS ARE THE BEST PROGNOSTIC FACTORS FOR PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES DURING AZACITIDINE TREATMENT.

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Introduction. In addition to the scoring risk systems, many efforts have been spent in order to find some genes whose expression could allow a good stratification of patients affected by high-risk myelodysplastic syndromes treated by azacitidine. Among these genes, some involved in the ribosomial biogenesis, such as RPS14, and WT1 have been reported as good candidates. Thus, we decided to analyze the impact of these two genes in addition to IPSS, R-IPSS, WPSS, R-WPSS, MDACC scoring systems on the outcome of 53 consecutive high-risk MDS patients treated with azacitidine at our center from 2007 to 2012. Patients and Methods. Bone marrow samples from 14 healthy donors were used as controls; the stratification in 2 categories (RPS14 low and high) was defined as the average value measured in the healthy donors (0.79 copies/104 copies of S18, the internal control gene) or as the average plus 2 standard deviations (1.39 copies). WT1 copies >180 were considered high, as stated by the manufacturer of the PCR commercial kit (Ipsogen). Patients' characteristics were recorded in the data base updated at March, 2013. Results. After 4 cycles, 9% of patients achieved a complete response (CR), 55% a partial response/ hematological improvement, 9% remained stable, and 27% progressed. After 6 cycles, the CR rate increased to 23%. Two-year overall survival (OS) was 62% and leukemia-free-survival (LFS) 36%. LFS was not significantly affected by the IPSS, R-IPSS, WPSS, R-WPSS, or MDACC risk scores. On the contrary, patients showing lower RPS14 levels than those measured in healthy controls presented shorter LFS (27% at 24 months *vs* 100%; p=0.013). The 64% of our patients presented WT1 levels higher than normal controls. Patients with levels >681 (mean value) presented a significantly reduced LFS (0% at 24 months *vs* 52%; p=0.038). OS was significantly affected by R-IPSS, with 38% of patients at high/very high risk surviving versus 75% of those at intermediate/low/very low risk (p=0.017). Patients showing RPS14 levels higher than mean values (>0.54 copies) presented a significant advantage in OS (74% at 24 months *vs* 51%; p=0.043). On the contrary, WT1 levels did not significantly condition OS. Conclusions. These results suggest that in our series the R-IPSS and quantitative PCR for RPS14 and WT1 are good prognostic factors in high risk MDS patients during azacitidine.

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PI-PLCBETA1 GENE METHYLATION AND EXPRESSION AS A RELIABLE AND DYNAMIC MARKER OF CLINICAL RESPONSE TO 5-AZACYTIDINE IN PATIENTS WITH LOW-RISK MYELODYSPLASTIC SYNDROMES

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Hypomethylating agents, such as 5-Azacytidine (AZA), significantly modified the therapeutic approach to MDS, primarily in older patients with higher risk disease for whom intensive chemotherapy and allogeneic stem cell transplantation are not an option. In low-risk MDS, 5-AZA aims to reduce transfusion dependency, improve quality of life and hopefully the survival, but it is still unclear if this therapeutic approach would be cost-effective. At a molecular level, the mechanisms underlying the effect of epigenetic therapy are not completely understood, although it is well known that DNA methyltransferase inhibitors can induce the expression of Phosphoinositide-Phospholipase C (PI-PLC) beta1 in high-risk MDS. Here, we prospectively investigated the efficacy and safety of AZA in low-risk MDS patients. AZA was administered at a lower intensity schedule, that is 75 mg/sqm/day subcutaneous for 5 days every 28, for a total of 8 cycles, and response was assessed at the 4th and 8th cycle of AZA. Moreover, PI-PLCbeta1 promoter methylation and gene expression levels were quantified before and after each cycle of 5-AZA. The study included 32 patients, and 26 cases completed 8 cycles of AZA. ORR was 47% (15/32) on intention to treat and 58% (15/26) for patients completing the treatment program. In this latter group, 5 (19%) cases achieved CR and 10 (38%) had HI, according to the IWG criteria. Interestingly, three patients have maintained their HI after 37, 34 and 33 months without other treatments. At a molecular level, although baseline PI-PLCbeta1 levels were not correlated to clinical response, 5d-AZA induced a statistically significant decrease in PI-PLCbeta1 promoter methylation in 14/15 responders, which corresponded to a significant increase in PI-PLCbeta1 mRNA. In 9/14 (64%) responsive patients, the first molecular increase in PI-PLCbeta1 level was observed between the 3rd and 4th cycle, therefore anticipating the clinical evaluation. In addition, 8 cases showed a loss of the response after the end of therapy (8th cycle) and these cases displayed a significant reduction of PI-PLCbeta1 levels, below the pre-treatment values, already before the clinical loss of the response. Taken together, our results show that 5d-AZA is safe and effective in a proportion of low risk MDS patients. PI-PLCbeta1 gene expression is a reliable and dynamic marker of response that can be useful to optimize AZA therapy.

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MANAGEMENT OF MYELODYSPLASTIC SYNDROMES (MDS) WITH DEL(5Q) ASSOCIATED WITH PURE RED CELL APLASIA (PRCA): ROLE OF LENALIDOMIDE

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Myelodysplastic syndromes 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 therapy. Nearby 25 cases of MDS with PRCA have been described until today and 5 of them were associated with del(5)(q14q34). WHO classification 2008 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 anemia in which del (5q) 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 emerged with fatal evolution.(F/35 y.o.) received diagnosis of PRCA after 1 year treatment with steroids and transfusions (2 U/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. In conclusion, 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.

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USE OF BIOSIMILAR EPOETIN IN MYELOID NEOPLASMS: PRELIMINARY EXPERIENCE WITH Z- EPOETIN (RETACRIT®)

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The recent availability of biosimilar growth factors in hematology is going to significantly decrease treatment cost in several diseases. However, some uncertainty still remains about their actual efficacy and safety compared to originator drugs. In Italy recombinant erythropoetins are approved for the treatment of chemotherapy-induced or renal failure-related anemia. Alpha and beta epoetins are not formally approved yet for the treatment of anemia in myelodysplastic syndrome (MDS); however their use in this disease is allowed by the regulatory authority provided a particular patient monitoring is exerted (648 law). Biosimilar Z-epoetin has been included among epoetins allowed by 648 law for MDS patients. However, very few data about its activity and safety in these patients are available. This study evaluates the efficacy and safety of Z-epoetin in a group of anemic patients with MDS or other myeloid neoplasms. We evaluated 18 anemic patients (median age 76, range 64-91 years) who received Z-epoetin (40- 80.000 U/week) from October 2011 to April 2013. We included 13 MDS patients (1 RA, 3 RARS, 4 RCMD, 4 RAEB-1, 1 RAEB-2) and 5 patients with other different myeloid neoplasms (2 primary myelofibrosis, 1 CMML, 1 atypical CML, 1 AML in consolidation treatment). Six patients received Retacrit® in addition to chemotherapy (1 RAEB-1 and the patients with other myeloid neoplasms). Nine patients were transfusion dependent at the beginning of therapy. Erythroid response was defined according to IWG criteria (Cheson *et al*,Blood 2006). Two patients were not evaluable for erythroid response because of their short follow-up (<3 months). Twelve of the 16 evaluable patients (75%) achieved an erythroid response after a median of 2 months (range 1-5 months). Four patients lost response (33%) after a median of 3 months of treatment (range 2-6 months), 2 of them for disease progression. At the median follow up of 6 month, 58% of the patients maintained the erythroid response (range of response duration 1-18 months). No significant toxicities were observed and no patient discontinued the treatment for side effects. In our preliminary casistics Z-epoetin was perfectly tolerated and its efficacy to induce erythroid responses in patients with myeloid neoplasms (mainly MDS) was comparable to that of alfa and beta epoetin in the same categories of patients. However, larger studies with longer follow up are needed to confirm the therapeutic role of Z- epoetin in myeloid neoplasms.

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PROGNOSTIC EVALUATION OF CONVENTIONAL CYTOGENETICS IN MYELODYSPLASTIC SYNDROME: A SINGLE INSTITUTION EXPERIENCE

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Background. Cytogenetic information in patients affected by myelodysplastic syndromes (MDS) is the most important factor in predicting prognosis and therapeutic direction [Greenberg *et al*., 1997]. Recently, a new prognostic model has been proposed to stratify patients according to specific cytogenetic abnormalities [Schanz *et al*., 2012]. The objective of this observational retrospective study is to validate this prognostic score. Patients and Methods. 106 patients affected by MDS, based on 2008 WHO classification, referred to our center from January 2007 to December 2011. Abnormal karyotype was found in 32/106 cases (30,2%), including 20,8%, 1,9%, 1,9%, 5,7% of single, double, three or more than three abnormalities respectively. The distribution of patients according to IPSS risk score was: Low 36,8%; Int-1 43,4%; Int-2 11%; High 8,8%, while according to R-IPSS risk score was: Very low 23,6%; Low 27,4%; Intermediate 23,6%; High 9,4%; Very high 13,2%. The median follow-up was 21 months, ranging between 1,7 and 70 months. At the time of analysis, 51 patients were died, 1 patient was lost-to follow-up. Overall, 14/106 patients progressed to AML, 5/14 had an abnormal karyotype, 3/5 with a poor and 2/5 with a favorable feature, while in 1 patient the baseline cytogenetic failed. Results. IPSS score preserved a clinical significance with a reduction of the median OS according to the risk class, *i.e.* 61 months for low risk, 37 for int-1, 21 for int-2 and 11,4 for high risk group. The survival analysis, according to R-IPSS, showed a reduced OS in higher risk groups, *i.e.* median not reached for very low, 56,6 months for good, 24 months for intermediate, 34 for high and 12 for very high risk group. OS according to IPSS cytogenetic risk was: 54,4 months for good, 24 for intermediate and 11,5 for poor. However, when we evaluate our series according to cytogenetic prognostic model described by Schanz, we found that OS was 24 months for very good, 56 months for good, 18 months for intermediate, 11 months for poor and 11 months for very poor group. Conclusion: In our single-Institution series the value of R-IPSS based cytogenetic risk was not useful in better define MDS risk group at diagnosis.

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A SINGLE INSTITUTION POPULATION-BASED EXPERIENCE WITH LENALIDOMIDE (LEN) IN DEL(5Q) MYELODYSPLASTIC SYNDROME (MDS)

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Published results of large multicentre trials with LEN in MDS with del (5q) are well known, but limited information on population-based experience is available. We evaluate safety, efficacy and long-term outcome in a strictly consecutive population-based series of MDS pts with anemia and del (5q), treated with LEN at our Institution. All consecutive MDS pts classified according to WHO criteria and IPSS, and treated with LEN 10 mg po days 1-21 on 28-day cycles between July 2007 and February 2013 were analysed. Responses were evaluated according to IWG criteria after 4 months. Twenty patients were recorded (7,5 % of 264 newly diagnosed MDS). Median age was 75,5 years (range 53-87) female were 65%. WHO categories included unclassificable (2), MDS del(5q) (12), RCMD (2) and RAEB I, RAEB II, RARS and RARS-T (one each). Eighteen cases (90 %) had IPSS Low/Int-1 and bone marrow blast count <5%. By conventional cytogenetics, 5 (q-) was isolated in 18 (90 %) and associated with one additional abnormality in 2 [+8 and del(17p)]. Median time from MDS diagnosis was 13,5 months (range 1-87), all patients were transfusion-dependent. Fourteen cases were pre-treated and non-responders to epoetin. Median number of cycles was 6 (range 1-43). Median follow-up was 33 months (1-152) after MDS diagnosis. Among the 18 cases evaluable for erythroid response (2 too early), TI was reached in 15 cases (83 %), haematologic improvement in 1, failure in 1; one case refused treatment after 2 cycles. Among the 9 pts evaluated there were 6 complete cytogenetic response (CCR: 66 %). Neutropenia and/or thrombocytopenia WHO grade 3-4 was observed in 55 % of cases. Non haematological toxicity caused LEN discontinuation in 2 cases in TI (cutaneous rash and deep venous thrombosis with pulmonary embolism). Two pts with isolated del(5q) evolved to acute myeloid leukemia (AML) during treatment, respectively after 1 and 7 months (the latter while in CCR). The 5-year actuarial risk of progression to AML was 15,6 % (SE + 11,42). The 5-year overall survival probability was 67,7 % (SE + 13,87). Causes of death were: congestive hearth failure in one, gallbladder carcinoma in one, infection in one, hemorrhage in one and AML in 2. At present 9 patients are still on treatment. In a population-based series of MDS del(5q) pts median age was higher than in clinical trials. TI was obtained at high frequency and was durable even after LEN interruption. LEN did not increase the risk of leukemic evolution.

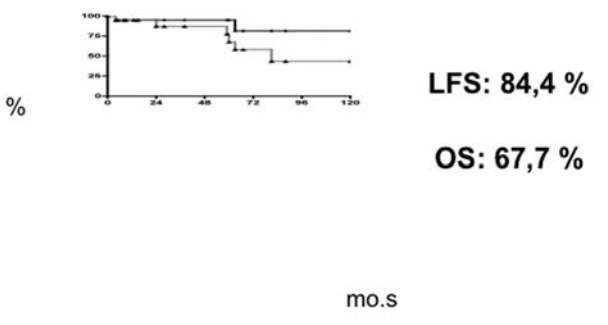

Figure 1.

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COMBINED OVEREXPRESSION OF WT1 AND BAALC GENES MAY PREDICT AML EVOLUTION IN MDS PATIENTS

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Current international Guidelines of Myelodysplastic Syndromes (MDS) indicate that management's strategy of MDS patients should be based on prognostic scoring systems such as the International Prognostic Score System (IPSS). Our goal was to verify if biological markers utilized in acute myeloid leukemia may have any predictive role in high and low risk MDS patients. We analyzed 102 consecutive MDS patients [58 low (LR) 21 intermediate-1 (INT-1) and 23 intermediate 2-high IPSS score (HR)] observed in our Hematology department. FLT3 gene ITD, NPM1 gene mutations, WT1 and BAALC expression were evaluated. Abnormal karyotype was observed in 20 cases (8 high and 12 intermediate risk karyotypes). Twenty-two patients were treated with azacitidine plus erythropoietin, 7 with chemotherapy , 30 with hematopoietic growth factors and 5 with low-dose cytarabine. The remaining patients received supportive care alone. A molecular analysis was performed at diagnosis on bone marrow samples in all the patients. No patients showed either FLT3-ITD or NPM mutations. WT1 overexpression was observed in 28 patients (5 LR, 10 INT-1, 13 HR), BAALC overexpression was present in 34 cases (7 LR, 10 INT-1, 17 HR). Neither WT1 nor BAALC overexpression were related to cytogenetic abnormalities. We observed a simultaneous overexpression of WT1 and BAALC genes in 18 patients (1 LR, 7 INT-1, 10 HR). An evolution to acute myeloid leukemia (AML) was reported at a median follow up period of 6 months (range 2-9) in 13/17 patients. In particular 6/7 INT-1 patients experienced evolution to AML. Among 58 low risk patients only 3 patients evolved to AML. All these patients had an aspecific diagnostic molecular profile. Nine more cases showed an isolated overexpression of WT1 and 3 of these experienced an evolution. The remaining patients are still leukemia free after a median follow-up of 15 months. An isolated BAALC overexpression was observed in 15 patients. In 3 cases we reported an evolution. The combined more than the isolate overexpression of WT1 and BAALC may be

associated to a MDS evolution. The prognostic value of this molecular pattern may overcome that of IPSS score especially in INT-1 subset. On the other hand the simultaneous low-expression of WT1 and BAALC seems to predict low AML evolution rate. We suggest that molecular evaluation at diagnosis of MDS should include WT1 and BAALC evaluation whereas there is no reason to perform FLT3 and NPM analysis in this setting.

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AZACYTIDINE THERAPY FOR MDS AND AML PATIENTS: RETROSPECTIVE MULTICENTRE REGIONAL EXPERIENCE IN PATIENTS NOT ENROLLED INTO CLINICAL TRIALS.

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Background and Aims. In higher-risk patients with myelodysplastic syndromes (MDS), the DNA methyltransferase inhibitors produce responses in 20% to 30% of patients and azacitidine (AZA) has demonstrated a survival advantage when compared with conventional therapies. AZA is increasingly used in low-risk patients failing to respond to recombinant human erythropoietin (r-EPO) and in acute myeloid leukaemia patients (AML) with low marrow blast count or considered not fit for intensive chemotherapy. We report a retrospective review on AZA treatment of MDS and AML patients in the common clinical practice of an Italian region (Liguria). Patients and Methods. Of 67 patients who started azacitidine therapy only 46 patients received at least 4 courses of therapy, and were therefore considered evaluable for response and included in the study. Median age was 74 years (56-84), male/female ratio was 25/21. Nine patients (19%) had untreated AML with marrow blasts ranging from 25 to 41%. MDS patients had RA or RARS (n.7, 19%), AREB-1 (n. 13, 35%), AREB 2 (n. 15, 40%), other forms (n. 2, 5%). In MDS patients the IPSS score was low / int-1 in 18 pts, int-2 / high in 13 pts, not assessed in 7 pts. Twenty-six patients had a transfusion-dependent anaemia and the median number of packed erythrocyte units transfused weekly was 1 (range 1-2). All low and int-1 risk MDS patients had transfusion dependent anaemia and were unresponsive to r-EPO. Results. After a median of 8 courses (range 4-44) 26 patients (63%) achieved a haematological response (CR in 26%, PR in 24%, HI in 6,5%) whereas 20 (43,5%) were unresponsive. According to diagnosis and IPSS score responders were 6 (66,6%) among AML patients (CR 1, PR 4, HI 1), 10 (55,5%) in low /int-1 risk MDS patients and 7 (54%) among int-2 / high risk MDS patients (CR 4, PR 3). Response was achieved after a median of 5 (range 3-6), 3 (range 2-12) and 5 (range 1-12) AZA courses in AML, low/int-1 risk and int-2 / high risk MDS patients, respectively. Grade 1-2 myelotoxicity was commonly observed but no life threatening infections were reported. Eight-teen patients concomitantly received r-EPO therapy. Response lasted a median of 16 months (range 4-40) and median survival was 6 months in AML patients (4-9) and 23 months (6-48) in MDS patients. Conclusions. These preliminary data confirm efficacy and feasibility of AZA therapy in the common clinical practice, for both AML and all risk MDS patients.

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EVALUATION OF ERYTHROPOIETIC ASPECTS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES RESPONDERS TO ERYTHROPOIESIS STIMULATING AGENTS

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Erythropoiesis stimulating agents (ESAs) are the first line therapy in low risk anemic MDS patients and an early inception of this therapy can delay the need for RBC transfusion, hypothetically by slowing the disease course. It is a matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation ofresidual normal erythropoiesis by ESAs. Evidence has been provided to support both views. Macrocytosis is one of the cytological hallmarks of dyserithropoiesis in MDS : in this work we have analyzed erythropoietic response to ESAs therapy in a cohort of anemic MDS prevalently "low risk" patients enrolled in a regional retrospective register, RECAMDS (Registro Campano Mielodisplasie), subgroup of the Italian MDS register. We focused on cytometric differences in Mean Corpuscolar Volume of erythrocytes during the observation period in order to speculate on the target of such therapy in responsive patients. 114 anemic MDS patients (43 RA, 17 RARS, 39 RCMD, 8 RAEB, 7 MDS del5q) not transfusion dependent, under standard ESA treatment (and Epo, 40000/80000 or 30000/60000 U/weekly respectively), were analyzed at the baseline, after three and six month of continuous therapy. The response rate was evaluated following IWG criteria. Statistical analysis was performed with 2 and Anova tests. ESA therapy was started at Hb concentration 9.56 g/dL±1,5, global response rate was 84% (96/114) , no difference among WHO subgroups was found. 88 patients responded after three months, 8 after six. In the responsive cohort, MCV was higher than normal at baseline in 52/96 (54%) patients, while 14/18 (77%) non-responsive patients exhibited macrocytosis. During the response at ESAs treatment, after 6 months from beginning of EPO therapy, 45/52 (86%) macrocytic patients showed permanently elevated values of MCV whereas 7/52 (13%) macrocytic responsive patients became permanently normocytic. In the group of 44 initially normocytic responsive patients 7/44 (15%) became macrocytic and contemporarily 4 of them showed an increase in their neutropenia and/or thrombocytopenia. These very preliminary data can suggest that in the majority of MDS patients responsive to ESA treatment the increase of hemoglobin level occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESA last even when the expression of dysplasia progresses.

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EVALUATION OF TET2 MUTATIONAL SCREENING BY HIGH RESOLUTION MELTING AND SANGER SEQUENCING IN THE CLINICAL SETTING FOR MYELODYSPLASTIC SYNDROMES (MDS)

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Background. TET2 is the most frequently mutated gene in MDS(12-26%). TET2(ex3-11) mutation status was analyzed in 90 newly diagnosed MDS pts enrolled in an Italian multicentre prospective study (O-MDS-protocol; ClinicalTrials.govIdentifier: NCT01291745), comparing cost effectiveness of novel HRM with traditional Sanger sequencing. Methods. Genomic DNA isolated from bone marrow was subjected to HRM analysis performed on the 7900HT ABI-platform (Applied Biosystems) in triplicate using primers designed on TET2 isoform A gene locus (Genbank ID: NM_001127208). Samples displaying aberrant melting curves were subjected to Sanger sequencing. Products were amplified and bidirectionally sequenced on the ABI3130 Genetic Analyzer (Applied). Results. Ten templates were first screened for known mutations to optimize the HRM analysis for each exon, variations in the melting curve were consistent with aberrations identified from direct sequencing. Subsequently, 80 previously sequenced patients were blindly subjected to HRM screening. Sequencing of buccal gDNA was used to confirm acquired mutations. Among the 90pts, we detected 2 previously described and 13 novel mutations of the TET2 coding sequence consisting of 8 InDel mutations, 5 aminoacid substitutions (A301V, H578R, S820G, M1028I, S1898Y) and 2 substitutions producing a STOP codon likely to alter or abrogate TET2 protein function. Notably, 3 mutations fell within the highly conserved LCX1 and one in LCX2. MDS amplicons with an aberrant melting curve, led to the identification of 75(83% of pts) previously described and annotated SNPs, whose individual frequencies reflected those of the general population. Our incidence of detectable TET2 mutations is 17%, all identified in low-risk patients (Int-1 or Low IPSS). Conclusions. HRM is a useful screening tool for screening genetic mutations and SNPs in the TET2 coding region in MDS. Advantages of HRM-based screening compared to traditional direct sequencing, include rapid evaluation of the extensive coding sequence of TET2 (9796bp), direct sequencing of the generated amplicon with no need of additional DNA, saving time (40%) and costs(30%), and 100% correlation with direct sequencing protocols. Using a combination of both techniques we identified 13 new heterozygous TET2

mutations. Although we consider HRM less feasible than direct sequencing for exons longer than 2000 base pairs, such as TET2 ex3 and ex11 given the number of PCR reactions required for HRM, yet it may be easily applied to mutation hotspots in longer coding regions.

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ERYTHROPOYESIS STIMULATING AGENTS (ESAS) IN THE MANAGEMENT OF MYELODYSPLASTIC SYNDROMES

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ESAs is considered first line therapy in anemic MDS patients, in earlier stage of the disease. Our aim is to evaluate retrospectively clinical response to ESAs in anemic MDS patients, not transfusion dependent, enrolled in a regional retrospective register, RECAMDS, subgroup of the Italian Register, focusing on WHO classification and IPSS risk stratification. 114 anemic MDS patients(M/F 55/59, median age 77±9.1,WHO 43 RA,17 RARS,39 RCMD,8 RAEB,7 MDS with del5q, risk assessment:53 low,58 Int-1,3 Int-2), not transfusion-dependent, treated with ESAs(or EPO 40000/80000 or 30000/60000 U/weekly respectively)from 2006 to 2012. We analyzed data at 3 and 6 months of treatment: responses were evaluated according to IWG criteria 2006; in the responders group we evaluated median time from diagnosis, serum EPO at the beginning of therapy, time to response, duration of response and relationship with WHO subgroups.28 patients were treated with standard dose EPO(40.000 IU/W) and 12 received high dose(80000/W), the remaining 70 were treated with EPO: 56 with a weekly standard dose of 30.000IU and 14 with high dose (60000IU/W). ESA therapy was started at mean Hb concentration of 9.50 g/dL±1.5 with EPO serum level of 64mU/L ± 79.6 after a mean of 6 months(1-118) from diagnosis. ORR was 84%(96/114):88/114 achieved response after 3 months, other 8 patients achieved a significant response after 6 months(4 AR,1RARS,2 RCMD,1MDS 5q-). 40 (38%) lost the response after a mean of 18 months(3-84) while 74(65%) are still on treatment without transfusion-need after a median time of 25 months(3-96).Non responders patients were 7 AR,4 RARS,5 RCMD,1AREB,1Del5q and their EPO level was 173 mU/L±143.93 while in the responders subgroup it was 47.28 mU/L±48.6, with statistical difference between the 2 groups. WHO subgroups did not exhibited different responses. It is noteworthy that in a subgroup of anemic patients (7) with del5q, not transfusion dependent, there was a good response to ESAs in the majority of them (6/7), 5/6 achieved response after 3 months and 1 after 6, all responder 5q- patients (3del5q-,3 RA,1RCMD) are still responsive with a median time of 34 months(r.8-96 months). ESAs therapy is a successful treatment in low risk MDS: we report a good response to ESAs also in advanced stage and in a subgroup of del5q generally reported as bad responders to such treatment, further studies will evaluate if this good response will delay transfusion requirement.

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IMPACT OF COMORBIDITIES ON THE "REAL LIFE" MANAGEMENT OF MYELODYSPLASTIC PATIENTS: AN EXPERIENCE FROM THE LIGURIAN REGISTRY OF MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDS) are an heterogeneous group of hematological disorders which mainly involve older patients. The management of these diseases can be difficult due to important comorbidities which often affect these subjects. Current international Guidelines indicate that management's strategy should be based on evaluation of prognostic score systems such the International Prognostic Score System (IPSS); however several different reasons might largely influence in the real life the actual clinical management of these patients. Materials and Methods. we evaluated the current management strategies on the series of MDS patients collected in the Ligurian MDS database estabilished within the framework of the Italian Network of regional MDS registries. Results. from 2010 to 2012 293 patients (158 (54%) males and 135 (46%) females) were registered into our database. Median age at diagnosis was 76 years (range 42-98 years); WHO categorization was as follow: 32% RA, 34% RCMD, 5% RARS or RCMD-RS, 3% 5q- syndrome , 6% RAEB-1, 8% RAEB-2 and 1% MDS-unclassified; 11% remained undetermined. IPSS was determined in 226 (77%) of the patients; LR (low and intermediate-1 risk) patients were 87%, while HR (intermediate-2 and high-risk) were 13%. Revised IPSS (r-IPSS) was calculated in 225 (77%) of the patients: very low risk 15%, low risk 43%, intermediate-risk 10%, high-risk 6% and very high-risk 3%. Transfusion-dependent patients at diagnosis were 56 (19%). The large majority of the patients, mostly older and with one or more comorbidities, received only supporting therapy (mainly transfusions) or erythropoietin, alone or with G-CSF; only 9% was treated with Azacytidine, 1% with lenalidomide (all 5q-syndromes) and 0,7% with immunosuppressants agents; as few as the 1% received AML-like chemotherapy. Only 1% were enrolled on some clinical trial. Very few transfusion-dependent patients (9%) received iron chelation therapy. The choice for supportive care only was correlated with the number of comorbidities and the poor performance status (PS) Conclusions. Older age, comorbidities and poor PS do not allow in the real life to have an always "ideal" management of MDS patients; the majority of MDS patients received just supportive care or ESA; only a small number was treated with iron chelator and even a lower number was enrolled in a clinical trial.

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AZACITIDINE IS SAFE AND WELL TOLERATED IN VERY ELDERLY PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES

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Introduction. Azacitidine (AZA) has be proven to be more effective than standard supportive care in patients with high-risk MyeloDysplastic Syndromes (MDS). However, because of lack of appropriate clinical trial, management of Very Elderly Patents (VEP), defined as age >80 years or more comorbidities,is still unsatisfactory even in MDS with low-intermediate risk. Here we report the experience of a single institution in the treatment of VEP affected by MDS. Patient and method: We analyzed 21 (17 males, 4 females) consecutive elderly patients with MDS treated with AZA over a period of 4 years; the median age was 78 years (range 65-92). Patients were classified as follows (WHO classification): 3 refractory cytopenia with unilineage dysplasia (RCUD), 2 refractory cytopenia with multilineage dysplasia, 4 refractory anemia with excess of blasts (RAEB-type 1), 6 RAEB type 2, 4 SMD/LAM and 2 chronic myelomonocytic leukemia type 2 (CMML-2). According to International Prognostic Scoring System (IPSS), 11% were low, 46% were intermediate-1, 11% were intermediate-2 and 6% were high IPSS. All patients were treated with AZA 75mg/sqm/die subcutaneously (sc) for 7 days, every 28 days. We collected data after 6 cycles based on International working group (IWG) response criteria. In patients with response or stable disease therapy was continued until progression. Results. Our results showed: 31% Complete Remission (CR) with HB >11, blasts <5%; 56% Partial Remission (PR) with reduction of transfusion requirements, 13% Stable Disease (SD), 2 patients died for progression to Acute Leukemia after two cycles. Hematologic toxicity accordingly to WHO criteria were experienced in 60% of patients (45% grade 3–4 thrombocytopenia, and 20% grade 3–4 neutropenia). All patients suffered of pruritus, edema and erythema in the site of injection. Nineteen patients completed the all course (6 cycles) without delays, serious adverse effects or hospitalization. Conclusions: In our experience, treatment of VEP suffering of MDS with AZA was safe; it was also well tolerated and allowed a relatively good quality of life. Although the lack of a direct comparison, response rate and adverse events seems not different than those observed in younger patients. These results should be confirmed in prospective clinical trials. It would be interesting to experiment AZA with new schedule or new route of administration aimed at improving adherence to treatment of VEP.

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MESENCHYMAL STEM CELLS OF MYELODYSPLASTIC SYNDROMES SHOW IMPAIRED HEMATOPOIETIC SUPPORT FUNCTION AND GLOBAL DNA HYPERMETHYLATION STATUS

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Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by ineffective hematopoiesis and risk to leukemic transformation. The bone marrow microenviroment promotes survival and manteinance of hematopoietic stem cell. Evidences exists that microenvironment of MDS marrow show functional abnormalities, which may be relevant to the incidence of such a disease. The possible involvement of bone marrow mesenchymal stem cells (BM-MSC) in the pathogenetic process of MDS has been recently suggested. Here, we characterized BM-MSC isolated from MDS patients (low and high risk IPSS) for their structural and functional properties, at diagnosis and in high risk-MDS after 5-azacytidine treatment. MDS-MSC were expanded in liquid culture and characterized for their immunophenotype and karyotype. These cells were studied also for their functional properties, analyzing the hematopoietic supporting role and global DNA methylation status, evaluating 5-methylcytosine level. All data were compared with healthy MSC donor. Moreover, methylation results were correlated with MDS-hematopoietic cells (MDS-HC) of the same patients tested. MDS-MSC (median age 67, range 36-87) achieved confluence at a significantly slower rate than MSC donor (median age 79, range 55-82) and some samples weren't able to grow *in vitro*. Briefly, these cells showed an average long term proliferation potential significantly lower than MSC donor (0.92±0.4 logs in 58 days *vs* 3.3±1.3 logs in 128 days) even after treatment (0.39±0.6 logs in 68 days) ($p<0.05$). Furthermore, MDS-MSC expressed the typical MSC surface antigens and the ability to support hematopoiesis in long term culture was less than MSC donor even if without significant difference. MDS-MSC expressed a hypermethylated status of global DNA 2.2 times higher than MSC donor ($p<0.05$). Just after the first 5-azacytidine treatment, the MDS-MSC methylation status changed and decreased strongly reaching MSC-donor methylation status. Similarly, we observed the hypermethylation status of MDS-HC with 5-methylcytosine level 1.3 times higher than HC donor ($p<0.05$). As above, these data decreased after treatment up to normal level. In summary our results showed that MDS-MSC have an impaired proliferation potential and hematopoietic support function. Notably, they displayed an hypermethylated status of their DNA, specially linked to the hypermethylated status of MDS-HC, that decreased to normal level with 5-azacytidine treatment.

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IRON CHELATION THERAPY IN MYELODYSPLASTIC SYNDROMES AND IN OTHER TRANSFUSIOND-DEPENDENT CHRONIC ANEMIAS. RETROSPECTIVE STUDY OF 57 PATIENTS

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To date, several guidelines 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. Moreover, 10-20% of pts show an improvement of peripheral cytopenia after ICT. However, several barriers may limit the initiation or the continuance of ICT in MDS pts: older age, comorbidities, poor tolerance and compliance. Therefore, with the aim of assessing the safety and efficacy 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, 57 pts (41 males), median age: 73 (23-96) yrs, with transfusion-dependent anemia, received 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. 31 pts (54.3%) were affected by lower-risk MDS (IPSS risk: low or intermediate-1), while 15 pts (26.3%) 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. 11 pts (19.3%) were affected by other diseases (idiopathic myelofibrosis: 3 pts; aplastic anemia: 7 pts; pure red cell aplasia: 1 pt). 34 pts (59.6%) received deferasirox (DFX), 9 pts (15.8%) received deferoxamine (DFO) (subcutaneous bolus injection), 11 pts (19.3%) received DFO and subsequently DFX, and 3 pts (5.3%) received DFX and subsequently DFO. Median time from diagnosis to the start of ICT: 18 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: 2131 ng/ml; median SF after ICT: 1960 ng/ml; median duration of ICT: 11 (range 1-192) months. Grade >2 adverse events occurred in 24 pts (42.1%): renal (transient increase of serum creatinine): 8 pts; gastrointestinal: 10 pts; cutaneous: 6 pts (5 receiving DFO). Permanent discontinuation of ICT: 28 pts (49.1%), because of toxicity (13 pts), worsening of clinical condition (3 pts), hematologic remission (6 pts), allogeneic transplantation (6 pts). 4 pts (7%) , (3 MDS and 1 PRCA) showed an erythroid response, according to IWG criteria: (Cheson, 2006) following ICT, one of them achieving complete remission. In conclusion, in our experience ICT appears feasible even in a population of elderly pts, if carefully selected.

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AZACITIDINE IN HIGH AND LOW RISK MYELODYSPLASTIC SYNDROMES: RETROSPECTIVE EVALUATION OF 57 PATIENTS TREATED WITH 4 DIFFERENT THERAPEUTIC REGIMENS

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Azacitidine (AZA) has proven effective in Myelodysplastic Syndromes (MDS), and the currently approved AZA regimen is 75 mg/sqm/die subcutaneously for 7 days every 28 days. Subsequently, other alternative and more convenient AZA dosing regimens have shown to be effective, in terms of hematologic responses (Lyons, 2009). From September 2004, in our Institution, 57 MDS patients (pts) (43 males), with a median age of 70 (37-84) yrs, were treated with AZA, following 4 different treatment regimens. Group 1 (10 pts), received the currently approved regimen (AZA 7). Group 2 (6 pts), received the AZA 7 regimen with valproic acid and all-trans-retinoic acid. Group 3 (29 pts) received the AZA 5-2-5 regimen: 50 mg/sqm/die SC for 10 days/28 days. Group 4 (12 pts) received the AZA 5 regimen: 75 mg/sqm/die SC for 5 days/28 days. Moreover, we quantified the degree of phosphoinositide-phospholipase C (PI-PLC) beta1 methylation and gene expression before and during AZA administration. At AZA onset, IPSS risk was: low: 3 pts; intermediate-1: 12 pts; intermediate-2: 33 pts; high: 9 pts. R-IPSS risk was: very low: 1 pt; low: 3 pts; intermediate: 7 pts; high: 10 pts; very high: 36 pts. WPSS risk was low: 3 pts; intermediate: 5 pts; high: 9 pts; very high: 40 pts. 9 pts had therapy-related MDS. ECOG-PS was poor (≥2) in 15 pts. Transfusion need was high (≥4 RBC units/8 weeks) in 34 pts. 6 pts presented circulating blasts. Following Itzykson's AZA prognostic scoring system, the risk was low in 7 pts (12.3 %), intermediate in 48 pts (84.1 %), and high in 2 pts (3.6 %). The pts received a median of 8 cycles of AZA (range: 1-59). 50 pts were considered evaluable for response (at least 6 cycles): 34/50 pts (68%) showed a favourable response following IWG criteria (Cheson, 2006): complete remission (CR) in 7 pts (14% %), hematologic improvement (HI): 27 pts (54%). The median duration of response was 12 (range: 1-88) months. Group 1: 5 responders (62.5%) (1 CR and 4 HI); Group 2: 3 responders (50%) (3 HI); Group 3: 19 responders (73.1%) (5 CR, 14 HI); Group 4: 7 responders (70%) (2 CR, 5 HI). A significant toxicity (grade >2) was observed in 23 (40.4 %) pts. 37 pts died, 13 for AML, 9 for infection, 17 for other causes. Median OS from the start of AZA was 18 (range: 5-108) months. The detection of an increase in PI-PLCbeta1 gene expression within the first three cycles of AZA therapy was significantly associated with a better clinical outcome and a longer hematologic response.

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PROLONGED AZACITIDINE TREATMENT IN THE MANAGEMENT OF ELDERLY PATIENTS WITH HIGH RISK MYELODYSPLASTIC SYNDROMES, CHRONIC MYELOMONOCYTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA NOT ELIGIBLE FOR STANDARD INTENSIVE THERAPY

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Clinical trials with Azacitidine (AZA), a cytosine analogue with hypomethylating activity, have documented an overall survival benefit and an improvement in quality of life for patients with high risk myelodysplastic syndromes (MDS). From October 2010 to April 2013, 37 consecutive elderly patients (17 males and 20 females, median age 76 years), with high risk MDS (RAEB-2, refractory anemia with excess of blasts type 2, n=20), chronic myelomonocytic leukemia (CMML, n=4) and acute myeloid leukemia (AML, n=13) not eligible for standard intensive chemotherapy were treated with AZA (75 mg/mq/day for 7 days every 28 days). All patients received at least 2 cycles of AZA, with a median of 6 cycles (range 2–19). Sixteen patients (13 MDS, 2 AML, 1 CMML) receiving at least 6 cycles of AZA were evaluable for response, according to International Working Group 2006 criteria. In this cohort of patients, 3 of them achieved complete remission (CR; 2 RAEB-2 and 1 CMML), 2 marrow CR (mCR), 2 partial response (PR), 4 patients showed stable disease (SD) and 3 progressive disease (PD), while two patients with AML showed PD after 6 cycles. However, 2 patients with RAEB-2 with mCR at the 6 months progressed in AML after 13 and 10 cycles, respectively. Hematological improvement was documented in the whole cohort of patients, with increase in platelet and neutrophil counts, as well as in hemoglobin after 7, 8, and 9 cycles of treatment, respectively. Hematologic toxicity (grade 3/4 neutropenia and thrombocytopenia) required growth factor administration and platelet transfusions in 35% and 15% of patients, respectively. No patient discontinued treatment due to toxicity and all responders after 6 cycles continued treatment until progression. Seventeen patients died: 7 with AML after a median of 3 months, 8 with RAEB-2 after a median of 8 months (7 due to AML transformation, 1 to infection), 2 with CMML after a median of 8 months (due to PD). With a median follow-up of 30 months, median overall survival for all patients treated with AZA was 13.1 months (14.1 months in MDS, 10.7 in CMML and 7.2 in AML; $p<0.05$). Our preliminary data further provide evidence that prolonged AZA treatment is effective and well tolerated in elderly patients with high risk MDS, CMML and AML not eligible for standard intensive treatment.

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PU001

MYELODYSPLASTIC SYNDROMES IN CARRIERS OF THALASSEMIA TRAITS: A REMINDER FOR HEMATOLOGISTS

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Concomitant myelodysplastic syndromes (MDS) in subjects carrying a globin gene mutation may result in an interacting hematological phenotypes which clinical and laboratory features may pose several diagnostic and therapeutic concerns. From our database, we retrieved 9 patients (6 female) with a median age of 80 (66 – 89) years, carrying an or a -thalassemic trait and diagnosed as having MDS. Five out of 9 patients kept under our attention after the occasional discovery of 1 or 2 cytopenias other than microcytic anemia. The remaining four patients were well-known carrier of heterozygous (1) and (3) thalassemic traits and presented a worsen anemia. The diagnosed MDS subtypes were refractory anemia (RA), refractory cytopenia with multilineage dysplasia (RCMD) and refractory anemia with excess blasts-1 (RAEB-1) in 4, 3 and 2 cases respectively. The blast percentages ranged from 1% to 7%. Standard cytogenetic and FISH analysis showed karyotipic abnormalities, such as the Y chromosome loss, the 20q12 deletion and trisomy 8 in 2, 1 and 1 cases respectively; five patients presented no cytogenetically detectable genetic changes. According to the IPSS, the MDS risk was classified as low and intermediate-1 in 7 and 2 patients respectively. Four patients received epo (endogenous epo: from 48 to 114 U/L), which was titrated according to individual targets (basic hemoglobin concentrations and red blood cell counts); two of them had required transfusions before (1 case) or soon after the initial phase (1 case) of the treatment. All four patients responded to epo and achieved transfusion independence. The remaining five patients presented no need for treatment. Considering all 9 patients, with a median follow-up of 21 (4 – 38) months, no disease progression or evolution in acute myeloid leukemia was observed. In conclusion, and thalassemia traits may be an incidental finding in cytopenic patients developing MDS; on the other hand, the worsening anemia in a subject carrying heterozygous - or thalassemia trait can be accompanied by myelodysplastic changes in the BM. Certainly, cytogenetic and molecular studies remain crucial to define diagnosis, as well as the study of all the possible factors, such as an enhanced apoptosis, implicated in the pathogenesis of MDS in this particular patient's population.

PU002

LONG-TERM SUSTAINED RESOLUTION OF ANEMIA DESPITE DISCONTINUATION OF EPOETIN AFTER EXCESSIVE THERAPEUTIC RESPONSE: AN UNEXPECTED OUTCOME IN THREE PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Human recombinant erythropoietin (epo) exerts its therapeutic effects by targeting multiple factors involved in the pathogenesis of anemia in patients with myelodysplastic syndromes (MDS). Although rarely, a treatment response over the optimal therapeutic hemoglobin (Hb) target may be occasionally observed. In these circumstances, dose reduction and/or a longer interval of administration are usually adopted. However, very long lasting and durable restored erythropoiesis in low-risk MDS despite the discontinuation of epo, as observed by us, represents an exceptional occurrence. There were three patients (two male) with median age of 76 (72 – 80) years; two had refractory anemia and one was affected by refractory cytopenia with multilineage dysplasia (RCMD). All other causes of anemia, including coexisting renal failure, were excluded. Two patients presented cytogenetic abnormalities (45, X0,-Y and 20q deletion, respectively); one patient had normal kariotype. The blast percentages ranged from 1 to 4%. According to IPSS patients were classified as low risk and the remaining as Int-1. Before starting epo (epoetin alfa), all patients required red blood cells (RBC) transfusion (2, 4 and

4 units of RBC respectively). The erythropoietin serum concentration measured in two patients before starting epo but after RBC transfusions, resulting as high as 20 and 89 mU/ml respectively. Epo at the dose of 40.000 IU was given twice a week in one and once a week in two patients, respectively. At the time of initiating epo, the median level of Hb was 7.8, 8.1 and 8.4 gr/dl, respectively. Within four weeks, a major erythroid response was achieved in all cases, with a median Hb level of 11.40, 11.80 and 12.50 gr/dl, respectively. Given the major erythroid response and the magnitude of the too rapid Hb improvement, the intensity of treatment was appropriately reduced. However, after eight weeks, an Hb level exceeding 13.00 gr/dl was reached and epo was withdrawn. Thereafter, the patients maintained near-normal Hb levels, ranging from 12.4 and 13.5 g/dL at 7, 10, and 21 months respectively from epo discontinuation. Interestingly, the two patients with cytogenetic abnormality continued to show the same original clonal alteration. In conclusion, some pathogenetic mechanisms other than the stimulating effects exerted by epo, such as the expansion of most responsive clones and/or proliferation of non-clonal erythroid matrix, can be supposed.

PU003

BENDAMUSTINE, LENALIDOMIDE AND DEXAMETHASONE VS BENDAMUSTINE, BORTEZOMIB AND DEXAMETHASONE FOR ADVANCED STAGE MULTIPLE MYELOMA (MM)

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Introduction. Despite the use of novel biological drugs, MM remains incurable cancer. Phase I/II studies suggest that bendamustine (B) variously combined with thalidomide (T), lenalidomide (L) or bortezomib (Bor) may be an effective treatment for relapsed/refractory MM. Cheson reported that doses used in MM ranged from 50 to 150 mg/m^2 on days 1-2 every 28 days. Patients and Methods. Ten patients (median age 58,5 years, range 43-80), affected by relapsed/refractory MM after the failure of at least one prior conventional salvage therapy, were treated with B variously combined with other drugs outside of clinical trials."BVD group": four patients were treated with B 150 mg/m^2 on days 1-2 in combination with Bor 1,3 mg/m^2 on days 1, 4, 8, and 11 and dexamethasone (D) 40 mg on days 1-2, 4-5, 8-9 and 11-12. "BRD group": two patients received B 150 mg/m^2 on days 1-2 in combination with L 25 mg on days 1-21 and D 40 mg on days 1-4, 15-18; after an initial experience with B 150 mg/m^2 on days 1-2, because of both severe neutropenia reported following higher dose of B and important comorbidities, three patients received B 80 mg/m^2 on days 1-2 in combination with L 10 mg on days 1-21 and D 40 mg on days 1-4, 15-18. One patient received B 60 mg/m^2 on days 1-2 in combination with L 10 mg on days 1-21 and D 20 mg on days 1-4 because of the severe deterioration of the clinical conditions and advanced age. Cycles were repeated every 4 weeks. Results. All patients completed at least three cycles of therapy. In"BVD group" 3/4 patients interrupted prematurely the treatment for non haematological response; only one patient achieved a haematological response (VGPR). In"BRD group" two patients achieved a CR, 1 patient a VGPR, 3 patients a PR. Grade 4 neutropenia occurred in 4 patients causing administration of GCS-F, delay of the treatment and reduction of daily dose. Only one patient developed septicaemia following grade 4 neutropenia. The different doses of B (150 mg/m^2 *vs* 80 mg/m^2) did not impact speed of haematologic response and decrease of myeloma protein value. The median time to the best hematologic response was 60 days (60–120). Median OS was 7 months (1-15). Responses were independent of B2M (P 0.5), albumine (P 0.2), disease stage (P 0.2), age (P 0.15), and number of prior therapies (P 1). Conclusions. In "BVD group" ORR was 25%, in "BRD group" was 100%. Maximum tolerated dose without severe hematologic side effects was B 80 mg/m2 on days 1-2 every 28 days.

PU004

CHRONIC LYMPHOCYTIC LEUKEMIA: MOLECULAR EVALUATION AND SEQUENCE ANALYSIS FOR MUTATIONAL STATUS OF THE IMMUNOGLOBULIN HEAVY CHAIN VARIABLE

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Background. The clinical heterogeneity characterizes chronic lymphocytic leukemia (CLL), with survival times ranging is the image of biological diversity. CLL was largely considered to be a disease of slow progression. Our understanding of the biology of CLL has helped us identify several markers of prognostic significance, by which CLL can be differentiated into several distinct diseases. Two subsets of CLL with different outcomes are identifies from the mutational status of the immunoglobulin heavy chain variable (IGHV) genes.The determination of IGHV mutation status may be performed in laboratories by PCR analysis and sequence gene study. The variation in immunoglobulin heavy chain variable region (IGHV) mutation, genetic aberration and variation in apoptosis and proliferation has had an influence on therapy initiation and follow up. AIM: To Standardize the diagnostic way for sequence study on mutational status in LLC patients, The evaluate VH-JH family in immunoglobulin heavy chain variable (IGHV). Methods. The mutational status of (IGHV) was performed by PCR and sequence study ; 37 untreated CLL patients , from Cancer Department of "Businco" Cagliari Hospital were analyzed retrospectively to evaluate independence and predictive power of mutational status of immunoglobulin heavy chain variable gene segments (IGHV). High-risk chromosomal alterations as 17p or 11q deletions, CD38, age, gender, Binet stage, 2-microglobulin levels, absolute lymphocyte count and number of lymph node regions was clinically evaluated. Mutational status was carried out by comparison with the germ line for VH families identifications (http://www.ncbi.nlm.nih.gov/igblast/). Results. IGVH rearrangement were amplified and analyzed using IMGT database. Were only evaluated correct and productive rearrangements. From sequence analysis, percentage of identify to germ line of family were analized .The 57% of cases were VH3-JH family mutated , while 14% were VH1-JH family mutated. VHFS4-JH, VHFS1-JH, VHFS3-JH were respectively family mutated for 1%. IGHV mutational status was the sole biological variable as independent prognostic indicator. Only two cases were unmutated , with a rapid clinical course, and lymphocytosis gradual increase. Conclusions. Data indicate that IGHV mutational status may be integrated with clinical variables in new prognostic tools to estimate overall survival and clinical course.

PU005

HIGH-RISK CHRONIC MYELOMONOCYTIC LEUKEMIA EVOLVED FROM A LONG-LASTING REFRACTORY ANEMIA: AN UNUSUAL OBSERVATION

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The current vision of oncogeny as a multistep process with different successive anomalies occurring in the homeostatic cellular control seems to itemize the disease history of a patient with chronic myelomonocytic leukemia (CMML) arising seven years after the diagnosis of refractory anemia (RA). Such a suite has been rarely reported (Breccia *et. al.* Leuk Lymphoma. 2008) in the setting of myelodysplastic syndrome (MDS), thus we think it worth of interest. A 74-year-old male with a history of prostate cancer and postoperative radiotherapy was admitted in our center on January 2005 because of severe macrocytic anemia. He received 4 units of red blood cells (RBC) concentrates. Bone marrow (BM) examination revealed a marked hyperplasia along with an evident erythrodysplasia and 5% of blasts. Conventional kariotype analysys and FISH analyses showed no abnormalities. A diagnosis of MDS (RA subtype according to FAB classification; IPSS: Intermediate-1 risk) was made. The patient received subcutaneous erythropoietin (epo) at a weekly dose of 40.000 units maintaining a good disease control until June 2012, when his hematologic status slowly deteriorated. Loss of response to epo, thrombocytopenia and leukocytosis with absolute monocytosis and 2% of circulating blasts, suggested a CMML transformation. In the BM (Figure 1), persisting the erythrodysplasia, the picture

had evolved to monocytosis with 15% blasts. A new karyotipic abnormality, such as Y chromosome deletion (45, X0,-Y) was present; this lesion was not detected at the onset of MDS seven years before; JAK2 V617F mutation was still absent. A diagnosis of RA coexisting with CMML type -2 was made. The MDAPSS was 3 (intermediate-2 risk). After a brief course of hydroxicarbamide, the patient was scheduled to receive azacitidine (75 mg/m^2, schedule 5+2). After the second cycle PB counts significantly improved, monocytosis disappeared and there was no more need for transfusions. Complete hematological and cytogenetic responses of CMML were achieved after six course of hypomethylating therapy without major toxicity. This case shows an atypical presentation of CMML, secondary to MDS. Above the rarity and anecdotal interest, we believe undisputable the evolution of a low-risk MDS to CMML, thus the existence of a "secondary" CMML as a distinct feature, never considered in any classification system. Moreover we underline the safeness and efficacy of hypomethylating therapy for both components of this complex malignancy.

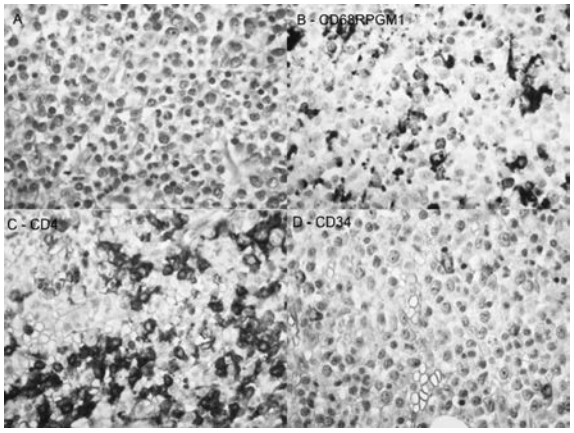

Figure 1. A: bone marrow biopsy (E/E – high magnification) show predominance of myeloid cells with myelo/monocytic differenziation; a lot of immature cells are detectable 10 – 15% suggesting a diagnosis of CMML-2 type for WHO 2008 classification. B-C: immunostains for myelomonocytic antigens such as CD68RPGM1 and CD4 antibodies, shows typical granular cytoplasmic (B) and intense cytoplasmic /membrane expression (C) by myelomonocytic cells (C). D: CD34 stain shows only a few immature CD34 positive cells , revealing that cells with immature-blastic morphology are blast-equivalent CD34 negative cells with myelomonocytic differentiation (monoblasts and promonocytes). These particular immunophenotypic features may be challenging about the border between a CMML-2 and acute myeloid leukemia.

PU006

ACUTE MYELOID LEUKEMIA (AML) ARISING FROM HIGH RISK CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) DURING HYPOMETHYLATING THERAPY (HMT)

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The introduction of HMT in the CMML management has provided some important advances, although the evolution in AML can be delayed but not avoided in this patients. With this regard, we describe here the characteristics of two high – risk CMML patients progressed to AML after HMT. The first, was a 60 years old man with a likely therapy-related CMML characterized by a hypercellular bone marrow (BM) with a 15% of blasts, a normal karyotype, the presence of the JAK2 V617F mutation and a MDAPS high risk. The patient was treated with azacitidine (75 mg/m^2,schedule 5+2). After the fourth course of HMT, a complete remission (CR) was achieved. However, soon after the sixth course of HMT, a sudden transition from a near normal blood counts to a very marked leukemic spread (WB =120.000/μl; 70% myelomonoblastic cells positive for HLA-DR,CD4,CD13,CD15, CD33,CD64, CD45,CD34,CD56 and CD117) was observed along the appearance of an abnormal kariotype, such as 46, XY, del (7) (q31) [7]/46, XY [13]. Molecular analysis showed an IDH2 R172K mutation whereas no others AML-related alterations, such as CBFb/MYH11, DEK/CAN, NPM1, FLT3, RUNX1/ETO were found. The patient received one course of AML-like chemotherapy but soon died because of progressive disease. The second case was that of a 72 years old man with a hypercellular BM and 18% of monoblasts. The mutation of JAK2 V617F was detected along a rare chromosomal abnormality, such as 46XY, inv (12) (p13.3 q15). The MDAPS was high. The patient was treated with HMT, as above reported, achieving a CR after the sixth course. However, before starting the fourteenth cycles of azacitine, he complained a sudden worsen of his general condition accompanied by a marked peripheral blastosis (WBC=140.000/uL), which immunophenotype was positive for HLA-DR, CD15, CD33, CD64, CD45, CD34, CD56 and CD117. The same kariotypic abnormality, such as the inv (12) was found. The molecular studies showed no abnormalities. The patient rapidly worsened and he died few days after the AML diagnosis because of cardiac and pulmonary complications without being able to receive any salvage treatment. Although the good efficacy of HMT, the CR achieved by our patients was transient (2 and 7 months respectively) and the evolution in AML was particularly devastating; high BM cellularity, elevated WBC counts, the rapidly fatal clinical course, the lack of response to an AML chemotherapy in one case, were the mainly prominent features recorded by us.

PU007

PRIMARY BONE MARROW DIFFUSE LARGE B-CELL LYMPHOMA IN A FRAIL AND OLDER PATIENT: COMPLETE AND LONG LASTING REMISSION BY R-CHOP CHEMOTHERAPY

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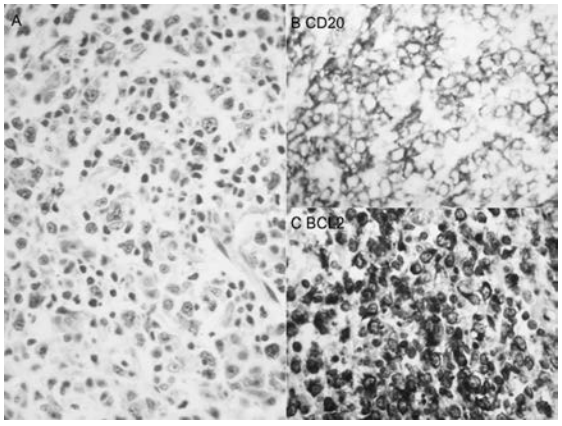
Primary bone marrow (BM) diffuse large B-cell lymphoma (DLBCL) is a rare type of extranodal lymphoma with poor prognosis, of which of which slightly more than 10 cases have been described until now (Chang H. *et al*. Ann Hematol., 2011). Here, we report a case of primary BM DLBCL successfully treated with R-CHOP chemotherapy in a frail 74-year-old woman who kept under our attention because of dyspnoea on exertion due to severe macrocytic anemia, requiring red blood cell (RBC) transfusions, and thrombocytopenia with prominent erythrocyte and platelet anisopoikilocytosis. The physical examination revealed pallor and tachycardia but neither lymphadenopathy nor hepatosplenomegaly was observed. A myelodysplastic syndrome (MDS) was suspected and a comprehensive work-up was performed. Initial BM aspirate revealed a dry tap; the examination of the BM specimen by trephine biopsy showed an involvement of large abnormal lymphoid cells (Figure 1) and fibrosis.



A: Bone marrow biopsy (Giemsa stain - high magnification) shows a neoplastic infiltrate composed by predominance of blastic cells with centroblastic morphology; minor component composed by small centrocytes-like cells. The findings are indicative of DLBCL with features suggestive for a centrofollicular origin.

Standard radiological work-up showed no other suspected DLBCL localizations; so that, an 18F-fluorodeoxyglucose positron emission tomography (PET) scan revealed a disseminated BM uptake without any evidence of disease involvement at other sites. A diagnosis of primary BM DLBCL was made. Laboratory evaluation showed high level of LDH but not other remarkable abnormalities.
The age-adjusted IPI was 3 (high risk). Our patient received R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy (3-week standard schedule) without any significant toxicity. The BM examination showed the disappearance of lymphoma cells after the eighth course; however, transfusion independence was achieved after the first two course of R-CHOP. To date, seven months after the completion of the eight R-CHOP course, she is well present no signs of disease, as demonstrated by a clinical revaluation, including a body PET scan and BM trephine biopsy, recently performed. Primary BM DLBCL is a rare but distinctive entity of extranodal lymphoma with a poor prognosis, being the reported and the 2-years survival approximately 30% and the median survival of 14.9 months. As immunochemotherapy R-CHOP has become the standard of care for elderly patients with DLBCL, our case indicated that this regimen can be an effective and well tolerated treatment able to induce long lasting remission also in the poor-prognosis primary BM DLBCL.

PU008

B-CELL LYMPHOPROLIFERATIVE DISEASE AND ACUTE MYELOID LEUKEMIA IN ONE PATIENT

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A 74-year-old man presented as outpatient at our Division of Hematology because of leukocytosis and thrombocytopenia. Blood cell count with differential showed WBC 15.9*10^3/L, lymphocytes 8600/ L, monocytes 1.400/ L, neutrophils 5900/ L, Hb 12.5 g/dL, PLT 84000/ L. Physical examination revealed splenomegaly and mild hepatomegaly. Laboratory workup showed hyperuricemia (9.5 mg/dL), 2-microglobulin= 6.3, and normal LDH. Anti-platelet antibodies were not detected. Bone marrow aspiration revealed 29% of atypical lymphocytes with no signs of myelodysplasia. Biopsy showed aggregates of small to medium size lymphocytes. These cells were CD19+, CD20+, CD10-, CD43-, CD23, CD5-, CD79b+, FMC7+, CD11c+, HLA-DR+. Karyotype was normal at cytogenetic analysis. Final diagnosis was splenic marginal zone lymphoma (MZL). Therapy with single agent rituximab 375 mg/m^2 weekly was started, for a total of 4 infusions. Complete resolution of lymphocytosis, anemia, and hepatosplenomegaly was achieved. Afterwards the patient was lost to follow-up. Eighteen months later the blood cell count with differential showed WBC= 42.000/ L (N= 75,8%; L= 10,7%; M=5,8%; E= 0,7%, myeloid blasts 3%). At bone marrow aspiration there were 40% blasts that at immunophenotyping were CD33+, CD11b+, CD11c+,CD 56+, CD64+, CD14+/-, CD38+, CD13-, CD117, CD34+ (49% of cells). The final diagnosis was acute myeloid leukemia (AML), FAB M4. Cytogenetic analysis was negative. Molecular biology revealed type A nucleophosmine gene mutation. Ultrasounds showed hepatosplenomegaly but no lymphadenopathy. Induction therapy was started and a complete remission was achieved. [IF: cell CD34+= 0,4% e CD45,CD34,CD33,CD13,CD14,CD56 (Minimal Residual Disease)= 0,2%]. Nevertheless, three months later AML relapsed and the patient died. Patients with chronic lymphoproliferative diseases (CLD) may develop a second malignancy, usually a solid neoplasm, because of impaired immune system or chemotherapy. Association between CLD and myeloid malignancy in one patient has been rarely reported. To the best of our knowledge this represents the first reported case of AML in a patient with previous diagnosis of MZL.

PU009

MULTIFOCAL EXTRAMEDULLARY PROGRESSION OF IGA MULTIPLE MYELOMA DURING BORTEZOMIB COMBINATION THERAPY

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Contemporary multifocal extramedullary (EM) involvement in Multiple Myeloma (MM) during the treatment is described only in cases report. EM spread may occur either at diagnosis or during the course in MM but generally it's very rare. In more recent years has been observed a overall 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 multifocal EM progression of IgA/kappa MM during bortezomib combination therapy. A 75 year old men was diagnosed IgA/kappa MM stage III A in June 2012. The patient was treated with bortezomib weekly, melphalan and prednisone (VMP). Was started zoledronic acid montly also. In January 2013 the patient presented a soft tissue swelling in the upper third of the thigh dx. ETG scan showed a solid nodulation of 4 cm with pathological vascularization to the power-doppler. In February appeared on the chest 2 very large subcutaneous nodular masses, at the same time the nodulation to the thigh extended to the skin surface causing ulceration. Chest Computed Tomografy scan demonstrated one solid masse 7 cm x 4 cm continues with bone tissue on sternal body and the other masse 4 cm x 3 cm on subcutaneous tissue of IX and X rib chest. Final-needle aspiration cytology (FNAC) revealed atypical plasma cell only. There was pancytopenia, since evidence of plasma cell in periferal blood, elevation of LDH, Beta2microglobulin and increase monoclonal protein. Was started salvage therapy with lenalidomide and dexamethasone (RD).After two cycles the EM massses diseappered. The introduction of bortezomib and lenalidomide has expanded the therapeutic options for MM. Some reports indicate good efficacy of bortezomib on EM disease. In addition, some patients had EM spread while on treatment with bortezomib such us in our case. It's possible that this novel agent (bortezomib) during its action on bone marrow microenvironment select plasma cell clone more aggressive. It is not possible to draw definitive conclusions due to the small number of patients with these condition. This was a rare case of multifocal EM spread of IgA MM occuring during bortezomib based therapy. The disease actually is responding to the other novel biological agent. We proposed that the role of novel agents for EM disease during follow-up must be investigated because it is associated with unusual aggressive course.

PU010

UNICENTRIC CASTLEMAN DISEASE PRESENTING IN THE NECK: REPORT OF A CASE

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Castleman disease is a uncommon lymphoproliferative disorder with two clinical entities: a unicentric presentation with a solitary mass confined to a single lymph node region and a multicentric clinic presentation with generalized lymphadenopathy, systemic symptoms, aggressive clinical course. Two histopathologic subtypes have been described: hyaline vascular and plasma cellular type. In unicentric Castleman disease (UCD) the most common localization is the mediastinum, rarely the head-neck and the surgery is considered the standard therapy with cure rates approach 100%. We report a case of 20-year-old man who presented with a 4-month history an slowly expanding mass in left cervical area. Physical examination revealed the presence of a isolated soft-tissue mass in the left neck measuring 5 cm in diameter. Serum biochemistry, complete blood count were normal and systemic symptoms were not present. Total body computed tomography showed a homogeneus well-defined mass in the neck and no other mass. Excisional biopsy identified the mass as a hypervascular lymphoid hyperplasia consistent with the hyaline vascular type of Castleman disease. The patient received diagnosis of UCD and none additional treatment was required considering the complete surgical resection of the mass. The patient is in complete remission after 18 months of follow-up. UCD most often presents as an isolated lymph node enlargement in a young

adult and approximately 90% of cases are of hyaline vascular subtype and 10% of the plasma cell subtype. The patients with UCD hyaline vascular subtype are asymptomatic, except in cases of compression adjacent structures and systemic symptoms are not present; some patients with plasma cellular subtype may have systemic symptoms. The etiology is unclear and various immunopathologic processes have been suggested. The prognosis for patients with UCD is generally excellent and surgical excision is curative; complete resection is the best chance of cure. No recurrence of the hyaline vascular variant of UCD has been reported in literature after complete resection and only one recurrence of the plasma cell variant of UCD has been reported. Anyway careful follow up is recommended due to rare association with lymphoid malignancy.

PU011

RAPID RESPONSE TO BORTEZOMIB IN A WOMAN OF 29 Y.O. WITH AL AMYLOIDOSYS WITH ACUTE RENAL DISEASE

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Background. Bortezomib is the first-in-class proteasome inhibitor,recently approved in Italy for treatment of amyloidosys (see 648/96). It inhibits the activation of NF-kappaB, which controls the genes encoding IL-6, TNF-alfa and other cytokines and growth factors. Case. A 29 years old woman , affected of ulcerative colitis disease treated with success with sulfasalazine, is admitted in hospital for acute renale disease and anemia. The laboratory tests, at the admission , show an acute renal disease without nephrotic syndrome, the presence of a beta 2 monoclonal peak with IFE positive for lambda light chains. Bone marrow biopsy shows a plasmacells infiltration of about 10% without morfological or cytometric clonality markers; total body CT scan is negative for bone lesions. There is no evidence of heart septum hypertrophy at echocardiography and values of pro BNP and C-troponine are in range. Renal biopsy is conclusive for cast nephropathy and subcutaneous fat biopsy is positive for amyloid AL. The patient start a dialytic treatment with Theralite membranes specific for light chains and contemporaneously therapy (bortezomib 1.3mg/sqm + dexamethasone 20 mg/d1-d4-d8-d11 q21) obtaining a complete remission of the renal disease after the first cycle. At this point we consolidate the result with three cycles of bortezomib + dexamethasone emended at weekly administration (d1-d8-d15-d22 for a 35 day cycle)and proceed to a PBSC harvest for autologous transplantation program. Conclusions. The increase in the number of complete remissions brought about by bortezomib therapies in patients with AL amyloidosis poses a question about which treatment should be used for younger low risk patients; *i.e.* high-dose chemotherapy with autologous hematopoietic cell transplantation or continous treatment with bortezomib. Additional comparative studies are required to answer that question.

PU012

COMBINED LIPOSOMAL AMPHOTERICIN B AND SURGERY AS SUCCESSFUL MANAGEMENT FOR PULMUNARY MUCORMYCOSIS IN A PATIENT WITH ACUTE MYELOID LEUKEMIA

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Mucormycosis is an increasingly recognized invasive fungal infection (IFI) in patients with acute myeloid leukemia (AML) and after allogeneic (allo) stem cell transplantation (HSCT); it is mainly due to the severe and prolonged neutropenia related to high-dose chemotherapy. Here we report a case of 32-year-old AML who developed ten days after the onset of neutropenia during induction therapy, clinical and radiological findings of a possible IFI, such as a non productive cough, broad spectrum antibiotic resistant fever, prolonged neutropenia associated to a rapidly progressive peripheral pleural-based nodule with surrounding ground-glass halo and a slight pleural effusion detected by high resolution computerized tomography (HRCT). During febrile neutropenia, serum aspergillus galactomannan (GM) antigens, tested twice a week, were not detected. Empirical treatment with liposomal amphotericin B (LamB, 3 mg/kg/day for 21 days) was started; three days later, fever disappeared concomitantly with the achievement of complete disease remission. Pulmonary HCRT scans sequentially performed 30, 37, and 45 days after starting induction chemotherapy showed at beginning an increase of nodular lesion (4.5 cm) and then a progressive infiltrate regression. At this time, a CT-guided fine needle biopsy of the pulmonary lesion and bronchoalveolar lavage (BAL) specimens, tested for aspergillus GM antigen, failed to document any fungal infection. As HRCT scan 45 days after first induction did not shown fully regression of lung nodule (2 cm), 7 days later, the patient underwent a limited toracothomy, leading to a complete surgical removal of the lung infiltrate. Fungal culture of lung specimens remained negative, but histological examination established a mucormycosis. Three weeks after surgery, the patient performed consolidation chemotherapy, and 98 days after first induction therapy, the patient received a myeloablative allo HSCT from a sibling HLA-matched related donor. Secondary prophylaxis with LamB, applied during both consolidation therapy and myeloablative sibling allo HSCT, was effective to prevent IFI recurrence despite the development of grade II acute graft-versus- host disease (GVHD) and limited chronic GVHD requiring immunosuppressive treatment. Our case report further provide evidence that the combined surgical and LAmB therapy is an effective and safe choice for the management of pulmonary mucormycosis in hematological immunocompromised patients.

PU013

CONTINUOUS MAINTENANCE THERAPY WITH ALTERNATE-DAY LOW DOSE LENALIDOMIDE IN MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Maintenance therapy with immunomodulatory drugs has shown to improve responses and delay relapse and progression in Multiple Myeloma (MM) patients after autologous stem cell transplantation (ASCT). Although maintenance therapy with Thalidomide (T) after ASCT increases progression free survival (PFS) in MM patients, it is associated with dose-limiting multiple toxicities. Lenalidomide (R), approved for relapsed and/or refractory MM, has been reported to be associated with lower rates of toxicities than T. We evaluated efficacy and safety of continuous maintenance therapy with alternate-day low dose R (LD-R, 10 mg/day), after high-dose melphalan (HD-MEL, 200 mg/mq) and ASCT, in 8 MM patients (6 male and 2 female) older than age 60 (median: 68 years, range 60-73), receiving pre-ASCT induction treatment with 4 cycles of conventional bortezomib, T and dexamethasone (VTD) regimen. Of these 8 MM patients, 2 and 6 patients were in complete remission (CR) and in very good partial remission (VgPR) after ASCT, respectively. After a median follow-up of 14 months (range 3-43) from the initiation of LD-R maintenance, patients in CR maintained their CR, and all patients in VgPR improved the depth of response except one who showed disease progression. In this LD-R group, PFS and overall survival (OS) at 24 months were 83% and 100%, respectively, and no significant specific R-related toxicity was encountered. LD-R maintenance therapy was retrospectively compared with a matched age, gender, disease stage cohort of 17 MM patients treated with low-dose T (LD-T) maintenance therapy (50 mg/day for 2 years) after HD-MEL ASCT. In this last cohort, after a median follow-up of 53 months (range 3-125), 8/17 (47%) patients relapsed, and median PFS and OS were 39 and 53 months, respectively. In LD-T group, grade I-III neurotoxicity was detected in 11/17 (65%) patients, increasing up to 80% after 2 years of therapy, leading to drug discontinuation in 4/17 (23%); in addition, grade I hematological toxicity was documented in 55% of patients. PFS and OS were not statistically significant between these two groups of patients. Our preliminary results provide evidence that continuous therapy with alternate-day LD-R is a feasible and effective maintenance treatment after ASCT for MM patients enabling a long-lasting maintenance therapy. These results require further validation in prospective larger studies.

PU014

BENDAMUSTINE, A REBORN AGENT. EXPERIENCE OF PARMA

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Premise. Treatment of patients with B-Cell Chronic Lymphocytic Leukemia (CLL) Relapsed/Refractory (R/R) to conventional treatments is particularly challenging and requires effective alternatives. Bendamustine, a bifunctional alkylator with unique properties, appears to exert its antineoplastic effects via different mechanisms to that of the other alkylating agents. Moreover this reborn agent, associated to rituximab, has recently demonstrated substantial efficacy not only in CLL R/R but also in front line patients with low-grade non-Hodgkin lymphomas (NHLs) with durable response and acceptable tolerability. Patients and Methods. We treated four patients in 2012, all females. Three of them were affected by CLL R/R and one by newly diagnosed Lymphoplasmacytic Lymphoma (Immunocytoma). Median age was 53.5 years (range 47-64). One CLL R/R patient had del (11q23) detected on peripheral blood by fluorescence in situ hybridization (FISH) involving the ataxia-telangectasia (ATM) gene while another patient had del (17p13), involving the p53 tumor suppressor gene. Treatment cycle included intravenous bendamustine (90 mg/sqm on days 1 and 2 of a 4-week cycle) and rituximab (375 mg/sqm on day 1 of each cycle). Results. One month after the end of three cycles of bendamustine plus rituximab (BR) the 50% of patients obtained complete remission (CR). The partial remission (PR) was due to persistent splenomegaly in one patient and persistent enlarged lymph nodes in the other one, patients evaluated by ultrasound or Computed Tomography scan, while haematologic remission was found in all of them. None of them developed neither haematological nor non-haematological toxic events and for this reason nobody has needed hospitalization. Furthermore no cases of alopecia was recorded and these patients enjoy an excellent quality of life (QoL). Conclusions. Our preliminary ongoing study suggests BR is effective and highly effective in the treatment of R/R CLL and front line low- grade NHLs. Furthermore, the absence of alopecia , a very important feature especially in female patients in our experience, is associated with an excellent QoL. However, due to its haematologic toxicity , bendamustine is associated with risk of infection which must be carefully monitored during time and managed. A longer follow-up is needed to fully establish long-term response duration.

Table 1.

PTS	AGE (Y)	HYSTOLOGY	CYTOGENETIC -FISH	ADENOPATHY/ ORGANOMEGALY	N. OF CYCLES	OUTCOME	HAEMOGRAM PRE-TREATMENT	HAEMOGRAM POST THIRD CYCLE	TOXICITY
1	48	LPL	NORMAL	Yes/ Liver	3	CR	WBC 12.83 x10³/L Hb 7,8 g/dl PLT 55 x10³/L	WBC 2.8 x10³/L (N 1.93 x10³/L) Hb 13,7 g/dl PLT 282 x10³/L	NONE
2	64	R/R CLL	del (17p13) / p53	Yes/ Spleen	5	PR	WBC 15.61 x10³/L Hb 13.5 g/dl PLT 100 x10³/L	WBC 4.75 x10³/L (N 2.94 x10³/L) Hb 11.6 g/dl PLT 96 x10³/L	NONE
3	55	R/R CLL	del (11q23) / ATM	Yes/ Liver	3	CR	WBC 31.75 x10³/L Hb 13,8 g/dl PLT 179 x10³/L	WBC 6 x10³/L (N 1.01 x10³/L) Hb 11,4 g/dl PLT 279 x10³/L	NONE
4	47	R/R CLL	NORMAL	Yes/ Spleen and liver	3	PR	WBC 81 x10³/L Hb 11,6 g/dl PLT 228 x10³/L	WBC 6.6 x10³/L (N 2.24 x10³/L) Hb 11,9 g/dl PLT 369 x10³/L	NONE

LPL: Lymphoplasmacytic Lymphoma; CLL: Chronic Lymphocytic Leukemia; R/R Refractory/Relapse; CR: Complete Remission; PR: Partial Remission.

PU015

HYPOMETHYLATING AGENT AZACITIDINA AS " BRIDGE TO TRANSPLANT " FOR YOUNG PATIENT WITH CHRONIC MYELOMONOCITIC LEUKEMIA

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Introduction. Chronic myelomonocitic leukemia (CMML) is a clonal disorder of a bone marrow stem cell. We describe a case of young woman with CMML treated with the hypomethylating agent azacitadine (AZA) as a bridge to allogenic bone marrow transplantation (HSCT). Case Report. A 26 year old girl came to our hospital for anemia and monocytosis. Bone marrow aspirate with the immunophenotype showed a picture compatible with MDS / MPC (CMML2). The kariotype was normal. The bone marrow biopsy confirms CMML-2. Clinically she hadn't splenomegaly. She was treated with erythropoietin 40.000 UI once a week and with azacitidine 75 mg/m^2 sc daily for 7 days every 28 days for a total of 8 cycles. After eight cycles the patient obteined the RC. Later on allogenic bone marrow transplantation was performed from HLA-identical family donor, conditioning with tepadina , busilvex and fludara; GVHD prophylaxis with cyclosporine and methotrexate. Neutrophil engraftment at + 26 days and platelets at +21 days. She had acute GVHD grade II. After six mounts from HSCT her clinical condition ore good with full haematological recover. Discussion. CMML is characterized by persistent monocytosis , fewer than 20% blasts in the blood or bone marrow with dysplasia. The median age at diagnosis of CMML is 65 to 75 and the median survival time is 12 to 24 months. Progression to acute leukemia occurs in 15-20% of cases. The natural course of CMML is rapidly fatal with 80% of patients surviving less than three years; 5-azacitidine is an inhibitor of DNA methyltransferase that has been approved for treatment of CMML. HSCT is currently the only curative treatment for juvenile-CMML. While awaiting transplant, most patients receive chemotherapy, and most clinicians will use cytarabine-based acute myeloid leukemia-like therapy. There is no agreement on the use of induction or hypomethylating therapy before HCT, but AZA are gaining increasing attention as a bridge to HSCT. A review showed that patients receiving pre-transplant AZA had outcomes comparable to patients undergoing high-dose induction chemotherapy. Conclusion: HSCT appears to be the only current treatment that alters the natural history of CMML. The approach of administering 5-Azacitidine before transplantation in young patient with LMMoC, is feasible, is a valid option for the control of disease and a good "bridge to transplant" but a prospective study would need to be performed to confirm it.

PU016

LENALIDOMIDE CONTINUOUS TREATMENT IN MULTIPLE MYELOMA ELDERLY PATIENTS

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Background. Lenalidomide represents an important treatment option for multiple myeloma patients either as first line therapy, either in resistant/refractory disease or consolidation/maintenance therapy. In this way Lenalidomide increases the available treatment options. Aim. In our Department, Lenalidomide was administered in resistant/relapsing myeloma patients and as continuous therapy in elderly myeloma patients. Methods. We treated 47 patients (26M and 21F) with median age of 73 years (range 66-81). We have evaluated 38 patients with a median follow-up of 30 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) for first four cycles and then alone in continuous treatment. We used Enoxaparin for prophylaxis of venous thromboembolisms. Clinical restaging was per-

formed after three, six and twelve months, in course of therapy. Results. At present we have not observed any progression of disease and in 25/47 cases we found a good impact on Monoclonal Component (MC). In all patient therapy was well tolerated and were not found significant adverse events or second neoplastic events. Conclusions. Role of Lenalidomide is established as continuous therapy in previously treated elderly myeloma patients. This therapy seems to lead an improvement in prognosis of these patients, without causing severe complications.

PU017

LENALIDOMIDE PLUS RITUXIMAB IN ELDERLY PATIENTS WITH RELAPSED OR REFRACTORY FOLLICULAR LYMPHOMA

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Background. Follicular lymphoma (FL) is the second most common subtype of lymphoma and despite recent chemotherapeutic advances up to half of all patients relapse. Rituximab plays an important role in the treatment of FL. In spite of high response rates achieved with this monoclonal antibody, however, many patients tend to relapse and become refractory to rituximab over time. Lenalidomide, an immunomodulatory agent, had direct tumoricidal and antiangiogenetic actions on tumor cells and was able to modulate tumor- cell microenvironment, with the restoration of impaired T-cell activity and the formation of immunosynapsis. Based on these actions, lenalidomide represented an active drug on FL. Aim. We report the results obtained in three patients, aged >70 years, affected by FL and treated with lenalidomide and rituximab. Patients and Methods. From January 2011 to January 2012, we treated 3 elderly patients (male, 71, 72 and 75 years) with relapsed/refractory FL who had been heavily pretreated (more than 5 lines of treatment, including ASCT). Oral lenalidomide (15 mg/d for 21 days of each 28-day cycle) was initiated for four cycles and 375 mg/m^2 intravenous rituximab was administered on day 1 and day 21 of each 28-day cycle for four cycles. After this induction phase, two patients achieved a complete response (CR) and one partial response (PR). Lenalidomide maintenance therapy was administered at the same schedule for another 6 months. At the end of treatment all three patients achieved complete remission. Results. To date (+14 months after the end of therapy) all three patients are alive and still in CR. Adverse events were manageable and the most common included neutropenia and thrombocytopenia. Conclusions. Our experience, although on a very small number of patients, seems to confirm the preliminary data of the literature showing good efficacy of lenalidomide plus rituximab in relapsed follicular lymphoma. In fact, oral lenalidomide in combination with rituximab seem to be active and well tolerated in elderly patients with relapsed/refractory FL with a high percentage of patients achieving a continuous CR after lenalidomide maintenance.

PU018

SERUM FREE LIGHT CHAINS QUANTIFICATION IN MONOCLONAL GAMMOPATHIES AND MULTIPLE MYELOMA PATIENTS

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Background. Identifying patients with optimal response and long term survival is important for clinical guidance because patients with these features are likely not need further therapy. Serum Free Light Chains (sFLC) are used for better assessment of treatment response, thus patients are considered to achieve stringent Complete Response (sCR) by having CR criteria plus normal serum Free Light Chains Ratio (sFLCR) and absent clonal cells in bone marrow. Moreover, sFLC are commonly assessed in patients with monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM) as indicators of disease progression. Aim. We evaluated serum free light chain (sFLC) and sFLC ratio to clarify the impact in patients with MGUS and MM. Methods. In the last two years, we have examinated 40 patients with Monoclonal Gammopathy (MGUS) and 30 patients with Multiple Myeloma (MM) in course of therapy. We have assessed serum Free Light Chains (sFLC) and serum Free Light Chains Ratio (sFLCR) for evaluation of progression disease and treatment response. Serum FLC concentrations were measured by nephelometry, using particle-enhanced, high-specificity, homogeneous immunoassays. Results. We observed an increase of sFLC in patients with monoclonal gammopathy in evolution, with simultaneous progression of monoclonal component (M-spike). In MM patients sFLC and sFLCR were evaluated for assessing the response to treatment, and we observed a strict correlation with disease status. Moreover, in MM patients sFLC monitoring proved to be the earliest indicator of disease relapse. Conclusions. Our results confirm the role of sFLC in the monitoring of MGUS and MM patients. Specifically in MM patients sFLC and sFLCR evaluation seems to be very useful in identifying patients response and early relapse.

PU019

VMP (BORTEZOMIB-MELPHALAN-PREDNISONE) REGIMEN FOR THE TREATMENT OF FRAIL ELDERLY PATIENTS AFFECTED BY MULTIPLE MYELOMA

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Background. 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 proven to be safe and effective in MM patients not only as monotherapy but also given in combination with cytotoxic agents. Aim. The objective of study was to evaluate the efficiency and safety of bortezomib in combination with melphalan and prednisone (VMP) as a starting regimen for the treatment of elderly frail patients affected by MM. Methods. In our institution we are following 25 elderly patients with stage II/III MM (13 F and 12 M, median age: 75 years, r.: 69-88 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). Results. 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 16 out of 25 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 4 out of 25 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 +36) only one patient shows a progression disease, while two patients are in CR and the remaining in PR or VGPR. Grade 3-4 non-hematological adverse events (AEs) included diarrhea (10%) and peripheral neuropathy (10%), and grade 4 hematological AEs included lymphopenia (20%), neutropenia (30%), and thrombocytopenia (22%). Conclusions. Our results suggest that the combination of melphalan-prednisone-bortezomib is effective and well tolerated in the treatment of MM in elderly "frail" patients. Several studies confirm the superiority of MP plus bortezomib combination over MP therapy in treatment-naive patients with newly diagnosed multiple myeloma who are ineligible for autologous stem cell transplantation.

PU020

AN UNUSUAL PRESENTATION OF B CELL LYMPHOBLASTIC LYMPHOMA AT DUODENUM WITH OBSTRUCTIVE JAUNDICE: A CASE REPORT IN YOUNG FEMALE

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Introduction. Cholestatic jaundice as a presenting symptom of precursor B Lymphoblastic Lymphoma (B-LBL) has been reported only in few cases in literature. Duodenal lymphomas are the least common variety of gastrointestinal lymphomas, which also are rare. We report a case of B-LBL of duodenum that presented with jaundice in a young woman. Case Report. A 25 year old female presented with a seven day history of jaundice. Physical examination showed jaundice, anemia, and palpable mass in right upper quadrant with direct hyperbilirubinemia. Complete blood picture was normal. Contrast-enhanced computed tomography of the abdomen showed a diffusely enlarged tumor mass and dilated common bile duct. PET CT scan again showed a large abdominal mass with diffuse duodenal involvement. Duodenal biopsy during EGDS showed infiltration of B-LBL. The biopsy of abdominal mass was compatible with B-LBL. Bone marrow aspirate and the examination of CSF were negative. The biliary drainage by the endoscopic or percutaneous route was impossible for the treatment of the jaundice of that patient. We started, for the high bilirubin, fractioned CHOP protocol without rituximab for one cycles followed by R- Hyper CVAD/MA cycles. A significant drop in bilirubin and alkaline phosphatase was noticed within seven days and they both returned to normal in two weeks. After fifteen days from induction chemotherapy an abdominal CT scan demonstrated significant reduction of the abdominal mass in size. As central nervous system prophylaxis, intra-thecal methotrexate and arabinoside-c was administered. Actually she is in complete remission after two R-HyperCVAD/MA cycles; she tolerated the chemotherapy fairly well, except that he required G-CSF and prophylactic intravenous antibiotics for severe neutropenia. Discussion: Acute lymphoblastic leukemia is known as lymphoblastic lymphoma when it involves lymph nodes rather than the blood and bone marrow. The gastrointestinal tract is a predominant site for extra-nodal lymphomas but B-LBL more frequently presents in the leukemic form than in the lymphomatous form. Lymphoma is a rare cause of biliary obstruction and, on cholangiography, may mimic other causes of obstructive jaundice. The optimum treatment for these patients is unclear. Conclusion: Duodenal involvement by L-LBL with cholestatic jaundice as initial presentation is exceedingly rare and only a few cases have been reported. We reported a rare case of L-LBL.

PU021

A RARE CASE OF MIXED PHENOTYPE ACUTE LEUKEMIA, T/MYELOID, NOS: DIAGNOSIS, THERAPY AND CLINICAL OUTCOME

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We describe a case of a 70-years patient affected with a rare case of mixed-phenotype acute leukemia (MPAL) referred to our haematological department on October 2012 for mild anaemia, severe thrombocytopenia and blast cells in peripheral blood. The bone marrow (BM) aspirates was performed and demonstrated >90% of a mixed population of myeloid and lymphoid blasts. Immunophenotyping by flow cytometry (FCM) showed two blast populations, the first one (60%) expressing T-lymphoid phenotype (CD7+CD13+CD34+CD43+CD99+CD3s-CD19-CD79a-CD33-MPO-CD5+CD3cy+TdT ±CD117-HLA-DR-), the second one expressing myeloid (40%) phenotype (CD7+CD13+CD34+CD43+CD99+CD3s-CD19- CD79a-CD33-MPO-CD117+HLA-DR+CD3cy-CD5-TdT-). Cytogenetic analysis showed normal karyotype. Polymerase chain reaction for BCR/ABL1 was negative. The patient was treated in according to acute lymphoblastic leukemia directed chemotherapy (methylprednisolone; cyclophosphamide; desametasone; vincristine; idarubicin). FCM showed a blastic population CD7+CD99+ with a prevalence of myeloid blast cells CD33-CD117+CD34+CD5- (40%). The lymphoid blast cells CD33-CD117- were 20% with a slight positivity for CD5 and an important reduction of CD34 expression. The patient was then treated with methotrexate and cytarabine. FCM on BM aspirate showed CD99+CD7+CD34+/- (in 50% of blasts) CD117 of about 3% and a minimal infiltration of myeloid blast cells CD117+CD7-CD99- CD34-, of about 0.2%. In consideration of patient's age, failure of achieve a complete response after reinduction chemotherapy, he was treated with palliative chemotherapy with vincristine and prednisone and then with mercaptopurine. The least FCM on peripheral blood showed the persistence of lymphoid blast cells (18%) and a minimal infiltration (0.2%) of myeloid blasts. Patient is still live at seven months from diagnosis, with acceptable blood count and quality of life. MPAL T/myeloid is rare, accounting for less than 1% of leukemias. It has a poor prognosis. It is possible that this leukemia arises in a very early hemopoietic progenitor with potential to undergo either on myeloid or lymphoid differentiation. There is little consensus as to whether induction therapy should follow myeloid and/or lymphoid acute leukemia chemotherapy. Our case report confirmed published studies which have documented poor outcome in terms of achieving complete remission in MPAL.

PU022

EFFICACY OF DASATINIB IN A PATIENT WITH SIMULTANEOUS CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND CHRONIC MYELOID LEUKEMIA (CML)

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Although CLL and CML are frequently diagnosed in elderly patients, their simultaneous occurrence in the same patient is rare. We present the case of M.P., a seventy-three years old male, with NIDDM and hypertension, that came to our attention with multiples adenopathies and hepatosplenomegaly. His blood cells count showed severe leukocytosis ($270x10^3$/L), anaemia (Haemoglobin 8.4 g/dL) and thrombocytopenia ($90x10^3$/L). Peripheral blood smear showed myeloid precursors and rise of lymphoid amount. Hyperplasia of myelopoiesis and erythropoiesis with prevalence of promyelocytes, myelocytes and metamyelocytes was found in the bone marrow with rise of lymphocyte count (about 10% of total cells count). Blasts were about 5%. Cytogenetic and molecular studies showed a classical LMC Ph + bcr/abl+. Immunophenotype evidenced a usual CD19+ CD5+CD23+sKappa+ CLL. After starting treatment with dexamethasone (20 mg/day for 4 days) and vincristine (2 mg p.o. once) for debulky and hydroxyurea for CML, following the recent evidences, we decide to shift the patients to dasatinib (100 mg/day p.o.). This because the latter is used routinely in case of CML and should be effective on CLL as demonstrated anecdoctically and on a theoretical basis. Leukocytosis disappeared after 3 weeks of treatment with persistence of relative lymphocytosis; platelet count resulted normal after 1 month with haemoglobin levels >10.5 g/dL. At the third month of dasatinib the bone marrow evaluation showed complete cytogenetic response with disappearance of Ph+ metaphasis. We registered also an excellent response on molecular biology evaluation (MR3). Superficial adenopathies and splenomegaly disappeared at the clinical examination. At the 4 month, as adverse event of dasatinib, the patient experienced pleural effusion treated with drug discontinuation, steroids and furosemide. Thus, the therapy was restarted and the haematological conditions are still stable. The rare association between CLL and CML in our case report provides the chance to confirm both the experimental data and the anecdoctical reports about the possible usefulness of dasatinib in the treatment of CLL.

PU023

LIPOSOMAL IRON IS BETTER THAN IRON SULFATE IN LOW-RISK MYELODYSPLASTIC SYNDROMES (LR-MDS) WITH MILD ANEMIA. MONOCERNTRIC STUDY

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Background. LR-MDS frequently shows a chronic inflammatory status, ferritin high values and impaired capacity of iron absorption and utilization. Liposome has a described anti-inflammatory effect and transports its content directly in blood, beyond gastric and enteric wall. AIM Aim of this study is to verify if liposomal iron support in refractory anemia (RA) and refractory cytopenia with multilineage dysplasia (RCMD) with mild anemia is safe and effective in increasing hemoglobin level. Patients and Methods. In group A 7 patients (5RCMD and 2RA), with normal cytogenetics, M/F:4/3, median age 65 years (R64-75), Hb 10.7 g/dL (R10-11.5), saturation of iron binding capacity >20%, with a median ferritin level of 480 ng/mL (R380-550), ESR 28 mm/1st hour (R20-32), CRP 6 mg/I (R4-7), normal B12 and folate, received liposomal iron 30 mg/day orally for 3 months. In group B 7 patients (3RCMD and 4RA), with normal cytogenetics, M/F:5/2, median age 63 years (R62-70), Hb 11 g/dL (R10.8-12), saturation of iron binding capacity >20%, with a median ferritin level of 430 ng/mL (R370-580), median ESR 30 mm/1st hour (R18-38), median CRP 7 mg/I (R5-7), normal B12 and folate, received support with iron sulfate 105 mg orally/day. Results. Group A showed a median hemoglobin increase of 1.5 g/dL (R0-2), a ferritin decrease to a median of 160 ng/ml (R 100-250), a ESR decrease to a median value of 15 mm/1st hour (R 8-20) and a median CRP 3 mg/I (R2-5). In group B no significative increase of hemoglobin or decrease of ferritin, ESR and CRP were recorded. 2 patients showed hepygastralgia, 2 stipsis, 2 diarrohea. Conclusion. Liposomial iron is safe, effective, well tolerated, effective in increase hemoglobin level and reduce inflammatory markers in low-risk MDS.

PU024

MELATHONIN PLUS DANAZOLE, PREDNISONE AND ERYTHROPOIETIN ALPHA IS EFFECTIVE IN TREATMENT OF MYELODYSPLASTIC SYNDROMES WITH ANEMIA AND THROMBOCYTOPENIA

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Background. Melathonin was reported effective in some cases of ITP because of its thrombopoietic effect. Aim. Aim of this study is to verify if danazole, prednisone, melathonin and erythropoietin alpha is effective and safe in patients with refractory cytopenia with multilineage dysplasia (RCMD) with anemia and thrombocytopenia. Patients and Methods. This study is a multicentric study. 20 patients with RCMD with IPSS intermediate or low showed anemia and thrombocytopenia. Cytogenetics was normal in 15 patients and not evaluable in 5 patients. In group A 10 patients received orally danazole 200 mg/day, prednisone 25 mg/day, melathonin 60 mg/day, B12 400 mg/day, calcium levofolinate 7.5 mg/day, liposomal iron 30 mg/day, erythropoietin alpha 40000 IU subcutaneous weekly (5 originator and 5 biosimilar) for at least 3 months. In group B 10 patients received the same treatment except melathonin. In group B 7 patients received originator erythropoietin alpha and 3 biosimilar. In group A M/F was 6/4, median age was 68 years (R62-80), median follow-up was 4 months (R2-6), median Hb 9 g/dl (R8.5-10), median PLT count 40000/mcl (R30000-50000). In group B M/F was 5/5, median age was 66 years (R60-84), median follow-up was 3 months (R2-5), median Hb 8.7 g/dL (R8-9.5), median PLT count 27000/mcl (R20000-45000). Results. In group A median platelet count after treatment was 55000/mcl (R40000-60000), median Hb 10 g/dL (R9-11). In group B median platelet count after treatment was 38000/mcl (R25000-50000), median Hb 10.2 g/dL (R9-10.5). In group A the 5 patients receiving biosimilar erythropoietin alpha showed a median platelet count of 55000/mcl *vs* a median platelet count of 40000/mcl in patients receiving originator molecule. No side effects were noted in the two group. Conclusion. Melathonin, danazole, prednisone and erythropoietin alpha is safe and effective in RCMD with anemia and thrombocytopenia.

PU025

METRONOMIC THERAPHY IN VERY OLD PATIENTS: WHEN TO TREAT AT HOME BED IT'S NOT SO BAD

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AIM Aim of this study is to verify if metronomic therapy is not inferior and less toxic than standard chemotherapy in treatment of aggressive lymphomas of very old patients. Patients and Methods. In 26 patients to calculate frailty of patients CHARLSON,CIRS-G,CRASH and GISL score were used. In groupA patients were treated at home with metronomic therapy with cyclophosfamide 50 mg days 1 to 5, etoposide 50 mg days 1-3-5, prednisone 25 mg days 1 to 7, lenalidomide 10 mg days 1 to 21, all orally, every 28 days for 9-12 cycles (Large B Cell Lymphoma and Mantle Cell Lymphoma), or with cyclophosphamide 50 mg days 1 to 3, fludarabine 25 mg days 1 to 3, etoposide days 4 to 6, prednisone 25 mg days 1 to 15, all orally, methotrexate 15 mg im day 15, every 28 days for 9-12 cycles (T cell Lymphoma). In group B patients received at hospital i.v. Rituximab 375 mg/sqm day 1, Cyclophosphamide 750 mg/sqm day 1, adryamicin 50 mg/sqm day 1, prednisone 50 mg/sqm orally day 1 to 5 (Large B Cell Lymphoma,T cell lymphoma and Mantle Cell Lymphoma). In group A M/F:8/8, median age was 85.5 years (R85-94), TNHL/DLBCL/MCL:5/4/1, median IPI 4 (R2-5), median follow-up was 6 months (R2-13), 9 patients showed 1 comorbidity (56%), 7patients 2 or more (44%;CHARLSON>5:12 pat (75%), CIRS-G=4:9 pat.(56%), CRASH>9:7pat (43%), GISL FRAIL:12pat. (75%). In group B M/F:4/6, median age was 85 years (R85-91), TNHL/DLBCL:2/8,median IPI 4(R2-5), median follow-up was 6 months (R1-24), 2 patients showed 1 comorbidity (20%), 2 patients 2 or more (20%), 6 patients no comorbidities (60%), CHARLSON>5:4pat (40%), CIRS-G=4:5pat.(50%), CRASH>9:3pat(30%), GISL FRAIL:5pat.(50%). SF8 questionnaire was used to evaluate quality of life of patients. Results. In group A median hospitalization was 0 weeks (R0-12),complete remission 4 patients (25%), partial remission 8 patients (50%), progression of disease 4 patients (25%), G3/G4 toxicities (hematologic 25%, not hematologic 25%, infection 37%, transfusion 19%, death 37.5%), days of hospitalization/days of global survival 5% (R0-25), cost per month of survival €5000 (R250-9100), SF8 60 (R40-100). In group B median hospitalization was 9 weeks (R3-17),complete remission 5 patients (50%), partial remission 2 patients (20%), progression of disease 3 patients (30%), G3/G4 toxicities(hematologic 70%, not hematologic 50%, infection 40%, transfusion 80%, death 60%), days of hospitalization/days of global survival 33% (R20-100), cost per month of survival €21000 (R5000-37000), SF8 40 (R20-50). Median survival at kaplan-mayer was 18 months for both groups. Conclusion. Metronomic therapy is cost-effective and warrants a good quality of life.

PU026

ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH MYELOFIBROSIS , CLINICAL OUTCOMES OF EXPERIENCE OF THE REP (HAEMATOLOGIC NETWORK PUGLIA)

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Myelofibrosis is a myeloproliferative neoplasm characterized by bone marrow fibrosis and extramedullary hematopoiesis which may be complicated by the evolution in leukemia, thrombotic events and hemorrhagic episodes that impair the life of patients. Recently, a small molecule inhibitor of JAK1 and JAK 2 has been approved for the treatment of symptomatic intermediate or high risk myelofibrosis. This drug reduces splenomegaly and myelofibrosis-related symptoms but it is not a curative drug. The allogeneic stem cell transplantation(allo HSCT) remains the only therapeutic curative option although it is feasible in a small

subpopulation of patients. We analyzed a retrospective group of patients (pts) with primary Myleofibrosis(PMF) who received allo-HSCT to evaluated the safety and the efficacy of the procedure and both the clinical outcome of patients. 9 pts underwent allo HSCT after myeloablative conditioning (MAC n=2) or reduced intensity conditioning (RIC n=7) The median age at transplantation was 51.7 years (range 40-69) At time of transplantation the pts were in stable disease(n=5) in progressive disease (n=4). Donor were HLA identical sibling (n=4) or matched unrelated donor(n=5) and the transplant consisted of unmanipulated peripheral blood stem cell in all patients. GVHD prophylaxis was performed with CSA plus MTX in HSCT sibling, anti- tymocyte globulin (ATG) was added in HSCT MUD. Engraftment was obtained in all of these recipients, the median days to reach a neutrophil count above 0.5 10(9) were 18.2 days (range 12-36). Post transplantation chimerism analysis showed mixed chimerism (n=3) , full donor chimerism (n=3) and not evaluable chimerism (n= 3) Acute graft-versus-host disease (GVHD) grades II to IV was observed in 5pts (55%),GVHD grades I in 1 pts(1%) and limited chronic GVHD in 4 ptz (44%). 4 pts died for aGVHD (2 HSCT MAC and 2 HSCT RIC) and 1 pts for relapse of disease with a transplant related mortality (TRM) the 44%. There are 4 patients alive(44%), median follow up was assessed 24,5 months after transplantation, and none of them are in relapse. Despite the small number of cases, our results suggested that ALLO-HSCT is an encouraging curative strategy for treating MF but it still has a high mortality, future studies could also address if the combination of this procedure (HSCT) with the new drug (ruxolinitib) could improve even more the outcome(result) and reduce the TRM in these patients.

PU027

MULTIDISCIPLINARY ASSESSMENT OF NEUROLOGICAL EFFECTS OF NEW DRUGS IN PATIENTS WITH MULTIPLE MYELOMA: A PROSPECTIVE STUDY

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Hystory of multiple myeloma (MM) has changed from the introduction of new drugs such as thalidomide, bortezomib and lenalidomide, but their toxicity could limit their use, compromising their effectiveness. In a single-center and prospective study, we evaluated the incidence, clinical characteristics and risk factors of neurological effects of these treatments, in consecutive patients with a diagnosis of symptomatic MM, for a median period of observation of 8 months and treated with bortezomib intravenously (iv) and subcutaneously (sc), thalidomide per os (po) alone and in combination regimens. Incidence of peripheral neuropathy (PN) in patients treated with bortezomib iv in first line was 11% after 2 cycles of therapy, and 25% after 4 cycles of therapy, like in literature data. Incidence of PN in patients treated with thalidomide in front line was 25% at 160 days (5 months and 10 days), after 4 cycles of therapy. Compared to the literature, incidence was lower in series analyzed. Concerning risk factors, we observed that preexisting PN is the most important one for development of PN during treatment. We also observed, in 32% of cases, development of deficit of muscle strength, compatible with myopathic disorder. In 87.5% of cases therapy included bortezomib iv. No studies described a skeletal muscle toxicity of the drug, even if it has been underline a cardiac muscle toxicity related to the administration of bortezomib iv. Moreover, *in vivo* sc administration of bortezomib reduces significantly the risk of PN and myopathy in patients treated in single agents and bortezomib-based combination treatments. Considering this, we perfomed an experimental study to evaluate the toxicity of bortezomib *in vitro* testing the drug on human muscle cell cultures. Therefore, an integrated multidisciplinary approach is important in patient's management. In fact PN could lead permanent neurological damage and limited subsequent treatment, significantly reducing patients' survival. Initial neurological evaluation also allows to recognize pre-existing neuropathy, clinically silent and to select patients at increased risk of developing PN during treatment. Identification of categories of patient should guide the choice of therapy and be useful to modify dosage during the treatment.

PU028

HAPLOIDENTICAL ALLOGENIC STEM CELL TRANSPLANTATION , AFTER SECOND RELAPSE OF FOLLICULAR NON-HODGKIN LYMPHOMA, GRADE IIIA. A CASE REPORT

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Background. The three year survival of advanced stage follicular lymphoma (FCL) with intermediate and high risk - FLIPI2 - is respectively 96% and 82%. Autologous and allogeneic stem cell transplants (SCT) are considered in patients who relapse or progress after first line therapy. However often patients continue on many courses of chemotherapy until they become refractory. Case Report. We report a case of FCL that summarizes the use of conventional therapy as first line, autologous SCT as second line after relapse, and allogeneic SCT after second relapse. Presentation.This is a 54 years old woman, who presented a swelling of left ear in April 2006. The histological examination documented FCL grade IIIA, stage IVE for bone marrow (BM) involvement, intermediate risk according with FLIPI2 score. She received 6 R-CHOP and achieved complete remission (CR). First relapse. In 2007 she presented a local relapse. She was treated with 3 IEV but failed stem cells harvest. After restaging, she was in partial remission (PR) We started the search for unrelated donor and started follow-up. In 2008 she was in relapse, left ear and cervical nodes was involved. BM was negative. The cervical node biopsy documented reactive lymphoadenopathy, while the biopsy of the ear lesion confirmed FCL grade IIIA. She received high dose chemotherapy, achieved the second CR and successfully completed stem cell harvest. Autologous SCT. In April 2010 she underwent autologous SCT. CT-PET after SCT confirmed CR. Second relapse. After one year of follow up she presented a bulky left cervical mass. The TC documented cervical, axillar and supraclavicular nodes compressing neurovascular structures. The nodal biopsy confirmed the relapse. BM was not involved. Maximum dose of anthracycline was reached, so we treated her with Rituximab and high dose ARA-C, followed by RT on bulky. She achieved PR. Allogeneic SCT. In March 2012 she underwent allogenic SCT from her HLA haploidentical daughter. The conditioning consisted of thiotepa, fludarabine, busulfan and GvHD prophylaxis consisted of high dose post-transplant cyclophosphamide, cyclosporine and mycophenolate. CT-PET after allogenic SCT documented CR. At present she is well, in CR, with no transfusion need and a very good quality of life. Conclusions. This case highlights the possibility of achieving control of lymphoma, despite several relapses and despite failing an autologous transplant, by integrating several different therapeutic approaches.

PU029

THE UNUSUAL INTESTINAL INTRAVASCULAR LARGE B CELL LYMPHOMA INVOLVEMENT IN A 70 YEARS-OLD-PATIENT

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Intravascular large B-Cell lymphoma (IVLBCL) is a rare, aggressive and extranodal B-Cell lymphoma characterized by localization of lymphomatous cells within small to medium size vessels.However other different sites should potentially being involved since the diagnosis time. IVLBCL is pathologically distinct with a broad clinical spectrum and immunophenotypic heterogeneity.Classically it primarily affects people from middle aged to elderly with a slight predominance in male. Here we described the clinicopathological features of a 70 years-old-man with a recent history of normocytic anemia (9.1 g/dL), thrombocytopenia ($66x10^9$/L) and leukopenia ($2.98x10^9$/L).Symptoms at diagnosis time were fever, fatigue, weight loss and central nervous symptoms such as meningoradiculitis, hypostenia, vertigo and myoclonus.No lymphoadenopathy were observed but hepatosplenomegaly was detected.The laboratory tests showed elevated serum lactate dehydrogenase (1562 U/L),beta2microglobulin (4.9 mg/L), eritrocyte sedimentatio rate (92 mm),C-reactive protein levels (198 mg/L) and a monoclonal M immunoglobulin serum component was remarked. The bone marrow

biopsy showed large atypical lymphoid cells aggregate wholly occluding the small sized blood vessels lumina. Large Lymphoma cells were characterized by vesicular chromatin and irregular nuclear perimeter with single or multiple small nucleoli.Mitotic figures were easily seen, with Ki-67 proliferation rates valued more than 90%. Immunohistochemical analysis revealed intense homogenous CD20 staining, lack of CD3 immunoreactivity and negativity for CD10. Brain Magnetic Resonance Imaging showed nonspecific parenchymal abnormalities, especially in the supratentorial regions and at the corpus callosum. Brain biopsies were performed and histological analysis revealed features of IVLBCL, involving both cortex and white matter.Furthermore a later colonoscopy showed an intestinal polyp in the sigmoid tract and the histological analyses confirmed the presence of IVLBCL also in this site. Finally total body Computed Tomography scan showed pulmonary nodules but no biopsy were performed even if high is the suspicion of site involvement. Currently the patient has been already preliminarily treated with steroid therapy obtaining the remission of clinical symptoms. The next step will be to undertake an immunochemotherapy such as anthracycline based regimens plus rituximab, nowadays the only therapeutic strategy for IVLBCL.

PU030

LONG-TERM CONTROL OF CENTRAL NERVOUS SYSTEM NON-HODGKIN LYMPHOMA RELAPSING AFTER HIGH-DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION, OBTAINED BY TEMOZOLOMIDE, RITUXIMAB AND HIGH-DOSE DEXAMETHASONE

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We report the case of a 41-yr old woman diagnosed with Central Nervous System (CNS) non-Hodgkin lymphoma (NHL) in August 2011. At diagnosis, a brain CT scan, performed after the appearance of aphasia and myoclonal twitching of the upper right arm, revealed a mass extended from the splenium of the corpus callosum to the left parietal region. Diagnosis was histologically confirmed. Staging did not reveal any secondary lesion. In her medical history, the patient had been diagnosed with benign littoral cell angioma and treated with splenectomy in 2007. Following diagnosis, she has been treated according to a Swiss-German protocol (Illerhaus *et al*. 2005 & 2008) consisting of high-dose MTX (8 gr/m^2 over 4 hr iv infusion,dd 1,11,21, with rescue - 3 cycles) followed by Ara-C (1x3 gr/m^2,dd 1-2) + Thiotepa (40 mg/m^2, d 2 - 2 cycles), CD34+ stem cell harvest and first-line ASCT using Carmustine and Thiotepa (5 mg/kg, dd 2-3) as conditioning. In our case, we substituted Carmustine with Ara-C (1x3 gr/m^2, d1) during conditioning. At day +7 (11.01.2012), 14.4 x 10^6 CD34+ cells have been transplanted. Following the start of therapy, the patient experienced a fast recovery, with the persistence of only a deficit in short-term memory. Serial brain imaging revealed the complete remission of the NHL. Unfortunately, she relapsed at day +80 from ASCT, with aphasia as main symptom, and confirmation by CNS imaging. We started CNS radiotherapy (50 Gy) with concomitant Temozolomide, as in Santisteban *et al*.2007, achieving fast control over symptoms, with little toxicity. Thereafter, we started therapy consisting of Temozolomide (150 mg/m^2 per os,dd 1-5), Rituximab (375 mg/m^2 iv,d 1) and high-dose Dexamethasone (40 mg/day,dd 1-4), administered in an Outpatient setting on a 4-week schedule. Toxicity was minimal, with grade 1-2 neutropenia and no infectious complications. We repeated brain imaging after 3 months from the end of radiotherapy, confirming the achievement of a second complete remission of the NHL, which is persisting to date, at 13 months from disease recurrence. The only long-term sign of disease was a slight persisting mnemonic deficit. Currently, the patient is still on treatment (11th cycle). Other two patients with similar history, both affected by CNS NHL refractory to the same high-dose treatment (including ASCT), are being treated in our Institution; while toxicity is similarly very low, follow-up to date is still too short to assess efficacy in their cases.

PU031

GEMCITABINE-BASED THERAPY IN REFRACTORY/RELAPSED NON HODGKIN'S LYMPHOMAS: A MONOCENTER RETROSPECTIVE STUDY

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Background. Refractory/relapsed non-Hodgkin's lymphomas (NHLs) management constitutes a problem and it is not yet standardized. High-dose therapy (HDT) with hematological stem-cell support is the standard for chemosensitive patients, but it is also characterized by poor responsiveness and significant toxicity. Thus new drugs and novel therapeutic approaches are needed. Gemcitabine has shown promising activity in heavily pretreated patients with NHL even after autologous stem cell transplantation. In the present retrospective study we described our experience about the use of gemcitabine, alone or in association with Cisplatin and dexamethazone, in relapsed/refractory lymphomas. Patients and Methods. Fourty-five patients affected by relapsed/refractory lymphomas were treated with Gemcitabine based regimens with or without Rituximab respectively in B-Cell or T-Cell Lymphoma. Results. Overall response rate was 48.8%. Complete response (CR) 15/45 (33.3%); partial response (PR) 7/45 (15.5%). Quality of response was influenced by the number of previous therapies administered. Patients pre-treated with less than two chemotherapeutic regimens had better overall response rate: in this subset (27/45 patients) 12 achieved CR, 5 PR and 10 a NR/SD, with an ORR of 17/27 (63%). In the subset of the patients pre-treated with more than two chemotherapeutic regimens (18/45 patients) 3 cases obtained a CR, 1 a PR and 14 a NR/SD with an ORR of 4/18 (22%). Conclusions. GDP and Gemcitabine alone represent a valid therapeutic strategy for aggressive relapsed/refractory lymphoma: the efficacy and good toxic profile configures these regimens as a valid therapeutic opportunity for patients which cannot benefit from HDT.

PU032

B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA/LYMPHOCYTIC LYMPHOMA AND SMOULDERING MULTIPLE MYELOMA IN THE SAME PATIENT: A COMMON CLONAL ORIGIN?

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Multiple Myeloma (MM) and B-cell Chronic Lymphocytic Leukemia (B-CLL) are two distinct lymphoproliferative disorders that are thought to arise from different stages of B-cell maturation pathway. The occurrence of both malignancies in the same patient is uncommon. A 64-year-old man was referred to our Hematology Unit because of weight loss and fatigue. Physical examination revealed generalized lymphadenopathy, hepatomegaly and splenomegaly (surface 100 cm^2). Routine investigations showed hemoglobin 8.8 g/dL, white blood cell 7.31x10^9/L (lymphocytes 4x10^9/L), platelets 77x10^9/L, creatinine 4.08 mg/dL, total protein 9.9 g/L. After blood immunofixation, IgA paraprotein was of 6,36 g/dL, while urinary -light chain was of 1.7 g/L with positive Bence Jones proteinuria. Skeletal survey resulted normal. Bone marrow aspiration showed hypercellular marrow with 55% mature lymphocytes. Immunophenotyping by flow cytometry showed that lymphocytes were CD5+ CD19+ CD23+ CD20+ CD22+ CD38+ CD138+ FMC7- CD10- CD56- sIg- CyIg-. This pattern was consistent with a diagnosis of either B-CLL/Lymphocytic Lymphoma with a coexistent plasmacytoma as well as extremely plasmacellular-differentiated B-CLL. This was considered an extremely differentiated B-CLL/Lymphoma and the patient was treated with 6 cycles of R-COP immunochemioterapy (rituximab-cyclophosphamide-vincristine-prednisone) with a good clinical response and reduction of spleen's surface (61 cm^2). After therapy, despite a significant creatinine and IgA- paraprotein reduction (2.23 mg/dL and 4.6 g/dL respectively), the M-component was still present. A subsequent bone marrow analysis identified 48% of -chain restricted CD138+ plasmacells and 10% of CD20+/- CD5+/- CD23- lymphocytes, consistent with a diagnosis of plasmacytoma. Considering the preexistence of chronic renal failure, the absence of CRAB and Amyloidosis AL criteria, this patient is actually affected by IgA- smouldering MM. CLL is known to be associated with transformation to various entities, such as prolymphocytic leukemia, which represents a dedifferentiation, whereas CLL transformation to MM represents a change to a more mature form. Whether the two diseases arise from the same progenitor

cell or are of biclonal derivation is still controversial. In our case, the identical light chains and immunoglobulin isotypes expression in both diseases suggest a common clonal origin. However, further genetic investigations are needed to validate our hypothesis.

PU033

A CASE OF CHRONIC LYMPHOCYTIC LEUKEMIA DEVELOPING CHRONIC MYELOID LEUKEMIA WITH HIGH PLATELETS AND FOLLOWED BY ACUTE MYELOID LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western Countries, with an incidence of 4.2/100.000/year. During the disease course there is a greater incidence of secondary malignancies compared to general population, possibly related to the known CLL-associated immunodeficiency and/or its therapy. Chronic myeloid leukemia (CML), concomitant, preceding or following CLL, is an extremely rare event (25 cases reviewed by the GIMEMA), with a different clonal origin of these neoplasms demonstrated in most cases. We describe a patient with CLL, who developed two different hematologic malignancies, namely CML and AML. A 69 year-old woman was diagnosed with CLL in 2004 at our Institution (67% CD20+CD5+CD23+ peripheral lymphocytes, 78% lymphocytes at marrow aspirate, 45% CLL interstitial and nodular infiltrate at marrow biopsy). Karyotype was normal in 24/31 metaphases and non-clonal chromosome abnormalities (not further identified) in 7/31. FISH analysis was negative for typical CLL abnormalities. CD38 and Zap-70 were positive and VHIg status unmutated. The patient was classified in stage A/0 Binet/Rai, intermediate risk. For the appearance of constitutional symptoms, splenomegaly and progressive lymphocytosis (LDT=9 months) she was treated from Nov 2005 to Sept 2009 with chlorambucil (overall dose 1060 mg) and then she underwent 4 FCR cycles, obtaining disease remission. On may 2012 an abrupt increase of platelets (1,365,000/mL) and basophils (1,009/mL) was observed. Bone marrow aspiration and biopsy displayed increased cellularity (90-95%), with granulocytes series hyperplasia and prevalence of intermediate and mature forms and eosinophilia. Megakaryocytes were increased, small and with hypolobated nuclei. Karyotype showed t(9:22)(q34;q11)[19/22]. RT-PCR was positive for BCR-ABL p210 b3a2. A diagnosis of CML Ph+ (HR Sokal) was established and CLL was confirmed to be in remission (CLL infiltrated 5%). Imatinib therapy was then started and after 3 months a CCyR was obtained. Two months later, clinical conditions rapidly worsened and peripheral blood showed 20% of myeloid blasts (CD34+, CD33+, CD117+/-), consistent with the diagnosis of AML although she was still in CCyR. The patient was then hospitalized but after few days died because of MOF. Based on clinical and biological data, we suggest that in this case the three haematological neoplasms had an independent origin.

PU034

BRENTUXIMAB VEDOTIN IN THE TREATMENT OF "GRAY ZONE LYMPHOMA: CASE REPORT

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A 71 year-old man presented with multiple enlarged mediastinal lymph nodes, the largest of which measured 3 x 4,9 cm , and multiple pulmonary nodules by CT and PET imaging, preceded by a few month's history of persistent or paroxysmal cough, fever and weigh loss. The biopsy of mediastinal adenopathy was consistent with gray zone lymphoma. The term mediastinal gray zone lymphoma (MGZL) has been used for lymphoid neoplasm included in the World Health Organization classification as "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma." Diagnostic criteria for MGZL are still challenging, and the optimal therapy is as yet undetermined. This lymphoid neoplasm is characterized by the heterogenic expression of CD30 in addition to CD20. Although several studies have evaluated different therapeutic strategies, there is a paucity of prospective experience in the treatment of MGZL, given its rarity and relatively recent recognition. After 4 R-CHOP cycles the patient have a partial response (PR) on PET/CT imaging but shortly thereafter he was experienced a recurrence of symptoms consistent with progressive disease: PET/CT scan revealed the presence of news mediastinal adenopathy in addition to hepatic nodules. Given progression disease, he started a salvage treatment with brentuximab vedotin, an anti CD30 conjugated antibody, because CD30 was strongly expressed on node biopsy. He received 1,8 mg/Kg/day as an IV infusion over 30 minutes every 3 weeks. The symptoms resolved promptly after first cycle and PET/CT scan after the fourth cycle showed a nearly complete remission (CRn). The patient tolerated brentuximab very well except mild leukopenia and moderate neutropenia without infections. The patient will continue brentuximab until 8 cycles before further assessment of disease.response Up to now the patient has continuous disease remission. We suppose that brentuximab might be beneficial in any CD30 positive lymphoid neoplasm as previously observed in systemic anaplastic large cell lymphoma and in Hodgkin lymphoma after failure of other therapies. In particular this immunotherapy may be targeted therapy for the treatment of "gray zone lymphomas".

PU035

THYMIC REBOUND AFTER SUCCESSFUL CHEMOTERAPY AND ROLE OF FDG-PET/CT IN PEDIATRIC LYMPHOMAS: A REPORT OF 3 CASES

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Thymic rebound is already reported in the literature, especially in pediatric patients, after special conditions that determine an involution of the thymic tissue, such as infections, prolonged therapy with corticosteroids and in particular after administration of chemotherapy in various malignancies. However in lymphoproliferative diseases with mediastinal localization, the reappearance of a mediastinal mass at the end of treatment can be a diagnostic dilemma between thymic rebound and disease recurrence. We report 3 cases of pediatric patients, 1 female, 2 males, with mean age at diagnosis of 11 (4-15), with lymphoma who presented the reappearance of mediastinal masses after successful chemotherapy. All patients were admitted for lymphadenopathy and CT scan documented anterior mediastinal mass. FDG-PET/CT showed measured standardized uptake values (SUV) max >4 in all patients. The histological diagnosis was T lymphoblastic lymphoma in 4 aged patient and hodgkin lymphoma in the other two cases. Chemotherapeutic treatments were performed according to AIEOP protocols and the CT scan checks made during the various stages of the protocol have documented progressive reduction of the mediastinal mass until obtaining complete remission in all patients. However, CT scan performed at the end of treatment showed the reappearance of an anterior mediastinal mass in all three patients and also of a cervical mass in one of them. PET/CT was subsequently performed and SUV max were respectively 2.8, 2.6 and 2.0. Therefore in two first patients a videomediastinoscopy biopsy of the mass was performed and histological examination confirmed normal thymic tissue. The third patient underwent close follow-up and after 6 months PET/CT had returned to normal. Our cases indicate such rebound in patients with lymphomas is not rare and underline the importance of knowing the age-related and treatment-related incidence of physiologic thymic hyperplasia in children, together with PET/CT features, in order to reduce the potential pitfalls and to avoid unnecessary invasive diagnostic procedures. Furthermore this small case series suggests the importance of the role of PET/CT in the differential diagnosis between thymic hyperplasia and recurrent disease: in fact it is reported in literature that SUV is a sensitive predictor for differentiation of thymic rebound from mediastinal lymphoma: SUV of 3 or higher is a strong predictor of mediastinal lymphoma as this report confirms.

PU036

EFFECTIVE SPLENECTOMY IN A THROMBOPHILIC PATIENT WITH A 21 YEARS-LASTING IMMUNE THROMBOCYTOPOENIA (ITP) AND MULTIPLE TREATMENT ITP-RELATED COMPLICATIONS

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The response to splenectomy, which traditionally represents the option of choice in the setting of steroid-refractory ITP, is quite rapid; the lack of increasing in platelet (PLT), may mean the failure of the procedure. However, although rarely, late response to splenectomy, as recently observed by us, may occur. A 42 years-old woman kept under our attention in 2011 because of refractory ITP, which has been diagnosed 21 years before at another center. At that time, a comprehensive clinical work-up has been unrevealing. She has received 1 mg/kg body weight/day prednisone (PDN); after about one year, PDN has been appropriately tapered until suspension. However, an ITP relapse has been recorded. The patient refused splenectomy; therefore, prednisone has been restarted and then continued until to 2006 at the lowest effective dosage (25-30 mg/day) in order to maintain the PLT count higher than 30.000/µL. In 2006, given the lack to response to PDN, the patient was revaluated; a heterozygous Factor V Leiden mutation, but not other abnormalities, was discovered. Cyclosporine (CSA) at a dosage of 200 mg/day was given and a stable increase of PTL count (> 50.000/µl) was achieved. However, from December 2010, the patient suffered from hypertension. In June 2011, she came to our observation. Moderate renal failure other than hypertension was diagnosed for which CSA was withdrawn and PDN restarted. After a hypertensive crisis, being the PLT count of 15.000/µL, the patient was admitted and IG as IV infusions (IVIG) was given. So that, in November 2011(21 years after the ITP diagnosis) laparoscopic splenectomy (LS) was performed under IVIG and enoxaparin prophylaxis. The procedure was followed by pneumonia and acute pulmonary embolism for which the patient was managed in ICU; after LS the PLT count did not increase and additional IVIG was required. The patient was discharged on full enoxaparin that was administered for the following six months. The PLT count spontaneously increased over 50,000/µL after about two months following the LS. To date, about 2 and 22 years after the LP and the ITP diagnosis respectively, she is well and active; her last PLT count was 550.000/uL for which she is receiving low-dose acetylsalicylic acid. Our case outline the effective role of splenectomy also in advanced and long-lasting ITP and the and the possible occurrence of a late response to the procedure, probably due in our case to the serious medical complications experienced by our patient.

PU037

BING-NEEL SYNDROME: 2013 UPDATE

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Background. Bing-Neel Syndrome (BNS) is a rare neurologic complication of Waldenström Macroglobulinemia (WM) caused by direct invasion of central nervous system (CNS) by malignant cells. Limited data exist regarding its clinical manifestations, diagnosis and treatment. We present a case of BNS occurring as the first manifestation of WM, and associated with severe peripheral neuropathy. In addition, we report an updated review of available literature. Case report A 54-year old man presented with night sweating, painful paresthesias of lower extremities, and IgM monoclonal gammopathy. Clinical examination revealed hepatosplenomegaly and generalized hyporeflexia. Lower limb EMG showed bilateral demyelinating sensorimotor polyneuropathy. Cerebrospinal fluid (CSF) examination disclosed lymphocytic pleocytosis, increased proteins, and clonal B cells. Microbiological tests were negative. Bone marrow biopsy revealed infiltration by lymphoplasmacytoid (LMP) and plasma cells, consistent with lymphoplasmacytic lymphoma. Anti-glycolipid antibodies were absent both in serum and CSF. The patient was treated with rituximab, cyclophosphamide and dexamethasone (RCD), craniospinal radiotherapy, intratechal chemotherapy, and plasma-exchange. Decreased consciousness and coma developed. Brain MRI revealed multiple, subcortical altered signal areas, suggesting disease progression. The patient died 6-months after the initial diagnosis. Literature review and discussion To date, only isolated reports and small case series of BNS have been published. BNS often occurs during the evolution of WN but can be its first manifestation in a minority of patients, as those reported here. Two clinical presentations of BNS are recognised: diffuse or intraparenchymal. Common symptoms are dementia, confusion, ataxia, headaches, or cranial nerve deficit. Peripheral nerve involvement has been also reported. The diagnosis is confirmed by the CSF finding of LMP, clonal B-cells or a monoclonal IgM. In intraparenchymal forms, LMP cells are absent within the CSF. Treatment options include systemic and/or intratechal chemoimmunotherapy, with or without CNS radiotherapy. The outcome is generally unfavourable. Conclusions Patients with diagnosed or suspected WM presenting with neurologic symptoms should be evaluated for possible BNS. Definitive diagnosis is mainly based on MRI, CSF analysis, and pathology studies. Further research is needed in order to improve management strategies of this uncommon condition.

PU038

PEGFILGRASTIM IN THE MANAGEMENT OF POST-CHEMOTHERAPY FEBRILE NEUTROPENIA IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF that extends the half-life and allows for once-per-cycle dosing, requiring less frequent dosing than nonpegylated G-CSF. Multiple Myeloma(MM) in advanced phases of disease may be managed by regimens combining agents not frequently employed in early phases of treatment, but myelotoxicity is the main expected side effect. Avoiding severe neutropenia by prophylactic pegfilgrastim seems particularly useful in these cases. Aim of the study was to compare the efficacy and safety of pegfilgrastim in patients in chemotherapy for MM in advanced phase. In order to determine whether a single subcutaneous injection of pegfilgrastim is as effective as daily injections of standard filgrastim, in terms of toxicity, febrile neutropenia, antibiotic usage and hospedalization duration. We studied 18 patients (10 M,8 F; median age 62y (range 54-82)) affected by MM, all relapsed and refractory to a median of 6 lines of therapy(r.4-8), treated with different regimens. Since first course, patients performed blood counts twice weekly and received prophylactic oral chinolonic antibiotics and anti-fungal drugs, from day 8 to d19. Filgrastim (5 gr/kg/day for 3 d) was given if neutrophils count was $<1500x10^3/mm^3$. Median number of filgrastim administrations was 4(r. 3-6); nadir neutropenia was registered after a median of 11 d (r.8-14); median of nadir neutrophil count was $1.2 x10^3/mm^3$ (r.0.4 – $1.8x10^3/mm^3$), with maximum duration of 10 d. After the first course of chemotherapy, patients received prophylactic pegfilgrastim (6 mg), injected subcutaneously with a single administration on d2 after last dose of chemotherapy, independently from the neutrophil count. Primary endpoint was the duration of neutropenia ($N<1.5x10^3/mm^3$), which was never longer than 10 d. Median nadir neutrophil count, registered 11 d after the end of the therapy (r.9- 15) was 1.576 (r.0.63-$2.25x10^3/mm^3$); two patients(11%) needed a second administration of pegfilgrastim one week after the first (d+9,+10,+11, respectively) and also a supplement of 3 administrations of filgrastim. Pegfilgrastim was well tolerated in all patients: main side effects in our patients were fever and bone pain(3/18,16%). In conclusions, in patients affected by MM exposed to myelosuppressive agents in advanced phases of disease, pegfilgrastim seems to reduce the incidence of neutropenia and may increase the possibility to maintain the scheduled time of treatment.

PU039

FIRST LOCALIZED LATE RELAPSE IN HODGKIN'S LYMPHOMA: SURGICAL TREATMENT IN A YOUNG PATIENT REFUSING ANY OTHER THERAPY

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We report the case report of a late localized ralapse in a young patient with Hodgkin's Lymphoma (HL) refusing chemo and/or radiotherapy. In June 2009, a 53 years old woman, was referred to our Hospital with chest pain and dry cough. Chest X ray showed a mediastinal enlargement; total body CT scan (TBCT) revealed the presence of a mediastinal bulky tumor, without any other lesions (fig.1). A diagnosis of classic HL, nodular sclerosing variant, was made on the mediastinal mass. The clinical stage was IIA; the patient received four courses of ABVD regimen, followed by 30Gy involved field radiotherapy (IFRT 30Gy), obtaining the complete remission, confirmed by TBCT and FDG-PET (PET). At 22 months from the end of therapy, PET scan showed a single abnormal pericardial uptake, confirmed by the cardiac Magnetic Resonance Imaging (MRI) (fig.2-3).

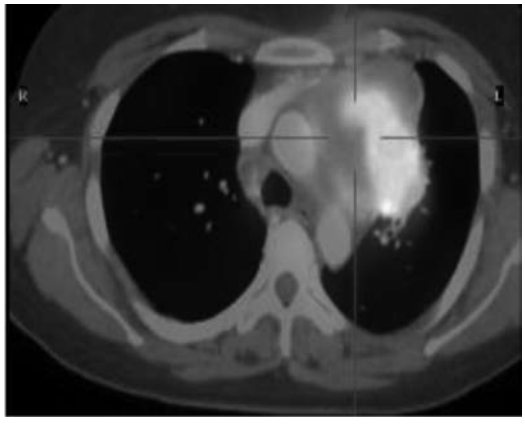
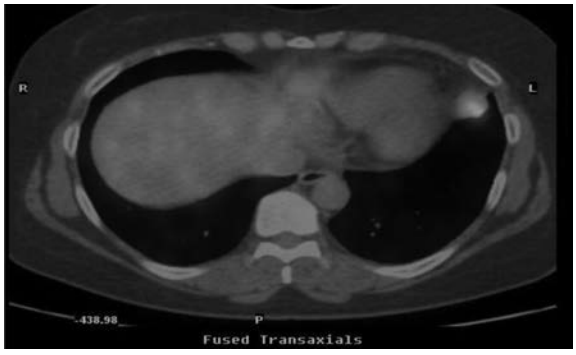


Figure 1.



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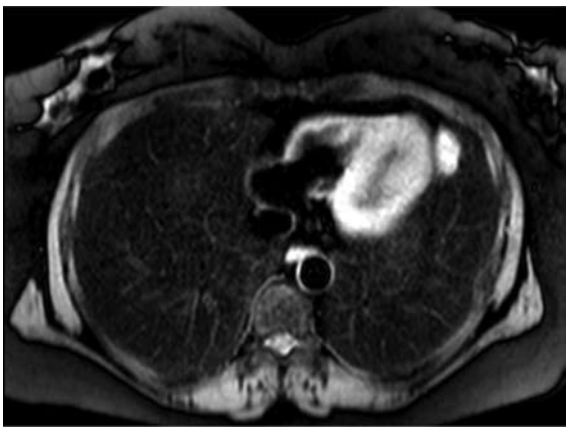

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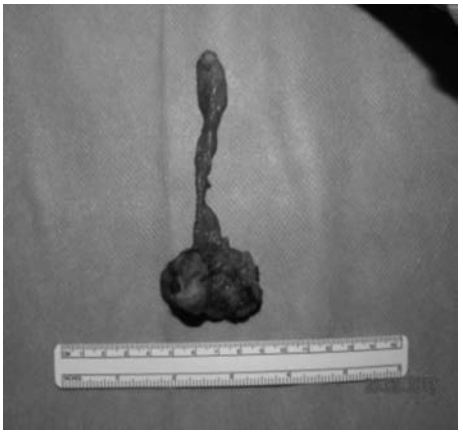

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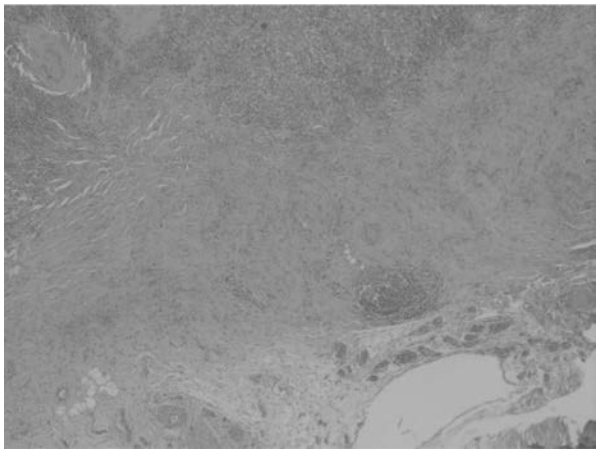

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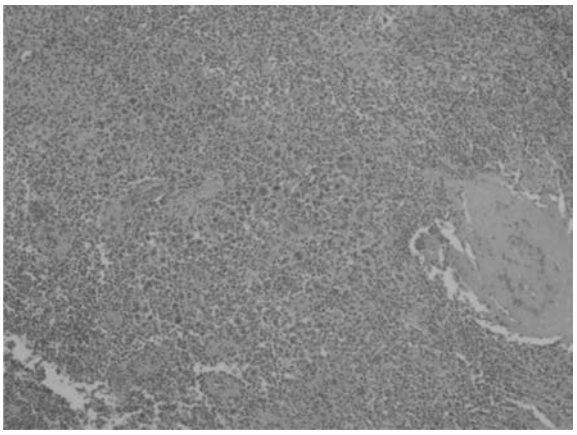

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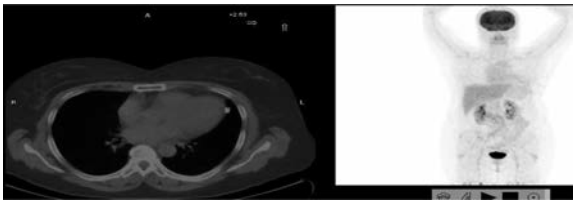

Figure 7.

The patient was asymptomatic. CT scan guided fine needle aspiration was not diagnostic, therefore a thoracoscopy was performed and a 3 cm nodular lesion was found, adherent to the pericardium into the pericardial fat (fig.4). Histology report showed neoplastic cells (CD15, CD30, PAX5 positive and CD20, CD3, EMA, CD5, CD68 negative) into the lesion; pericardial fat and pericardium were not involved (fig.5-6). A diagnosis of late relapse of classical HL in early extranodal stage (IEA) was made. Two courses of ABVD regimen followed by IFRT 30Gy were planned but the pt refused any therapeutic options. Therefore, we decided to start a strict follow up program including clinical and instrumental examinations. Thirteen months after the surgical excision of the lesion, 18F-FDG PET/CT scan is negative (fig.7). CONCLUSION: High Dose chemotherapy followed by Autologous Transplantation is considered the best treatment for relapsed HL and, in cases of late relapses, it is possible to re-treat with the same initial therapy. Anyway, in particular cases, such as early stages (IEA), can surgical debulking be considered sufficient, without any other therapeutic approach? Could it be an alternative option in the management of this kind of patients?

PU040

SAFETY AND EFFICACY OF AN OUTPATIENT DHAP SCHEDULE: AN EXPERIENCE ON 101 RELAPSED LYMPHOMA PATIENTS

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Background. Relapse after first-line therapy occurs in about one third of Non-Hodgkin(NHL) and Hodgkin lymphomas(HL).In these patients, standard treatment consists of 2nd line chemotherapy for re-induction, followed by autologous Stem Cell Transplantation(SCT).DHAP regimen(Dexamethasone-Cytarabine-Cysplatin), performed as inpatient schedule,is widely used in this setting, attaining an Overall Response Rate(ORR) between 58 and 75% in published experiences, and of 77% in an historical cohort from our centre. AIM OF THE STUDY:To evaluate the feasibility, efficacy and toxicity of an outpatient schedule of DHAP regimen in the salvage therapy of relapsed NHL and HL. Patients and Methods. We retrospectively evaluated 101 lymphoma patients(28 with HL,73 with NHL) relapsed after 1st line treatment, consecutively treated at our Hematology Clinic with DHAP(or R-DHAP) in an outpatient setting, from 2001 to 2011. Median age was 55 and 34 years for NHL and HL, respectively. 13 patients were >65 years. Patients with NHL diagnosed before 2005(n=35) received DHAP, while after this date R-DHAP was the standard treatment for CD20+ NHL(n=37). Results. The mean number of DHAP courses was 2.1. ORR was 78%; 82% in HL, 75% in NHL (not significantly different in those treated with or without rituximab). There were no toxic deaths. Toxicity was mainly hematological with 33%, 34% and 5% of patients showing severe(grade 3-4 WHO) neutropenia, thrombocytopenia and anemia, respectively.Grade 3-4 infections were reported in 14% of patients. Extrahematological severe adverse events were almost exclusively gastrointestinal (12 with grade 3 and 1 with grade 4). 64 patients underwent autologous SCT, 34 of them after high-dose sequential therapy. Of the 37 patients not undergoing autologous SCT, 8 failed the collection, in 10 indication for autologous SCT was changed to allogeneic SCT and in 8 to follow-up(high risk for SCT and/or complete response after re- induction),11 died because of early progression or other comorbidities. With a median follow-up of 3 years, the 5 years overall survival is 57%,69% for those achieving CR after DHAP.Disease-free survival at 3 years is 55%.61 patients are alive at last follow-up. Conclusions. Our results regarding the efficacy and toxicity of an outpatient DHAP schedule favourably compare to historical data with the same regimen.We conclude that DHAP performed on an outpatient basis is feasible and safe and achieve a similar efficacy to the inpatient schedule.

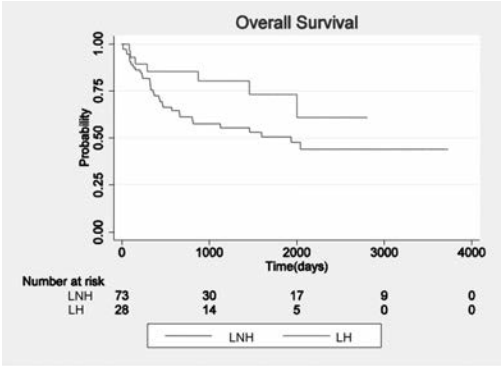

Figure 1.

PU041

OCULAR RELAPSE IN A CASE OF PH+ ACUTE LYMPHOBLASTIC LEUKEMIA AFTER ALLOGENIC PERIPHERAL STEM CELL TRANSPLANTATION

Santoro L,[1] Volpe S,[2] Alfieri G,[2] Urciuoli E,[2] Pensa M,[3] Palmieri F,[1] Palmieri R,[1] Storti G,[1] Cantore N[1]

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A 42 year old women on January 2011 received diagnosis of Ph+ ALL. Induction treatment with imatinib for 30 days resulted in complete haematological and cytogenetic remission and 1-log decrease of BCR/ABL transcripts. After consolidation treatment with HAM, Bcr/Abl % IS was 0.474, then therapy with imatinib was restarted. About five months from diagnosis the patient was in complete haematological and cytogenetic remission and Bcr/Abl % IS was 0.1. Matched related donor allogeneic hematopoietic peripheral stem cell transplant was performed on August 2011. After transplant the patient was in full chimerism with CMR (MR 4.5). On April 2012 the patient had headache and edema in her right eye. Ophthalmic exploration revealed scotoma areas on right eye. Brain TC scan and MRI were negative. CSF cytospin examination was negative. CSF microbiologic cultures were negative. She presented in our department in Jun 2012 and reported decrease of visual acuity in the right eye. Ophtalmic exploration revealed hypopyon and retinal detachment. Brain and orbits MRI were negative. Peripheral blood and a bone marrow smear examination were both normal. CSF examination was negative. Bone marrow FISH analysis with BCR/ABL probes was

negative with MR (Bcr/Abl % IS: 0.025). Aspiration of 100 ul of aqueous humor from the anterior chamber was gave for a flow cytometric immunophenotypic analysis. The sample showed a cellular count of 380/ul with 88 % of mononuclear cells. The percentage of populations were : CD19+CD10+:93% , CD19+CD10+CD45+:30%, CD3+:4%, CD3+CD4+:3%, CD3+CD8+:1%. Leukemic cells showed the same immunophenotype as initial diagnosis. Diagnosis of extramedullary ocular relapse was made. Treatment with dasatinib 140 mg once a daily was started. After 2 months from treatment the visual acuity had improved. Aqueous humor was clear and no leukemic cells were found at flow cytometer analysis then surgery for retinal detachment was performed. To our knowledge this is the first case of extramedullary ocular primary relapse in a patient with Ph+ ALL after allogeneic PSCT treated with dasatinib. We emphasize the diagnostic usefulness of flow cytometer analysis on small samples from organs in which it is difficult to make biopsies and that Dasatinib can be an effective treatment in ocular relapse.

PU042

PRESENCE OF GENETIC ABNORMALITIES IN A CASE OF ESSENTIAL THROMBOCYTHEMIA (TE)

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TE is a chronic clonal myeloproliferative disease ph negative characterized by abnormal proliferation clone of megacakaryocytes with the platelet lineage progenitors in the circulation and/or the spontaneous growth *in vitro*, of unknown etiology. Clinical course is characterized by the tendency to thrombosis and to bleeding with long-term risk to evolve into aggressive myeloid disorders. Few and rare chromosomal abnormalities are reported: trisomy 8, 9 and (13q). Recently between the diagnostic criteria for a diagnosis of TE has assumed diagnostic value also the search for the mutation of JAK2V617F present in 30-40% of cases. This communication aims to focus attention on the need to always make the diagnosis of bone marrow cytogenetic evaluation. In november 2011, a young woman affected by TE JAK2 mutated, was referred to our observation for progressive platelets increase. Initial theraphy was interferon, poorly tolerated by the patient, then began therapy anagrelide.In course of therapy for the progressive increase in platelets (up to 2,000,000 /mm3) and suspicion of evolution in chronic myelogenous leukemia, looking for bcr / abl on peripheral blood and mielobiopsy with karyotype examination.was performed. Results. karyotype 46, xx, + der (1; 15) (q10, q10) -13/46, xx-14. Conclusions: constitutionally normal karyotype with the presence of a pathological clone characterized by the presence of a derivative chromosome consisting of the long arms of a chromosome 1 and the long arms of a chromosome 15. Are presents two normal chromosomes 1 and only one normal chromosome 15, therefore the clone is trisomic for the long arm of chromosome 1. The patient maintains a value of platelets around 700.000/mm^3 with anagrelide therapy. After the patient showed an increase of hematocrit. Unexpected genetic abnormalities and hematocrit increase shall be construed so as signs of considerable instability of disease. Therefore the patient continues monthly hematologic checks.

PU043

EFFICACY OF ALEMTUZUMAB PLUS EXTRACORPOREAL PHOTOPHERESIS IN ADVANCED STAGE SEZARY SYNDROME: A CASE REPORT

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Sézary syndrome (SS) is one of the most common clinical variants of primary cutaneous T cell lymphomas (CTCL). Advanced SS have a poor prognosis and may be beneficially affected by a multi-disciplinary approach. We report a 70-year-old man presented with a 4-year history of SS diagnosed on the basis of clinical and pathologic features of CTCL with leukemic involvement and treated by other medical officers over the years with extracorporeal photophoresis (ECP) and alpha interferon with only limited partial clinical responses. His condition gradually deteriorated coming to our observation with a poor performance status (ECOG: 3), intractable itching erythroderma involving the whole body, palmar and plantar hyperkeratosis, supraclavicular, axillary and inguinal lymph node enlargement, confirmed by PET-CT scan. He had a white blood cell count of 9.520/mL with 87% of clonal T-cell receptor gene rearranged lymphocytes CD3+, CD4+, CD5+, CD7-, CD8-, identical with those detected in neck lymph node, in the skin and bone marrow (BM). According to the current WHO–EORTC classification, all diagnostic criteria matched with SS, 4th stage. The patient was first treated with 4 doses of pentostatin without any response and 2 months later with alemtuzumab at a dose of 30 mg two times a week. The patient concomitantly received cotrimoxazole, acyclovir and itraconazole prophylaxis. Cytomegalovirus (CMV) infection was monitored weekly using polymerase chain reaction (PCR). After the first week of alemtuzumab, itching disappeared and after 2 weeks of treatment a marked improvement of erythroderma, concomitant with complete clearing of circulating Sezary cells, was observed, associated 2 weeks later with almost complete regression of lymphadenopathies. Alemtuzumab had to be discontinued at the fourth week (total dose 270 mg) due to cytomegalovirus DNA reactivation, successfully resolved after standard dose gancyclovir treatment. Two months post alemtuzumab discontinuation, reappearance of 17,1/mL circulating Sezary cells prompted us to start again ECP (two session per month in the first year and then two session every two month in the second year) leading to a progressive tapering of circulating Sezary cells and complete resolution of disease signs and symptoms. This case report further supports the efficacy and safety of alemtuzumab plus ECP in inducing prolonged responses in patients with refractory late stage Sezary syndrome.

PU044

CO-EXISTENCE OF CHRONIC MYELOPROLIFERATIVE SYNDROME AND CHRONIC LYMPHOCYTIC LEUKEMIA-B: REPORT OF A CASE

De Falco G, Esposito D, Graziani F, Iovane E, Di Maria D, Martone C, Caparrotti G

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A 67 year-old patient came to our attention in April 2007 for leukocytosis with lymphocytosis moderate. Diagnosis of lymphocytic leukemia-B classical (CD 19 + CD23 + CD5 + CD20 + and restriction k chains low intensity of fluorescence), was performed and staging was definited 0 Rai. The patient practiced periodic laboratory and instrumental checks every three-six months which showed stationarity of blood chemistry panel until June 2010. In October 2010 initial and progressive increase in platelet count, while hypochondriac organs and lymph nodes in normal stage. Progressive increase of thrombocytosis and in the absence of secondary causes the patient was admitted to day-hospital recovery. The presence of exon 14 V617F mutation of the JAK2 gene and cd 34+ equal to 0.04% of circulating leukocytes, was showed. Therefore we concluded for simultaneous onset of chronic myeloproliferative syndrome JAK2 mutated in a patient with chronic lymphocytic leukemia-B.The patient continues monitoring clinical laboratory quarterly and considering the stability of the platelet count (about 500,000/mm^3), none specific therapy is in course.

PU045

DOUBLE HIT LYMPHOMA(DHL): CASE REPORT

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The 2008 WHO classification lists the category of B-cells lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma(DLBCL) and Burkitt lymphoma(BL).Genetic abnormalities of this intermediate category include c-MYC with BCL2 and/or BCL6 translocation that identify"double/triple hit lymphoma".We describe

the case of an aggressive B-cell lymphoma that meets the criteria of a double-hit lymphoma. We observed a 55 years old woman with a 3 months history of dyspnea,sporadic chest pain and dysphagia;paresthesia of the upper right, night sweats, increase of supraclavicular lymph node (LN). Laboratory: WBC:2260/μL, Platelets: 126.000/μL,LDH:715U/L, 2 microglobulin: 3.81mg/L, -globulin:0.55g/dL. IPI:2. TB-CT: supraclavicular, mediastinal,abdominal lymphadenopathy. Splenomegaly. PET-CT: hyperaccumulation of over and under diaphragmatic LN(SUV Max: 18.5, supraclavicular,SUV Max:18.96,abdomen). LNbiopsy: Follicular B-cell NHL(FL) gr.IIIa (50%)+DLBCL (50%), MIB-1>90%. Bone Marrow (BM): infiltration of FL. Diagnosis of high-grade B-cells lymphoma evolved from FL. We treated the patient with R-CHOP. After a cycle, the patient's condition improved and the superficial LN reduced. Cytogenetic analysis by Fluorescence in situ ibridization (FISH): c-MYC and BCL-2 translocations. Definitive diagnosis: DHL. We changed the treatment with BL-like chemotherapy (CT) according to GITIL protocol. Induction phase: cyclophosphamide 500 mg/m^2,d0-1; vincristine (VCR) 1.4 mg/m^2, d0,35; methotrexate (MTX) 150 mg/Kg,d7; MTX250mg/Kg,d21; rescue: folic acid; etoposide 250 mg/m^2, bid,d14; Rituximab 375 mg/m^2 + Dexamethasone (DEX) 8 mg, bid, d15,29,36; doxorubicin 50 mg/m2, d28. Weekly lumbar puncture: MTX 10 mg alterning to Aracytin (Ara-C) 60 mg. PET-CT at the end of treatment: complete remission (CR). BM: negative. It followed a consolidation phase (CF): Ara-C750 mg/m^2 bolus+1 g/m^2 c.i., d42-45; Cisplatin 20 mg/m^2, d42-45; Rituximab 375 mg/m^2+ DEX 8 mg bid, d75,82. Haematological toxicity (grade IV-WHO) overall after CF. Sepsis from E.Coli. Diarrhea and vomiting grade II-WHO. Supportive care:eritropoietin,transfusion of packed red blood cells and platelets. The attempt to stem cell mobilization (with G-CSF from d+8 to d+17) failed, twice. Follow-up(18 months) confirms a CR.DHL is clinically aggressive,associated with a poor prognosis.FISH should be performed on all high grade lymphomas for early identification of DHL to consider upfront HD-CT.This case represents a good clinical outcome after treatment with high doses sequential chemotherapy without ASCT.

PU046

BENDAMUSTINE IN COMBINATION WITH RITUXIMAB FOR ELDERLY PATIENTS WITH AGGRESSIVE B NON HODGKIN LYMPHOMA NOT ELIGIBLE FOR ANTHRACYCLINE-BASED THERAPY

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Bendamustine is an alkylating agent with favorable clinical activity against indolent lymphomas as single-agent or combined with rituximab. Up to now, data on clinical efficacy of bendamustine in aggressive non Hodgkin lymphomas (NHL) are extremely limited. More than one third of the elderly patients are not fit to receive front-line high-dose chemotherapy for a newly diagnosed aggressive B cell lymphoma due to pre-existing comorbidities, and outcome for these elderly and frail patients not eligible for rituximab, cyclophosphamide, doxorubicin, vincristin and prednisone (R-CHOP) standard treatment is poor with a median survival of less than 6 months. Therefore, for these "slow go" patients with aggressive NHL, alternative effective treatments with less toxicity are required. From January 2012 up to now, we evaluated efficacy and safety of bendamustine plus rituximab (R-B; R at a dose of 375 mg/m^2 i.v. on day 1, and B at a dose of 90 mg/m^2 on days 1 and 2 of each cycle, every 4 weeks), as frontline treatment in 13 (7 males and 6 females) elderly and frail consecutive patients with aggressive B-NHL (3 mantle cell lymphomas, 2 anaplastic and 8 diffuse large B-cell lymphomas) not eligible for R-CHOP. All patients gave their written informed consent. The median age of patients was 80 years (range, 71-87), and the median Karnofsky performance status was 65% (range, 40-90%). Three patients had stage II and 10 patients had stage III/IV disease. Response (complete remission -CR-, partial remission -PR- and progressive disease -PD-) was assessed according to revised international workshop response criteria. Up to now, a total of 40 cycles of R-B were administered, with a median of three cycles (range, 2–6 cycles) per patient. No patient discontinued or delayed treatment due to treatment-related toxicity. Grade III hematologic toxicity (mainly anemia) was documented in 2/13 (23%) patients, and only 1 patient needed 50% dose reduction of bendamustine. No organ toxicity >grade I was detected. Overall, 8 of the 13 patients were assessable for response. The overall response rate was 62%. Of these 8 patients, 4 achieved CR, 1 PR and 3 showed PD. Two of these 13 patients died for PD. Our preliminary data provide evidence that R-B is an effective and well tolerated frontline therapy in elderly and frail aggressive B-NHL patients. These results require further validation in prospective randomized studies.

PU047

CO-OCCURRENCE OF MULTIPLE MYELOMA AND CHRONIC MYELOGENOUS LEUKEMIA: A CASE REPORT

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Background. Simultaneous presentation of multiple myeloma (MM) and chronic myelogenous leukemia (CML) is an extremely rare event. Here we report a case of synchronous occurrence of light-chain MM and CML and discuss relative pathophysiological and therapeutic implications. Case Report. A 67-year-old man was referred to our department in January 2011 with light-chain monoclonal gammopathy and multiple osteolysis. Bone marrow biopsy revealed a hypercellular marrow with granulocytic hyperplasia, increased eosinophils, and hypolobated megakaryocytes. Furthermore, there was an infiltrate of atypical plasma cells accounting for 35-40% of nucleated cells, leading to the diagnosis of light- chain MM. The patient was initially treated with thalidomide, dexamethasone and involved-field radiotherapy. However, extreme thrombocytosis developed, requiring cytoreduction. Conventional and molecular cytogenetic analysis performed at diagnosis was then available, which showed the presence of t(9,22). Reverse transcriptase polymerase chain reaction detected the p210 BCR-ABL fusion transcript, confirming the diagnosis of CML. Treatment with bortezomib, dexamethasone and imatinib was then started, followed by autologous peripheral stem cell transplantation. At 12-month follow-up, our patient maintains a complete molecular response for CML and stringent complete response for MM, while continuing therapy with imatinib. Discussion Limited data have been reported about diagnosis, clinical features and treatment of coexisting active MM and CML. This association has been considered to be mostly coincidental. However, the development from a common malignant pluripotent stem cell has been proposed, as supported by our report of synchronous occurrence of both diseases. Imatinib has become the cornerstone of therapy for CML. Furthermore, recent evidence suggest interesting effects on marrow microenviroment, angiogenesis, and plasma cell proliferation, which may potentiate the effect of common antimyeloma agents. We believe that this synergistic action could have contributed to the relatively favorable outcome observed in our patient, and further studies are warranted to explore these mechanism.

PU048

EFFICACY OF 5'-AZACITIDINE AS LONG-TERM TREATMENT IN INTERMEDIATE AND HIGH-RISK MYELODISPLASTIC SYNDROMES

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Background. Despite the improvement of biological data on high-risk myelodysplastic syndromes (MDS), their prognosis remain poor for advanced age of patients, high incidence of evolution in acute leukemia, poor response to standard intensive cytotoxic treatments. Hypomethylating agent 5'-Azacitidine (AZA) is now the drug for the standard of care in patients with intermediate and high-risk MDS according to the R-IPSS. Aim. To report a single Institution experience on the efficacy and toxicity of long-term AZA treatment in a small cohort of high risk MDS patients. Patients. 15 patients, 8 male and 7 female, median age 72 years (range, 65-84), classified by R-IPSS as intermediate-2 (n=11) or high risk (n=4) received AZA at the standard dose of 75 mg/sm for 7 days, every 28 days. Erythropoietic Stimulating Agents were added to the treatment

until the hemoglobin level was <11 g/dL. Before starting therapy, all patients were supported with PRBC. Results. At diagnosis, median blood counts were: Hb 8,4 g/dL (range, 6,8-10,9 g/dL); ANC 800/mmc (range, 300-1800/mmc); Plt 77.000/mmc (range, 4.000-235.000/mmc). Median LDH value was 620 U/L (range, 296-996 U/L). Cytogenetics study showed a normal karyotype in 12 patients, del5q in one patient, Ch 8 and Ch 9 trisomy in two different patients. A median of 12 courses (range, 3–36) were administered. Three patients died while in treatment because of severe infection, without any response. After six courses, seven patients showed a median hemoglobin increasing of 2,5 g/dL. Nine patients were transfusion dependent at diagnosis, and seven of them reached transfusion independence. Hematological toxicity was easy manageable, while no extra-hematological toxicity was observed. FUO or documented infection was observed in 5 patients, and was resolved with appropriate antibiotic therapy in two cases. After a median follow-up of 24 months (range, 5-41), four patients showed a disease progression in AML, eight patients showed a stable disease and are still in treatment having received a median of 20 treatment courses. Median OS is 30 months with a PFS of 53% at 26 months. Conclusions. AZA therapy is effective in patients with high-risk MDS inducing blood counts improvement in almost half of the patients treated. Long-term treatment is well tolerated without the emergence of toxicity.

PU049

UPAR SOLUBLE FORMS AND HEMATOPOIETIC STEM CELL TRANSPLANTION

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The urokinase (uPA)-type plasminogen activator receptor (uPAR) is expressed on the surface of various cell types, both in full-length and cleaved forms, lacking the N-terminal DI domain. uPAR binds uPA and vitronectin (VN) and regulates integrin activity. uPAR can be shed from the cell surface, generating full-length and cleaved soluble forms (suPAR and DIIDIII-suPAR, respectively). suPAR is still able to bind uPA and VN, unlike DIIDIII-suPAR, which, however, if exposing the sequence SRSRY (aa 88-92) at its N-terminus, is able to bind the chemotactic receptors for fMLF. Soluble forms of uPAR have been detected in human fluids. We previously demonstrated the involvement of uPAR soluble forms in G-CSF-induced human CD34+ hematopoietic stem cell (HSC) mobilization. Further, we demonstrated that DIIDIII-suPAR can induce mobilization of hematopoietic stem/progenitor cells in mice. Since HSC mobilization and homing to bone marrow (BM) are specular processes which utilize same mediators and similar signaling pathways, we investigated whether the soluble forms of uPAR could be also involved in HSC homing and engraftment to the BM. Firstly, we examined suPAR and DIIDIII-suPAR expression in cultures of human BM stroma cells. Interestingly, stroma cells produced suPAR and high amounts of the chemotactic DIIDIII-suPAR. We then evaluated the levels of both suPAR forms in sera from four healthy donors and from five patients before and after the pretransplant conditioning with chemotherapy. We found a significant increase only in DIIDIII-suPAR levels in sera from patients before conditioning, as compared to healthy donors; however, the chemotherapy conditioning significantly decreased circulating DIIDIII-suPAR levels. We also examined the potential effects of the different soluble forms of uPAR in long term cultures (LTC) of G-CSF-mobilized CD34+ HSCs, in the presence of suPAR or of the uPAR84-95 peptide, corresponding to the active site of DIIDIII-suPAR. Both suPAR and the uPAR84-95 peptide increased the number of adherent clonogenic progenitors in LTC of G-CSF mobilized HSCs. Altogether, our results suggest that BM stroma produces soluble forms of uPAR which could contribute to the engraftment of CD34+ HSC to BM. According with our previous observation on the mobilizing effects of DIIDIII-suPAR, the circulating cleaved suPAR seems to be lowered by pretransplant conditioning, probably to allow CD34+ HSC homing.

PU050

EXTRAMEDULLARY MULTIPLE MYELOMA: CASE REPORT

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A 64 years old man was admitted to our Institution in November 2010 for a lumbar vertebrae acute pain due to L3 Lysis with low IgGk monoclonal component. Somatectomia of L3, osteosynthesis of L2-L4 and stabilizing radiotherapy treatment were effected. The L3 histological examination showed multiple myeloma infiltration in absence of other osteolitic lesions. When the painful symptomatology and the perfomance status (PS) were improved, the restaging showed multiple myeloma (MM) IgGk, stage II A, ISS III. Low ß-2microglobulin. Cytogenetics: absense of unfavorable karyotype. Patient refused the autologous stem-cell transplantation (ASCT) and he was treated with biphosphonate intravenous and nine cycles of chemotherapy of bortezomib plus melphalan and prednisone, ended in February 2012 with VGPR of the desease. In April 2012 he was admitted to the hospital for acute renal failure, hypercalcemia and progressive painless bilateral testicular swelling. Testicular mass measured 10 cm of max diameter. It was highly vascular on ultrasound and suggestive of multiple myeloma infiltration. Total body CT scan with contrast revealed lung involvement, liver involvement, adrenal involvemnent and psoas muscles involvement. Hepatic biopsy confirmed the infiltration of multiple myeloma. Bone marrow examination showed normal hematopoiesis. It was decided to treat the patient with biological therapy with oral Lenalidomide (10 mg/die to 21 days/month) plus dexamethazone with improvement of the renal function and decreasing of the testicular mass. CT scan of November 2012 confirmed significantly regression of the extramedullary lesions and reduction some lesions in the liver. In March 2013, after eight cycles of biological treatment, patient complained persistent stomac-ache and melena. Esophagogastroduodenoscopy showed multiple myeloma lesions in absence of medullary infiltration. Instrumental revaluation confirmed the progression of the extramedullary multiple myeloma with abdominal skin masses, without a rase of monoclonal component. Systemic chemotherapy was renstituted, this time with VAD (vincristine plus doxorubicin and dexamethazone), induction regim in preparation for ASCT. VAD-chemotherapy was given at 75% for grade IV neutropenia, with a real improvement of the PS. Based on the published literature extramedullary multiple myeloma is a poor prognostic marker in relapsed/refractory MM patients and therefore is a therapeutic challenge, even in the era of novel agents.

PU051

HAEMOLYTIC ANAEMIA AFTER VALVULOPLASTY AND ANNULOPLASTY WITH RING: A CASE REPORT AND REVIEW OF THE LITERATURE

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Introduction. Haemolytic mechanical anaemia associated with valvular replacement is well known. Rarely, haemolysis is due to valvuloplasty and annuloplasty with or without ring. We report a case of haemolytic anaemia due to periannular regurgitation after disruption of the suture of the ring, in a patient who received mitral valvuloplasty and annuloplasty with ring. Case Report. In November 2012, a 60-year-old man underwent mitral valvuloplasty and annuloplasty with ring (future band) for severe valvular incompetence. The patient was in good clinical conditions at discharge. In December 2012, he was admitted to hospital for haemolytic anaemia. Physical examination revealed pale skin and jaundice, mitralic systolic murmur (3/6) and red-brown urine. Laboratory tests showed: Hb: 7.5g/dL, MCV: 95.7fl, total bilirubin: 2.1mg/dL (indirect bilirubin: 1.5 mg/dL), LDH: 2190U L, haptoglobin: 0.1g/dL, reticulocytes: 3%, schistocytes: 3%, Coomb's test (direct and indirect): negative, haemoglobinuria, B12 vitamin: 150 pg/ml. Cardiac ultrasound scan revealed repaired mitralic flaps. Colour doppler imaging showed moderate mitral valve regurgitation. Transesophageal echocardiogram (TEE) revealed a moderate-severe (3+/4) periannular posterior regurgitation of the mitral prosthesis and the presence, in the same place, of thin and mobile threads: the disruped suture of the ring. As other possible causes of haemolysis were excluded, we diagnosed mechanical haemolytic

anaemia caused by periannular regurgitation. B12 vitamin and transfusion of red blood cells were administered with mild improvement of clinical conditions. The patient refused a corrective surgical treatment. Conclusions. Mechanical haemolysis can be rarely seen after valvuloplasty and annuloplasty with ring and it's caused by regurgitation linked to defective positioning of the prosthesis. Other causes of haemolysis must be always excluded. TEE imaging studies play a key role in the diagnosis. Surgery is the treatment of choice. Alternatively, vitamin support, administration of erythropoietin or red blood cells transfusions can be useful.

PU052

TREATMENT WITH AZACITIDINE INCREASES OVERALL SURVIVAL, REDUCES INFECTIONS AND HOSPITALIZATIONS IN ELDERLY PATIENTS WITH ACUTE LEUKEMIA COMPARED WITH INTENSIVE CHEMOTHERAPY: A MONOCENTRIC STUDY CHEMOTHERAPY: A MONOCENTRIC STUDY

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Background. Survival in older patients(>65 years old) with acute leukemia is shorter than young patients. Aims: We reviewed the baseline characteristics and outcome after therapy of 40 consecutive patient aged 65 years and older with acute leukaemia admitted and treated in our institution from 2009 to 2013.20 patients with performance status 2 or 3,or some older patients that did not accepted intensive chemotherapy were treated with azacitidine (Aza group), while 20 patients with performance status 1 or 2 younger than 75 years were treated with intensive chemotherapy (IC group). Methods. Azacitidine was administered subcutaneously (75mg/m^2/d) for 7 days of every 28-day cycle until progression,while IC consisted of 1 induction course with mitoxantrone,cytarabine and etoposide followed by 2 course in patients in complete remission. In aza group (7f/13m) the median age was 76.5 (range 65-83), median white blood cells was 3.3 (1.1-48), median hb was 7.85 (6.2-12.2), median platelets 70 (17-245), median bone marrow blasts counts was 47% (21-90%). Karyotype risk stratification was normal in 11 (55%), intermediate in 2 (10%) and high risk in 7 (30%).The median number of cycle was 10 (range 1-29).The fever infections that requiring IV antiobiotics werein7 patients (35%), but 5/7 (71%) of infected patients not reduced next cycle,while 1 (5%) patient presented an Early Death(ED). 15 patients in Aza group achieved response to treatment in fact overall response rate (ORR) was 75% (complete remission 45%+ partial remission 30%) with a median duration of 9 months (range1-29), the median overall survival (OS) was 12 months (range 2-29). In IC group (10f,10m) the median age was 70.5,median white blood cell was 5.3,median hb was 8.05g/dL, median platelets 33 ,median bone marrow blasts counts was 65% (range 35-90%). Karyotype risk stratification was normal in 15 (80%), and high risk in 5 (20%). The median number of cycle was 1.5. The fever infections 3-4 grade that requiring IV antimicrobics were in 15 patients (75%) and in 9 (45%) were observed ED.9 patients in IC group achieved response to treatment: ORR was 45% (complete remission 35%+ partial remission 10%) with a median duration of 3.5 months (range1-11) the median overall survival (OS) was 1.5 months (range2days-19 months). Results. Treatment with azacitidine in older patients with acute leukaemia prolongs OS (p<0.01), expecially in patients with worse PS (p<0.001),reduces ED (p.0.008) for lower toxicity and lower incidence infections and improve ORR(p0.66 compared with IC.

PU053

HEMOPHAGOCYTIC SYNDROME HYDING T-ACUTE LYMPHOBLASTIC LEUKEMIA: A CASE REPORT

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Introduction. We report clinical case of hemophagocytic syndrome (HPS) concealing T-cell lymphoblastic leukaemia. Case report. Female, 49 years-old is admitted to our hospital with weakness, fever, nausea. Complete blood count reveals severe pancytopenia. Other abnormal blood tests are: creatinine 5.3 mg/dL, uric acid 40 mg/dL mEq/L, LDH 7600 U/L, ferritin 607 ng/dL, fibrinogen degradation product> 20 µg/mL, D- Dimer 5720 ng/mL. Blood smear and immunophenotype analysis are negative. We decide for a bone marrow biopsy showing prominent proliferation of reactive macrophages with cellular debris and absence of malignant cells. Immunohistochemical report and clinical history are compatible with hemophagocytic syndrome. Patient receives supportive therapy with transfusion, antibiotics, haemodialysis and rasburicase. At twenty-four day blood tests are in normal ranges. After other twenty days, patients shows leucocytosis, serum LDH is 1882 U/L. Blood smear analysis shows lymphoid cells with leukemic characteristics. Cytofluorimetry on peripheral blood shows a 7% cellular population CD7+, CD2+, CD5+, CD4+, CD1a+, TdT+, CyCD3+, CD3-, CD8- . We performs diagnosis of T-cell lymphoblastic leukemia. Patient is treated with conventional induction chemotherapy and allogeneic transplant. After four years, she is alive in complete remission. Discussion. Described case shows a T-cells lymphoblastic leukaemia beginning with a severe HPS. HPS is a rare condition characterized by deregulation of immune system and triggered by hyperactivation of macrophages. Symptoms are fever, hepatosplenomegaly, pancytopenia, liver dysfunction, coagulopathy, and hyperferritinemia. HPS may be primitive or secondary to other pathologies. Lymphoproliferative disorders commonly associated with HPS are T/NK cell, intravascular, anaplastic large cell lymphomas and T-lymphoblastic leukaemia. Median survival for untreated patients was 11 days. High- dose chemotherapy followed by stem cells transplantation is only treatment able to give chances of complete remission (Han AR *et al*.; Ann of Hematol 2007). Long term complete remissions and long survivals are rare. Conclusions. Lymphoproliferative diseases must be suspected when HPS's signs are present with no evidence of infections or autoimmune pathologies. An early biopsy (bone marrow or lymph node) can simplify diagnosis of hidden disease revealing itself only after a long time.

PU054

SAFETY AND EFFICACY OF LENALIDOMIDE/DEXAMETHASONE TREATMENT IN ELDERLY PATIENTS WITH REFRACTORY/RELAPSED MULTIPLE MYELOMA

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Introduction. Lenalidomide is an immunomodulatory agent that has tumoricidal and immunomodulatory effects in Multiple Myeloma (MM). The combination of lenalidomide and dexamethasone (Len/dex) is indicated for patients with relapsed or refractory MM (RRMM). Two subanalysis of MM-009/010 have evaluated the impact of patient age on the efficacy and safety of Len/Dex and have noted that, for elderly patients with RRMM, Len/Dex is an effective treatment option. We report a single-center series of elderly patients RRMM treated with this combination. Patients. Since 2009 16 patients (pts) with RRMM who were not candidates for autologous stem cell transplantation were treated with Len/Dex at our center. All pts at start of therapy had an ANC of less than 1500, a platelet count of less than 75,000, clearance creatinine > 60 ml/min and peripheral neuropathy of grade 0-2. Pts received Len 10-25 mg on days 1-21 and Dex 20-40 mg on days 1-4, 9-12 and 17-20 for four cycles and than 20-40 mg on days 1-4 or 20-40 mg weekly. All pts received DVT prophylaxis with LMWH and antibacterial prophylaxis. Response to treatment were assessed according EBMT criteria. Results. All pts (16) had received at least 1 prior therapy with Bortezomib. The median age was 72 years (65-85), 7 man and 9 female. Five, 6, 4 and 1 pts (31%, 38%, 25% e 6%) had ECOG PS 0, 1, 2 e 3, respectively. The median number of cycles was 12 (range 1-28). 10 pts (62%) experienced grade 3 or 4 toxicity. Neutropenia (44%) and thrombocytopenia (32%) were the most common grade 3-4 adverse events followed by neuropathy (12%), thrombosis (6%) and hyperglycemia (6%). Dose reductions occurred in 7 pts, due to neutropenia or thrombocytopenia and 6 pts required G-CSF treatment. Median number of cycles before achieving a response was 3 (1-9 cycles). Four pts (20%) interrupt treatment within 4 cycles (2 progression MM, 1 thrombosis, 1 neuropathy) and 12 pts (75%) received Len/Dex until disease progression. A very good partial response (VGPR) and a partial response (PR) were reported in 5 (31%) and 7 (44%) pts respectively. The median progression free survival (PFS) was 14 months. In our data six pts were older than 75 years of age and in this group 4 pts (66%) obtained a VGPR. In this setting the median

PFS was 18 months. Conclusions. Our experience confirm that Len/Dex is an effective combination in elderly patients with RRMM and that this combination had a manageable safety profile also in very elderly patients.

PU055

WITHDRAWN

PU056

WITHDRAWN

PU057

PROGRESSION OF THROMBOEMBOLISM AND RENAL FAILURE IN PNH PATIENT WITH REDUCED HEMOLYSIS

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Background. Paroxysmal nocturnal hemoglobulinuria (PNH) is characterized by a somatic mutation in the PIG-A gene in the hematopoietic stem cell causing chronic uncontrolled complement activation ultimately leading to hemolysis, platelet activationand inflammation. Thromboembolism (TE) and renal failure are dangerous complications of PNH.Aims:To present a case highlighting severity and progression of disease in a PNH patient with low transfusion need, and subsequent successful treatment with eculizumab. Results. Patient, male 55 years old, presented in 1999 with mild hemolytic anemia and a negative Coombs test. PNH was diagnosed and treatment with chronic prednisone (25 mg/day), acetylsalicylic acid (100 mg/day) was initiated. Danazol was added in 2006 (50 mg/day). Blood transfusion need has been low (15 packed red blood cells over 14 years). The patient experienced several hemolytic exacerbations that were generally responsive to an increased dose of prednisone, but any attempt to reduce the amount of the drug caused return of hemoglobinuria and asthenia.Two exacerbations leading to hospitalization can be noted; three years after diagnosis he was admitted for acute renal failure (LDH 2773 U/L, haptoglobin 9 mg/dL, Hb 8.2 g/dL, creatinine 3,5 mg/dL), and a few years later he was admitted with renal failure, pneumonia, pulmonary hypertension, arterial hypertension. Flow cytometry showed a PNH clone of 97%, 97% and 57% on the granulocyte, monocyte and erythroid lineages respectively. In February 2012, the patient developed sepsis with hemoglobinuria, anemia (Hb 8.4 g/dL) and hemolysis (haptoglobin 10 mg/dL, LDH 485 U/L). CT scan showed thrombosis of the portal and splenic vein. Treatment with eculizumab and warfarin was initiated. The CT scan performed two months after the start of therapy showed complete resolution of the thrombosis. A year after therapy hemoglobin values have improved (Hb 10.9 g/dL), LDH levels have decreased (333 U/L), the patient shows good tolerance of the treatment, subjectively feels better, and prednisone and danazol have been permanently suspended. Conclusions: Our case confirms that in PNH patients immunosuppression does not protect against TE, which can occur also in patients with reduced hemolysis, and that eculizumab should be indicated in such cases. TE can be life-threatening and can occur also a long time after diagnosis. Eculizumab seems effective and well tolerated also in elderly patients with a long history of PNH.

PU058

STRONG EOSINOPHILIA CAUSED BY LENALIDOMIDE IN THE TREATMENT OF MYELODISPLASTIC SYNDROME. A CASE REPORT

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Eosinophilia is not mentioned among the side effects of lenalidomide. A single case of DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms) is reported in a patient treated for multiple myeloma (Foti *et al*, 2012). We observed a case of eosinophilia with no other signs or symptoms, caused by lenalidomide in a patient with myelodysplastic syndrome. R.R., male 75 years old, referred to our hospital in September 2011 for anemia. A bone marrow biopsy confirmed the diagnosis of refractory anemia with ringed sideroblasts (RARS), with trisomy 8 in cytogenetic analysis. The FISH study showed also deletion of 5q region in 6% of examined nuclei. The IPSS was intermediate-1. High transfusion requirement was present and the patient began treatment with alfa erythropoietin (EPO) 40000 U per week; the dose was increased at 40000 U twice a week after 2 months. Treatment had initial benefit of about 6 months, then transfusion requirement began to increase again. For the presence of a cell clone with 5q-, in September 2012 EPO was stopped and a treatment with lenalidomide was started at a dose of 5 mg for 21 days every 28. At the beginning of the new treatment, the blood count showed 5.5x1000/mm^3 WBC with 12% eosinophils (0.47x1000/mm^3); after 21 days WBC were 6.9 with 17.5 % eosinophils (1.21). The patient did not show any organ signs or symptoms. During the week of discontinuation of lenalidomide, the eosinophil count returned to normal. In all subsequent cycles of treatment, the patient had a normal eosinophil count on day 1 (mean 0.41, range 0.29-0.62), and a significant increase of the count on day 21 (mean 1.46, range 0.84 - 2.6). No signs or symptoms correlated with eosinophilia has never been observed. Therapy was always well tolerated but the dose was not increased further, in the fear of allergic reactions. After 5 cycles of treatment any benefit in terms of reduction of transfusion requirements was not observed. A new bone marrow examination in January 2013, showed increase of blasts to 10%. The new FISH study confirmed trisomy 8, while 5q deletion was no longer detectable. Treatment with Lenalidomide has been stopped and the eosinophilia was no longer observed. New IPSS was intemediate-2 and then patient started new treatment with azacitidine.

PU059

HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR OLDER PATIENTS WITH RELAPSED AGGRESSIVE NON HODGKIN LIMPHOMA (NHL). A SINGLE CENTER EXPERIENCE

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Background. In patients with relapsed aggressive NHL, High Dose Therapy (HDT) followed by ASCT, has the standard and more effective treatment. However as demonstrated in accord with International Prognostic Index that age above 60 years, is a poor prognostic factor for survival. Today is possible a better selection of elderly patients with relapsed NHL means specific Geriatric Score (MPI: Multifactorial Prognostic Index) and ASCT can be safely carried out with acceptable toxicity and Transplant Related Mortality (TRM). Aims. In this study we assessed the efficacy and safety of HDT and ASCT in older patients with relapsed aggressive NHL. Patients and Methods. From 2005 to 2012 we treated twenty-five patients with relapsed aggressive NHL, 16 male (64%); 9 female (36%); median age 61 yrs (range 60-71), 16 (64%) DLBCL, 5 (20%) Mantle Cell Limphoma (MCL), 4 (16%) transformed Follicular Limphoma (FL), 18 (72%) IPI score 0-1, 7 (28%) IPI score 2-3, at ASCT 5 (20%) Partial Remission (PR), 20 (80%) Complete Remission (CR). Number of regiments before HDT: 1 (20 patients, 80%), 2 (3 patients, 12%) and 3 (2 patients, 8%). All the patients were evaluated for ASCT by MPI (ADL, IALDS, SPMSQ, MNA, EXON–SMITH scale). Myeloablative chemotherapy consisted of 13 BEAM, 8 FEAM, 3 TEAM and 1 Mitoxantrone plus Melphalan. Attenuate dosing in 10/25 patients (40%). Results The median time to neutrophyl engraftment › 500 l was 9 days (7-13), and platelets › 20.000 12 days (9-15). Median of febrile neutropenia was 7 days (0-11). Median of packed red cell blood and platelet units transfused was respectively 2 (0-9) and 4 (1-13). Mucositis appeared in all patients: 2–3 WHO 20 (80%) and 3-4 5 (20%). Treatment–Related Mortality was 8%. One patient died of Multi Organ Failure and one of unknown causes after 10 days from the ASCT. Tree patients died after 60, 59 and 36 months after ASCT, of Myelodysplastic Syndromes (2) and unknown causes (1). Median follow-up was 36 months (12-96) with OS 61.1% and PFS 49.4%. Conclusions. HDT/ASCT can improve PFS and OS in older (› 60 years) patients with aggressive NLH compared to salvage chemotherapy alone. This study showed that in our experi-

ence, ASCT can be performed in older patients with relapsed NHL, carefully selected by IPI and MRI. Toxicity and TRM are acceptable. However in this patients ASCT may increase the secondary Myelodysplastic Syndromes.

PU060

DIFFUSE LARGE B-CELL LYMPHOMA PRESENTING DURING ACQUIRED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS OF UNKNOWN ORIGIN MANTEINANCE THERAPY: A CASE REPORT

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Hemophagocytic lymphohistiocytosis syndrome (HLH-S) is a rare immune dysregulatory disorder, resulting from prolonged and intense activation of macrophages, histiocytes and CD8+ T-cells. Hyperinflammation in HLH is accompanied by systemic manifestations. This condition might occur as either a familial disorder or a sporadic condition. We report a patient undergoing manteinance therapy for HLH-S who developed aggressive B-cell lymphoma (DLBCL). On February 2012, a Caucasian 58 years old man was admitted to the Medical Division of Padua Hospital, for persistent fever and severe fatigue. Preliminary blood tests showed marked normocitic anemia and increased levels of LDH without evidence of hemolysis. Bacterial, viral sierology and autoimmunity screening tests were negative and lymphocyte subpopulations were normally distributed. TC scan documented only a mild splenomegaly and PET-CT scan was negative for pathological uptakes. The suspect of HLH was further confirmed by the high levels sIL2-R, hyperferritinemia and hypertriglyceridemia. The bone marrow examination showed some activated macrophages and rare pictures of hemophagocytosis. The expression of perforin was decreased but mutational analysis excluded familial HLH-S, so we concluded for acquired HLH-S of unknown origin. The patient was initially treated with high dose steroid and intravenous high-dose immunoglobulin, immediately stopped for neurological and respiratory disturbances. A cerebral MRI documented vasculitis. Therefore the patient underwent to HLH 94 protocol treatment (etoposide and dexamethasone) with immediate clinical improvement. After induction therapy, anemia and neurological symptoms reappeared; thus etoposide treatment was resumed as a bridge to allogenic HSCT. During manteinance therapy, HLH-S related symptoms reappered and the patient developed a bulky inguinal lymphadenopathy. Histology was consistent with DLBCL. A combined therapy (R-CHOEP21) induced prompt clinical and bio-humoral improvement and resolution of lymphadenopathy. Although there was no clear evidence of lymphoma at the time of HLH-S presentation, the close time of its occurrence suggests a possible correlation between the two diseases. Etoposide probably controlled the cytokine storm, but did not affected or might even have favoured lymphomagenesis. In conclusion, during work-up and follow-up of HLH, occult malignancies should be carefully ruled out as they can manifest at diagnosis or appear during treatment.

PU061

WITHDRAWN

PU062

EFFICACY AND SAFETY OF PONATINIB IN MOLECULAR RELAPSE AFTER STEM CELL TRANSPLANT IN ADVANCED PHASES CHRONIC LEUKAEMIA PATIENTS. TWO REPORTS FROM HEMATOLOGY OF FLORENCE

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Ponatinib is a novel, next-generation, small-molecule tyrosine kinase inhibitor with potent activity against the BCR-ABL fusion oncogene as well as all other ABL kinase domain mutations that confer resistance to earlier generation TKIs. Due to its unique structure, it is the only tyrosine kinase inhibitor with the capability to counter the highly resistant T315I or gate-keeper mutation in leukemic cells that express the Philadelphia chromosome. We report two cases of CML-AP successfully treated with Ponatinib 45 mg/day for molecular relapse after performing SCT. A 43 years-old, male, had CML-BC on December, 2011. No ABL mutations were identified, while cytogenetic assay documented the presence of 3;6 translocation with EVI1 gene involvement. Extramedullary involvement (CNS) was present at diagnosis. Patient started with Dasatinib 140 mg/day and intratecal administration treatment. He achieved CHR after two weeks and he maintained this response for 3 months without achieving any cytogenetic response. Sibling donor was identified, so patient received High dose Ara-C and SCT received (June 2012). At that time bcr/abl ratio was 1%. At the engrafment, acute grade 2 GvHD occurred. On +47 days after SCT bcr/abl ratio increase to 13%. Then Ponatinib 45 mg/day, as compassionate use, started. Meanwhile patient had grade 3 hepatic GVHD, so DLI infusion was not indicated. After 30 days from Ponatinib administration bcr/abl ratio was 0,023% and after 30 days more undetectable. Ponatinib was well tolerated without any clinical impact on liver function or GVHD manifestation. Patient is alive, in MR 4,5, with extensive GVHD in Ponatinib treatment after 12 mths from SCT. A 67 years-old, male, was affected by Acute Lymphoblastic Leukemia (ALL) (Ph1+)(March 2011).He received chemotherapy (NILG protocol)with High dose of Imatinib. 0,06% bcr/abl ratio was obtained. The patient did not have sibling donor, then 4/6 cord blood unit identified. Unfortunately bcr/abl ratio increased to 50% and t315I mutation was found.By the way SCT was performed on December 2011.At +80 days from SCT aGvHD occurred (skin and lung), then immunosuppressive therapy was incremented.At +100 days bcr/abl ratio was 17%.Ponatinib 45 mg/day,as compassionate use, started.This therapy was well tolerated and bcr/abl ratio was undetectable(+130).Because of GVHD patient reeceived 3 lines of therapy.Patient died at +180 days from SCT because of bilateral pneumonia in undetectable bcr/abl.

PU063

COAGULATION DISORDERS IN ELDERLY PATIENTS WITH NON HODGKIN'S LYMPHOMA

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Acquired hemophilia is a rare disorder characterized by autoantibodies against coagulation factors. This condition may be associated with autoimmune disease, solid tumor, lymphoproliferative disorders and pregnancy. We describe three cases of indolent NHL who showed isolated prolonged aPTT and PT. 1) A 72 year-old man referred to our Institution because of recurrent epistaxis and abnormalities of coagulation tests PT INR 2.5, aPTT ratio 2.73. No history of bleeding disorders or new drug intake were reported. Splenomegaly with a focal lesion and pancytopenia were observed. Laboratory test showed a reduction of various coagulation factors activity: FVIII C 16% FII 44% FV 8% FVII 11% FIX 10% FX 30% FXI 27% and appearance of antibodies against many of them. Bone marrow exam showed a lymphoid infiltrate. PET/TC revealed high uptake in spleen and in a supraclavear lymphonode. FNAB enabled the NHL CD5-CD22+ + diagnosis. The patient made 6 R-CEOP courses and achieved complete remission with coagulation parameters and factor activity normalization. 2) A 62 year-old woman came to our observation for lymphoadenopathy, hepatoplenomegaly, anemia and lymphocitosis. She didn't present any bleeding disorders and didn't take drugs. Laboratory tests showed: PT INR 3.26, aPTT ratio 4.92; there was also a reduction of several coagulation factors activity: FVIII C 2.3% FII 32% FVII 47% FIX 1% FX 43% FXI 1% and appearance of antibodies against many of them. PET/TC revealed increased uptake at axillary and inguinal lymphonodes and spleen. Bone marrow analysis showed a lymphoid infiltrate and enabled the diagnosis of NHL CD5-CD22+ +. The patient made 6 courses of R- Fludarabine, and was observed a PT and aPTT normalization. 3) A 80 year-old man came to our Istitution because of pancytopenia and abnormalitites of coagulation tests: PT INR 2.28, aPTT ratio 3.2. No story of bleeding disorders. Laboratory tests showed reduction of various coagulation factors activity: FII 54% FV 68% FX 60%. PET/TC documented splenomegaly with

increased uptake. Bone marrow exam enabled the NHL CD20+CD19+CD5-CD22+CD23- + diagnosis. The patient made 6 doses of R-Leukeran and was observed a normalization of PT and PTT. All patients underwent to maintenance with Rituximab. The onset of an acquired coagulation disorder can be used as diagnostic marker of an immunological impairment due to an underlying lymphoprolipherative disease. Maintenance therapy may control the neoplastic clone reducing the risk of bleeding.

PU064

THIOTEPA AND MELPHALAN AS A NEW CONDITIONING REGIMEN FOR AUTOLOGOUS TRANSPLANTATION IN MULTIPLE MYELOMA

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Introduction. High-dose chemoterapy followed by autologous stem cell transplantation (ASCT) is actually considered the standard of care for younger patients affected by Multiple Myeloma (MM). There is no consensus both on the timing of a second ASCT (in tandem with first or delayed to disease relapse), and on the possible role of alternative alkylating drugs currently used in the allogeneic setting, such as Thiotepa (TT). Objectives. We investigated a new conditioning regimen including TT (10 mg/kg day -3) associated with Melphalan (Mel, 140 mg/m^2 day -2) in patients with MM and relapsing disease or not responding to first line therapy; endpoints of the study were feasibility in terms of toxicity, response rate and survival. Other eligibility criteria included age between 18 and 70 years and adequate cardiac, hepatic and renal function. Results. Between February 2010 and February 2013, 19 consecutive patients (male 8; female 11; median age 61,5) were conditioned according to this scheme; 9 were receiving TT/Mel as first ASCT while 10 were receiving it as second. At the time of transplant 3 patients were in CR, 4 in VGPR, 6 in PR, while the other 6 showed stable or progressive disease; median number of prior treatment lines was 3 (range 2–8). All patient received peripheral blood stem cells (Median number of nucleated cells reinfused was 2,6x10^8/kg) and engrafted, with a median number of days to neutrophil count ≥1000/mm 3 of 10 (range 9–11), and a median number of days to platelets count ≥20.000/mm3 of 12 (range 8–17). No patients died for transplant related mortality; median number of days of hospitalization was 11 (range 9-15). Most common adverse events included standard toxicities: 5 patients (27%) experienced oral mucositis (2 grade II/III), 10 patients (52%) gastrointestinal mucositis (4 of grade II/III) and only 1 patient experienced a mild liver toxicity, which was self-limiting. Febrile episodes, responding to empirical antibiotic therapy, was observed in 8 patients (42%). The overall response rate was 42% (7CR/1VGPR), 5 patients maintained PR (26%), while the other 6 showed active disease after ASCT. At a median follow-up of 18 months, 5 patients died for disease progression, while 14 were still alive. Conclusion. TT/Mel regimen was feasible and well tolerated in terms of toxicity; response rate and survival were as expected for MM, but efficacy in terms of disease control should be investigated in larger studies.

PU065

CONCOMITANT TRANSFORMATION TO MULTIPLE MYELOMA (MM) AND ACUTE BIPHENOTYPIC LEUKEMIA FROM 37-YEARS LASTING MGUS AND ESSENTIAL THROMBOCYTHEMIA (ET): A REPORT ON A VERY RARE OCCURRENCE

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The association of MGUS with ET in the same patient is quite rare. With an anecdotal purpose, we herein report the long-lasting clinical history of an our patient who presented the simultaneous evolution to MM and to acute biphenotypic leukemia from MGUS and ET respectively, have been the latter conditions diagnosed 37 years before. In 2010, a 77 years-old man came to our center because of increasing thrombocytosis and monoclonal paraproteinemia. In 1975, at another center, he was diagnosed as having MGUS associated with ET. The patient was managed according the clinical guidelines of that time and received low-dose acetylsalicylic acid (LD-ASA). The patient then came to our attention 35 years after reporting that from a few years ago he has not been attended the prescribed periodic laboratory evaluations and hematologic visits. Therefore, a comprehensive work – up was performed. An Ig-G lambda MGUS and a Jak-2 negative ET were found. Given the remarkable thrombocytosis, hydroxyurea was added to LD-ASA. Two years later her hemogram showed pancytopenia concomitantly to the increase of monoclonal protein concentration higher than 4 gr/dL. The examination of bone marrow (BM) aspirate revealed a 30% of plasma cells (PC) which immunophenotype was positive for CD45, CD38, CD138, CD56 and Ig-G lamba along 20% of blastic cells; the latter, showed the coexpression of lymphoid and myeloid markers, being positive for CD34, CD13, CD33,HLA-DR, CD19 and CD22. The BM threphine biopsy (Figure 1) confirmed the BM infiltration by PC and blasts. The karyotype was normal. Laboratory and radiologic evaluations revealed moderate Bence Jones proteinuria (type K) and mild pancytopenia but not other abnormalities. In particular, renal failure and bone lesions were ruled out. So that, the diagnosis of MM coexisting with blastic transformation of ET was made. The patient was evaluated as a possible candidate for treatment with hypomethylating but he sudden deteriorated and soon died because of pneumonia. Lacking practical therapeutic implications and reliable indications in order to manage this uncommon occurrence, for its rarity our description has an anecdotal value. As for speculation, the synchronous evolution of TE and MGUS along the coexpression of lymphoid antigens by blastic cells may raise the debate about the common origin of two malignancies which may be evolved by a progressive transformation, until the final development of more aggressive disorders, from a common precursor, although this question remained unresolved.

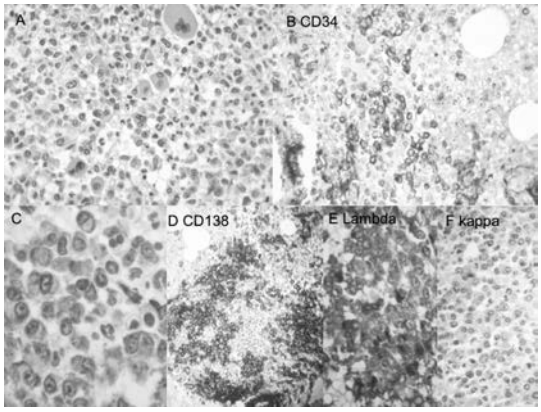

Figure 1. A: BM biopsy shows increased of immature precursor and dismegakariocytopiesis. B: immunostaining for CD34 antibody reveals 20% of blasts. C: clusters of plasma cells (PC) with mature-like morphology. D: immunostaining for CD138 shows strong membrane staining by sheet of PC, representing 25/30% of BM cells. E – F: immunostainining for light chains reveals monotypic cytoplasmic expression of lambda light chain, suggesting the neoplastic nature of PC

PU066

PLERIXAFOR AND G-CSF AFTER CHEMOTHERAPY TO MOBILIZE PERIPHERAL STEM CELLS FOR AUTOLOGOUS TRANPLANTATION. ANALYSIS OF COLLECTION AND ENGRAFTMENT CHARACTERISTICS OF 25 PATIENTS WITH LYMPHOMA AND MULTIPLE MYELOMA

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Background. High-dose chemotherapy and autologous stem cell transplantation (ASCT) has become a standard procedure for many patients (pts) with non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HD) and Multiple Myeloma (MM). A requirement to undergo ASCT is adequate mobilization and collection of hematopoietic progenitor cells from the peripheral blood (PB). Chemotherapy plus granulocyte colony-stimulating factor (G-CSF) is a common strategy to obtaining peripheral blood stem cells (PBSCs). Neither less, up to 30% of pts fail to collect a minimum number of CD34 cells to support ASCT. Plerixafor (AMD) in

combination with G-CSF results in increased mobilization of CD34+ cells compared to G-CSF alone, also in poor mobilizer pts. Patients and methods Between October 2009 and November 2012, 25 pts (16 NHL, 1 HD, 8 MM) underwent mobilization with chemotherapy plus G-CSF. Patients' characteristics are shown on Table 1. 4 pts (16%) were identified as predicted poor mobilizer and 21 pts (84%) as proven poor mobilizer (if PB CD34+ cells count ≤10/ L on 2 consecutive days or PB CD34+ cells after first apheresis too low and CD34+ yield <2x10^6/kg). Results In this report we compared our results with a control group of 25 consecutive pts with NHL and MM undergoing mobilization with disease specific chemotherapy plus G-CSF, referred to the same period. After AMD administration and before the first apheresis, the median number of circulating CD34+ cell was 25.3/ L (7.6-55.0) with a median of 4.3-fold increase (2.01-11.7) with a significant difference (p=0.022) with the control group (47.1; 12-302). Time to collection was 17 days (5-22) versus 14 days (10-21) for control group (P= 0.008). After AMD, all pts successfully reached the target dose of CD34+cells for ASCT (median 5.1; range 2.3-7.8), with a median number of 2 apheresis, in comparison with the control group (1; P=0.0001). 19 pts (76%) mobilized with AMD received ASCT with a CD34+ median dose of 2.77 x10^6/kg (1.8-6.2) and a significant difference (P=0.0186) with the control group (3.86; 1.52-6.77). There was no significant difference in terms of both neutrophil engraftment (>500 and >1000/L) and platelet (>20000 and >50000/L). Conclusion In this study we confirm the efficacy of AMD to mobilize and collect an adequate dose of CD34+ cells in pts with lymphoma and multiple myeloma without significant difference in term of engraftment in respect of pts mobilized with G-CSF and chemotherapy.

Table 1.

Patients' characteristics	N° (%)	Median (min-max)
N°	25	
NHL	16 (64)	
HD	1 (4)	
MM	8 (32)	
Età		54.5 (22.0 – 73.0)
Sesso m/f	19/6	
Proven poor mobilizer	21 (84)	
Predicted poor mobilizer	4 (16)	
Disease status at mobilization		
Complete Remission	11 (44)	
Partial Remissione	10 (40)	
Advanced phase disease	4 (16)	
Previous chemotherapy courses		2 (1 – 5)
Mobilization regimen		
ARA-C containing regimen	12 (48)	
EDX-HiD	10 (40)	
Others	3 (12)	
Plerixafor (AMD) mobilization results		
WBC (x 10e3/µL) after AMD		24.3 (8.08-84.4)
Hgb (g/dL)		9.80 (7.80-14.9)
PLT (x 10e3/µL)		37.0 (15.0-339.0)
CD34/ µL before-AMD		5.9 (2.8-12.0)
CD34/ µL before-apheresis		25.29 (7.63-160.98)
CD34/ µL after-apheresis		17.39 (7.21-193.76)
N. of apheresis		2 (1-3)
Time to collection		17 (5-22)
Apheresis data		
Blood volume processed (Lt)		13,16 (8,68-18,89)
Procedure time (min)		314 (236-390)
CD34 efficiency (%)		71.17 (24.82-93.83)
MNC efficiency (%)		56.90 (17.74-73.66)
Platelet efficiency (%)		4.7 (2.09-15.5)

PU067

THERAPEUTIC EFFICACY AND GOOD TOLERABILITY OF LENALIDOMIDE IN REFRACTORY ANEMIA WITH RINGED SIDEROBLASTS AND THROMBOCYTOSIS (RARS-T) WITHOUT DELETION 5Q

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Case Report. D.F.,76 years, male; since 2007 affected by not deficiency macrocytic anemia (hgb 11>10 g/dL, MCV 115) with thrombocytosis (plts 480.000-510.000/µl).In 2008, a diagnosis of low-grade MDS (refractory anemia, endogenous EPO high levels, normal spleen) was made. At this time, the patient was treated with high doses recombinant epoetin, prednisone low doses and danazol. During the follow up, anemia progressively worsened(hgb 9.9>8.1) always with mild thrombocytosis (plts 470.000-565.000/µl) and mild splenomegaly (ultrasound bipolar diameter cm 15). April 2011: the patient was revalued at our institution because symptomatic anemia (hgb 7.1 g/dL, MCV 126, plts 565.000/µl, wbc 4400/ul). Bone marrrow was hypercellular with trilinear dysplasia, marked erythroid hyperplasia, blasts <5%, grade 2 fibrosis; PERLS: iron deposits well represented, many ringed sideroblasts(65%). Cytogenetics, FISH, PNH phenotype evaluation, JAK2 V617F, bcr-abl were negative. Epoetin treatment was suspended and RBC transfusion treatment began (9 RBC packed units through December 2011, 8 units from January to April 2012) with deferasirox 10 mg/kg/day. In December 2011, during a routine echocardiographic monitoring, a severe pulmonary hypertension (PAPS 59 mmHg) with pulmonary scintigraphic pattern of chronic venous microembolism (subclinic?) was diagnosed and the patient was started on anticoagulant treatment(warfarin, INR target 2.5-3.0). March 2012: due to worsening of symptomatic anemia, the high transfusion requirements (up to 3 RBC units/month) and the ineffectiveness of high-dose epoetin treatment, off-label treatment with lenalidomide 10 mg per day was started, on the basis of several clinical reports demonstrating the efficacy of lenalidomide even in cases of transfusion-dependent low grade MDS and RARS-T without deletion 5q. Treatment with lenalidomide resulted in a rapid and steady improvement of the anemia with a progressive rise of hemoglobin rate up to 11.5 g/dL (April 2013), an early abolition of RBC transfusions need already after the 2nd treatment cycle (April 2012) and a normalization of platelet counts. The treatment was well tolerated without any type of both clinic and haematological toxicity nor thromboembolic complications; the echocardiographic indeces of pulmonary hypertension have improved. Due to rapid correction of anemia, the patient (with dynamic lifestyle, still active in the professional) achieved a marked and stable clinical improvement.

PU068

PRIMARY PULMONARY LARGE B CELL LYMPHOMA MEDIASTINAL TYPE: A CASE REPORT

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Primary Mediastinal B-Cell lymphoma (PMBCL) accounts for 2-4% of non Hodgkin Lymphoma most commonly affecting young adults and usually presents with antero-superior mediastinal mass. In this report we present a case of PMBCL occurring outside the mediastinum. In November 2012 a 28-year old female patient previously diagnosed with scleroderma presented with a six months' history of mild fever and night sweats. The patient underwent X-ray chest radiogram showing left basal parenchymal infiltration for which she received different unsuccessful antibiotic treatments. In January 2013 a whole body PET-CT scan showed an extensive area of increase glucose uptake (SUV 27) in the basal left lung parenchyma. The chest CT-scan showed a massive solid mass of the left lower lobe of the lung (cranio-caudal diameter 11 cm), a minimal left pleural effusion and a mediastinal lymph node of 12 mm. The patient was admitted to Pneumology Department and underwent a core biopsy of the mass. The histological features were compatible with Primary Mediastinal B-cell Lymphoma (PMBCL) with the following immunostaining pattern: CD20+, CD23+/-, MUM1/IRF4 +/-, CD30-, CD3-, Ki67 80%. The abdominal CT

scan and trephine bone marrow biopsy were normal. LDH tested within normal range value. The final diagnosis was Primary Mediastinal B-Cell non Hodgkin Lymphoma with exclusive pulmonary involvement, defined as stage IE Bulky. The patient received 6 R-CHOP 14 cycles along with 6 intrathecal prophylaxis with methotrexate until April 2013 without major complications. An intermediate CT scan performed after the third R-CHOP 14 cycle showed marked reduction of the basal lung lesion (2.5 cm x 2.8 cm) and complete resolution of the pleural effusion. The post-chemotherapy assessment is scheduled for the end of May. The present case reports on a rare extra-mediastinal presentation of Primary Mediastinal B-Cell Lymphoma. Previous case reports have shown that Gene Expression Profile pattern of PMBCL with extra mediastinal involvement is found similar to PMBCL cases. Considering our patient's good intermediate response to the chemotherapy schedules commonly used in PMBCL patients, we would expect a similar outcome. Further studies are warrented to better define this anatomical entity of PMBCL as far as treatment and outcome, with particular concern on the use of PMBCL-like treatments or more dose-intensity drug regimens.

PU069

BISGLYCINATED CHELATED IRON IN SIDEROPENIC ANAEMIA TREATMENT OF CELIAC PAZIENTS WITH REFRACTORINESS TO THE OTHERS IRON TERAPIES AND IMPROVEMENT OF LABORATORISTIC HAEMOGLOBINIC PARAMETERS

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Sideropenic anaemia is very frequent in Celiac patients. The Celiac disease, diagnosed with a safety intestinal biopsy of the second duodenal part, is characterized by a damage of the proximal intestinal mucous membrane with a bad absorption for its insufficient action, and alterations of epithelial surface absorpting digestive's products; this can be related to the enzymes of the enterocyte's brush-border or to the alterations of the intracytoplasmatic mechanisms transferring and metabolizing digestive products, iron included. The goal standard of this randomized study is to value the efficacy of bisglycinated chelated iron in secondary sideropenic anaemia in Celiac patients. The iron chelated with two glycina molecules (AA with lower molecular weight) is agree with the organism; its chemical properties are characterized by a best farmacological profile with better absorption, better gastrointestinal tolerability, no interference with diet; and his aminoacidic bond makes it available and assimilable. Consecutively in 2011, 20 patients are chosen by this study (15 Female/ 5 Male) with a Celiac disease: -the continuous presence of anaemia, after irregularly treatment with iron sulphate for os in cyclic way for time inadequate to obtain therapeutic answers, needed a change of therapy and this iron was prescribed as first line with a progressive mitigation and disappearance of symptomatology after one month; -everybody was valuated at the start of therapy (T0), after a first period of therapy (T1), generally 2 months and at the end of eighteen months of follow-up, with a diet and a regular way of life and the observation of three parameters: Hb, Haematic Iron and Storage Iron. The numbers clear a great percentage increase of 30,02% for Hb, 139,1% for Haematic Iron and 313,5 % for Storage Iron. Sideropenic anaemia is common in celiac patients. It's efficacious remedy performed for right time permits to correct peculiars symptoms of this disease and assures a good quality of life. These results following a follow up period of 18 months confirm the capacity of this Iron to re-establish quickly the iron supplies and to keep in time physiologic levels of the iron parameters. This study is very important for its meaning and the great percentage gain of laboratoristic parameters makes conspicuous the validity of this product, if it is dispensed with adequate record; these data confirm the possibility to open a new therapeutic scenary in management and control of sideropenic anaemia in Celiac disease.

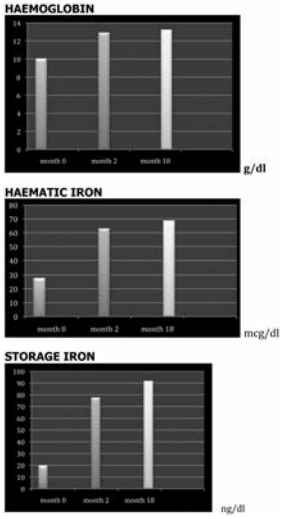

Figure 1.

PU070

EFFICACY OF A TREATMENT WITH AZACITIDINE IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Azacitidine improves overall survival in patients affected by higher risk myelodisplastic syndromes as shown by previous studies, but more recent evidences are growing up about its efficacy in acute myeloid leukaemia (AML). Here we report three cases of older patients affected by AML de novo who obtained response after azacitidine. At diagnosis the characteristics of the patients were: median age 81 (range 79-86); male/female ratio 1/2; median WBC count x10^9/L was 13 (range 5,3-23); median time from diagnosis to azacitidine was 2 months (range 1-5); median bone marrow blast count was 40% (range 20-40); Cytogenetic risk group according MRC was intermediate (normal karyotype) in a patient, not valuable in the remaining two patients. Azacitidine was administered subcutaneously at conventional dose until progression disease; the median number of cycles received was 9 (range 8-10). After six cycles of azacitidine all patients achieved a response (one complete response, two complete responses with incomplete recovery); all patients reduced the transfusion need; at a median follow up of 15 months (range 9-16), only one patient was alive; the median overall survival was 11 months (range 8-14); the median overall response duration was 9 months (range 7-11). Adverse effects were irritation at injection site, febrile neutropenia (for overall three episodes) and persistent G3 neutropenia (in a patient, until progression). Here we have shown: all patients treated with azacitidine showed an increased response rate and improved median overall survival. These data compare consistently with the other reports in which the median overall survival varies from 10 to 24 months. Throughout azacitidine therapy transfusion need decreased and the quality of life ameliorated. This drug is a good chance, although further and larger randomized studies are needed to define the role of azacitidine as first line for the elderly patients.

PU071

ITP IN A PATIENT WITH CROHN'S DISEASE TREATED WITH MESALAZINE: CASE REPORT

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Mesalazine, used in the treatment of Crohn's disease, has been associated with a number of non-common hematologic complications, including aplastic anemia, neutropenia and immune thrombocytopenia. However, no clear association has been found so far. We describe the

case of a 32 years old girl with active Crohn's disease (diagnosis on 2007) who developed immune thrombocytopenia (ITP). From September 2011 to January 2012, she had several hospitalizations for exacerbations of her Crohn's disease; she was treated with prednisone and subsequently (from November 2011 to January 2012) with Mesalazine. Her blood counts were normal until January 2012 when her platelets count dropped down to 6.000/mmc. The patient was then admitted to our Division for bleeding appearance of skin and mucosa membranes; she was also symptomatic for fatigue, fever and pain in the lower limbs. A peripheral blood smear showed marked thrombocytopenia with occasional giant platelets and no evidence of microangiopathic haemolytic anaemia. A bone marrow biopsy specimen revealed normocellularity with megakaryocytic hyperplasia with widespread distribution and discrete signs of dysmegakaryocytopoiesis, compatible with peripheral platelet destruction. The viral serology for virus related to ITP and the search for H. pylori infection on stool was negative. Mesalazine was discontinued and thrombocytopenia was managed, in order, with: Prednisone (1 mg/kg/die), high dose-intravenous immune globulins, Rituximab 375 mg/mq one dose a week for 4 weeks and finally with splenectomy We conclude that in patients who develop bleeding, anemia or fever of unclear etiology during treatment with Mesalazine, haematological investigations should be conducted and therapy discontinued, especially in case of blood dyscrasia. The correlation between Crohn's disease and ITP has been so far investigated without clear association. It would be interesting to determine if Crohn's disease and Mesalazine, in conjunction with particular HLA-alleles, can promote the onset of ITP particularly with resistant phenotype.

PU072

ANAPLASTIC CD30+ ALK- (MICRO)LYMPHOMA ARISING OUT OF MEDIASTINAL PLASMA CELL CASTLEMAN'S DISEASE IN IMMUNOCOMPETENT PATIENT TREATED BY RITUXIMAB-DEXAMETASONE AND CONTINUOUS LENALIDOMIDE

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Case Report. D.O, female, 50 years; from February 2011 dry tickly cough, night sweats, mild dyspnea (no fever nor weight loss); laboratory: mild anemia (hgb 10.9), ESR 107, CRP 227, b-2-mg 3.3, polyclonal hypergammaglobulinemia (29.9%), LDH 361. A chest X-ray and a subsequent whole body CT-PET showed mediastinal limphoadenopathy (aortopulmonary window) 3-5 cm, SUV 6,35. A CT-guided microbiopsy proved suggestive of a non neoplastic, chronic atypical lymphoproliferative disorder. In June 2011, at our institution, we performed a thoracoscopic lymphnode biopsy thar showed histologic features of plasma cell variant Castleman's disease. In August 2011, due to the unique location of mediastinal lymphadenopathy confirmed by a new CT-PET, we performed a thoracotomic excision of lymphnode package (max diameter 6.5 cm). The histology confirmed the framework of angiofollicular lymphoid hyperplasia plasma cell variant, but revealed in all the sections the presence of parafollicular clusters or sheets of anaplastic large cells with large eosinophilic cytoplasm, vesicular nucleous, single eosinophilic macronucleolus or multiple nucleoli, rare RS/H like cells, with immunophenotype CD30+ ALKC/ALK1- CD15- MUM1+ CD20 +/- CD79a +/- Ki67+ 40%. CD68PGM1- CD138- K/Lambda- CD10- CD23- bcl-2- bcl-6- CD3- CD56- HHV8-, CK-, EMA- (the molecular analysis by microdissection was non-contributory). The bone marrow was trilinear with a polyclonal plasmacytosis (10%), normal amount of T/B lymphocytes and blasts. In December 2011, a whole-body CT-PET showed the persistence of active mediastinal lymphadenopathy (4 cm, SUV 10.36). Therefore, we started a treatment with rituximab 375 mg/sqm for 4 weeks plus high-dose dexamethasone (40 mg days 1-2, 8-9, 15-16, 22-23) followed by the complete normalization of laboratory indices of disease activity. Since January 2012, the patient was then treated with lenalidomide 25 mg/day until April 2013 (12 cycles) plus ASA, dexamethasone 120 mg/month for 6 months and rituximab every 2 months (until June 2012) The treatment was well tolerated with minimal toxicity: mild leucopenia, marked hypogammaglobulinemia (no infectious complications), superficial phlebitis at right lower limb treated with temporary lenalidomide discontinuation and LMWH. The CT-PET monitoring carried out until December 2012 showed a marked progressive reduction in lymphadenopathy and PET uptake (1.76 *vs* 10.36). The patient is currently asymptomatic, in good general clinical condition.

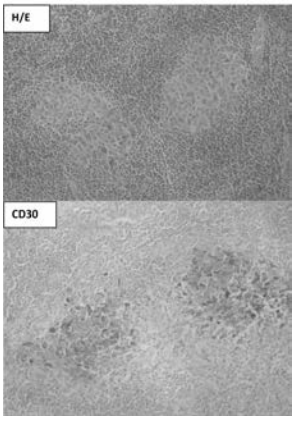

Figure 1

PU073

INFLIXIMAB FOR STEROID REFRACTORY GASTROINTESTINAL ACUTE GRAFT *VS* HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Treatment of pts with corticosteroid-refractory acute graft versus host disease (GVHD) following allogeneic stem cell transplantation (SCT) is a major challenge since their prognosis is negatively affected by an excess in transplant-related mortality (TRM). Due to the possible involvement of -TNF in the pathogenesis of GVHD, the anti-TNF monoclonal antibody infliximab has been proposed as a possible effective agent in different settings of GVHD management with contradictory Results. In this study, we retrospectively analyzed data about pts who were treated with infliximab for refractory intestinal GVHD in our BMT Center. From 2002 to 2012, 11 pts with corticosteroid-refractory grade III intestinal GVHD received infliximab 10 mg/kg/week over four consecutive weeks. Diagnosis of intestinal GVHD was histologically proven in all but one case. Steroid refractoriness was defined as a failure to achieve a response after 10-14 days of 2 mg/kg methylprednisolone. Response included resolution of the clinical signs, normalization of the laboratory signs of malabsorption and regression of hypervascularization at ultrasound scan. Nine pts received infliximab after one to three additional lines of immunosuppressive therapy. Median time from SCT to start of infliximab was 74 days (range 31-500). Nine pts had isolated intestinal GVHD and two hepatic/intestinal GVHD. At the end treatment, 9 pts (82%) had complete remission (CR) of the clinical signs of GVHD and laboratory signs of malabsorption. At ultrasound scan of the bowel, hypervascularization had resolved, with or without residual thickening of the bowel wall. One patient had a partial response (PR) and an additional patient had no response. At the last follow-up, 9 pts had died: four of aspergillosis, two of progressive hepatic GVHD, one of cerebral toxoplasmosis, one of graft failure and one of relapse. Median time from start of infliximab to death was 6 months (range 4-12). None of the responding pts had signs of relapsing bowel GVHD at the date of death. The patient achieving a PR is still alive and well on combined therapy with cyclosporine and rapamycin.Only one patient achieving a CR is alive and well 30 months after discontinuation of infliximab. Altogether, while confirming the ineffectiveness of infliximab on extra-intestinal GVHD, our data strongly support the design of prospective trial to definitely assess the efficacy of infliximab for intestinal GVHD after failure of first line corticosteroid therapy.

PU074

RITUXIMAB, IFOSFAMIDE AND VINORELBINE IN PRIMARY RESISTENT CUTANEOUS DIFFUSE LARGE B CELL LYMPHOMA LEG TYPE. A ICONOGRAPHIC CASE REPORT

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Cutaneous lymphomas represent a unique group of lymphomas and are the second most frequent extranodal lymphomas. B-cell lymphomas account for the majority of nodal lymphomas, whereas primary cutaneous B-cell lymphomas (CBCLs) represent 20-25% of all cutaneous lymphomas. Primary cutaneous diffuse large B-cell lymphoma, leg-type (PCBCL-LT) is less common than other CBCLs, but is usually more aggressive (fast-growing), developing over weeks or months. This lymphoma usually appears as red or bluish-red lesions on the lower legs, although lesions can occur on any part of the body. The lesions frequently grow into large tumors that extend deep into the body. The lesions may become open sores and spread outside the skin more frequently than the slow-growing CBCLs. This distinct entity has a poor prognosis, particularly in patients with multiple tumors on the legs. We present the case of a female patient, 76 years old, diagnosed CBCLs leg type (Ki67 70%, BCL2 positive) with leg and nonleg multiple lesions. First line therapy was R-CHOP but already during the third cycle was observed a rapid progression of lesions in nonleg site (see photos). We then decided to treat the patient with the combination of intravenous (IV) rituximab 375 mg/m^2 day 1, ifosfamide 1,000 mg/m^2 as a continuous infusion days 1 to 3, IV vinorelbine 25 mg/m^2 day 1 and MESNA 1,000 mg/m^2 as a continuous infusion days 1 to 3, and oral prednisone 1 mg/kg days 1 to 3, repeated every 28 days for 6 cycles. Pegfilgrastim support (6 mg at day 7) and epoietin alfa 40,000 IU/week subcutaneously was used. The treatment was repeated for six cycles and was well tolerated, with obtaining a complete remission of the disease and skin lesions disappeared already after three cycles (see photos). The patient maintains complete remission after 20 months from the end of the therapy and skin biopsy performed after treatment does not view lymphocytic infiltrates. Recently, EORTC/ISCL consensus recommendations for the management of CBCLs have been formulated, but the best treatment in refractory/resistant PCBCL-LT is unknown. The combination of ifosfamide and vinorelbine is used in treatment of nodal lymphomas, but has not been used in refractory cutaneous lymphomas. This case report suggest a good efficay in relapsing PCBCL-LT and appears as an attractive combination, more specially for a manageable toxicity even in advanced age patients.

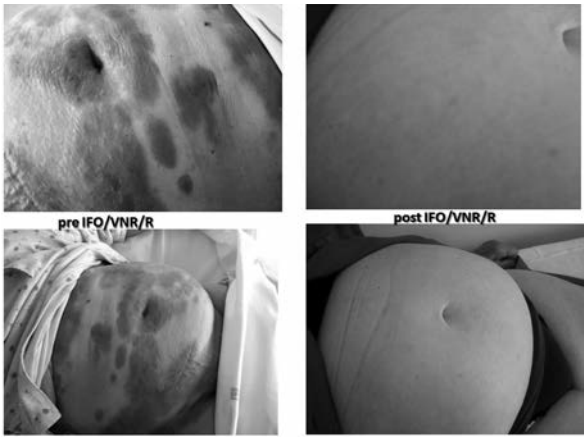

Figure 1.

PU075

HIGH DOSE CYCLOPHOSPHAMIDE FOR THE TREATMENT OF SEVERE MILLER-FISHER SYNDROME, FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Miller-Fisher syndrome is a variant of Guillain Barre' syndrome. It involves the cranial nerves, and can be rapidly progressive leading to respiratory failure and death. Aim of the study. We report 2 cases of Miller Fisher syndrome in patients after an allogeneic stem cell transplant (SCT). Cases. A 56-year-old male presented with visual impairment, on day +150 after an unrelated donor transplant. The patient in one week rapidly developed hearing loss, facial palsy and respiratory insufficiency. He was intubated, sedated and transferred to the intensive care unit. Neurologists made the diagnosis of Miller Fisher. Patient was refractory to high dose steroids and high dose intravenous immunoglobulin. We were unable to convince the neurologist to administer high dose cyclophosphamide. The patient died with respiratory insufficiency and multi organ failure after 69 days on a respirator. Shortly after this patient died, a second case was recorded in a different transplant unit: this was a 19 year, on day +300 after an allograft from an HLA identical brother for CR1 AML. At that time, the patient developed mild dysphagia and dysphonia, followed one month later by emesis and rhinolalia, and then by overwhelming dysphagia and dysphonia. At that stage soft palatal palsy could be appreciated upon neurologic examination. The patient was transferred to the intensive care unit due to acute respiratory failure. Prostigmin test was positive so that pyridostigmine and plasma exchange were started. Despite an initial clinical improvement, neurological symptoms and respiratory function rapidly worsened to life-threatening level and tracheal intubation with mechanical ventilation were required. After discussion of the outcome of case n.1, the decision was made to treat case n.2 with cyclophosphamide single dose, 4 gr/m^2. The improvement was fast and dramatic: 3 days later mechanic ventilation was discontinued and the patient returned to the hematologic ward and twenty-five days later was discharged home. The patient has been free of neurologic symptoms for the following 4 years. Conclusions. These two cases illustrate a Miller Fisher like syndrome, occurring after an allogeneic HSCT. The dramatic and lethal course of case n.1 prompted early treatment of case n.2, with high dose cyclophosphamide, which proved effective, and lifesaving also in keeping with the hypothesis of an immune pathogenesis of the disorder.

44° Congress of the Italian Society of Hematology
Verona, Italy, October 20-23, 2013

MAIN PROGRAM

REALITY AND ISSUES IN CHRONIC LYMPHOCYTIC LEUKEMIA TODAY

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Epidemiology

Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in Western countries, representing about 30% of all adult leukemias, with an incidence of about 4 new cases per 100.000 individuals per year in the US. Its epidemiology is intriguing, as in Asia CLL it is almost 10 times less frequent, with an estimated incidence of around 0.48 per 100.000 individuals per year, which does not change in Asians living/born in the Western world. The majority of patients are elderly at diagnosis; indeed, the updated median age at first presentation in the US is ~70 years.

The epidemiology of the disease has changed over the years and, as a consequence, the prevalence of CLL continues to increase. First, the incidence of CLL increases with age, with about 9% of patients diagnosed between 45 and 54 years, 20% between 55 and 64 years, 27% between 65 and 74, 29% between 75 and 84, and 13% above 85 years of age. Second, nowadays CLL at diagnosis is more frequently in early stage, due to an anticipated identification as a consequence of a broader use of routine blood tests. Third, as most early stage patients after the diagnosis are observed for years before requiring treatment, this means that more than half of the patients who require therapy are above the age of 70. Fourth, due to the extended life expectancy in Western countries, the burden of people diagnosed and living with CLL is continuously increasing. This trend is geographically-related due to the differences in median life expectancy (and in the resulting aging of the population), that in Europe is in general over the age of 80, while it is about 10 years younger in the Gulf area and 30 years younger in central Africa. Finally, there are marked geographic differences in the median age of the local population. Italy, for instance, is a country of elderly individuals. In 2007, the over 65 were calculated to be 11.8 millions, representing 19.9% of the entire population. It has been estimated that this could increase to 26.5% by 2030. Between 1980 and 2005, the number of over 65 increased by 50%, while that of the over 80 by more than 150%. A continuous and conspicuous growth in the elderly population is a reality.

Thus, young hematologists are expected to see an increasingly higher number of patients with CLL and always older patients. The understanding of the reasons of the different geographic distribution will contribute to elucidate the pathogenesis of the disease.

Diagnosis and prognosis

A correct diagnosis of CLL still remains an important starting point, even in 2013. First, the morphology of circulating lymphocytes should always be associated to an immunophenotypic characterization. Second, if the classic immunophenotype - CD5+, CD20+, CD23+, CD200+, kappa or lambda+ (weak), CD79b-, FMC7-, CD18-, CD11a- - is not fulfilled, the immunophenotypic characterization should be supported by other integrated techniques (*i.e.* histopathology, FISH, molecular biology) for a precise differential diagnosis with similar indolent conditions, nowadays managed and treated with an often completely different approach (*i.e.* mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma, etc). Third, immunophenotype at diagnosis/progression should also include a dedicated extended panel in cases who will undergo an assessment of minimal residual disease (MRD) during and after treatment.

The prognostic work-up of a CLL patient can be based nowadays on a broad variety of assessable markers. The role and impact of the biologically-based prognostic factors is still a matter of debate and their use is extremely variable according to local practice, research interests and financial restraints. Different key issues still need an answer. Within these: which biologic prognostic markers are really useful today? When should they be performed (at diagnosis, at the time of treatment, at re-treatment)? For which patients? Does it depend on age and/or performance status? Moreover, large trials focusing on biologic prognostic factors in the elderly are lacking. Could they change according to age? It is likely that their impact is less relevant than the clinical features such as the burden of comorbidities, although this has to be proven. In addition, how and where should they be performed? Standardization of complex technologies and/or network of specialized laboratories is and will be essential, especially for those markers that can guide treatment decisions. Finally, could there be differences in the prevalence and impact of the various prognostic markers in different geographic populations? In recent years, our knowledge of the molecular genetics of CLL has significantly broadened, offering potential new prognostic factors (for example the mutational status of the variable region of the Ig heavy chain genes) and clinical implications. In some instances, the clinical implications of these molecular lesions are well established. This is the case for patients with TP53 disruption, who should be considered unsuitable for alkylator- and purine analog-based therapies in order to avoid toxicities associated with therapies that have a low likelihood of efficacy in favor of alemtuzumab, steroids, possibly ofatumumab or alternative terapies and allogeneic stem cell transplant in selected patients, or for patients with ATM disruption, who are candidates to rituximab-based immunochemotherapy. Next generation sequencing technologies have allowed to identify previously unknown genetic alterations in CLL, although not revealing a unique and specific genetic abnormality with pathogenetic relevance. Mutations of NOTCH1, SF3B1 and BIRC3 have been associated with short time to progression and survival. Each of these lesions recognizes a different distribution across different clinical phases and biological subgroups of the disease. Their clinical value is currently being validated, *i.e.* association to Richter syndrome transformation for NOTCH1 mutations and short progression-free survival (PFS) after treatment for SF3B1 mutations. Certainly, these new lesions have helped clarify the molecular bases of CLL aggressiveness beside TP53 disruption. Prof. Gianluca Gaidano will specifically cover the relevance and challenges of the new technologies in CLL research and management.

Treatment

Traditionally considered a chronic disease of the elderly, for decades CLL has been managed with a conservative approach and limited therapeutic options. Since the nineties, the therapeutic options have progressively broadened. However, many of the improvements in the prognostic and therapeutic management of CLL do not apply to all patients with this disease, since the majority of CLL patients are elderly and suffer from comorbidities, which tend to progressively increase with age. This patient population is underrepresented in clinical trials, where the median age is in the late 50s to early 60s; even in trials specifically designed for the elderly, a very small proportion of patients enrolled is 75 or older. In fact, the only recommendation of the new IWCLL guidelines on future clinical trials involving elderly patients is to assess their comorbidities and/or functional activity. Management of elderly patients has remained a primary unmet clinical need until recently, when evidence-based therapeutic strategies exploring the association between chlorambucil and anti-CD20 monoclonal antibodies are being reported

in Europe by the Italian and British trials. Age has been defined as a progressive loss of tolerance to stress, due to a decline in the functional reserve of multiple organ systems (*i.e.* decreased renal excretion of drugs and increased susceptibility to myelosuppression, mucositis, cardiotoxicity and neurotoxicity), high prevalence of comorbidities, limited socioeconomic support, reduced cognition and higher prevalence of depression. The majority of individuals who are physiologically old are over the anagraphic age of 70 years, which does not imply that all individuals above 70 are physiologically old. Therefore, the assessment of the physiologic age after the age of 70 is necessary for optimal clinical decisions, to distinguish the so-called "fit" and "non-fit" patients. Novel and key clinical issues related to this increasingly large proportion of patients need an answer. Within these: 1) How to dissect between the anagraphic and biologic age? 2) In a chronic and so far incurable disease, how to live with the leukemia? 3) Which are/should be the goals of treatment? 4) How should the benefits and risks of treatment be balanced in individuals with a reduced life expectancy and treatment tolerance? 5) What are individual expectations of this special category of patients? 6) Quality of life should be taken into account. Three categories of patients have been proposed, with different treatment aims: 1. Medically fit patients functionally independent, with no or mild comorbidities and a normal life expectancy (so called "Go Go"). The most effective treatment should be the standard of care, irrespective of age. This today has been represented until last year by chemoimmunotherapy (*i.e.* fludarabine, cyclophosphamide, rituximab (FCR) or alternative regimens, ideally within clinical trials, such as bendamustine + rituximab (BR), fludarabine + rituximab (FR)); the aim is to prolong PFS and, possibly, overall survival (OS). 2. Medically less-fit patients ("Slow Go"). This category of patients should be recruited into specifically designed clinical trials. They can receive chlorambucil +/- rituximab or alternative regimes within clinical trials such as bendamustine, chlorambucil + ofatumumab or GA101, dose reduced fludarabine + cyclophosphamide (FC) or FCR, pentostatin + rituximab +/- cyclophosphamide (PR or PCR), adapted to the comorbidity burden and a balance between toxicity and benefits of therapy. Response and symptoms relief are the primary goals of treatment. A limitation in toxicity should influence treatment decisions. The first trial specifically dedicated to CLL patients with comorbidities has been opened in 2010. The CLL11 trial (GCLLSG) randomized previously untreated CLL patients to one of the three arms: chlorambucil alone, chlorambucil + rituximab or chlorambucil + GA101, a new type II glycoengineered humanized anti-CD20 monoclonal antibody. Inclusion criteria to define unfit patients included a CIRS total score >6 or a creatinine clearance <70 ml/min. 3. Medically frail patients with severe comorbidities, *i.e.* three or more comorbid conditions, dependence in one or more activities of daily living, and a very short life expectancy ("No Go"). They should undergo palliative treatment only. This successful categorization could rapidly be overcome by the broad use of the new inhibitors claimed to control the disease with no toxicity. The long-term efficacy and toxicity of these new agents is waited (see below). In the meantime, we have no uniform and conventional performance scale in order to assess the fitness of patients. As the aging of the population has been associated with an increased incidence and prevalence of neoplastic diseases, during the last decade oncologists and geriatricians have begun to integrate the principles of geriatrics into oncology care. Whilst oncologists assess the functional status of patients by the Karnofsky or ECOG performance status (PS), geriatricians assess it by patients' ability to complete activities of daily living (ADL) and instrumental activities of daily living (IADL). In older cancer patients, evaluation of ADL or IADL adds information to ECOG or Karnofsky PS. ADL and IADL are integral part of a CGA (comprehensive geriatric assessment), a multidisciplinary evaluation of an older individual's functional status, comorbid medical condition, cognition, nutritional status, psychological status and social support, and a review of the patient's medications. The application of CGA to older patients with cancer has been shown to predict morbidity and mortality, as well as chemotherapy toxicity, providing a common language to classify older cancer patients and the recognition of potentially treatable conditions such as depression or malnutrition, that may reduce the tolerance of cancer treatment. Therefore, efforts to develop a brief and feasible CGA into the oncology practice, as a baseline evaluation for older patients in cancer clinical trials, are ongoing. One of the scales capable of measuring the number and severity of comorbid medical conditions is the Cumulative Illness Rating Scale (CIRS). Comorbidity is generally associated to a worse survival in patients with solid tumors. Comorbidity and functional status can be independent in older cancer patients; they should therefore be independently assessed and routinely included in clinical trials. Interesting data from the National Institute on Aging (NIA) and the National Cancer Institute (NCI) showed that comorbidity increases with age in elderly patients with cancer: the mean number of comorbidities for patients aged 55-64 years is 2.9, for those aged 65-74 years it is 3.6 and for those aged >75 years it is 4.2. Hypertension, heart-related conditions, arthritis and gastro-intestinal problems are individually detected in more than one third of cases. The frequency and spectrum of comorbidities in elderly patients affected by CLL has not been specifically addressed. A retrospective study from the Mayo Clinic investigated the prevalence and prognostic implication of comorbidities at the time of CLL diagnosis. Median age was 68 years (range 58.8-75.1) and most patients had Rai 0-I CLL (84%). Patients had a median of two comorbidities; 89% had at least one comorbidity of any type and 46% at least one of the major comorbidities: coronary artery disease/peripheral vascular disease, cerebrovascular disease, other cardiac disease (cardiomyopathy, valvular heart disease, atrial fibrillation), diabetes, respiratory disease, second malignancy (other than non-melanomatous skin cancer). Moreover, about 9% of patients was not independent in at least one ADL (eating, bathing, dressing, walking, toilet, housekeeping). In this cohort, 26% of patients would not have been eligible for participation in clinical trials at the time of their CLL diagnosis for poor PS or organ dysfunction. Indeed, in the CLL-8 trial only around 10% of patients over the age of 70 were enrolled in the protocol. Patients ineligible for clinical trials had a shorter survival in comparison to those eligible and an inferior probability to receive purine analogs or purine-analogs chemoimmunotherapy, whilst the time to first treatment was similar among the two groups, indicating the same frequency of treatment. More efforts are needed to explore the pattern of comorbidities at the time of CLL progression and treatment, as many medical conditions can develop over time, and to dissect their impact on CLL-specific survival more than OS. The picture is even more complicated when considering prognostic markers beside age and fitness. Do we need the most aggressive treatment for all fit patients, even those with good prognostic markers? Today we can identify CLL patients with del(13q) devoid of any adverse gene mutation who have a life expectancy almost as long as that of an age-matched healthy population. Would control of the disease not be sufficient? In this prospective, the potential benefit of maintenance in CLL still remains an open issue. Few experiences mainly employing rituximab have been conducted with promising although preliminary results. On the other hand, emerging data indicate that the MRD status (evaluated by flow-cytometry and/or molecular techniques) during and at the end of treatment is one of the most powerful predictors of PFS and OS. This predictor seems to be independent of clinical response, type or line of therapy, and known biological markers. In the near future, clinical trials will determine whether MRD assessment can be used to guide treatment, either to improve quality of responses through maintenance/consolidation or to prevent relapses through preemptive therapies based on the reappearance of MRD. Despite the broadening of therapeutic options, CLL still remains an incurable disease. Beside chemotherapy and monoclonal antibodies, new drugs with different modes of action have recently entered the CLL treatment scenario (*i.e.* immunomodulatory agents, bcl-2 inhibitors, SYK, BTK and PI3Kδ inhibitors). With a mechanism-driven approach, they target the intrinsic CLL cell resistance to apoptosis or the anti-apoptotic and pro-proliferation stimuli that CLL cells receive in lymph nodes and bone marrow, mainly through the B-cell receptor (BCR) activation. BCR signaling, which may be constitutively expressed, antigen-induced, or both, plays a critical role in driving CLL cell proliferation and survival through the cascade of protein kinases. Prof. Antonio Cuneo will extensively cover the role and potential impact of the new drugs in CLL. The orally administered tyrosine kinase inhibitors fostamatinib and ibrutinib, and the phosphatidylinositol 3-kinase inhibitor GS-1101 have induced impressive responses in relapsed and refractory CLL patients, mostly with moderate side effects. Reductions in lymphadenopathy and splenomegaly are seen within weeks and are frequently accompanied by a transient rise in absolute lymphocyte count that is asymptomatic and probably the result of changes in CLL cell trafficking. In addition, even if the preliminary results need to be confirmed, these compounds appear to be active also in TP53 disrupted cases. These results are a further advance in the ever-changing management of hematologic cancers, which is shifting from a chemotherapy-based approach to treatments aimed at targeting the underlying biologic mechanisms of disease occur-

rence and progression. New inhibitors certainly represent an important step forward and a potential turning point in the treatment of CLL. The challenges today are to define their effectiveness as front-line treatment both alone and in combination with other agents, as well as their long-term effects. The possibility of a future long-term control of CLL for all patients regardless of age, comorbidities and prognostic markers is certainly a most stimulating challenge, which however underlines once more the growing problem of drug accessibility and overall sustainability.

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THE IMPACT OF NEW TECHNOLOGIES ON THE UNDERSTANDING OF CHRONIC LYMPHOCYTIC LEUKEMIA

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General concepts. In the Western hemisphere, chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. The clinical course of CLL ranges from a very indolent condition, with a nearly normal life expectancy, to rapidly progressive leading to early death. In a proportion of patients (~10%), CLL undergoes transformation into an aggressive lymphoma, a complication known as Richter syndrome (RS). The detection of recurrent chromosomal aberrations has been of key importance for understanding the biology of CLL and the mechanisms driving the variable clinical phenotype of this disease, and has enabled the construction of a first hierarchical model of genetic lesions that correlates with outcome and is commonly applied in the clinical practice.[1-3] However, cytogenetic lesions do not entirely explain the molecular pathogenesis and the clinical heterogeneity of CLL. In addition, from a clinical standpoint, the aggressive phenotype of high risk CLL cannot be recapitulated solely by TP53 disruption, that accounts for not more than 40% of this unfavorable subset of patients.[1,2] Whole genome/exome sequencing has disclosed the genetic landscape of several hematologic tumors, providing comprehensive catalogues of somatic mutations and new insights into the genes that contribute to cellular transformation.[1,2] Thanks to these technical progresses, research on the molecular pathogenesis of CLL has also advanced at a sustained pace in recent times. NOTCH1, SF3B1, BIRC3, and MYD88 are the most recurrently (>5%) mutated genes that have been identified through the application of whole genome/exome sequencing to CLL (Table 1). [1,2,4-6]

Table 1. Prevalence of CLL recurrent lesions stratified according to the disease phase.

	TP53 disruption	*NOTCH1* mutations	*SF3B1* mutations	*BIRC3* disruption	*MYD88* mutations
MBL	1-2%	3%	1-2%	0	n.a.
Diagnosis	5-10%	8-11%	4-7%	5%	3%
First treatment	10-11%	10-15%	17%	n.a.	n.a.
Chemorefractoriness	40-50%	15-20%	17%	25%	0
Richter Syndrome	50-60%	30-40%	6%	0	0

CLL, Chronic lymphocytic leukemia; MBL, Monoclonal B-cell lymphocytosis, n.a., not available

Beside their contribution to leukemic transformation, a number of observations point to NOTCH1, SF3B1, BIRC3 and MYD88 mutations as attractive prognostic markers since they identify poor survival patients among newly diagnosed CLL, are recurrent in chemorefractory cases, and are frequently exclusive with TP53 disruption, suggesting that they represent alternative mechanisms contributing to chemorefractoriness. [1,2,4-6] For all these reasons, genetic alterations are indisputably key players in CLL leukemogesis, yet do not fully explain the pathobiology of this disease. In fact, growing evidence acknowledges the intricate interplay of genetic and epigenetic events shaping the complex molecular landscape in CLL.[7] Epigenetic aberrations, such as anomalous histone and DNA methylation, mark and dysregulate the CLL genome. [7] In particular, differences in DNA methylation between CLL subtypes seem to be associated with epigenetic imprints of their putative cell of origin, and the cellular origin affects the biological features and clinical evolution of the disease. [7] By performing large-scale analysis of the DNA methylome in normal B cells and CLL samples, it has been shown that widespread hypomethylation targets mainly the gene body and enhancer sites. This seems to be a major epigenetic change in both B-cell differentiation and CLL development, indicating that DNA methylation is functionally relevant beyond promoter regions.[7] These notions represent an initial step toward the full characterization of the complete genome and epigenome of CLL, provide new insights into the pathogenetic mechanisms of CLL and may harbor clinical implications. NOTCH1 mutations. The NOTCH1 gene encodes a class I transmembrane protein functioning as a ligand-activated transcription factor that plays an important role in a number of cellular functions during embryogenesis and in self-renewing tissues of the adult organism, including maintenance of stem cells, cell fate specification, proliferation, and apoptosis.[1,2,4] NOTCH1 is a heterodimeric complex composed of an N-terminal extracellular subunit (NEC) and a C-terminal transmembrane and intracellular subunit (NTM). [1,2,4] The NEC subunit interacts with Delta-like and Jagged ligands through 36 epidermal growth factor (EGF)-like repeat domains. In addition, it contains a negative regulatory region (NRR) composed of three Lin12/NOTCH repeats (LNRs). These LNR domains fold over and stabilize the NOTCH1 heterodimerization domain (HD), which consists of the C-terminus of NEC and of the N-terminus of NTM in close interaction, to prevent the spontaneous activation of the receptor in the absence of ligand. The NTM subunit contains a transmembrane sequence followed by a series of cytoplasmic domains, including a RAM domain, a series of ankyrin repeats, a transactivator domain, and several nuclear localization signals, which collectively function as a ligand activated transcription factor. The NTM of NOTCH1 also contains a C-terminal PEST [proline (P), glutamic acid (E), serine (S), and threonine (T) rich] domain, that is responsible for the proteosomal degradation of activated NOTCH1 in the nucleus.[1,2,4] When membrane-bound NOTCH1 receptors interact with cognate ligands on an adjacent cell, two consecutive proteolytic cleavages of the receptor are initiated (Fig. 1).[1,2,4] These proteolytic cleavages allow the NOTCH1 intracellular domain to translocate to the nucleus, thus leading to transcriptional regulation of multiple target genes, including MYC activation, TP53 suppression, and deregulation of genes of the NF-κB pathway (Fig. 1).[1,2,4] Recent studies utilizing next generation sequencing have identified NOTCH1 mutations as a recurrent molecular lesion of CLL.[4] NOTCH1 mutations recur in ~10% unselected newly diagnosed CLL and preferentially associate with specific groups of CLL patients (Table 1).[4] In fact, NOTCH1 mutations are significantly more common in the subgroup of CLL with unmutated, rather than mutated, immunoglobulin heavy variable (IGHV) genes and are significantly enriched in CLL harboring +12 as the sole cytogenetic abnormality.[4,8] In contrast, NOTCH1 mutations are uncommon in CLL cases in which +12 is associated with other chromosomal aberrations.[4,8] Consistently, up to 40-50% CLL with isolated +12 and unmutated IGHV genes are characterized by NOTCH1 mutations.[4,8]

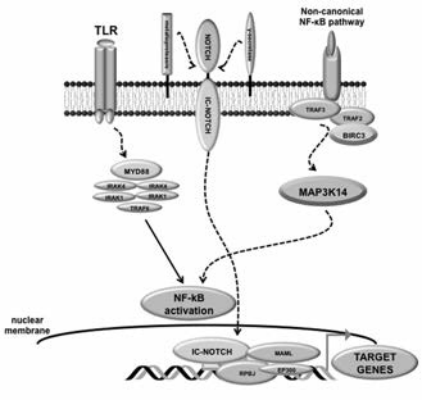

Figure 1. Molecular pathways involved in the pathogenesis of CLL. Upon ligand binding, the TLRs recruit the TIR-domain containing protein MYD88. In turn, MYD88 binds IRAK1 and IRAK4, which associate with TRAF6 leading to the subsequent activation of NF- B signaling. Upon ligand binding, NOTCH signaling is initiated by a series of proteolytic cleavages that ultimately lead to the release of the intracellular domain of NOTCH (IC-NOTCH) from the membrane to the nucleus. In the nucleus, IC-NOTCH recruits MAML, RBPJ and EP300, thus forming a complex that drives the transcription of its target genes. NF- B activation is achieved through either the canonical or non-canonical pathways. In the non-canonical pathway, TRAF2, TRAF3 and BIRC3 are recruited to the active receptors, allowing the release and stabilization of MAP3K14 that, in turn, leads to the activation of NF- B signaling. Genes harboring somatic lesions in CLL are highlighted in yellow.

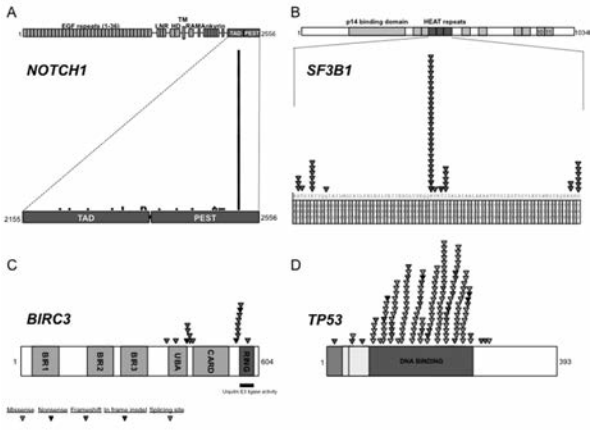

Figure 2. NOTCH1, SF3B1, BIRC3, TP53 mutation type and distribution in CLL. Schematic representation of the human NOTCH1 (A), SF3B1 (B), BIRC3 (C), and TP53 (D) proteins, with their key functional domains. Color-coded symbols indicate the type and position of the mutations.

NOTCH1 mutations in CLL are almost exclusively frameshift or nonsense events, clustering within a hotspot in exon 34, and are commonly represented by one single 2-bp deletion (c.7544_7545delCT) that accounts for ~80-95% of all NOTCH1 mutations in this leukemia (Fig. 2A).[4,8] The predicted functional consequence of NOTCH1 mutations in CLL is the disruption of the C-terminal PEST domain of the NOTCH1 protein.[1,2,4] Removal of the PEST domain results in NOTCH1 impaired degradation and accumulation of an active NOTCH1 isoform sustaining deregulated signaling.[1,2,4] Consistent with this notion, a number of cellular pathways are specifically deregulated in CLL harboring NOTCH1 mutations. The functional relevance of NOTCH1 mutations in the pathobiology of CLL is further suggested by the observation that constitutively active NOTCH1 signaling in CLL confers resistance to apoptosis through downstream activating effects on the NF-κB pathway.[1,2,4] By impairing the degradation of the intracellular domain of the protein, NOTCH1 stabilizing mutations conceivably prevent a regulated switch off of NOTCH signaling, thus boosting a growth promoting effect that is initially activated by microenvironmental interactions. NOTCH1 is preferentially targeted in specific disease phases of CLL. The prevalence of NOTCH1 mutations increases with disease aggressiveness, being exceptional (~3%) in monoclonal B-cell lymphocytosis, a pre-malignant entity that often precedes CLL, rare in unselected CLL at diagnosis (10%), and frequent in relapsed and fludarabine-refractory cases (20%) and in patients who have transformed to Richter syndrome cases (30%) (Table 1).[4,8] The observation that NOTCH1 mutations accumulate in the more advanced phases of the disease suggests that they represent second step genetic lesions progressively selected or acquired during the evolution of the clone. Beside their pathogenetic role, NOTCH1 mutations may also represent a new biomarker for the identification of poor risk CLL patients. NOTCH1 mutated patients have a rapidly progressive disease and a significantly shorter survival probability (~30% at 10 years) compared to NOTCH1 wild type cases.[4,8] SF3B1 mutations. SF3B1 is a core component of the U2 snRNP, that recognizes the 3′ splice site at the intron-exon junctions.[1,2,5] The SF3B1 protein interacts with RNA sequences in the vicinity of the branch point, as well as with the early 3′-splice-site recognition factor U2AF65 and the branch point-binding protein SF3B14.[1,2,5] Structurally, the SF3B1 protein has two well-defined regions: i) the N-terminal amino acid region, that contains several protein-binding motifs and functions as a scaffold to facilitate its interaction with other splicing factors such as U2AF65 and SF3B14; ii) the C-terminal region, that contains 22 non-identical tandem repeats of the HEAT motif that meander around the SF3b complex, enclosing SF3B14.[1,2,5] Whole genome/exome sequencing studies have revealed that SF3B1 is a recurrently mutated gene in CLL.[5] SF3B1 mutations occur with a prevalence that ranges from 7% to 15% of CLL, depending on the composition of the CLL cohort and on the inclusion of relapsed patients in the case mix (Table 1).[5,8] SF3B1 mutations in CLL are generally represented by missense nucleotide changes that cluster in selected HEAT repeats of the SF3B1 protein, and recurrently target three hotspots (codons 662, 666 and 700), with a single amino-acid substitution (K700E) accounting for ~60% of all SF3B1 mutations (Fig. 2B).[5] At variance from NOTCH1 mutations, SF3B1 lesions do not seems to consistently cluster with any specific cytogenetic subgroup of CLL.[5] Instead, SF3B1 mutations preferentially target specific aggressive phases of disease, since they are virtually absent in monoclonal B cell lymphocytosis , occur at a low rate at CLL presentation, while are enriched in ~20% relapsed and chemorefractory CLL (Table 1).[5] Consistent with the results of these analyses is the finding that newly diagnosed CLL patients harboring SF3B1 mutations are characterized by a short survival probability (~30-40% at 10 years).[5] Overall, these observations, along with a trend toward a mutually exclusive distribution of SF3B1 and TP53 abnormalities, prompt SF3B1 mutations as a potential novel biomarker for the early identification of high risk, but TP53 wild type, CLL patients. BIRC3 abnormalities. The Baculoviral IAP repeat containing 3 (BIRC3) gene cooperates in a protein complex that negatively regulates the MAP3K14 serin-threonine kinase, that represents the central activator of non-canonical NF-κB signaling (Fig.1).[1,6] BIRC3 is recurrently disrupted in CLL by mutations, deletions or a combination of mutations and deletions (Fig. 2C).[1,6] At the biochemical level, BIRC3 inactivating mutations and a fraction of BIRC3 deletions cause the truncation of the C-terminal RING domain of the BIRC3 protein, whose E3 ubiquitin ligase activity is essential for switching off MAP3K14 through proteosomal degradation, thus leading to constitutive non-canonical NF-κB activation (Fig. 1).[6] Identification of BIRC3 involvement in CLL may be important for elucidating the molecular genetics of 11q22-q23 deletion.[1,3,6] In fact, although ATM has been regarded as the relevant gene of this chromosomal abnormality, biallelic inactivation of ATM does not exceed ~30% of cases with 11q22-q23 deletion.[3] On these bases, a second tumor suppressor in the 11q22-q23 region has been postulated along with ATM. In this respect, the BIRC3 gene, that maps on 11q22.2 approximately 6Mb centromeric to the ATM locus, might represent an attractive candidate.[6] From a clinical standpoint, BIRC3 lesions contribute to clinical aggressiveness and chemorefractoriness in CLL.[6] BIRC3 disruption selectively occurs in ~25% fludarabine-refractory CLL (Table 1), while is consistently absent in progressive CLL that require treatment but prove to be sensitive to fludarabine-based regimens.[6] Consistent with these findings, BIRC3 lesions are absent in monoclonal B cell lymphocytosis and occur at low rate (4%) in unselected newly diagnosed CLL where they identify a subgroup of high risk patients displaying poor survival similar to that associated with TP53 abnormalities (Table 1).[6] Fludarabine refractoriness in CLL may be explained by TP53 disruption in ~40% of patients, while ~60% high risk

CLL are devoid of TP53 abnormalities. BIRC3 abnormalities recapitulate the genetics of ~40% chemorefractory and TP53 wild type CLL and, along with SF3B1 mutations, may contribute to expand the panel of biomarkers for the early identification of chemorefractory cases.[6] MYD88 mutations. In B-cells, Toll-like receptors (TLRs) are central to the B cell receptor (BCR)-independent response to antigens by sensing a variety of pathogen-associated molecular patterns. Upon ligand binding, TLRs aggregate and initiate intracellular signaling by engaging various cytoplasmic adaptors, including MYD88 (Fig. 1).[1] After stimulation of the TLRs, MYD88 is recruited to the activated receptor complex as a homodimer and forms proteins complexes that trigger activation of NF-κB (Fig. 1).[1] The MYD88 gene encodes a cytosolic adapter protein that consists of an N-terminal death domain, a linker region, and a C-terminal Toll-interleukin-1 receptor (TIR) domain, which may mediate contact with the TLRs upon signaling activation. Although many different MYD88 mutations exist in B-cell tumors, the most prevalent in CLL is the L265P missense substitution that occurs in ~3% of cases (Table 1), affects the evolutionarily conserved B-B loop of the TIR domain, and, in B-cell tumors, promotes cell survival by constitutive activation of NF-κB signaling.[1,8] TP53 abnormalities. The tumor suppressor TP53 gene is a tetrameric nuclear phosphoprotein that maps on the short arm of chromosome 17 (17p13) and encodes for a central regulator of the DNA-damage-response pathway.[1,2] Activation of TP53 leads to cell-cycle arrest, DNA repair, apoptosis, or senescence via both transcription-dependent and transcriptional-independent activities.[1,2] Consistently, TP53 plays a central role in mediating the pro-apoptotic and antiproliferative action of several DNA-damaging chemotherapeutic agents, including alkylators and purine analogs.[1,2] The human TP53 protein comprises 393 amino acid residues and three main functional regions, namely: i) the N-terminal activation domain, which is able to interact with a variety of proteins; ii) the C-terminal domain, responsible for tetramerization; and iii) the core domain that constitutes the sequence-specific DNA binding domain (DBD) of the protein. More than 90% of the point mutations in TP53 that are related to malignancy are found in the DBD segment (Fig. 2D).[1,2,8] In CLL, the TP53 gene may be inactivated by deletion and/or somatic mutations. Most cases with 17p13 deletion also carry TP53 mutations on the second allele (~60%), while the remaining cases have a monoallelic 17p13 deletion in the absence of TP53 mutations (~10%), or TP53 mutations in the absence of 17p13 deletion (~30%).[1,2,3,8] In line with the genetic instability associated with defective DNA-damage checkpoints, TP53 abnormalities frequently couple with complex cytogenetic abnormalities.[3] At the molecular level, approximately 75% of all TP53 mutations are missense substitutions, while the remaining lesions (~25%) are represented by truncating events, including frameshift insertions or deletions, non-sense substitutions and splice site mutations (Fig. 2D).[1,2,8] Most missense mutations are localized within exons 5-8, which encode the central DBD of TP53, thus impairing DNA binding and targeting gene transactivation (Fig. 2D).[1,2,8] From a clinical standpoint, genetic lesions affecting the TP53 gene are significantly enriched in high risk CLL (Table 1), and represent the only established biomarker of chemorefractoriness in this leukemia.[1,2,8] Clonal evolution in CLL. Analyses of several genomes and hundreds of exomes from CLL patients identified novel oncogenes and tumor suppressors of therapeutic interest for this disease. However, the mechanisms that trigger CLL progression are still poorly understood, despite some recent advances.[4-10] Integration of data derived at the high depth of coverage yielded by next generation sequencing with data generated through the analysis of copy number alterations allowed to accurately estimate the fraction of cancer cells harboring each mutation [9]. Following this procedure, driver mutations can be classified in two main groups: i) clonal mutations, which are present in all tumor cells and represent early events in the transformation process; and ii) subclonal mutations, usually present in a small population of leukemic cells and representing late events in CLL.[9,10] The identification of these two categories of driver mutations in CLL has also facilitated the analysis of leukemia progression. Three periods during the history of the disease can be identified.[9,10] The first pre-transformation period involves the age-dependent accumulation of clonal passenger mutations in a cell that will eventually be the founder of the future CLL population. The next stage is driven by a malignant transformation event in the founding CLL B cell, which is recurrent across patients. Finally, the third and last period is characterized by the expansion of subclonal mutations in response to intrinsic or extrinsic pressures. According to this model, transformation of normal B cells into CLL results from the accumulation of early driver somatic mutations, such as chromosome 13q deletion, chromosome 12 trisomy and mutations in MYD88 or NOTCH1, whereas mutations responsible for CLL progression would target other cancer drivers such as TP53, ATM, SF3B1 and NRAS, which expand in response to intrinsic or extrinsic pressures.[9,10] These findings may have important implications for the clinical management of CLL. Notably, chemotherapy can trigger the clonal evolution of the disease in a process where highly fit clones colonize the new habitat.[9,10] Thus, for most untreated patients, clone composition is stable over time, probably owing to the slow growing capacity of CLL B cells.[9,10] However, chemotherapy-treated patients experience a clonal evolution characterized by the expansion of highly fit clones.[9,10]

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IMPACT OF NOVEL TREATMENTS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. The treatment of chronic lymphocytic leukemia (CLL) has dramatically changed in several respects over the last years thanks to the convergence of basic research and a number of well conducted clinical studies, leading to a much clearer understanding of basic mechanisms underlying the natural history of the disease and to the discovery of effective treatment regimens (table 1). Meanwhile molecular cytogenetic studies,[1,2] allowed for the identification of genetically-defined disease subsets with different response to treatment,[3] and guidelines for disease management and treatment were developed,[4] that represent the standard of care for this heterogeneous disease.[5]

Table 1. Efficacy of the principal options for first line treatment of CLL.

	Catovsky Lancet 2007	Foà ASH 2011	Hillmen ASH 2010	Catovsky Lancet 2007	Byrd Blood 2003	Catovsky Lancet 2007	Flinn JCO 2007	Hallek Lancet 2010	Keating JCO 2005	Hallek Lancet 2010	Fisher JCO 2012	Hillmen JCO 2007
Regimen	*Chl*	*R-Chl*		*F*	*FR*	*FC*			*FCR*		*BR*	*A*
% CR	7	16,5	9	15	47	38	23.4	24	70	44	23.1	24
PFS (months)	20	na	23.5	23	70%*	43	31.6	48	47	65%**	33.9***	23.3

Modified from Cuneo A, Rigolin G: La leucemia linfatica cronica, available at http://www.ematologiainprogress.net/web/
PFS is reported as median values (months), unless otherwise indicated with asterisk
* at 2 years; **at 3 years; *** event free survival
Chl: chlorambucil; *R:* rituximab; *F:* fludarabine; *C:* cyclophosphamide; *B:* bendamustine; *A:* Alemtuzumab

Improved survival in CLL

When retrospectively comparing CLL overall survival in different time periods, it should be taken into account that i) life expectancy in the general population largely improved in the last decades, ii) the widespread use of automatic blood counters may lead to earlier diagnosis, ii) more precise diagnosis allow for the exclusion of lymphoma in leukemic phase in patients diagnosed in recent years, iii) supportive treatment improved. The following observations, however, indicate that the overall outlook of CLL improved in all age groups over the last 20 years, thanks to the introduction of effective regimens in first and subsequent lines of therapy.

Data from single centres and registries

Brenner and coworkers (2008) assessed 5- and 10-year relative survival rates, specifically reflecting excess mortality attributable to CLL, and found a slow but steady improvement in survival in patients <80 years of age between 1980-1984 and 2000-2004). In the >80 years age group improvement was seen in the first 5 years after diagnosis only. Thus, in 2000-2004, patients younger than 70 years of age reached a 10-year relative survival close to 65%, while a 55% 10-year relative survival was reached in the 70-79 age group. After adjusting for the expected survival in the general population Abrisqueta and coworkers (2008) observed improved 5- and 10-year relative survival in stage B/C patients younger than 70 years, due to a decrease in CLL-related mortality occurring in the 1995-2004 period as compared with the 1980-1994 period. These data suggest that more effective treatment produced longer survival in young patients with intermediate-advanced stage, whereas no obvious improvement was noted in this analysis in limited stage disease and in the elderly, which represent the vast majority of patients diagnosed with CLL in our countries. Using population-based data in a very well organized Swedish registry, Kristinsson and coworkers (2009) assessed variations in survival among all CLL patients reported from 1973-2003 and found significantly improved 5-, 10-, and 20-year relative survival ratio for the entire cohort during the study period. Improved 5- and 10-year relative survival ratio was found for the majority of the age-groups. An unexplained observation in this study was that the 5-year relative survival ratio improved only in the 1973-1980 period and was stable thereafter in the youngest CLL population, including 491 CLL <50 years of age (4% of the total population).

Comparisons with historical controls

The above mentioned studies refer to a period antecedent the widespread use of effective monoclonal antibodies in CLL treatment. Interestingly, chemoimmunotherapy upfront was found by Tam (2008) to prolong survival at 6 years (77%) with respect to historical controls using fludarabine alone (54%) or in combination with mitoxantrone and/or cyclophosphamide (59%). More recently, PFS and overall survival (OS) were retrospectively assessed in 4 successive frontline Cancer and Leukemia Group B (CALGB) studies trials by Woyach (2012). With a median follow-up across studies of 92 months, OS was improved with fludarabine over chlorambucil (31% reduction of risk of death) among patients younger than 70 years, but not in older adults. Importantly, a 35% reduction of death risk was observed with the adjunct of rituximab to fludarabine, irrespective of age.

Randomized trials

In the CLL8 trial (6) the demonstration was provided that the FCR regimen was able to improve survival in CLL with respect to FC in fit patients. Inclusion criteria in this protocol precluded enrolment many elderly patients and when restricting outcome analysis to the 40% study population ≥65 years, improved CR rate and PFS were maintained in the chemoimmunotherapy arm, whereas no significant advantage in survival was noted in this age subset. A prospective trial was designed by Eichhorst (2009) for the elderly population to compare the efficacy and tolerability of fludarabine vs chlorambucil in 196 patients. Although fludarabine attained a higher CR rate, no significant difference in terms of PFS and survival was recorded in this 70-year median age population due to the efficacy of second line regimens.

Novel treatments

The growing body of information on the genetics and on the biology of CLL has relevant impact in the management the disease in that different treatment approaches are being offered to patients with genetically-defined aggressive disease and novel agents interfering with unique biologic features are being rapidly introduced in clinical practice (figure 1). An exhaustive overview of novel treatment options in CLL was recently published;[5] in the following section, the efficacy of the most promising novel treatments supported by solid data from phase II-III studies will be discussed.

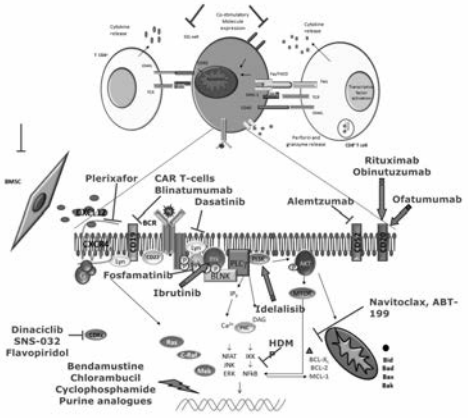

Figure 1. Relation between CLL biology and drugs in current use or under development in CLL. Interactions of the CLL cells with the microenvironment and activity of lenalidomide is shown in the upper part; surface antigens and biologic pathways representing targets for novel therapies are shown in the lower part. The drugs representing possible mainstay for modern CLL treatment are indicated in red, HDMP refers to high-dose steroids.

CLL with 17p-/TP53 mutations

This subset of CLL shows, with few exceptions, a very poor prognosis in patients with progressive disease, with expected median survival of a few years even with intensive regimens. Because the anti CD52 monoclonal antibody alemtuzumab and high dose steroids kill CLL cells through a p53 independent mechanism the efficacy of combination regimens using these two drugs was assessed and Pettitt and coworkers

(2012) reported a 65% CR rate, 36% MRD- and a 18.3 months median PFS in 17 untreated patients. This regimen showed greater efficacy than other combinations, however its toxicity raises some concerns about its applicability outside experienced centres. Furthermore, virtually all patients are expected to relapse and allogeneic bone marrow transplantation is an important option in this subset, as suggested by the EBMT consensus panel (2007). Interestingly, the efficacy results reported by Dreger (2013) were independent of the presence of unfavourable genetic features, including 17p-, in a study of 90 allografted patients, 49% of whom were fludarabine resistant. The efficacy of BCR-targeted therapy in this genetic subset is discussed below.

i) Rituximab-based combinations

The combination of rituximab (R) with fludarabine (F), with or without cyclophosphamide (C), with bendamustine and with chlorambucil achieved high overall response rates, with prolonged PFS in several studies (table 1). After the excellent results described by MDACC investigators who tested the FCR scheme in fit patients, episodes of late cytopenia were reported by Strati (2013) who observed grade 2 to 4 cytopenia 3 months after the completion of therapy in 35% of the patients and a 10% and 4% risk of late infection for the first and second year of remission. These data, along with the notion that the majority of CLL patients are elderly prompted Foon and coworkers (2009) to design of an attenuated schedule of administration of the FCR combination rendering this schedule relatively safe in the community setting. The efficacy of the addition of rituximab to fludarabine therapy was assessed in 104 untreated patients enrolled in the CALGB 9712 protocol, randomized to receive or not concurrent rituximab with 6 cycles of fludarabine, followed by four once-weekly doses of rituximab in both arms. Complete response rates were higher in the concurrent group (47% vs. 28%) with an estimated 2-year progression-free survival of 70% for each regimen, as reported by Byrd in 2003. Several studies using rituximab in combination with other purine analogues didn't show any consistent advantage over fludarabine. Following the demonstration of an increased efficacy over chlorambucil at the price of greater though manageable toxicity, the association of bendamustine and rituximab was investigated by Fischer and coworkers (2012) in 117 CLL patients, 25,6% of whom were age 70 or older and 46,2% were stage C. This regimen was effective (table 1) and appeared less toxic than FCR, with grade 3 or 4 neutropenia, thrombocytopenia, and anemia occurring in 19.7%, 22.2% 19.7% of the patients, respectively and severe infections in 7.7%. Chlorambucil is generally well tolerated in the elderly patient and its combination with rituximab is being explored in phase 2 studies including patients unfit for FCR in the U.K. (presented by Hillmen at ASH 2010) and in Italy (presented by Foà at ASH, 2011). A 9-16.5% CR rate, along with dose reductions occurring in a minority of cases and unfrequent grade 3-4 neutropenia and infections indicate that this combination is relatively safe and effective in the elderly population. Interestingly an 8,3% CR rate was observed in the chlorambucil + rituximab arm of the CLL11 trial (vide infra), using lower cumulative doses of chlorambucil as compared with the UK and Italian study.

Second generation anti CD20 monoclonal antibodies and anti CD52 Alemtuzumab

Ofatumumab

Ofatumumab was recognized as an active single agent, with greater CDC activity compared to rituximab (table 2). Interestingly, its activity was independent of previous rituximab exposure and of CLL genetic profile, with a 41% response rate in 17 double refractory patients with 17p- reported by Wierda (2010). Wierda (2011) found that the combination of ofatumumab with fludarabine and cyclophosphamide was active and manageable as frontline therapy for CLL. Further studies are required to fully appreciate the potential of this agent in the treatment of CLL.

Obinutuzumab (GA101)

In the CLL11 trial, 589 treatment-naïve CLL pts with a median age of 73 years, who were unfit for FCR treatment, were randomized to receive chlorambucil alone, chlorambucil with GA101, or with rituximab. No CR was observed in patients receiving chlorambucil alone, as compared with 8,3% in the rituximab arm and 22% in the GA101 arm. Grade 3-5 adverse events included 15%, 25%, 34% neutropenias, 11%, 8%, 6% infections in the chlorambucil arm, in the arm with rituximab and in the arm with GA101, respectively. After a median observation time of 14 months PFS was 10.9 months in patients receiving chlorambucil, 15,7 months and 23 months in patients receiving chemoimmunotherapy with rituximab or GA101, respectively). The results, presented at the ASCO meeting by Goede (2013) demonstrate that GA101 in association with chlorambucil is very active and manageable in this population comprising a majority of elderly patients.

Table 2. Salient data on efficacy and safety of some classical and novel treatment options in relapsed refractory CLL.

	Various regimens at MDACC in FA refractory and F refractory with bulky adenopathy (*)	FCR (**)	Ofatumumab in FA refractory and F refractory with bulky adenopathy (***)	Lenalidomide + rituximab	Ibrutinib (****)	Idelalisib + R or +B or + RB (*****)
No. of patients	99	276/284	138	59	85	51
No. previous regimens (median)	NA	1/2	4-5	2	4	1-10 range
Percentage CR PR	 0 23	 24/30 45/44	 0-1 47-58	 12 54	 2 69(i)	 78-87% (overall)
Months PFS Survival	 2-3 9	 30/21 NR/47	 5,7-5,9 13,7-15,4	 17,4 (ii) 71%(iii)	 75% (iv) 83% (iv)	 74-87% (v) NA
Grade 3/4 AE infections neutropenia	 54% NA	 18/16% 89/81%	 8-12% 6-14%	 24% 73%	 17% 15%	 0-29% (vi) 32-67%

(*)Tam, 2007; (**)Robak, (2010) / Badoux, 2011; (***)Wierda, 2010; (****) Byrd, 2013; (*****) Coutre, 2012; (see text);
NA: not available, NR: not reached, R:rituximab, B:bendamustine,
(i) additional 18% patients had PR with lymphocytosis; (ii) time to treatment failure, months; (iii) at 33 months; (iv) % at 26 months; (v) % at 1 year; (vi) pneumonia

Alemtuzumab

This recombinant, fully humanized, monoclonal antibody targets the CD52 antigen which is strongly expressed on normal and malignant CLL lymphocytes. Monotherapy with alemtuzumab was shown to be superior to chlorambucil in untreated CLL by Hillmen (2007). With the exception of the 17p- subset, its use is limited by its strong immunosuppressive activity, requiring CMV infection monitoring by PCR. Attenuated dose of alemtuzumab proved to be effective and better tolerated both as single agent and in combination with fludarabine and cyclophosphamide as reported by Cortelezzi (2012) and Montillo (2012).

ii) Agents targeting BCR downstream signaling

The neoplastic lymphocyte respond to BCR stimulation by activating intracellular signaling and this biologic process is more pronounced in the prognostically unfavourable IGHV unmutated subset where unfavourable clinicopathologic and genetic features are frequently encountered (7). The biologic basis of BCR signaling inhibition has been recently reviewed by Burger (2011) and Stevenson (2013) and the following drugs have shown exciting results in recent trials (table 2).

Ibrutinib (PCI-32765)

The Bruton tyrosine kinase (BTK) is a cytoplasmic tyrosine kinase that is essential for BCR signaling, inducing calcium release, cell proliferation, and activation of the NF-KB pathway. Ibrutinib is an oral agent which binds covalently to Cys-481 of BTK, causing its inhibition. The publication of a phase 1b–2 multicenter study to assess the safety and efficacy of ibrutinib in 85 relapsed-refractory CLL who had received a median of four previous lines of treatment,[8] was welcomed as the first mechanism-driven treatment for chronic lymphocytic leukemia.[9] Toxicity was modest (table 2), with grade 1-2 diarrhea, fatigue and upper respiratory tract infection being the most common events. The drug induced rapid shrinkage of lymph nodes with concomitant increase of the absolute lymphocyte count, reflecting a compartment shift. Over time, this lymphocytosis gradually resolved in the majority of the cases. Responses (table 2) were independent of stage, number of previous therapies, and 17p13.1 deletion. At 26 months an impressive 75% progression-free survival and 83% overall survival were observed. Disease progression occurred in 11 patients, 10 of whom had 17p- or 11q-. Interestingly, in a recent analysis presented at the ASCO 2013 meeting by Chang, single nucleotide variations of the BTK gene and in PLCg2, a substrate of BTK, were detected in 3 patients in the relapse, suggesting possible mechanisms of ibrutinib resistance. The favourable therapeutic index and tolerability may facilitate the use of ibrutinib in combination with other agents to limit the increase of peripheral lymphocytosis

and to further improve its efficacy. Ongoing trials are exploring the efficacy of anti CD20 monoclonal antibodies in association with ibrutinib.

Idelalisib (GS1101 - CAL101)

Phosphoinositide 3-kinases (PI3K) transmit signals from diverse surface molecules, such as the BCR, chemokine receptors, and adhesion molecules, thereby regulating growth, survival, and migration. CLL shows constitutive activation of the PI3K pathway that is dependent on the B-lineage restricted isoform PI3K-delta. *in vitro*, CAL-101 was able to sensitize CLL cells to the effects of cytotoxic drugs and steroids and to interact with BCR signaling, possibly reflecting a dual mechanism of action. Following the demonstration of its activity and pharmacodynamic effects in relapsed or refractory CLL at the ASH 2010 annual meeting, Coutre and coworkers reported at ASH 2012 durable responses in the majority of patients using GS1101 in combination with rituximab (R) and/or bendamustine (B) (table 2). As with ibrutinib initial nodal response was associated with lymphocytosis; this effect was limited by adding ofatumumab in one study of 15 patients producing a 94% overall response rate as reported by Furman at ASH (2012). The favorable safety profile of GS1101 allowed the administration of this oral PI3Kdelta inhibitor at the full single-agent dosage with concomitant chemoimmunotherapy and provided the basis for the initiation of phase III studies evaluating the efficacy of GS-1101 in combination with rituximab or with bendamustine plus rituximab.

iii) Treatment interfering with the interactions of CLL lymphocytes in the microenvironment and the immune system

Lenalidomide

Lenalidomide treatment promotes CD154 expression on CLL cells and enhances production of antibodies by normal B cells through a PI3-kinase–dependent pathway. CD154-positive CLL cells become sensitized to TRAIL–mediated apoptosis. Furthermore, lenalidomide may reverse impaired T-cell immunologic synapse in CLL, a phenomenon described *in vitro* consisting of molecular and functional defects in previously healthy T cells *in vitro* and *in vivo* after contact with CLL cells. The proof-of-principle that lenalidomide could have a beneficial immunologic role *in vivo* was provided by Shanafelt and coworkers (2013) who reported on a trial of pentostatine, cyclophosphamide and rituximab as induction regimen followed by lenalidomide consolidation in 34 untreated CLL. Twenty-four% of the patients who received lenalidomide improved their quality of response and, interestingly, antitumor T-cell immune synapse activity improved after PCR and was further enhanced after lenalidomide consolidation. The role of lenalidomide in the treatment of CLL is currently under investigation. Its activity as initial therapy for CLL was investigated in 60 elderly patients (median age 71) by Badoux and coworkers (2011), who reported durable remissions and reasonably good tolerability in this population of elderly, symptomatic patients. Lenalidomide at the dose of 10 mg/daily in combination with rituximab was effective in relapsed/refractory CLL (table 2), including the 17p- subset, where a 53% overall response rate was observed by Badoux (2013).

Chimeric antigen receptor-modified T cells

This novel approach consists i) in the construction of a chimeric receptor recognizing on the one side a surface antigen of CLL cells (usually CD19) and endowed on the other side with a costimulatory receptor in T cells and a signal-transduction component of the T-cell antigen receptor), ii) its introduction in T-cells followed by, iii) their infusion *in vivo*, leading to their expansion and immune reaction against the presence neoplastic B-cells expressing the specific antigen. Porter (2011) used CAR engineered T-cells in one patient with refractory CLL who developed tumor lysis syndrome and subsequent complete remission, the only other grade 3/4 toxic effect being lymphopenia and hypogammaglobulinemia. At the 2012 ASH meeting, 9 adults with refractory CLL were reported to have been treated by chemotherapy followed by CAR T cell infusion, attaining continuous CR in 3 patients at a median observation of 5.6 months, with mild infusional toxicity and persistence of the anti-CD19 CAR for up to two years. This fascinating approach requires standardization and further efforts to make it amenable to large scale therapy.

iv) Agents inducing apoptosis and targeting cell-cycle proteins

The Bcl-2 family include several proteins providing the cell with a balance between proapoptotic and prosurvival signals. CLL cells express in the vast majority of cases high levels of bcl-2, due to trans-regulatory mechanisms, involving partial loss of the negative effect played by miR-15a and miR-16-1 which are frequently deleted due the 13q- anomaly. CLL with higher levels of Bcl-2 protein survived longer in culture than CLL with lower Bcl-2 levels. Thus Bcl-2 has been used as a target for treatment using the BCL2 gene antisense nucleotide oblimersen, which however did not produce significant survival advantage in an intent to treat analysis published by O'Brien (2009). More recently, the Bcl-2 antagonist navitoclax showed activity in CLL with dose-limiting thrombocytopenia due to concomitant Bcl-xL inhibition, whereas a single dose of the reengineered compound ABT-199 targeting more specifically Bcl-2 resulted in potent tumor lysis within 24 hours without significant effect on platelet count in 3 patients with refractory CLL reported by Souers (2013). Flavopiridol showed some encouraging results as cyclin-dependent kinase (CDK) inhibitor. However, there have been difficulties in identifying an acceptable schedule of administration due to the frequent appearance of severe tumor lysis syndrome. The second-generation CDK inhibitors SNS-032, a potent and selective Cdk2 inhibitor and dinaciclib, promoting apoptosis and abrogating microenvironmental cytokine protection in CLL, are promising agents.

Conclusion

Modern CLL treatment is a remarkable example of how biologic studies and clinical expertise may converge, providing a rationale basis for the development of effective treatments, resulting in a significant improvement of the number and quality of responses, quality of life and survival in the majority of age groups. Guidelines for treatment were recently updated,[5] with the important consideration that the management of CLL is undergoing a rapid change which may modify our approach in the near future. With aging of the population and increased overall health-related costs, the burden of hemopoietic neoplasms on the national health systems is becoming an issue in high-income countries. In a recent analysis the costs attributable to CLL were calculated as the difference between annual costs of a CLL patient in 2007-2008 and annual costs of an average individual with the same age and sex. The economic impact of each incident CLL case was €4946 from the payer's perspective and €7910 from a societal perspective, with hospital stays and drugs being the main cost drivers. The global economic burden of disease in Germany was €201 million per year for the sickness funds (10). It is reasonable to anticipate that with new healthcare technologies and the increasing incidence of the disease the economic burden of CLL will continue to grow. However it is worth noting that the pharmacoeconomic analysis performed by the National Institute of Health and Clinical Excellence in the U.K. (www.nice.org.uk/nicemedia/pdf/TA174FullGuidance.pdf) recognized that FCR, a regimen improving survival in a direct comparison with the best chemotherapy combination was a cost-effective use of NHS resources. The predicted efficacy of very potent, targeted and non-chemotherapeutic drugs in CLL along with the development of sensitive predictors of response offer a unique opportunity to intensify coordinated research programmes aimed at providing compelling evidence of the positive cost/efficacy ratio of these novel agents.

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ACUTE MYELOID LEUKEMIA: TO TRANSPLANT OR NOT TO TRANSPLANT?

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The treatment of acute myeloid leukemia (AML) in adults remains a challenge. A highly heterogenous disease, with respect to both its biologic and clinical characteristics, AML is difficult to cure in younger patients and nearly impossible to cure in elderly patients. Currently, standard frontline therapy in fit patients consists of dose-intensive combination chemotherapy (*e.g.* cytarabine plus anthracyclines) given during induction and consolidation. Although on average 50-80% of patients achieve a complete remission (CR), most patients will relapse and ultimately die of their disease or associated complications. This is the reason why allogeneic stem cell transplantation (AlloSCT) represents an important option for eligible patients with high-risk AML during first remission (CR1), as well as for any patient in second or subsequent remission. Unfortunately, the benefit of AlloSCT can be considerably offset by the complications of the procedure, particularly in the elderly population where the transplant-related mortality associated with myeloablative conditioning (MAC) regimens remains a major limitation. Furthermore, most patients lack an HLA-matched sibling donor. These limitations have prompted the search for alternative stem cell sources and for reduced-intensity conditioning (RIC) regimens to decrease toxicity. Cytogenetics is the most robust prognostic marker for risk stratification of AML at the time of diagnosis as well as for selection of post-remission treatments. With this background, several prospective trials were undertaken in the late 90s and early 2000s to clarify the role of AlloSCT for AML in first complete remission (CR1). The design of these studies typically involved genetic assignment substituting for randomization on the basis of the availability of a matched-sibling donor. Overall, these donor versus no-donor trials and their meta-analyses have shown that AlloSCT results in superior disease-free and overall survival rates in adults with intermediate- and unfavorable-risk cytogenetic profiles who are in CR1. In contrast, AlloSCT performed in CR1 did not improve outcome in patients with favorable-risk cytogenetics. The reasons for this lack of benefit are two-fold in that such patients have a lower risk of relapse when treated with conventional post-remission therapies and a high chance of being effectively salvaged if they do relapse. However, it is now clear that cytogenetic and molecular risk profiling in adult AML is an evolving field and new patient subsets are increasingly being identified, especially within the large group of patients with intermediate-risk cytogenetics (mainly those with normal karyotype). While most of these leukemias lack a specific chromosomal abnormality, molecular markers such as gene mutations and deregulated gene expression can be identified in the majority and may be associated with a more specific prognosis. For some patients with cytogenetically normal AML, there is now a clear indication for AlloSCT in CR1 (those with FLT3 ITD mutations). For others, there is evidence that AlloSCT does not have an advantage over traditional post-remission therapies (NPM1- or CEBPA-mutated in the absence of FLT3 ITD mutation). As the molecular characterization of AML improves, it is likely that a more widespread use of genetic markers will change the landscape in the near future. Furthermore, there is now mounting evidence that the incorporation of available techniques to measure and continue to monitor minimal residual disease (MRD) after induction and consolidation therapy will further refine risk assessment in AML, because an increasing MRD signal is a recognized strong predictor of early relapse. Such information, in turn, might affect the decision whether or not to offer AlloSCT in CR1. Historically, older patients with AML have been excluded from treatment with AlloSCT due to concerns of excessive treatment-related morbidity and mortality. However, transplantation outcomes have significantly improved over the last decade due to advances in supportive care, better patient selection, and the widespread use of nonablative and reduced-intensity conditioning regimens. Such regimens are sufficiently immunosuppressive to allow engraftment of allogeneic cells and rely largely on graft-versus-leukemia effects rather than high-dose therapy to eliminate malignant cells. These have all led to an increasing acceptance of AlloSCT as a plausible option to consider for older patients with AML in CR1. As suggested by recent data, these transplants are feasible in selected patients up to 75 years of age and may yield better outcomes than standard consolidation therapy, but prospective trials are necessary. While the focus remains mostly on patients in CR1, there will continue to be AML patients with active disease who require AlloSCT as optimal therapy. Based on available data, it is reasonable to argue that: 1) for patients who fail to achieve first complete remission following frontline induction therapy, AlloSCT offers the best, and likely the only, chance for cure; 2) recurrent disease in any risk group is generally an indication for allotransplantation. What is currently debated is whether a trial of re-induction chemotherapy prior to transplantation is beneficial. Several studies have documented 20-30% cure rates for patients transplanted at first relapse without re-induction. Although the cure rate for transplantation in CR2 may be higher, salvage chemotherapy is only successful in roughly 50% of the patients and 15-20% of them will die during attempted re-induction with most regimens. Recently developed prognostic risk scores may be useful in predicting those AML patients who are unlikely to respond to salvage chemotherapy and could be considered for prompt transplantation. Autologous stem cell transplantation (AutoSCT) for AML in CR1 has been extensively investigated as an alternative strategy for adult patients without matched family member donors, but its role remains undefined. Pooled analyses of prospective randomized trials showed that AutoSCT is consistently associated with a modest improvement in disease-free survival but not benefit in overall survival, relative to consolidation therapy or no further treatment. However, the picture may change in the future given the fact that novel stratification methods (based on genetics and minimal residual disease assessment) may allow for identification of patient subsets which could benefit from autografting as a post-remission therapy.

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TIMING OF ALLOGENEIC STEM CELL TRANSPLANTATION IN CHRONIC MYELOPROLIFERATIVE DISORDERS: NOT TOO EARLY BUT NOT TOO LATE

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The discovery of key genetic lesions such as the JAK2617F mutation in Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Myelofibrosis (MF) has opened the way for the development of new innovative pharmacologic treatments of these chronic myeloproliferative neoplasms (CMNs). However, none of the new drugs is able to show a curative effect for primary MF or when PV or ET progress to MF or AML and allogeneic hematopoietic stem cell transplantation remain the only treatment which may eradicate and cure these diseases. Unfortunately, the non-relapse mortality associated with transplant is still remarkably and often unacceptably high and this poses burning questions to the physician's decision making process. Among the issues which may induce divergent opinions for referral a patient to transplant, the most commonly discussed remain the age limit, the risk definition according to modern risk stratification systems, the optimal conditioning regimens and the role of prior splenectomy. The Dynamic International Prognostic Scoring System (DIPSS) and DIPPSplus (including also transfusion dependency, low platelet count and adverse cytogenetics) may partially help physicians to offering a transplant choice to the patient 1,2. Indeed, the expected median survival of patients with low or intermediate 1 risk MF is about 15 and 6 years and the risk of allogeneic transplantation may not be justified in these patients. In the GITMO experience published in 2008, the unrelated donor, and a long interval between diagnosis and transplantation were associated to a worst outcome while there was a trend towards longer overall and relapse-free survival in patients receiving peripheral blood stem cells rather than bone marrow as the source of their graft3. Nonetheless, for younger patients (less than 50 years) this algorithm may be challenged and other clinical data should be considered to define the risk profile and the expected benefit form transplant. Among these the total amount of transfusions and the spleen size may be of crucial importance. 4. A peculiar clinical situation in this setting is represented by the evolution to MF or AML which is characterized by an almost invariable early death if transplant is not performed rapidly. We recently reviewed the EBMT data base and we could observed that the main parameters that negatively affected post-alloHSCT outcomes are older age (>55 years), a diagnosis at transplant of AML and donor type (mismatched and unrelated vs. related). Patients younger than 55 years had an OS and EFS significantly better compared to those older than 55 years, due to a significant higher NRM observed in elderly patients. The reduced OS observed in patients with a secondary AML was due to a higher RI in AML (53%). Patients transplanted earlier in the course of the disease are those who most benefit from the procedure, suggesting, once more, that an appropriate timing for alloHSCT before leukemic transformation is crucial to obtain better results. In CML Tyrosine kinase inhibitors have completely changed the therapeutic strategy for this disease but allogeneic transplantation remains the best option for all patients who failed second-line TKIs, with mutations T315I or with progressive disease. The most recent published experience with allo indicate that for patients still in chronic phase, the expected 3 years survival is above 80% with a transplant related mortality usually below 15%5,6. Since the survival of patients in blast crisis remains very poor, even in the TKI era, the transplant option should be offered before this dismal hematologic progression of the disease will eventually occur.

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PATHOGENESIS OF EXTRANODAL LYMPHOMA

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Introduction

Although almost any non-Hodgkin lymphoma (NHL) type can affect extranodal sites as part of more widely disseminated disease, there is a group of lymphoid tumors that specifically arise in these locations. A few number of B-cell malignancies accounts for ~80% of all primary extranodal NHL in Western countries, including extranodal marginal zone lymphoma (EMZL), splenic marginal zone lymphoma (SMZL), primary mediastinal large B-cell lymphoma (PMBL), and diffuse large B-cell lymphoma of the central nervous system (CNS-DLBCL).[1] The molecular pathogenesis of these tumors offers paradigmatic exemplifications of a number of mechanisms that are more generally involved in lymphomagenesis, namely deregulation of signaling pathways (*i.e.* B-cell receptor, NF-kB, NOTCH, toll-like receptor, JAK-STAT), epigenetic deregulation, and immune escape.

Extranodal marginal zone lymphoma

EMZL is an indolent tumor arising in the mucosa-associated lymphoid tissue (MALT) and accounting for ~10% of NHL. EMZL is considered an antigen driven lymphoid malignancy associated with protracted antigenic stimulation by microbial pathogens, auto-antigens or other unknown stimuli, which trigger a sustained lymphoid proliferation at sites normally devoid of lymphoid tissue.[1] With the progression of disease, chromosomal aberrations may occur, resulting in aberrant activation of signaling pathways which lead to the lymphoproliferation becoming independent of antigenic stimulation. Long term antigenic stimulation sustained by infections or autoimmune inflammation explains how lymphoid infiltrates may appear in extranodal sites that are normally devoid of lymphoid tissue (*i.e.* stomach, lungs, salivary glands, thyroid and lachrymal glands) and conceivably represents the first step of the lymphomagenesis process. Bacteria, or at least immune response to bacterial antigens, have been implicated in the pathogenesis of EMZL. These include H. pylori in gastric EMZL, B. burgdorferi in cutaneous EMZL, C. psittaci in ocular adnexa EMZL, and C. jejuni in intestinal EMZL.[2] Beside epidemiological and *in vitro* evidence, the importance of this stimulation *in vivo* has been consistently documented, at least for H. pylori-associated gastric EMZL, by the induction of remission of EMZL with antibiotic therapy tailored at eradication of the bacteria. Epidemiological evidence points also to a role of autoimmune-based chronic inflammation in the pathogenesis of EMZL of certain anatomical sites. Indeed, Sjiögren syndrome and Hashimoto thyroiditis may precede EMZL of the salivary glands and thyroid, respectively. Consistently, patients affected Sjiögren syndrome have a ~40-fold increased risk of developing an EMZL of the salivary gland, while patients affected by Hashimoto thyroiditis have a ~40-fold increased risk of thyroid EMZL.[2] The acquisition of genetic lesions is associated with progression of EMZL and resistance to treatments tailored towards bacterial eradication, thus suggesting that lymphoma cells gain independence from the microenvironment trough the development of genetic abnormalities surrogating the same stimuli that in the early phases of the disease are provided by antigen stimulation and inflammation. Consistently, EMZL is genetically characterized by different, usually mutually exclusive, genetic abnormalities that surrogate chronic B-cell receptor (BCR) activation towards the constitutive deregulation of NF-kB. In normal B-cells, when the BCR is ligated by an antigen, a signaling cascade is initiated that ultimately results into CARD11 phosphorylation and activation. CARD11, along with BCL10 and MALT1, takes part into the CBM signaling complex that is required for triggering NF-κB signaling downstream of the BCR receptor. Upon BCR engagement-induced phosphorylation, CARD11 acquires an open conformation that allows CARD11 to recruit MALT1 and BCL10 into the CBM multiprotein com-

plex and activate the IKKB kinase, thereby initiating NF-kB signaling (Fig. 1).[3] Three mutually exclusive chromosomal translocations have been identified in EMZL, including t(1;14)(p22;q32), t(14;18)(q32;q21), and t(11;18)(q21;q21).2 These translocations are recurrent, although at considerably variable frequencies in EMZL at different sites, lead to the up-regulation of either BCL10 or MALT1 or the generation of the BIRC3-MALT1 fusion protein, and induce aberrant activation of NF-kB through the deregulation of the CBM multiprotein complex (Fig. 1). The t(1;14)(p22;q32) translocation brings the entire BCL10 gene under the regulatory control of the immunoglobulin gene enhancer and hence causes BCL10 overexpression. Physiologically, BCL10 acts as an adaptor protein linking its upstream protein CARD11 to its downstream molecule MALT1 to form of the CBM complex and activate NF-kB. Pathological overexpression of BCL10 is capable of activating the NF-kB pathway through BCR signaling-independent oligomerization of the CBM complex.[2] The t(14;18)(q32;q21) translocation brings the entire MALT1 gene under the regulatory control of the immunoglobulin gene and hence deregulates its expression. In contrast to BCL10, the overexpression of MALT1 alone is insufficient to induce NF-kB activation, probably due to a lack of structural domain that can mediate MALT1 self-oligomerization, but requires to synergize with BCL10 in the activation of NF-kB via CMB complexing.[2] The t(11;18) (q21;q21) translocation fuses the N-terminal region of the BIRC3 to the C-terminal region of the MALT1 and generates a functional chimeric fusion which gains the ability to activate the NF-kB pathway through at least two mechanisms. First, unlike wild-type MALT1, the BIRC3-MALT1 fusion oligomerizes through heterotypic interaction between the BIR1 domains of the BIRC3 moiety and the C-terminal region of MALT1 in the absence of any upstream stimulation, and such oligomerization bypasses the CBM complex in constitutively activating NF-kB. Second, oligomerization of the BIRC3-MALT1 fusion protein stimulates the proteolytic activity of the caspase-like domain of the MALT1 component. Normally BIRC3 interacts with the kinase MAP3K14, a potent activator of non-canonical NF-kB signaling, causing its ubiquitination and degradation. The BIRC3-MALT1 fusion lacks the ubiquitin ligase domain of BIRC3 but still binds MAP3K14, which allows the MALT1 caspase-like domain to cleave MAP3K14 near its amino terminus, creating a stable, active kinase that initiates NF-kB signaling.[2,3] The NF-kB pathway is also governed by a number of negative regulators. Among these TNFAIP3 can specifically inactivate several molecules that are critical for NF-kB signaling by targeting these protein for proteasome degradation (Fig. 1). Inactivation of TNFAIP3 by deletions or disrupting mutations is recurrently associated with EMZL and might be involved in promoting NF-kB signaling in translocation-negative EMZL.[3]

Splenic marginal zone lymphoma

SMZL is an indolent tumor arising in the spleen and accounting for ~2% of NHL. Epidemiological and molecular evidence point to protracted antigenic stimulation by microbial pathogens or auto-antigens as a mechanism of SMZL initiation/promotion. Indeed, SMZL development is associated with HCV infection in ~15%-20% of cases.[1] The contribution of antigen stimulation to SMZL pathogenesis is also suggested by the highly restricted immunoglobulin gene repertoire, including selective usage of the immunoglobulin heavy variable 1-2*04 allele in ~20-30% of SMZL and by the stereotyped B-cell receptor in an additional ~10% of cases.[4] SMZL transcriptional signature is characterized by constitutively deregulation of NF-kB and NOTCH signaling. The underlying bases of the SMZL transcriptional program are genetic alterations predominantly involving signaling pathways that regulate the physiological marginal zone (MZ) development, including NOTCH signaling, NF-κB signaling, BCR signaling, and TLR signaling. Thus, the finding that ~60% of SMZL cases display the alternative deregulation of these pathways suggests that a major component of SMZL pathogenesis is the constitutive activation of signals normally deputed to the differentiation and homing of B-cells into the splenic MZ.[5] The NOTCH pathway, especially when engaged by NOTCH2, represent a master regulator of MZ differentiation in normal B-cells, and, consistently, is the most frequently mutated pathway in SMZL, with NOTCH2 mutations representing the genetic hallmark of this lymphoma type.[6] The NOTCH receptor genes encode a family of heterodimeric transmembrane proteins (NOTCH1 to NOTCH4) that function as ligand-activated transcription factors. When the NOTCH receptors interact with their ligands through the extracellular subunit, two consecutive proteolytic cleavages of the NOTCH proteins are initiated and lead to pathway activation (Fig. 2). Upon activation, the cleaved intracellular portion of the NOTCH receptors translocate into the nucleus where they form a complex with the RBPJ transcription factor. In the absence of NOTCH signaling, RBPJ binds DNA in a sequence-specific manner and acts as a repressor of transcription. In the presence of NOTCH signaling, displacement of co-repressors bound to RBPJ by the active intracellular NOTCH allows the recruitment of co-activators, such as MAML1 and MAML2, to create an activation complex and to modify the expression of a number of target genes, including NF-kB signaling components.[6] The most prominent mechanism of NOTCH signal suppression is operated through its PEST domain. The PEST domain of activated NOTCH is recognized by the FBXW7 ubiquitin protein ligase that terminates NOTCH signaling by directing NOTCH towards proteasomal degradation. Other negative regulators of NOTCH signaling include SPEN and DTX1. SPEN represses NOTCH signaling by competing with the active intracellular NOTCH for binding to RBPJ. DTX1 represses NOTCH signaling by binding the NOTCH family proteins and inhibiting their recruitment of transcription coactivators.[6] Genes of the NOTCH pathway are mutated in ~40% of SMZL (Fig. 2).[5]

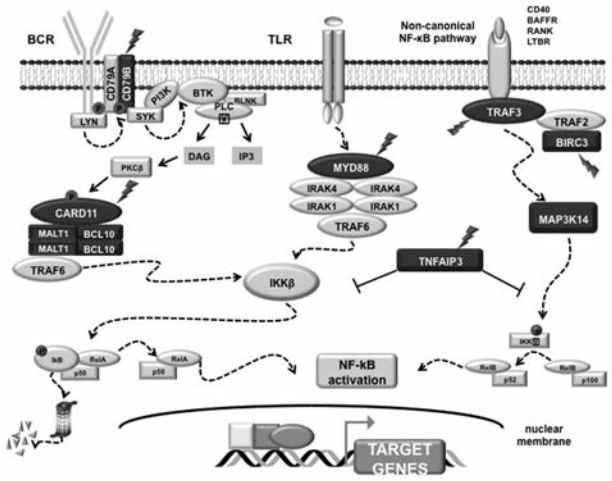

Figure 1. NF-kB activation through the B cell receptor (BCR), toll-like receptor (TLR) and non-canonical NF-kB signaling pathway. Genes harboring somatic lesions in extranodal B-cell tumors are highlighted in blue and marked by an arrow.

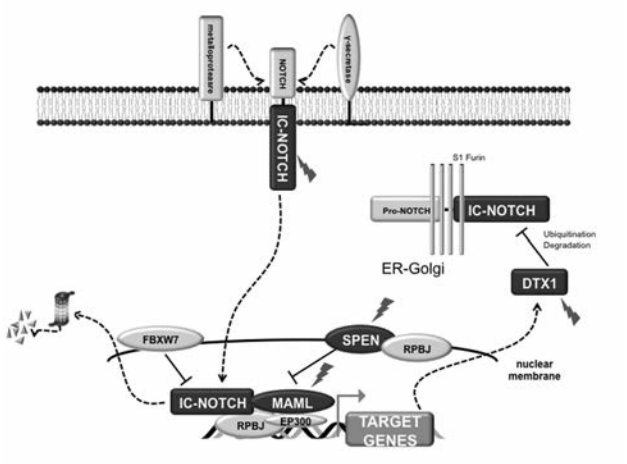

Figure 2. NOTCH signaling pathway. Genes harboring somatic lesions in extranodal B-cell tumors are highlighted in blue and marked by an arrow.

NOTCH2 shows recurrent mutations in ~20-25% SMZL, establishing NOTCH2 as the most frequently mutated gene in SMZL. NOTCH1, a paralog of NOTCH2, is also mutated in additional ~5% SMZL. NOTCH2 and NOTCH1 mutations in SMZL are selected to truncate the PEST domain of the protein, thus causing impaired degradation of the NOTCH2 and NOTH1 proteins and, as a consequence, sustained NOTCH signaling. In addition to NOTCH2 and NOTCH1, other genes involved in NOTCH signaling and known to be relevant for normal MZ B-cell differentiation are affected by genomic lesions in SMZL, including SPEN, DTX1 and MAML2. SPEN physiologically acts in the immune system as a negative regulator of B-lymphocyte differentiation into MZ B-cells by counteracting NOTCH activation. SPEN mutations occur in ~5% SMZL, are mostly represented by inactivating lesions that truncate the C-terminal domain of the protein, which is involved in the interaction between SPEN and RBPJ, and is required for NOTCH signaling inhibition. DTX1 and MAML2 mutations occur in ~2% SMZL.[5] NF-kB activation, which is physiologically required to reprogram mature B-cells towards the MZ, is driven in SMZL by genetic lesions targeting a few key regulators of the non-canonical NF-kB signaling (BIRC3, TRAF3, MAP3K14) in ~25% of cases.[7] In normal B-cells, the non-canonical NF-kB pathway is engaged by CD40 and BAFF receptors. Upon receptor binding, the TRAF3/MAP3K14-TRAF2/BIRC3 negative regulatory complex of non-canonical NF-kB signaling is disrupted, allowing the cytoplasmic release and stabilization of MAP3K14, the central activating kinase of non-canonical NF-kB signaling (Fig. 1).[6] BIRC3 is recurrently disrupted by mutations, deletions or a combination of both in ~10% SMZL.[7] BIRC3 inactivating mutations cause the truncation of the C-terminal RING domain of the BIRC3 protein, whose E3 ubiquitin ligase activity is required to prime MAP3K14 towards proteasomal degradation. TRAF3, another component of the TRAF3/MAP3K14-TRAF2/BIRC3 negative regulatory complex of non-canonical signaling, is targeted in ~5% SMZL. TRAF3 inactivating mutations cause the elimination of the C-terminal MATH domain of the protein that provides the docking site for MAP3K14, and is required for MAP3K14 recruitment to BIRC3 degradation. On these bases, the functional consequence of BIRC3 and TRAF3 mutations in SMZL is MAP3K14 stabilization in the cytoplasm and the constitutive activation of non-canonical NF-kB signaling.[7] The identification of BIRC3 inactivating mutations in SMZL points to BIRC3 disruption as a common mechanism across MZ B-cell lymphomagenesis. In fact, disruption of the BIRC3 RING domain, that in SMZL is produced by inactivating mutations, in EMZL is caused by t(11;18) leading to the formation of the BIRC3-MALT1 fusion protein that lacks the RING domain of BIRC3. In addition to genes specifically attributed to the NF-kB pathway, mutations also affect CARD11 (7% of SMZL) and MYD88 (5% of SMZL), which, among their many functions, act as positive regulators of NF-kB in signaling from the BCR and TLR (Fig. 1). Overall, mutations of positive and negative NF-kB regulators accounted for ~30% SMZL cases, implicating activation of NF-kB as the second major contributor to the pathogenesis of this disease after NOTCH deregulation.[7]

Primary mediastinal large B-cell lymphoma

PMBL is an aggressive tumor arising in the mediastinum from putative thymic B-cells and accounting for ~4% of NHL.1 PMBL cells must avoid immune surveillance imposed by thymic microenvironment. Consequently, under the selective pressure of thymic T cells, PMBL cells accumulate genetic lesions to prevent T cell recognition by blocking T cell activation and/or by impairing MHC class II expression. On these bases, immune escape is the mainstay of PMBL cell survival. PMBL has a unique transcriptional signature characterized by constitutively activated JAK2. Consistently, cell line models of PMBL die when the JAK2 tyrosine kinase is genetically or pharmacologically inhibited, suggesting that PMBL signature and survival stem from the action of JAK2.8 The underlying genetic basis for these observations is the recurrent amplification involving JAK2 on chromosome band 9p24 seen in 50-70% of PMBL and representing the genetic hallmark of this lymphoma type.8 Another mechanism of JAK2 activation in PMBL is the disruption by deletions or inactivating mutations of SOCS1, a suppressor of JAK signaling. SOCS1 inactivation co-occur with JAK2 amplification in a fraction of PMBL and the cooperation between these two lesions in PMBL contributes to JAK2 stabilization in its constitutively phosphorylated and active form.[8] The minimally amplified region at 9p24 in PMBL includes, beside JAK2, also JMJD2C. JAK2 and JMJD2C are coordinately overexpressed in PMBL and function in concert to epigenetically modify the PD-L1 and PD-L2 genomic loci, which encodes inhibitors of T-cell response essential for the malignant PMBL clone to escape immune surveillance of the thymus microenvironment (Fig. 3).[8] Indeed, heterochromatin formation and gene silencing is associated with recruitment of HP1-alpha, which binds to the histone H3 at the unphosphorylated tyrosine 41 (H3Y41) and at the heterochromatin mark H3K9me3. Activated JAK2 directly phosphorylates the histone H3 at H3Y41, leading to displacement of HP1-alpha from chromatin and increased gene transcription. JMJD2C is a chromatin-modifying enzyme that demethylates trimethylated lysine 9 of the histone H3 tail (H3K9me3), thereby reducing heterochromatin formation. Thus, JAK2-mediated phosphorylation of H3Y41 and JMJD2C-mediated demethylation of H3K9me3 synergize in blocking HP1-alpha recruitment, reduce heterochromatin formation and positively regulate the expression of hundreds of genes in PMBL, including PD-L1 and PD-L2.[8] Translocations involving CIITA, a transactivator of MHC class II genes, occur in ~40% of PMBL and represent a second mechanism of immune escape of this lymphoma. CIITA translocations invariably fuse the N terminus of CIITA in frame with a variety of other genes. As a result, one copy of CIITA is inactivated, and the fusion protein can also act in a dominant-negative manner to extinguish MHC class II expression, thereby limiting the ability of the tumor cells to interact with T cells. In some cases of PMBL, CIITA is fused to PD-L1 or PD-L2. The resultant fusion protein is displayed on the cell surface and functionally impairs T cell activation.[8]

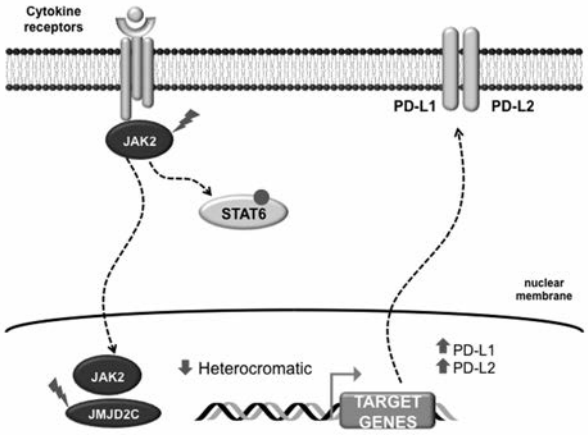

Figure 3. JAK2 signaling pathways. Genes harboring somatic lesions in extranodal B-cell tumors are highlighted in blue and marked by an arrow.

Diffuse large B-cell lymphoma of the central nervous system

CNS-DLBCL is an aggressive tumor arising in the central nervous system and accounting for ~1% of NHL.[1] Consistent with its predominant activated B-cell like (ABC) phenotype, CNS-DLBCL rely on constitutive NF-kB activation, which is one of the main downstream effectors of BCR and toll-like receptor (TLR) signaling. Various alternative genetic mechanisms of NF-kB activation have been identified in CNS-DLBCL affecting the TLR and BCR pathways. Mutations of MYD88, the central integrator of TLR signaling, is the genetic hallmark of CNS-DLBCL, where they occur in ~50% of cases.[9] In B-cells, TLRs are central to the BCR-independent response to antigens by sensing a variety of pathogen-associated molecular patterns derived from bacteria, viruses, and fungi. Upon ligand binding, TLRs aggregate and initiate intracellular signaling by engaging various cytoplasmic adaptors, including MYD88. After stimulation of the TLRs, MYD88 is recruited to the activated receptor complex as a homodimer and forms complexes with IRAK4, leading to activation of IRAK1 and IRAK2. TRAF6 is then activated by IRAK1, and catalyzes polyubiquitination of the MAP3K7 kinase, which in turns phosphorylates IKKB and triggers activation of NF-kB (Fig. 1).[9] The MYD88 protein consists of an N-terminal death domain, a linker region and a C-terminal TIR domain, which may mediate contact with TLR TIR domains upon signaling activation. Almost all MYD88 mutations in CNS-DLBCL affect the TIR domain. Although many different MYD88

mutations exist, the most prevalent is the L265P missense substitution.[9] The L265P mutation, as well as other MYD88 mutations, cluster in the evolutionary conserved B-B loop of the TIR domain, suggesting that they are selected to change the structure of MYD88 to allow spontaneous and activation-independent interaction with IRAK4 and IRAK1. Consistently, B-cell tumors harboring MYD88 mutations show constitutive and deregulated nucleation of a signaling complex that includes phosphorylated IRAK1 and leads to active NF-kB.[9] In ~20% CNS-DLBCL, the constitutive NF-kB activity is sustained by mutations affecting CD79B, a component of the BCR complex.[3] In normal B-cells, when IgM is ligated by an antigen, tyrosine residues in the cytoplasmic ITAM portion of CD79B are phosphorylated by the Src family kinases, including LYN. The tyrosine kinase SYK is activated by binding to the phosphorylated ITAM domains of CD79B, triggering a signaling cascade that involves BTK. BTK forms a complex with the adapter BLNK and PLC-gamma-2. PLC-gamma-2 then produces the second messenger diacyl glycerol, which activates PKC-beta, leading to CARD11 phosphorylation and NF-kB signaling (Fig. 1).[3] CD79B mutations mostly consist of non-synonymous missense substitutions that change the ITAM N-terminal tyrosine of CD79B. CD79B mutations result into two distinct functional effects, both able to enhance BCR-mediated NF-kB signaling. First, mutated CD79B render the BCR resistant to negative regulation by the LYN kinase. Second, CD79A and CD79B mutants enhance surface BCR expression due to diminished BCR internalization, conceivably prolonging BCR-dependent signaling upon stimulation.[3] In ~15% CNS-DLBCL, the constitutive NF-kB activity is sustained by mutations affecting CARD11, a key component of the CBM complex that activates NF-kB upon BCR engagement.[3] CARD11 encodes a multidomain signaling scaffold protein consisting of an N-terminal CARD motif, a coiled-coil domain, an inhibitory domain, and a C-terminal MAGUK domain that contains multiple protein-protein interaction sub-domains. CARD11 mutations in CNS-DLBCL exclusively affect the coiled-coil domain of the protein, and disrupt the association of the coiled-coil domain with the inhibitory domain that, in resting conditions, keeps CARD11 inactive in the basal state. As a result, mutations spontaneously convert CARD11 into an active signaling scaffold in a manner that is independent of BCR engagement. In this way, mutations promote spontaneous CARD11 multimerization and association with other components of the CBM complex as BCL10 and MALT1, thus leading to IKKB activation and NF-kB program deregulation.[3] A second genetic hallmark of CNS-DLBCL is the recurrent loss of chromosome 6p21.32 involving the HLA locus, that is observed in ~80% of cases.[1] This feature seems specific to DLBCL of immune-privileged sites, including CNS-DLBCL and testicular DLBCL, and might represent a mechanism of immune escape from T cells via the down-regulation of HLA class II expression, which is a phenotypic features uniformly observed in virtually all CNS-DLBCL.

Clinical relevance of genetic aberrations and future directions

New insights into the genes that contribute to cellular transformation in extranodal NHL provide molecular clues useful for addressing a number of unmet clinical needs in the management of these tumors. Genetic lesions that are highly recurrent and/or specific for a given clinicopathologic entity may behave as biomarkers for disease diagnosis and classification improvement. Because of the absence of disease defining clinico-pathologic markers, the diagnosis of SMZL may be only achieved after excluding other mimickers. These uncertainties affect the daily clinical practice by making the diagnosis of this tumor laborious and not easily reproducible. In this regard, NOTCH2 mutations, which are enriched in SMZL, while rare or absent in other mimicking conditions, are increasingly implemented as biomarkers for improving disease recognition and classification. The identification and functional characterization of the molecular bases of deregulated NF-kB, TLR, NOTCH and JAK-STAT signaling provides the preclinical rationale for therapeutic inhibition of these pathways in extranodal NHL. The NF-kB pathway represents a therapeutic target in many extranodal lymphoid malignancies (*i.e.* EMZL, SMZL, CNS-DLBCL) in which abnormalities affecting NF-kB genes cause tumor cells to be addicted to NF-kB. Inhibition of IRAK4 downstream of MYD88 effectively blocks NF-kB activation and survival of B-cell tumors harboring MYD88 mutations, thus representing an ideal target for CNS-DLBCL. The consistent identification of deregulated NOTCH signaling in SMZL provides the preclinical rationale for therapeutic inhibition of the NOTCH pathway in this tumor with antagonists such as inhibitory antibodies and gamma-secretase inhibitors. The notion that PMBL relies on JAK2 constitutive activation provides a strong rationale for the clinical evaluation of JAK2 inhibitors in PMBL. Finally, the notion that PMBL and CNS-DLBCL accumulate genetic lesions to prevent T-cell recognition and escape the immune system provides the rationale for the application of anti-CD19/CD3 bi-specific antibodies in these tumors to re-attract T-lymphocytes to eliminate lymphoma cells.

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MARGINAL ZONE LYMPHOMAS

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Introduction

Marginal zone lymphomas (MZLs) represent a group of lymphoma that originate from memory B lymphocytes normally present in a distinct micro-anatomic compartment, the so-called "marginal zone" of the secondary lymphoid follicles. According to the sites involved and to characteristic molecular findings the last lymphoma classification distinguished three subtypes of MZLs: extranodal MZL of mucosa-associated lymphoid tissue (MALT) type, splenic MZL (SMZL), and nodal MZL (NMZL). In adults, MZLs account for 5-17% of all non-Hodgkin's lymphoma (NHL), depending on the series. MALT lymphoma comprises 7-8% of all B-cell lymphomas and MALT lymphoma is the third most common NHL. Most of the cases occur in adults, with a median age of approximately 60 years and a slight female preponderance; it seems to be a geographical variability in the incidence of gastric MALT lymphomas, with a higher incidence in some areas as in the north-east of Italy. Splenic and nodal MZLs represent 20% and 10% of MZLs, respectively, and account for less than 2% of all NHLs. The median age of occurrence for SMZL is 65 years and between 50 and 60 years for NMZL.

MALT lymphoma (or extranodal MZL)

MALT lymphoma differs from its splenic and nodal counterparts as it arises in organs that normally lack lymphoid tissue (like stomach, lung, and salivary and lachrymal glands) but have accumulated B-cells in response to chronic infections or autoimmune processes.

Clinical features

MALT lymphomas mostly present as Ann-Arbor stage IE disease (extranodal disease limited to the site of origin) as bone marrow and peripheral lymph node involvement is rather uncommon. The stomach is the most common site of localization, accounting for about one-third of cases. Other typical presentation sites include the salivary glands, the ocular adnexa, the thyroid, the lung, the skin, the breast, other gastrointestinal sites, and the liver. Advanced disease at diagnosis appears to be

more common in MALT lymphomas that arise outside the gastrointestinal (GI) tract. Up to one quarter of patients with gastric lymphoma – but nearly half of those with non-GI lymphoma – presents with disseminated disease. Within the stomach, MALT lymphoma is often multifocal, possibly explaining the rates of relapses in the gastric stump after surgical excision. Concomitant GI and non-GI involvement can be detected in approximately 10% of cases. Bone marrow involvement is reported in up to 20% of cases. The clinical aspects and presenting symptoms of extranodal MZL are generally related to the primary location. Specifically, for gastric MALT lymphomas the most common presenting symptoms are non-specific dyspepsia, epigastric pain, nausea, and chronic manifestations of GI bleeding, such as anemia. MALT lymphoma most often involves the antrum but may occur in any part of the stomach. It can appear as intragastric nodularities or enlarged rugal folds. In other cases, the aspect is that of superficial irregularly shaped erosions or shallow ulcers. Overt distant dissemination is not common in cases of gastric MALT lymphomas; sites of involvement include the bone marrow, small intestine, liver and spleen. A special variant of MALT lymphoma is the IPSID that occurs mainly in the Middle East, especially in the Mediterranean area where the disease is endemic, affecting young adults and predominantly the males. IPSID usually manifests with severe unremitting malasorption; the lymphoma is characteristically confined to the upper intestine and regional lymph nodes and may rarely spread beyond the abdomen only in advanced stages of the disease, when high grade transformation has occurred.

Treatment and outcome

Approximately 30% to 50% of patients with Hp-positive gastric MALT lymphoma will show persistent or progressing lymphoma even after eradication of Hp with antibiotic therapy. Of the complete responders, almost 15% will relapse within 3 years, suggesting that about half of patients with gastric MALT lymphoma will eventually be considered for additional therapies. Patients that present with no evidence of Hp infection are unlikely to respond to antibiotics and should be considered for alternative treatments. The choice should be based on the epidemiology of the infection in the patient's country of residence, taking into account the locally expected antibiotic resistance. The most commonly used regimen is triple therapy: a proton pump inhibitor in association with amoxicillin and clarithromycin. The role of additional chemotherapy after antibiotics was reported in a randomized study comparing chlorambucil versus observation after anti-Hp treatment; chlorambucil did not increase progression-free survival and overall survival rates. There is no consensus for the treatment both of patients with gastric MALT lymphoma requiring further treatment beyond Hp eradication and of patients with non-gastric MALT lymphoma. For early stage MALT lymphoma of the stomach without evidence of Hp infection, or for those with persistent lymphoma after antibiotics, as well as for most non-gastric localized presentations a modest dose of involved-field radiotherapy (25 to 35 Gy) gives excellent disease control. In the last decade, the role of surgery in gastric lymphoma has been questioned: gastric MALT lymphoma is a multifocal disease, and adequate gastrectomy needs to be quite extensive, severely impairing quality of life, and residual disease at the margins may still require additional radiation and/or chemotherapy. Table 3 summarizes relatively large series of patients with gastric MALT lymphoma treated with chemotherapy/immunotherapy. Among non-gastric MALT lymphoma fludarabine has demonstrated some anti-tumor activity. The efficacy of the combination of rituximab with chlorambucil has been evaluated in a randomized study (comparator was chlorambucil alone) by IELSG in gastric MALT lymphomas that had failed antibiotics and in non-gastric MALT lymphomas. The final report showed that the 5-year event-free survival was significantly better for patients treated with chlorambucil plus rituximab. MALT lymphoma usually has a favourable outcome, with overall survival at 5 years higher than 85% in most series. The median time to progression has been reported to be better for GI than for non-GI lymphomas, but with no significant differences in overall survival between the two groups. Histologic transformation to large cell lymphoma is reported in about 10% of the cases, usually as a late event and independent from dissemination. Regarding antibiotic treatment in localized non-gastric MALT lymphomas, the finding that C. psittaci has a potential pathogenic role in the development of MALT lymphoma of the ocular adnexa and has been detected in about 80% of Italian patients may represent a strong rationale for antibiotic treatment of localized lesions. At the same time, the prevalence of C. psittaci infection in ocular adnexal lymphoma varies among countries and among different regions within the same country. With respect to the United States, C. psittaci was not detected in any case included in four North American series. A prospective phase II study was conducted by IELSG, and this has recently reported final interesting results, showing lymphoma regression in more than 60% of patients after doxycycline treatment. It is important to remember that lymphoma regression after doxycycline therapy has been observed in some lymphomas with no evidence of C. psittaci as well as in cases in which this treatment failed to eradicate C. psittaci infection.

Splenic MZL

SMZL is a B-cell neoplasm consisting predominantly of small cells and involving the white pulp follicles of the spleen, splenic hilar lymph nodes, bone marrow, and, often, the peripheral blood.

Clinical features

Most of the patients seek medical attention because of an abnormal blood cell count, especially anemia and/or thrombocytopenia, more related to splenic sequestration than to bone marrow infiltration, constantly associated with lymphocytosis. Patients are asymptomatic, but splenomegaly is detectable at clinical examination. In advanced cases, the typical clinical presentation is with massive splenomegaly and small splenic hilar lymph nodes are frequently associated. SMZL is also associated with autoimmunity. The neoplastic B cells can produce autoantibodies, and a haemolytic autoimmune anemia or autoimmune thrombocytopenia is present in a subset of cases (10-15% of patients). A relevant percentage of patients (10% to 40% of cases) has a serum monoclonal paraprotein (M-component). Splenic lymphomas with numerous basophilic villous cells in the peripheral blood, formerly denominated as splenic lymphoma with villous lymphocytes, are characterized by a peculiar histology with atrophic white pulp and a monomorphic diffuse infiltration of a congested red pulp, reminiscent of HCL variant. Few differences have been found in the clinical presentation, including a significant older age and an absence of immune disorder.

Treatment and outcome

The median overall survival is ranging between 5 to 10 years, but in cases with aggressive disease (25-30% of cases) median survival is less than 4 years. The Italian Foundation on Lymphomas (FIL) has developed a prognostic model based on the tracking of three factors (haemoglobin level less than 12 g/dL, lactate dehydrogenase level greater than normal, and albumin level less than 3.5 g/dL) in more than 300 patients, leading to a prognostic index. This index allows to separate patients into three subsets, each with a different 5-year survival rate: 88% in the low-risk group (no risk factors), 73% in the intermediate-risk group (one risk factor), and 50% in the high-risk group (more than one risk factor). So far, this index has not yet been demonstrated to have any therapeutic implications. Histologic transformation to large B-cell lymphoma is reported in 10-20% of patients. A treatment is required only in symptomatic patients with large splenomegaly, associated or not with cytopenia due to hypersplenism. Asymptomatic patients may be followed for several years by clinical examination and blood counts. The absence of treatment does not influence the course of disease. When a treatment is indicated due to the occurrence of clinical symptoms, the recommended front-line therapy is splenectomy. These patients have only partial response with a persisting bone marrow and blood lymphocytosis but with a correction of anemia, thrombocytopenia, and neutropenia; this improvement can be maintained for years with a free of treatment period lasting 8 years in median. Chemotherapy may be proposed to patients with contraindications to surgery, to elderly patients, or to those who have progression after surgery. Regimens are based on alkylating agents (chlorambucil, cyclophosphamide), fludarabine, and rituximab single agent or combined with chemotherapy. Recently, bendamustine has shown an interesting activity also in MZLs.

Nodal MZL

The present WHO lymphoma classification retains NMZL as a distinct clinico-pathologic subtype within the wide spectrum of marginal zone-derived lymphomas. "Conditio sine qua non" for this diagnosis is a primary lymph node localization in absence of prior or concurrent extranodal site of involvement, with the exception of bone marrow.

Clinical features

Given the recent identification of NMZL, few reports present detailed patients' clinical and outcome data. Only nine clinical series are available. The vast majority of the patients presents with disseminated peripheral and abdominal nodal involvement. Bone marrow involvement occurs in less than half of the patients in major part of series; peripheral blood involvement is quite rare. Performance status is generally good and presence of B symptoms is reported in percentages ranging from 10% to 40%. A serum M-component is infrequently detected in nearly 10% of patients. HCV infection is reported in association with NMZL.

Treatment and outcome

The average 5-year overall survival of NMZL is approximately 60-70%, with an estimated 5-year event-free survival of about 30%. Relapse at extranodal sites is rare. No treatment consensus guidelines have been developed for NMZL, but patients may be managed according to guidelines established for follicular lymphoma. In limited stage disease, surgery and radiotherapy seem appropriate. In advanced stage disease, immunochemotherapy is a relevant option. Among new drugs, bortezomib has demonstrated activity in NMZL. Use of veltuzumab, a humanized anti-CD20 antibody, has been required in few cases of NMZL. In relapsed young patients high-dose therapy and autologous transplant could be considered. In patients with NMZL and HCV-related chronic hepatitis who do not need immediately chemotherapy for the lymphoma, an antiviral treatment with pegylated interferon and ribavirin is recommended.

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PRIMARY MEDIASTINAL LYMPHOMA

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Summary

Primary mediastinal large B cell lymphoma (PMBCL) is a unique type of B-cell lymphoma probably arising from a putative thymic medulla B cell. It constitutes 6-10% of all diffuse large B-cell lymphomas (DLBCL), occurring more often in young females. PMBCL is characterised by a diffuse proliferation of medium to large B-cells associated with sclerosis and a degree of compartmentalisation. PMBCL expresses B-cell–related antigens such as CD19, CD20, CD22, CD79a and CD45. CD30 staining is observed in the vast majority of cases (~80%), although it is weaker and less homogeneous than in classic Hodgkin Lymphoma and anaplastic large cell lymphoma (ALCL). Molecular analysis shows it to be distinct from other types of DLBCL. PMBCL is characterized by a locally invasive anterior mediastinal bulky mass with pleural or pericardial effusions in a third of cases, often producing cough, chest pain, dyspnea, and superior vena cava syndrome. Some retrospective analyses suggests that it may respond better to third-generation chemotherapy regimens than to the more commonly used CHOP. The addition of rituximab reduced these differences and R-CHOP is now the most widely used chemotherapy regimen for PMBCL. The role of consolidation with radiotherapy (RT), which is often used to treat residual mediastinal masses, remains still unclear. Treatment with R-CHOP regimen followed by RT was associated with a 5-year survival of 75-85% The real role of FDG-PET scanning requires prospective studies, and it is hoped that this may allow the de-escalation of RT accordingly to yield reliable prognostic information.

Introduction

Primary mediastinal large B cell lymphoma (PMBCL) was first described in the 1980s. It is a relatively uncommon clinicopathologic entity specifically recognized in the WHO classification of Lymphoid Malignancies.[1] This malignancy is characterized by aggressive and locally invasive behaviour. Although in some respects it resembles nodal DLBCL, it has distinct epidemiologic, morphologic, immunophenotypic, and clinical features. This lymphoma is a DLBCL that arises in the thymus from a putative thymic peripheral B cell.

Epidemiology

PMBCL constitutes 2-4 % of non-Hodgkin lymphoma (NHL) and 6-10% of DLBCL.[1] It is more common in young adults (median age 35-40 years) with a female predominance and originates in the mediastinum, where it frequently presents with features of local invasion. No particular genetic or environmental risk factors have been clearly identified.

Pathology and biology

The diagnosis of PMBCL is based on the integration of morphologic, immunophenotypic, genetic, and clinical data, according to the WHO classification, the differential diagnosis mainly including classical Hodgkin lymphoma (cHL), mediastinal grey zone lymphoma (MGZL) and other DLBCL subtypes, from which it cannot be reliably distinguished in some cases. It is postulated that PMBCL derives from the small subset of thymic B cells with asteroid shape located around the Hassall's corpuscles in the medullary thymus which share with PMBCL a CD10-,CD21-,CD23+ - phenotype. The clinical presentation within the anterior mediastinum and the identification of normal thymic cells that express the MAL protein supports this hypothesis.[2] PMBCL has distinct morphological and phenotypic features. It is typically associated with compartmentalizing alveolar fibrosis in the vast majority of cases. The fibrosis tends to surround groups of lymphomatous elements, producing compartmentalization of the neoplastic growth. In cases when thick collagen bands enclose clusters of neoplastic cells, the sclerosis is readily appreciated on hematoxylin and eosin–stained sections. Tumor cells are large and polymorphic with rather abundant clear cytoplasm, and nuclei may be lobulated with prominent eosinophilic nucleoli. Not infrequently, Reed-Sternberg-like cells may be seen. In such instances, careful immunohistochemical evaluation is warranted in order to exclude the diagnosis of cHL . In this regard, it should also be noted that "grey zone" borderline cases combining features of PMBCL and cHL or cases of composite PMBCL and cHL can rarely be encountered.[3] On immunophenotypic analysis, despite generally lacking surface and cytoplasmic immunoglobulin (Ig), PMBCL expresses B-cell–related anti-

gens such as CD19, CD20, CD22, CD79a, PAX5 and CD45. CD30 staining is observed in the vast majority of cases (~80%), although it is weaker and less homogeneous than in cHL and ALCL. CD15 is occasionally present. Tumor cells are more frequently positive for IRF4 (75%), BCL2 (55–80%), and CD23 (70%), while BCL6 expression is variable (45–100%) and CD10 is more often negative (8–32%). Tumor cells are often MAL positive, as a consequence of MAL gene overexpression (4). Furthermore, PMBCL usually expresses BOB1, PU1, and OCT2, co-expresses TRAF1, and presents with nuclear REL.

Diagnostic criteria

The main differential diagnoses are cHL and DLBCL. (Table 1). Classical Hodgkin lymphoma can be distinguished from PMBCL by histological features such as abundant infiltration with granulocytes and lymphocytes as well as histiocytes in the former. In addition, cHL expresses CD15 and less often a full set of B-cell markers. MAL has been reported to be specifically expressed in PMBCL, but is rather a difficult marker to stain for in routine practice. Some cases with either morphological features of PMBCL but immunophenotypical features of cHL or vice versa do not allow a final diagnosis and are classified as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL or so-called mediastinal grey zone lymphoma (MGZL).[3] The differential diagnosis DLBCL- NOS is not always easy. The distinct morphological features of PMBCL, such as clear cell proliferation and sclerosis, may be difficult to evaluate on small biopsies and there is a lack of well defined diagnostic criteria that can be routinely applied. The expression of CD23 in PMBCL may be useful in that respect. Gene expression analysis allows for an improved distinction between PMBCL and DLBCL-NOS but can as yet not be used in clinical practice.

Table 1. Comparison of the pathological and immunophenotype features of: primary mediastinal large B cell lymphoma (PMBCL); diffuse large B cell lymphoma (DLBCL); nodular sclerosis classical Hodgkin lymphoma (NScHD); mediastinal gray zone lymphoma (MGZL).

Features	*PMBCL*	*DLBCL*	*NScHL*	*MGZL*
Morphology	Sheets of large cells; clear cells ; no inflammatory	Sheets of large cells with variable aspects	Lacunar Hodgkins Reed-Sstenberg cells Inflammatory polimorphous infiltrate	Sheets of pleomorphic large cells; Lacunar Hodgkins Reed Stenberg cells; sparse inflammatory infiltrate
Sclerosis	70-100% (alveolar, fine bands)	Absent	100% (large bands)	Focal fibrous bands
CD45	positive	positive	negative	positive
CD30	Positive weak (70-80%)	Rare (anaplastic variant)	positive	positive
CD15	negative	negative	positive	positive
CD20	positive	positive	negative	positive
CD79a	positive	positive	usually negative	positive
PAX-5	positive	positive	weak positive	positive frequently
Immunoglobulin	negative	positive	negative	negative
BOB-1	positive	positive	negative	positive frequently
OCT-2	positive	positive	negative	positive frequently
MAL expression	60-70%	<10%	<20%	30-40%

Clinical presentation

PMBCL normally presents with a bulky tumor in the anterior mediastinum that is rapidly progressive and gives rise to local compressive effects including dyspnea, cough, dysphagia, and superior vena cava obstruction. Up to one half of patients have symptoms and signs of superior vena cava syndrome, thoracic and neck vein distension, facial edema, conjunctival swelling, and occasionally arm edema. This results in relatively early presentation so that at diagnosis, most patients (around 80%) have stage I or II disease. The mediastinal tumor is frequently bulky, being over 10 cm in two-thirds of patients, and infiltrating the lung, chest wall, pleura, and pericardium. Pleural or pericardial effusions are present in one-third of cases. Breast edema is common and hoarseness may reflect recurrent laryngeal nerve damage. Figure 1. Despite the local invasiveness, distant spread is infrequent at the outset, and even spread to the supraclavicular nodes is unusual at presentation. Extranodal sites may, however, be involved, particularly in cases of disease recurrence, with an unusual propensity for involvement of the kidneys, adrenal glands, liver, and ovaries. Systemic symptoms, mainly fever or weight loss, are present in a minority of cases. Bone marrow infiltration at presentation is rare but elevated LDH levels are observed in two thirds of patients. MGZL shows similar clinical features, but compared to PMBCL is more common in young men, and more often has extranodal involvement. (Table 2)

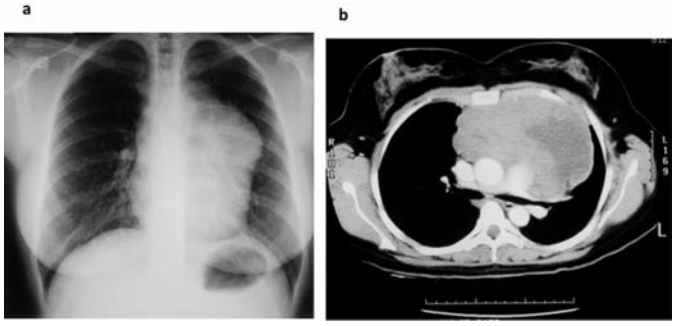

Figure 1. CXR (a) and CT (b) scan from a female patient presenting with PMBCL. Note is the large anterior mediastinal mass, with areas suggestive of central necrosis.

Table 2. comparison of the clinical features of: primary mediastinal large B cell lymphoma (PMBCL); diffuse large B cell lymphoma (DLBCL); nodular sclerosis classical Hodgkin lymphoma (NScHD); mediastinal gray zone lymphoma (MGZL)

Features	PMBCL	DLBCL	cHL	MGZL
Female/male ratio	2:1	1:1	1:1	1:2
Median age	35	55	28	35
Stage I-II	70-80%	30%	55%	70-80%
Mediastinal invol.	ALL	20%	80%	80%
Extranodal sites	uncommon	common	uncommon	uncommon
Bone marrow	2%	10-15%	3%	3%
Elevated LDH	70-80%	50%	rare	70-80%
B symptoms	< 20%	50%	40%	40%
Bulky disease	70-80%	10-15%	50%	70-80%

Diagnostic and staging procedures

The complete staging workup for PMBCL is the same as that routinely used for nodal lymphoma. It includes an accurate physical examination, complete hematologic and biochemical examinations, total body computerized tomography, and bone marrow biopsy. The staging system used is the standard Ann Arbor classification. A diagnostic tissue sample can be obtained by mediastinoscopy, biopsy of the tumor mass through the supraclavicular fossa, anterior mediastinotomy or minithoracotomy. It is important to consider the anaesthetic risk for patients with critical airways narrowing by anterior mediastinal tumours: sometimes may be preferable to obtain a needle core biopsy by a percutaneous route under local anaesthesia, than to obtain a large biopsy but have a patient who cannot be extubated following the procedure because of airway compromise. PMBCL shows almost universal avidity for [18F]-2-fluoro-2-deoxyglucose, making positron emission tomography (FDG-PET) an effective means to assess disease extent and to characterize residual masses at the completion of treatment. The extent of experience with this technique is however too limited to permit major changes to therapy based upon FDG-PET scans at present, pending the results of prospective trials.

Prognostic factors

The utility of the International Prognostic Index (IPI) in PMBCL is limited by the age distribution of the disease and its usual confinement to the mediastinum. This is reflected in the observation that half of patients have low IPI scores at presentation. The age-adjusted IPI has similarly been reported to be of limited predictive value in PMBCL. This may reflect differences between studies, assigning patients as either stage IV or stage 2E when contiguous extranodal sites such as the lung are involved. Elevated LDH to more than twice the upper limit of normal, age over 40, and performance status ≥2 correlated with reduced survival in a population-based series from British Columbia.

Treatment and outcome

The first line of treatment and its outcome is critical in managing PMBCL. Therapy for recurrence or progressive disease is of strictly limited efficacy making curative therapy at the first attempt even more important for this type of lymphoma. It is however important to strike an appropriate balance between delivery of the highest possible cure fraction and minimising the long term morbidity for this young population. A number of choices have to be made, including the initial immunochemotherapy and whether there might be a benefit from high-dose therapy (HDT) in first remission. The role of consolidation radiotherapy to the mediastinum is especially controversial. There is broad agreement that for conventional DLBCL the standard of care is the R-CHOP regimen. Prior to the introduction of rituximab some retrospective and prospective series of PMBCL suggested that a superior outcomes might be achieved with more intensive third generation regimens. The largest series was from the IELSG, which reviewed the outcomes of 426 previously untreated patients with PMBCL (5). Most of the patients that were treated with a third generation regimen received MACOP-B (n=204), the rest either VACOP-B (n=34) or ProMACE CytaBOM (n=39). Although the complete response rate was similar between the third generation subgroup and those treated with conventional CHOP or CHOP-B, the relapse rate at 3 years was significantly lower in the third generation group (12% vs 23%; P=0.02) and the projected 10 year OS and PFS were superior at 71% and 67%, compared to 44% and 33% (P=0.0001 and P=0.0003 respectively). The British Columbia group carried out a population-based retrospective analysis of 153 patients with PMBCL whose treatment was determined by era-specific guidelines. Between 1980 and 1992 MACOP-B or VACOP-B was used, switching to CHOP between 1992 and 2001 and then to rituximab with CHOP (R-CHOP) thereafter. The overall survival for the cohort was 75% at 5 years, with the overall survival at 5 years being 87% for those treated with MACOP-B/VACOP-B, significantly higher than the 71% for those patients treated with CHOP (P=0.048). In the multivariate analysis for OS the type of chemotherapy regimen showed a trend towards improved outcomes but this was not statistically significant.(6) It is generally accepted that the addition of rituximab to chemotherapy for PMBCL yields superior results. The MiNT study compared the outcomes for 824 patients with low risk large B-cell lymphoma randomized to receive CHOP-like chemotherapy with or without rituximab, which included a subset of 87 patients with PMBCL. The difference in OS did not reach statistical significance owing to the small number with PMBCL (3-yrs OS 78 vs 89%, p=0.16), but was of the same order as that seen for the whole trial (85 vs 93%, p<0.001) (7). In a small series from Israel, the addition of rituximab appeared to improve PFS, particularly in those patients receiving CHOP, whilst there was no difference in outcomes in a comparison between either a third generation regimen with rituximab (R- MVACOP-B) or R-CHOP (84% and 74% respectively; P=0.44) The addition of rituximab to dose-adjusted EPOCH in 51 patients with PMBCL has also been reported showing a very favourable outcome with a 3-yrs EFS =93% and OS = 97% in a non-randomized phase II trial (8). Overall, it appears likely that the use of rituximab removes the distinction between different chemotherapy regimens, and R-CHOP is now the most widely used for PMBCL, as it is for other types of DLBCL.

Assessment of the response to initial therapy

The presence of bulky masses at the time of diagnosis, together with the extensive fibrotic elements of PMBCL often results in a residual mediastinal mass being present at the completion of initial chemotherapy. It may be difficult to distinguish inert fibrous tissue from viable residual lymphoma on conventional cross-sectional imaging, and for this reason functional imaging has been extensively investigated. The FDG-PET scan has become the investigation of choice for residual masses in PMBCL, although there is some uncertainty about its positive predictive value in particular. A systematic review of FDG-PET studies has examined post therapy response assessment in lymphoma. In the studies reporting evaluation of residual masses in aggressive lymphomas, the demonstrated sensitivity of PET ranged from 33% to 87% and the specificity from 75% to 100%. A prospective study of FDG-PET scanning in patients with PMBCL after 4 cycles of accelerated R-CHOP-14 performed at Memorial Sloan Kettering Cancer Center showed that among 14 patients with interim positive PET scans, none had viable lymphoma present on biopsy, and all remained in remission after completing consolidation R-ICE chemotherapy (9). A prospective study of FDG-PET scanning of 125 patients with PMBCL conducted by the IELSG yielded a relatively low rate of negative scans (47%) after immunochemotherapy with very low positive-predict value (PPV= 18%) despite excellent clinical outcomes, albeit after the use of consolidation radiotherapy in 102 cases (10). These data indicate that further evaluation is required before modifying planned therapy based upon FDG-PET evaluation alone in PMBCL. The false positive rate in particular requires definition, although de-escalation of therapy based upon the finding of a negative FDG-PET scan is entering clinical practice and is the subject of a prospective randomized trial.

The role of consolidation radiotherapy

Irradiation of the mediastinum is one of the most controversial aspects of the management of PMBCL. It is not attractive to administer radiation extensively to a group dominated by younger subjects, who may be put at increased risk of second malignancies, especially breast cancer, and accelerated coronary artery disease. On the other hand, the chances of cure following recurrence of PMBCL are relatively poor, so that any approach which puts patients at increased risk of relapse is to be strenuously avoided. The best outcomes historically have been reported with regimens that incorporated radiotherapy as part of the primary treatment. It is clear from the IELSG series that many patients completing chemotherapy in PR may be converted to CR following radiotherapy or result in long term remission after a positive FDG-PET scan. Univariate and multivariate analysis in two retrospective series have suggested that the use of radiotherapy was correlated with better EFS and OS. Those who would prefer to avoid irradiation of the mediastinum can however point to good results in studies that have used chemotherapy alone. In British Columbia, the introduction of routine radiotherapy to consolidate response after chemotherapy was not accompanied by any improvement in PFS and OS , even for initially bulky disease. The study from Memorial Sloan Kettering Cancer Centre which used radiotherapy in only 7% of patients treated with the NHL-15 regimen (comprising intensified doxoroubicin, vincristine and cyclophosphamide) had excellent results, with OS= 84% at a median follow up of over 10 years (11). The results recently reported with dose adjusted R-EPOCH also claimed to negate the need for irradiation.(8) High dose therapy and ASCT as intensification of first remission or treatment of recurrent disease. At present there is no good evidence to support its use in this context for PMBCL. The results now obtained with R-CHOP and mediastinal consolidation radiotherapy or with more intensive chemoimmunotherapy regimen as dose adjusted-EPOCH appear comparable with the reports of HDT as consolidation first line therapy. The exception to this may be those patients whose lymphoma progress or obtain an inadequate response during primary therapy . These group of patients have a very poor outlook and it is appropriate in this setting to test chemosensitivity to a second line regimen prior to myeloablative treatment, proceed in those fit enough to do so and consolidate the response with involved field radiotherapy. The probability of recurrence after successful initial therapy for PMBCL appears to be lower than that of DLBCL in general, although this may reflect the earlier stage at presentation, the younger age or possibly the biology of the disease. Most recurrences occur within the first year, and they are rare beyond two years from completion of therapy Extranodal sites of recurrence are not uncommon, especially the kidneys and spleen, but spread to the central nervous system is highly unusual. Second line treatment strategies are similar to those used for DLBCL, attempting reinduction with non-cross resistant agents, followed by consolidation with HDT-ASCT in those with a good response who remain fit enough. In general the outcomes have been disappointing. The general use of rituximab in first line therapy has made recurrence less frequent but harder to manage successfully.

Open issues and future studies in PMBCL

The principal clinical open questions in the management of PMBCL remain: the role of consolidative mediastinal RT and if it can be omitted in selected patients with PMBCL; if PET-CT scan can drive this selection. For the above reasons a phase III randomized IELSG trial is ongoing to assess the role of radiotherapy in PMBCL patients with PET-negative mediastinal masses after immunochemotherapy. The trial should be able to demonstrate a non-inferior outcome in patients not receiving RT. The study may eventually allow to individualize treatment for each patient by adapting it to the PET response limiting the indication for additional radiotherapy only to the patients who show an inadequate response to immunochemotherapy.

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MANAGEMENT OF CRITICAL BLEEDING IN PATIENTS WITH PLATELET DEFECTS

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Inherited platelet disorders (IPDs) are abnormalities of platelet function and production which give rise mainly to mucocutaneous bleeding syndromes of variable intensity.[1-3] IPDs pathophysioloy encompasses defects of platelet receptors (Glanzmann thrombasthenia (GT); Bernard-Soulier Syndrome; Platelet-Type or Pseudo-von Willebrand Disease; Deficient Collagen Receptor Functions) or Enzyme Deficiencies (Deficiency of Granule Stores: Storage Pool Disease (SPD); Macrothrombocytopenias or Giant Platelet Syndromes). Usually, IPDs manifest early in life with bleeding immediately after injury, primarily in skin (petechiae), from mucous membranes and the nose. Bleeding following invasive procedures is almost inevitable.[1,2] In contrast to patients with haemophilia, IPDs usually do not present with haemarthroses or muscle bleeds and the bleeding occurs at the time of trauma rather than after a delayed onset. Some patients develop lifethreatening blood loss in the gastrointestinal or genitourinary tracts while intracranial haemorrhage can occur, but critical bleeding is usually rare in IPDs subjects. Platelet transfusion is frequently needed for controlling spontaneous bleeding manifestations such as menorrhagia, epistaxis, and gastrointestinal bleeding, and is always needed when trauma occurs or surgery is performed. Childbirth is also a high-risk period for severe bleeding.[3] Antifibrinolytic agents, such as tranexamic acid, are effective in the management of bleeding in patients with thrombocytopathy/-penia. For the treatment of mucocutaneous bleeding, menorrhagia, or gastrointestinal bleeding tranexamic acid is given orally (usually 2–3 times 1,000–1,500 mg daily) or intravenously as monotherapy or as adjuncts of other treatment modalities when severe bleeding occurs.[1,3] Desmopressin (1-desamino-8-Darginine vasopressin, DDAVP) stimulates the release of endothelial vWF and enhances platelet adhesion to the vessel wall, thus shortening the bleeding time in some forms of IBDs. DDAVP can be administered parenterally either via intravenous or subcutaneous injection or intranasally as drops or spray. The recommended parenteral dose is 0.3 μg/kg body weight. The effectiveness of DDAVP for prevention and treatment of bleeding was shown in some forms of IBDs, but response to DDAVP is highly variable among patients with different platelet disorders and may not be sufficient, notably in cases of lifethreatening bleeding. In patients with GT, therapy with DDAVP is not effective.[1,3,4] rFVIIa (NovoSeven®, Novo Nordisk Inc., Bagsvaerd, Denmark) is often used in thrombocytopathic/-penic patients suffering from bleeding that cannot be stopped by the previously described treatments. The mechanism by which rFVIIa stops bleeding is probably related to an increased thrombin generation by a tissue factor-independent process, an enhanced adhesion of platelets to extracellular matrix and a restoration of platelet aggregation.[5-7] Best reported data concerning the efficacy of rFVIIa is documented in patients affected by GT. Poon *et al.*[8] analyzed the data of an international registry for the treatment and prophylaxis of patients with GT and found that prophylaxis with rFVIIa was effective in 29 out of 31 thrombasthenic patients undergoing surgery. On the basis of this study, EMA approved the use of rFVIIa for thrombasthenic patients with antibodies against GPIIb/IIIa and/or HLA and with refractoriness to platelet transfusion therapy. Patients with GT receive at least 3 bolus injections at doses of 80–120 μg/kg body weight of rFVIIa every 1.5–3 h until hemostasis, and the same dosage (at least 3 bolus doses of 80–120 μg/kg) is also used for the treatment of severe bleeding complications in patients with other IBDs as well as in several acquired thrombocytopathies. Early use of rFVIIa seems to increase its efficacy. rFVIIa has been used also in managing otherwise untreatable bleeding complications in patients with thrombocytopenia. In these patients, hemostasis was achieved even at platelet counts <20,000/μl, although the efficacy of rFVIIa increases at higher platelet counts.[9] However, this use is largely empirical, as there are only a few casuistic reporting about successful treatment with rFVIIa alone or in combination with PCs. Therefore, the use of rFVIIa in thrombocytopenia should be limited to life-threatening or conservative non-treatable bleeding complications. When rFVIIa is used, a transfusion of platelet concentrates should be considered taking into account the risk-benefit ratio.[10] Although IPDs are extremely rare diseases, they can offer the clinician an excellent in-vivo experimental model for understanding acquired defects of platelet function, which are on the contrary exceedingly common. Indeed, millions of patients are prescribed antiplatelet agents (APAs) for a variety of clinical conditions such as coronary artery syndrome, stroke, transient

ischemic attacks, and peripheral arterial disease and for patients who are at risk for arterial thromboses. While older APAs have a low bleeding risk profile, the newer APAs currently used in clinical practice appear to have a higher bleeding risk profile. Elderly patients treated with APA are at a higher risk for falls with subsequent development of intracranial hemorrhage (ICH). When patients on APA present with a critical bleeding or when they need an urgent major surgical intervention, treating physicians and transfusion medicine specialists are often challenged with the task of reversing or counteracting the effects of APA [10] To date, no randomized clinical trials have been conducted to address this dilemma, nor are there known specific antidotes available. As a consequence, there are no evidence-based guidelines for APA reversal and the treatment of such compelling patients is performed in and empyrical way. Specific tests to assess anti-PLTdrug effect and platelet transfusion therapy can assist clinicians in the management of patients on antiplatelet treatment who present with bleeding complications or trauma or who require urgent surgery. Prospective clinical trials to optimize the dose and timing of PLT transfusion will greatly add to our knowledge of the efficacy of PLT transfusion. In the meanwhile, the knowledge of pathophysiology and treatment of IPDs is an essential background on which the Haematologists can rely for their clinical decisions.

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HEMORRHAGIC EMERGENGIES IN INHERITED BLEEDING DISORDERS

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Abstract

Hemorrhagic emergency in inherited bleeding disorders (IBD) is characterized by laboratory and clinical situations potentially life-threatening or resulting in a permanent functional damage if not promptly recognized and appropriately prevented or treated. Iin some circumstances bleeding can be expected as a consequence of an inadequate or sub-optimal prophylaxis (*e.g.* orthopedic surgery in hemophilic patient with inhibitor) and alternative therapeutic options are designed in advance. Unexpected bleeding can be the first sign of a novel severe disorder during the neonatal period or in early infancy or can represent the clue for an overlooked IBD, thereafter identified even at elder age. The risk of inhibitor appearance in patients with hemophilia must always be considered during the first exposure days in severe patients but also late in life in those with mild disease, sporadically treated with replacement therapy. Routine monitoring and scrutiny for these well-known complications should be always permanently adopted not only in patients with severe inherited bleeding disorders, but also in those with milder forms who may carry characteristics at risk (*e.g.*, particular gene mutations).

Introduction

Hemorrhagic emergency in inherited bleeding disorders (IBD) is characterized by laboratory and clinical situations potentially life-threatening or resulting in a permanent functional damage if not promptly recognized and appropriately prevented or treated. In some circumstances bleeding can be expected as a consequence of an inadequate or sub-optimal prophylaxis (*e.g.* orthopedic surgery in hemophilic patient with inhibitor) and alternative therapeutic options are designed in advance. Unexpected bleeding can be the first sign of a novel severe disorder during the neonatal period or in early infancy or can represent the clue for the presence of an overlooked IBD, thereafter identified even at elder age. Although the wide use of screening tests and the knowledge that the vast majority of significant IBD are easily suspected and confirmed by specific assays allow the identification of patients at risk, nevertheless careful clinical monitoring of at risk patients and prompt laboratory evaluation and appropriate treatment in case of unexpected bleeding in a previously undiagnosed patient are recommended to minimize the risk of potential life-theratening bleeding.

Clinical emergencies in IBD

Three large categories of situations can be identified as causes of hemorrhagic disorders in IBD. In the first group, bleeding may be the presenting symptom unraveling the existence of an IBD. In the most severe deficiencies, the symptom may be very peculiar suggesting the clue for the diagnosis of a specific IBD. The most feared and severe manifestation is neonatal cerebral bleeding. This can be present immediately after delivery, as a consequence of trauma during vaginal delivery or being caused by the use of mechanical device like forceps or ventouse, or occurring within a few days from parturition. Cerebral bleeding occurring at delivery or in the few days after may be the presenting symptom of patients with severe hemophilia A or B, X-linked bleeding disorders manifested almost exclusively in males. However, although less frequent compared to hemophilia, autosomal recessive disorders like afibrinogenemia, FVII or FXIII deficiency present a relatively higher risk of cerebral bleeding during this period.[1] Of interest, FXIII deficiency is characterized by the highest rate during life-time, if the patient is not undergoing prophylactic treatment. While the laboratory diagnosis of hemophilia, FVII deficiency or afibrinogenemia takes advantage of evidence of an abnormality easily detected by using the usual screening tests (PT, APTT) followed by specific factor assay, the diagnosis of FXIII requires a high suspicion in presence of the normality of these assays. For this disorder, only the results of a screening test (clot solubility assay in urea 4M or monochloroacetic acid 1 %) can suggest the requirement of a specific FXIII assay, which however nowadays is more easy to perform than in the past. Bleeding from umbilical stump is a second early important symptom suggesting the presence of a severe bleeding disorder and is usually associated with severe FXIII deficiency, followed by afibrinogenemia and FX deficiency.[1] Early diagnosis is important because the very long half-life (approximately 11-14 days) may allow patients with FXIII deficiency to start as soon as possible prophylaxis with the concen-

trate of FXIII, usually 10-20 U/kg every three-four weeks.[2] Since half-life of fibrinogen is similarly good (approximately 4 days), prophylactic treatment with fibrinogen concentrates has also been used with good results in some patients with afibrinogenemia and cerebral bleeding or recurrent bleeding.[3] However, recent data suggests that prophylaxis may be of benefit also for FVII deficient patients, although the half-life is very short (around 3 hours). The second group includes the occurrence of severe spontaneous bleeding in a patient already diagnosed with a bleeding disorder. In addition to patients with afibrinogenemia, FXIII or FVII deficiency, hemophilia patients may be burdened with a 5-10 % rate of intracranial hemorrhage, which is particularly difficult to manage in patients with inhibitors.[4] Hemophilic patients may also suffer from spontaneous hematoma of ileo-psoas muscle. This type of bleeding manifestation should be diagnosed and treated as soon as possible and for several days to minimize the risk of severe femoral nerve compression with loss of function. With the widespread use of prophylaxis, this kind of bleeding is declining in frequency and is now rare in children. Occult bleeding from the throat during inflammatory/infection disorders (*e.g.*, tonsillitis) may go undetected as well as pharyngeal hematoma from foreign body trauma in toddlers and they can cause severe anemia, delayed treatment with the risk of respiratory obstruction. Careful monitoring is required especially in very young children. Some patients with von Willebrand disease (VWD) may suffer from recurrent bleeding from gastrointestinal tract due to angiodysplasia. This complication is particularly frequent and recurrent in those patients with the lack of high molecular weight von Willebrand factor (VWF) multimers in plasma (type 2A and type 3 VWD) and prophylaxis with VWF/FVIII concentrate may not always control bleeding efficaciously.[5] Large amounts of packed red cells may also be required. Bleeding at menarche in women with VWD, especially with the more severe forms, may be so severe as to prompt immediate treatment with replacement therapy. In the third group, the emergency bleeding may be triggered by trauma or surgery. Table 2 summarizes the different situations which may concur to result in an inappropriate control of bleeding in these situations. In addition to inadequate timing and dosage of replacement therapy, surgically amenable reasons or delayed treatment for trauma, the onset of alloantibodies against the deficient factor represents the most critical issue in this group. Most of these antibodies are of IgG class directed against the clotting activity of FVIII and FIX. The prevalence vary across the different severity of the disorder, approaching 30 % of cases with severe hemophilia A compared to 5 % of moderate/mild cases.[6] The risk of inhibitor is very high within the first 20 infusions in severe hemophilia (FVIII/FIX < 1 U/dL) and then declines. No different influence of recombinant or plasma-derived concentrate in this regard is clearly evident so far. Persistence of bleeding despite theoretical adequate replacement therapy or failure of prophylaxis to prevent bleeding during surgery may represent the initial clue to inhibitor development. Sometimes the inhibitor may occur at surgery, complicating the healing and outcome. The prevalence in severe hemophilia B is by far lower (around 5 %) but the onset may be particularly abrupt and alarming since in some cases inhibitor development is associated with severe allergic/anaphylactic reactions.[7] Strict laboratory monitoring is advised especially during the first infusions in severe hemophilia patients to detect early the onset of inhibitor in order to plan the most appropriate treatment (rFVIIa or FEIBA to control bleeding when inhibitor is higher than 5 Bethesda Units and Immunotolerance regimen to eradicate the inhibitor). While no case of inhibitor has been reported in patients with mild or moderate hemophilia B, some patients with mild/moderate hemophilia A may develop such alloantibodies. Most of these patients have FVIII gene missense mutations clustered in a region at the junction between the C1 and C2 domain and in the A2 domain. Arg593Cys, Tyr2105Cys, Arg2150His and Trp2229Cys are the most common reported mutations.[8] At variance with what occurs in severe cases, it has been demonstrated that the risk increases with age, often after a period of intensive treatment with FVIII concentrate, reaching a 13.3 % of risk after 100 exposure days. This amount of infusions could take several years to be reached because bleeding is less frequent and replacement therapy less used in these patients compared to the severe ones. Most of these inhibitors may cross-react with the FVIII of the patients and in these cases the basal level drop to < 1 U/dL, thus contributing to the occurrence also of spontaneous bleeding. Since a large proportion of these patients may benefit from desmopressin, with no risk of inhibitor, this treatment should be used whenever possible to reduce the risk and sometimes it may be useful also for treatment when the antibody does not react with patient's FVIII. Apart from hemophilia, occurrence of an inhibitor in patients with severe IBD is very rare, with the only exception of FXI deficiency. Recently, it has been demonstrated that inhibitor may occur in patients with severe FXI deficiency associated with the so-called type II Jews mutation (homozygous Glu117stop), when treated with fresh frozen plasma. Since the risk of and the severity of bleeding may often be anticipated depending on the actual clinical circumstance[9], caution should be used when deciding to adopt replacement treatment and the type of product for anti-hemorrhagic prophylaxis in these patients. Tranexamic acid with or without rFVIIa has proven useful in these patients. It should also be borne in mind that mild disorders of clotting system could go unrecognized even at adult age if no specific hemostatic challenge occurred in the past. Mild hemophilia or some cases of VWD may become manifest only after invasive procedure (including minor surgery or tooth extraction), since in these cases often screening tests may be normal.

Table 1. Causes of bleeding

Clotting abnormalities	Congenital	Acquired Secondary to other disorder(s) Apparently idiopathic
Quantitative/qualitative platelet defects		
Acquired hemostatic defects (e.g, drugs)		
Trauma or surgery		

Table 2. Hemorrhagic emergencies triggered by trauma or surgery

In patients already diagnosed	Inadequate anti-hemorrhagic prophylaxis
	Anatomical reasons
	Inhibitor occurrence
In patients with mild inherited bleeding disorder still undiagnosed and manifest after surgery or trauma (*e.g.*, mild hemophilia)	

A frequent "Functional" hemorrhagic emergency: treatment of hemarthrosis in hemophilia

A large body of evidence now suggests that joint bleeding, the typical clinical manifestation of the hemophilic syndromes, should be prevented as much as possible rather than be treated after its occurrence. After the results of the initial experience from Sweden and of recent studies, it is now clear that late severe arthropathy may occur even after a few joint bleedings and that prophylactic regimens after the first episode of joint bleeding or after two or more have been largely implemented to prevent such complication, with clear satisfactory results.[10] The future introduction for clinical use of recombinant products with prolonged half-life seems extremely promising in determining a more safe coverage with adequate circulating factor levels able to prevent joint bleeding while reducing the number of infusions, an issue particularly critical in young children. When occurring, this symptom should be immediately treated with adequate amount of the deficient factor (at least 25-50 U/kg body weight), even for a couple of days or longer. Home-treatment programs have significantly contributed to reduce time intervals prior to treatment.

Conclusions

The management of patients with inherited bleeding disorders should foresee the risk of emergency bleeding. Regular surveillance allows the identification of patients particularly at-risk (*e.g.*, inhibitor development). Surgery should be always coordinated with hemostasis staff to plan treatment for unexpected bleeding. Treatment of hemarthrosis requires adequate factor dosage and should be timely to minimize long-term disabilities. Prophylaxis in children should be started as soon as possible after the occurrence of a first bleeding into joint to minimize the risk of long-term orthopedic disabilities.

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MANAGEMENT OF BLEEDINGS IN ACQUIRED HAEMOSTATIC DEFECTS

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Introduction, Definitions and Classification

Acquired haemostatic defects (AHD) may arise according to different causes and may involve the entire cascade of interactions among vascular endothelium, platelets and the multiple plasma proteins that ultimately results in the conversion of fibrinogen to fibrin, and cross-linking of fibrin by activated FXIII, which stabilizes the formed clot. Qualitative or quantitative deficiencies of any of these haemostatic factors involved in these cascade reactions may be associated with clinical significant bleeding disorders. Differently from inherited haemostatic diseases, the AHD are complex and usually involve more than one haemostatic mechanism: the most common forms of AHD are those associated with liver insufficiency as well as those caused by antithrombotic drugs (anti-platelets agents) including indirect (vitamin-k-antagonists) or direct (heparins and novel direct oral anticoagulants or DOAC) anticoagulants. The acquired haemostatic inhibitors (AHI) are relatively rare forms of AHD but they should be immediately diagnosed because of their severe bleeding tendency. These AHI are circulating antibodies (mainly immunoglobulin) that specifically neutralize the activity of the various haemostatic proteins and result in a deficiency state; they may develop in the plasma of individuals whose haemostatic mechanisms were previously normal and may function as a circulating anticoagulant when directed against a specific haemostatic protein. In this scenario, the immunoglobulin are designated as auto-antibody inhibitors, in contrast to alloantibody inhibitors, which arise in individuals with congenital factor deficiencies as a consequence of replacement therapy. The most well-characterized inhibitor is directed against factor VIII (FVIII) and occurs with a prevalence of 15-25% in patients with inherited hemophilia A (alloantibody): the clinical condition caused by the auto-antibodies is named Acquired Hemophilia A (AHA). Another acquired haemostatic defect caused by the interaction with the glycoprotein von Willebrand factor (VWF) is known as Acquired von Willebrand Syndrome (AVWS) to be distinguished from the inherited defects of VWF defined as von Willebrand Disease (VWD). AHA and AVWS are the two relatively most common forms of AHD even though very rare AHI have been also reported against Factor V, VII, X, XI, XII and XIII. Management of patients with AHD is difficult and costly and the attention of an experienced hematologist consultant is always required. Acquired Hemophilia A (AHA) is a potentially life-threatening bleeding disorder occurring in patients without a previous personal or family history of bleeding caused by the immune-mediated development of acquired FVIII auto-antibodies. The demographic, the clinical presentations, the management of bleeding episodes as well as the eradication of auto-antibodies with immune therapies have been extensively investigated in the European Acquired Hemophilia (EACH2) Registry. The incidence of AHA has been reported to be between 1.3 and 1.5/million/year and has a biphasic age distribution: a small peak occurs in 20- to 40-year old patients with a female predominance due to the high prevalence of the post-partum period and a large peak in patients aged over 65 year with an incidence of 14.7/million/year in people aged over 85. Clinical presentation of patients with AHA is quite different from that of inherited HA. Patients usually present with subcutaneous bleeds, which are often extensive. Soft tissue bleeds such as muscle hematoma, retroperitoneal bleeds and intracranial haemorrhage are also common, whereas joint bleeds are seen less commonly than in congenital HA. Gastrointestinal bleeding can be life-threatening and haematuria may occur. Some patients are diagnosed with bleeding at the time of invasive procedures while postpartum haemorrhage and bleeding following Caesarian section is the usual presentation of AHA associated with pregnancy. Bleeding episodes are unpredictable and may be very severe: about 8% of AHA patients have fatal bleedings. In contrast, about 25% of these patients have a relatively mild bleeding and do not usually require haemostatic therapy. The diagnosis of AHA is often delayed but it should be suggested by the recent onset of typical bleeding symptoms and the finding of a prolonged activated Partial Thromboplastin Time (PTT). AHA diagnosis is confirmed by the finding of a reduced FVIII activity level and the presence of a time- and temperature-dependent inhibitor. The kinetic of this anti-FVIII inhibitor (auto-antibody) is usually different (type 2 kinetic curve) from that of the alloantibody found in a patient with inherited HA (type 1 kinetic curve). After confirming the presence of a anti-FVIII inhibitor, the Bethesda assay with Nijmegen modification is used to evaluate inhibitor titre levels: it is very important to know the levels of anti-FVIII inhibitors because patients with low (<5) or high (>5) inhibitor titres must be managed with different therapeutic options. A lupus anticoagulant (LAC) can mimic an AHI, although typically the LAC should be differentiated from AHI in the laboratory by the absence of increasing neutralization of clotting factors activity after prolonged incubation in mixing studies. Clinically, the LAC is not associated with the same type of extreme bleeding symptoms which characterizes AHA. Management of patients with AHA is complex and ideally should be always coordinated by an expert hematologist with the help of other specialists according to the specific clinical sites of bleedings. Treatment of AHA is directed at bleeding control, inhibitor eradication to prevent subsequent bleeding events and treatment of any underlying causative disease. No randomized control clinical data is available to guide appropriate intervention and therefore selection of appropriate therapeutic approaches has been based primarily on expert opinion. Recent data from the European Acquired Hemophilia (EACH2) Registry have been used to prepare recommendations to guide selection of initial therapeutic intervention. Optimal treatment involves protection of the patients against trauma; invasive procedure should not be undertaken unless unavoidable. Bleeds are usually treated acutely with FVIII bypassing agents such as activated Prothrombin Complex Concentrates (aPCC) or recombinant activated Factor VII (rFVIIa): the most widely used aPCC is FVIII Inhibitor Bypassing Agent (FEIBA) and the rFVIIa is NovoSeven. The safety and efficacy data of FEIBA and NovoSeven was derived from congenital HA with alloantibody but numerous case reports and retrospective analyses indicate that both FEIBA and rFVIIa are safe and effective in controlling bleeding episodes in AHA patients. However, these drugs are associated with potentially life-threatening side effects. Such as myocardial infarction, disseminated intravascular coagulation, arterial and venous thrombosis, pulmonary embolisms and stroke. Human recombinant or plasma derived FVIII concentrates are rarely efficacious. Porcine plasma-derived FVIII concentrate has been used in the past with some success but the data about efficacy and safety are scanty Some patients with a low-tire autoantibody inhibitor and measurable baseline FVIII may respond to a desmopressin (DDAVP) infusion: therefore this approach should be tested. The inhibitor eradication therapy is necessary in patients with AHA to reduce morbidity and mortality because the risk of recurrent bleeding events still persists until the anti-FVIII inhibitors are present. Eradication of the autoimmune inhibitor antibody with immunosuppression is indicated as soon as the diagnosis is confirmed and the bleeding problems have been contained. Steroids, alone or combined with citotoxic agents such as cyclophosphamide or azathioprine, induce remission in about 70% of patients. Current evidence does not support the use of intravenous immunoglob-

ulin (IVIG) to suppress the AHA inhibitors except perhaps for low titer autoantibody. Acquired Von Willebrand Syndrome (AVWS) is an acquired bleeding disorder, first reported in 1968, with clinical and laboratory features similar to inherited von Willebrand disease. This rare bleeding disorder occurs mainly in patients with underlying lymphoproliferative, cardiovascular, myeloproliferative and immunologic disorders. However abnormalities about the levels, structure and function of circulating von Willebrand factor (VWF) can be found in many other clinical conditions. Among hematologic diseases Monoclonal gammopathies of uncertain significance (MGUS) and Essential Thrombocythemia (ET) are considered to be the relatively most frequent conditions associated with AVWS but other acquired VWF defects can be also found in many other chronic and acute Lympho- and Myeloproliferative disorders. In most instances, AVWS is identified because of bleeding complications: in fact more than 80% of the patients with this syndrome are active bleeders. Recurrent bleeding episodes occur in about 20-33% of patients with AVWS, especially following major trauma and surgery. Because of the heterogeneous mechanisms of AVWS, more than one therapeutic approach is often required to prevent or treat acute bleedings. Remission from some forms of AVWS can be obtained when the underlying disorders are treated. It has been challenging to collect data on AVWS as even large centres do not have sufficient patients with AVWS to comprehensively evaluate this rare bleeding disorder and there have been no large prospective studies of AVWS. Consequently, the actual prevalence of AVWS in the general population is somewhat uncertain. Between 1998 and 1999, a retrospective survey was conducted and published as an official communication of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Hemostasis (ISTH), which described information on cases in the ISTH-SSC registry. Since then, a number of major reports on AVWS cases have been published by single institutions. The prevalence of AVWS is probably underestimated because few physicians search for VWF abnormalities among patients with hematological, cardiovascular and immunologic disorders. Recently, a panel of experts proposed recommendations for haematologists on the treatment of AVWS. In contrast to inherited VWD, in AVWS, VWF is synthesized in normal or even increased quantity in most patients. In patients with AVWS, low plasma levels of VWF can result from accelerated VWF removal from the plasma by three main pathogenic mechanisms: a) specific or non-specific auto-antibodies that form circulating immune complexes with, and inactivate, VWF (these complexes are cleared by cells bearing Fc-receptors that bind immunoglobulin G (IgG); b) adsorption of VWF by malignant cell clones; and c) loss of high-molecular-weight (HMW) VWF multimers under conditions of high shear stress. Compared with AHA (which is always caused by auto-antibodies against FVIII), AVWS has more heterogeneous pathogenic mechanisms. None of the proposed mechanisms appear to be disease-specific, and the same mechanism can be responsible for AVWS in different underlying disorders associated with the syndrome. Additionally, in some patients, the basic mechanism is unknown. Another important mechanism sometimes forgotten of the acquired VWF defects is the increased VWF proteolysis by specific proteases which cleaves the VWF HMW forms. There are two main clinical situations in which the diagnosis of AVWS should be considered: (i) bleeding patients whose laboratory finding suggests abnormalities of VWF, and (ii) patients known to have a disorder associated with AVWS who are seen before undergoing procedures that are associated with a high risk of bleeding. Distinguishing AVWS from inherited VWD is important because the approaches to treatment of these conditions can be quite different. An onset of bleeding later in life, and a negative family history, should prompt the suspicion of AVWS, but additional workup can be required since mild VWD can be asymptomatic for decades and it may not be associated with a remarkable family history due to its low penetrance. On the other hand, the presence of an AVWS-associated disorder does not prove AVWS is present since these disorders may occur together coincidentally. When it is difficult to ascertain if the cause is inherited or acquired, it can be helpful to gather additional evidence, for example by testing of family members, performing genetic analysis for VWD mutations, and testing for VWF-specific antibodies and inhibitors. The tests used for the diagnosis of AVWS and of other acquired defects of VWF are the same as those used to assess inherited VWD. When used properly, VWF multimer analysis can be a very sensitive tool for detecting structural abnormalities of VWF and the pattern of abnormalities can help to distinguish AVWS from inherited VWD. Autoantibodies play a role in the pathogenesis of some forms of AVWS, in particular those associated with lymphoproliferative disorders, and their presence appears to be associated with a more severe bleeding tendency. In a minority of patients, inhibitory (neutralizing) antibodies can be detected in mixing studies evaluated by VWF:RCo or VWF:CB as endpoints. In contrast to AHA, where FVIII inhibitors are virtually always detectable with standard laboratory assays, the frequency of inhibitor detection is low in AVWS. The treatment goals in AVWS are: to control acute bleeds, to prevent bleeding in high-risk situations, and to obtain long-term remission. The strategies utilized to obtain these goals depend on the underlying disease mechanisms. Whenever possible, treatment should address the underlying disorder, which can treat the AVWS as well. However, it is not always possible to treat the underlying disorder. Furthermore, achieving a partial remission of the underlying disorder does not always result in an improvement of the bleeding symptoms of AVWS. The available evidence for efficacy and safety of the commonly used haemostatic treatments is summarized below by single therapeutic approach. Desmopressin, a synthetic analogue of vasopressin, can be used to prevent and control bleeding in some patients with AVWS. Desmopressin is usually administered in doses of 0.3 microgram (μg)/kilogram (kg) of body weight, given intravenously over 30 minutes, once or twice daily. In the only prospective clinical trial of desmopressin therapy, performed in 10 patients with monoclonal gammopathy of uncertain significance (MGUS), all subjects had improved VWF levels 30 minutes after treatment, whereas VWF levels were close to baseline by 4 hours after desmopressin treatment. Therefore VWF:Ag and VWF:RCo, along with FVIIIC, should always be closely monitored when desmopressin is used for prophylaxis and treatment of bleeds. Caution must be exercised with this therapy in patients that have cardiovascular disorders and/or are elderly and measures need to be taken to prevent fluid overload and hyponatremia, which are the most common adverse effects of desmopressin. VWF/FVIII concentrates can be used for replacement therapy. In our own clinical practices, we start with doses between 30 and 100 VWF:RCo units/kg, depending on the patient's residual VWF activity, severity of bleeding and presence of inhibitors. Similar to desmopressin, the half-life of infused VWF can be very short in AVWS, in particular in patients with AVWS associated with MGUS or inhibitors. Close monitoring of the clinical response, with measurements of VWF activities, are needed for tailoring doses and dose intervals. Intravenous gammaglobulin (IVIG) for AVWS were assessed in an open-label cross-over study in patients with AVWS associated with MGUS of the IgG class (IgG-MGUS): doses of 1 gram/kg body weight per day were used for 2 days. An increase of VWF and FVIII, and shortening of the bleeding time, were observed the day after the second infusion, with levels reaching their maximum after 4 days and slowly returning to baseline within 21 days. IVIG was not effective in AVWS patients with MGUS of the immunoglobulin M (IgM-MGUS) class. Repeated doses of IVIG every 3 weeks are effective to induce long remission from AVWS but lower doses (0.5-0.75 mg/Kg) are not sufficient to correct these VWF defects. Activated recombinant factor VII (rFVIIa) as a hemostatic agent has been also used in patients with AVWS and VWD, particularly for those who have significant bleeding manifestations and alloantibodies against VWF. rFVIIa is usually administered at a dose of 90 μg/kg body weight (range 40 to150 μg/kg), for a median of 3 doses (range 1-54). Treatment is usually effective, with responses reported in 96% of patients. Adverse events appear to be uncommon although myocardial infarction was reported in one patient with type 2A VWD. Thromboembolic complications are rare among hemophilia patients receiving rFVIIa, but it is unclear if this is also true for patients receiving this therapy for AVWS or VWD. Caution should be exerted, particularly when treating elderly patients and others at increased risk for thromboembolism. Plasmapheresis can be used to reduce the levels of autoantibodies and paraproteins of any immunoglobulin class although the treatment is more effective in reducing the levels of IgM antibodies. Plasmapheresis has been reported as therapy for patients with AVWS due to IgM-MGUS. When this treatment is given, fresh frozen plasma replacement should be used, instead of albumin, to prevent depletion of fibrinogen and other coagulation factors that could worsen bleeding from AVWS. When the treatment is used for managing severe bleeding, the restoration of VWF levels can be accelerated by concurrent treatment with VWF-containing concentrate or desmopressin.

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AUTOIMMUNE NEUTROPENIA

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Definition and classification

Neutropenia is a disorder characterized by a reduction of the absolute count of circulating neutrophils (ANC) below the lower limits which vary according to race and age. In Caucasians new-borns and toddlers up to the age of 1 year the lower limit is $1.0x10^9$/L whereas is $1.5x10^9$/L from >1 year to adulthood.[1] However black populations have lower normal inferior limits (0.2–0.6 $x10^9$/L circulating neutrophils)[3] than Caucasians (which thing accounts for a different threshold for definition of neutropenia). In Caucasians after the first year of life neutropenia is defined as mild if circulating neutrophils are between 1.0 and $1.5x10^9$/L, as moderate if between 0.5 and $1.0x10^9$/L, and severe if below $0.5x10^9$/L. Autoimmune neutropenia (AIN) can be classified in two fundamental forms: primary and secondary AIN. In primary AIN, neutropenia is the sole abnormality with no evidence of underlying disease-specific cause. The disease is far more frequent in children and generally occurs in the first 3 years of age. Secondary AINs are associated with an underlying, sometimes smoldering, autoimmune condition although malignancies, infections, immunodeficiencies and drugs may also be associated (Table 1).[2] Secondary AINs, though possible also in childhood, are usually more common in adults.

Infection prophile and clinical outcome of primary AIN

The clinical picture related to neutropenias is that of infections. In secondary forms symptoms and signs linked to underlying disorder may also be evident. A recent survey from the Italian Registry of Neutropenias has depicted the infection prophile of different types of neutropenias including primary AIN. Over 3.2 year follow-up, each AIN patient, experienced 0.66 infectious episode vs 6.35 of CSN (Chronic Severe neutropenia) subjects. The rate of infections before diagnosis of neutropenia was 0.48/patient/1000 days at risk and was significantly lower than that of CSN (P<0.001). After diagnosis, usually coinciding with the start of a follow up and surveillance program, the rate of infections was reduced. Most frequent infection sites were skin/soft tissue, lung, blood stream and tonsils. Even if there were no lethal infectious episodes, in 40% of cases hospitalization was required, thus indicating that the infection load of AIN is not negligible.[3] Truly primary AIN spontaneously resolve in a median of 3 years. Sometimes, especially in childhood, to the best of performed diagnostic work-up, it may not be possible to differentiate primary AIN from neutropenia due to ALPS or to CVID that in turn may have a fluctuating course with active phases alternated to quiescent periods. In case of re- appearance of neutropenia or other symptoms in a patients formerly diagnosed as having primary AIN, investigations for ALPS and CVID are mandatory (see specific paragraphs). Consistent with the link between neutropenia and immune deregulation, it is worthy noting that a recent study showed that NK cells from AIN patients are less reactive to antibody-coated cells and in responding to some target cells (K562). This reduced NK reactivity, that has been observed also in genetic forms of neutropenias, recovers when neutrophils count returns to normal.[4]

Table 1. Classification of immune neutropenias

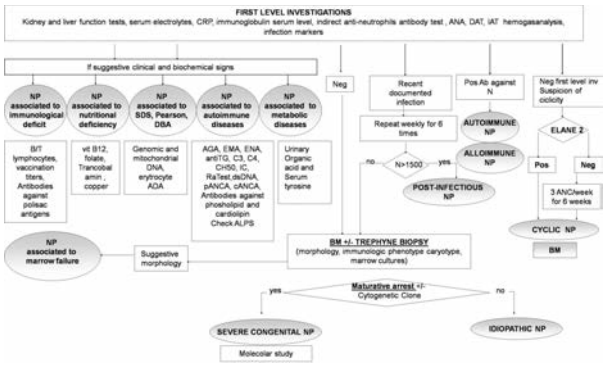
Primary immune neutropenia
Secondary immune neutropenia
Felty syndrome (FS)
Systemic lupus erythematosus (SLE)
Rheumatoid arthritis (RA)
Scleroderma
Sjogren syndroem
Alps (Autoimmune lymphoprolipherative syndrome)
Immune.deficiency (CVID, X-linked Ipo-gamma globulinemia)
CIN (Chronic Idiopathic Neutropenia)
Coeliac Disease
Large granulocytic lymphocytic leukemia
Primitive biliary cirrhosis
Crohn disease
Associated to activation of C5
Hyperthyroidism (Graves disease)

Modified from : F.Fioredda et al, Pediatr Blood Cancer 2011;57:10–17

Diagnosis

Diagnosis of AIN is a challenge whose main goal is to identify primary from secondary forms. In this respect a comprehensive approach should include full history to determine race, ethnicity, new medications (including over-the-counter and complementary medications), and potential infectious exposures. Evaluation should focus on fevers, chills, night sweats, weight loss, excess bleeding or bruising, recurrence of infections.[5] A comprehensive physical examination should be performed, with a focus on signs of infection, hepato-splenomegaly and lymphadenopathy. In children attention needs to be posed also on weight, stature, psychomotor development, somatic dysmorphisms, signs of infections (skin, mouth), hearth function, joints, neurological symptoms and other symptoms compatible with autoimmune, metabolic, gastrointestinal, nutritional diseases.[2,5] Initial work-up consists of a CBC, with a differential count to evaluate the severity of the neutropenia and detailed review of the peripheral smear to look for neutrophil abnormalities such as Dohle bodies (infection), immature neutrophil precursors (infection, myelodysplasia, myelopthisis), dysplastic changes in the neutrophils (myelodysplasia), hyperlobulation (nutritional deficiencies), and white cell inclusions (eg, anaplasmosis, bartonellosis). Review of red cell morphology on peripheral smear may also be helpful since dacrocytes (teardrop cells) and nucleated red cells (myelodysplasia, fibrosis, myelopthisis) in addition to red cell inclusions (eg, babesiosis, malaria) may all be seen in disease associated with neutropenia.[5] Subsequent investigations may include kidney and liver function test, serum electrolythes, CRP, serum immunoglobulin levels, indirect antineutrophil test, ANA, IAT, DAT, hemogasanalysis, infection markers, LDH, thyroid hormones. The aim of this investigations is to confirm/exclude the commonest causes of neutropenia and to direct further diagnostic steps in case a firm diagnosis can not be reached. If history, clinical findings and first level investigations suggest a form associated to other pathological conditions, than investigations can proceed, to further more targeted analyses as indicated by patient's history and clinical-laboratory data.[2] (Fig.1) Generally at the end of this extensive diagnostic work-up it should be possible to come to the diagnosis of primary AIN. Primary AIN is defined as neutropenia co-existing with anti neutrophil antibodies, in the absence of any other demonstrable cause of neutropenia. Consequently diagnostic criteria of primary AIN still remains elusive and essentially this can be considered an exclusion diagnosis. Although not

conclusive, the presence of a normo/hyper cellular marrow with generally a late maturational arrest may support to the diagnosis of primary AIN.[2,5]



From: F. Fioredda et al, Pediatr Blood Cancer 2011;57:10–17

Figure 1.

The anti neutrophil antibody test

Demonstration of anti-neutrophil antibodies is a key element of the diagnosis of primary AIN. Unfortunately the currently available tests for detecting anti-neutrophil antibodies still exhibit limited efficiency. A recent study showed that AIN patients have higher relative fluorescence intensity (RFI) as compared to other non AIN and normal controls on direct testing (identification of antibodies on the surface of the neutrophils). Although this test may be useful as auxiliary diagnostic tool, still retains limited discriminating power as non AIN and normal controls still tested positive.[6] This limit was also observed in other studies.[7] The main drawbacks of direct test are the low number of target cells and the frequent non antigen-specific binding of antibodies on neutrophil surface that remarkably affect the specificity increasing the false positive rate.[8] Indirect testing is based on the detection of antibodies in the serum of the patients directed towards surface neutrophil antigens. The most frequently implicated antigens are called HNA (Human neutrophil antigens). HNA consists of five groups numbered 1 to 5 with HNA1-1a and 1b being the isoforms of the FcγRIIIb most frequently implicated. Technically, sera of the patients are incubated with a suspension of granulocytes expressing the antigens. The Ag-Ab link is read out by fluorescinated anti human Ig monoclonal antibodies in flow citometry as RFI. This method enables a more precise and reproducible semiquantitative evaluation of the antigen presence. Lack of coverage of the full HNA pattern in the neutrophil suspension is the major determinant of the limited sensitivity of this test. The availability of granulocytes tested for the expression of all the target antigens would be optimal but in routine practice this is highly demanding and rarely feasible. However in the largest study ever conducted so far including 240 patients, sensitivity of indirect testing was 74% at first assessment and further increased on repeated determination.[9] Recently the use of a panel of granulocytes from 10 different HNA un-typed donors was shown to reach a specificity up to 85% and, in spite of a sensitivity of 62.5%, a predictive value of 91.8%.[10] The possibility to combine both indirect and direct testing to reach the diagnosis of AIN avoiding unnecessary and sometimes invasive investigations has been suggested by some group. Although sensible, this option did not enter the routine practice probably because of practical limits and of the lack of standardization of the interpretation of the double test.[10] On the whole, still in the absence of conclusive supports, it looks that indirect anti neutrophil Ab testing in the diagnosis of AIN is probably supported by better evidence over direct testing. Based on this, the diagnostic guidelines elaborated by Italian Marrow Failure Group of the AIEOP (Associazione Italiana Ematologia ed Oncologia Pediatrica) agreed to rely for the diagnosis of AIN on indirect anti-neutrophil antibodies detection by flow cytometry. The limited sensitivity of the test suggested to consider as "likely autoimmune neutropenia" those cases with at least one border-line positivity of indirect anti-neutrophil antibodies coexisting with compatible neutrophil count and clinical phenotype. In case the first test is negative or border-line, still in the presence of clinical picture consistent with AIN, it has been recommended to repeat the test up to four or more times over a time-span of 4–6 months or longer.[2]

Treatment

In most cases of primary AIN no continuous treatment is required. G-CSF is not generally necessary on a daily base but only in case of severe infections.[11] However some forms of AIN with severe and/or recurrent infections may require long-term daily treatment. In these cases, an initial low dose of G-CSF (1–2 mcg/kg/die) is recommended to achieve a neutrophil count between 1 to $5x10^9$/L. If this goal is not achieved, increase of another 1–2 mcg/kg/ is suggested.[11] The guidelines elaborated by Italian Marrow Failure Group of the AIEOP agreed on the concept that there is no major advantage to perform antibiotic prophylaxis rather than to treat the infection at its occurrence.[12] In this circumstance empiric antibiotic therapy has to be a broad spectrum irrespective of ANC and G-CSF treatment.[12]

Special form of AIN

Due to some specific peculiarity, certain forms of autoimmune neutropenia deserve more detailed description. They include: chronic idiopathic neutropenia; pure white cell aplasia; late onset neutropenia; neutropenia of autoimmune lymphoproliferative syndrome; nutropenia of common variable immuno deficency; levamisole-related neutropenia in cocaine abusers; chronic idiopathic neutropenia (cin). Patients without another clear cause of neutropenia are frequently labeled as having CIN. The clinical hallmarks of CIN are neutropenia with a relatively stable, reduced neutrophil count without recurrent infections. Diagnostic criteria for CIN are (12):1. ANC less than 1800 cells/mL in whites or less than 1500 cells/mL in individuals of African ancestry for greater than 3 months.[2] No clinical, serologic, or imaging evidence for another cause of neutropenia. 3. Absence of radiation exposures, chemical compound use, or drug intake associated with neutropenia. 4. Normal bone marrow karyotype; 5.No antineutrophil antibodies detected in the serum (a minimum of 2 methods such as the granulocyte agglutination and granulocyte immunofluorescence test should be used for confirmatory purposes). Alhough this still looks an exclusion diagnosis, recently some light has been shed on the pathophysiology of this disease. Experimental evidence suggest that CIN belongs to the spectrum of acquired bone marrow failure syndromes associated with immune deregulation as Aplastic Anemia and hypocellular MDS. Actually CIN patients have activated, oligoclonal T cells producing inflammatory cytokines like IFN-γ, TNF-α, Fas-ligand, CD 40-ligand and TGF-b1. Also bone marrow microenvironment contributes to the disease by producing TGF-b1that suppresses interleukin 10, an anti-inflammatory cytokine. These changes create a pro-inflammatory marrow milieu that leads to increased apoptosis within neutrophil progenitor compartment.[13,14]

CIN essentially affects middle aged subjects and poses the issue of differentiating it from MDS presenting with isolated neutropenia (refractory isolated cytopenia-RC-) or Refractory Cytopenia with Multilineage Dysplasia (RCDM) of the WHO classification. Bone marrow morphology, trephine biopsy, karyotype and flow cytometric analysis of the granulocytic series are mandatory in the initial work-up. It is of note that CIN was shown so far to have a limited tendency to evolve to MDS. Given the low infectious risk CIN patients rarely require anti-infections treatment. G-CSF can be used in those subjects suffering from severe or recurrent infections. Treatment for the underlying disease is generally not necessary but CIN patients need to undergo a systematic monitoring program for surveillance on aplastic evolution. The pro-inflammatory nature of the disease might represent the background to design clinical trials with anti-cytokine agent.

Pure white cell aplasia (PWCA)

In this condition neutropenia is due to the lack of neutrophil precursors in the bone marrow whereas red blood cell and platelet precursors are normal. PWCA is a rare cause of neutropenia, for which only case reports are available. There seems to be an association with thymoma. Thymoma removal and/or immunosuppressive agents like rituximab and cyclosporine have been used. The mechanism of action is immune mediated by antibody development to neutrophil progenitor cells that spares pluripotent stem cells, erythroid precursors, and mature neutrophils.[15]

Late onset neutropenia

Late-onset neutropenia (LON) is an emerging adverse effect to rituximab a chimeric anti-CD20 monoclonal antibody, widely used in the

treatment of B-cell lymphomas and autoimmune diseases. In LON neutrophil counts decrease 3 to 4 weeks after the last rituximab infusion. The incidence has been reported as between 3% and 27% of patients, with those most at risk for LON being patients after autologous stem cell transplant, HIV-positive patients, and those patients who have received purine analogues such as fludarabine. The infection rate is close to17% and tend to be higher in autoimmune patients. Recent reports pointed to factors like polymorphisms in FCGR3, as having a role in the development of LON. Also marked B-lymphocyte depletion and low serum IgM, may affect the mechanisms and risk of occurrence of LON.[16]

Neutropenia of autoimmune lymphoproliferative syndrome (ALPS).

ALPS consists of a chronic lymphadenopahy and/or splenomegaly in an otherwise healthy child. Multilineage cytopenias is also part of the clinical picture with neutropenia scoring third (19%) for frequency after ITP (23%) and AIHA(29 %). A variety of other autoimmune symptoms involving kidney, liver, lung and eyes can be associated.[17] Diagnosis of ALPS is a challenge because clinical and laboratory features overlap with those of other childhood hematologic disorders, including lymphoma, hemophagocytic lymphohistiocytosis, hereditary spherocytosis, Evans syndrome, and Rosai-Dorfman disease. Also diseases such as common variable immunodeficiency (CVID) and Wiskott-Aldrich syndrome, must be distinguished from ALPS. Currently diagnosis of Alps is based on criteria revised at the first international ALPS workshop held at National Institutes of Health in 2009 (Table 2).

Table 2. Revised diagnostic criteria for ALPS based on First International ALPS Workshop 2009.

Required criteria	
1.	Chronic (> 6 months), nonmalignant, noninfectious lymphadenopathy and/or splenomegaly
2.	Elevated CD3+ TCR+a/b+CD4-CD8- DNT cells (>1.5% of total lymphocytes or >3+ 2.5% of CD lymphocytes) in the setting of normal or elevated lymphocyte counts
Additional criteria	
Primary	
1.	Defective lymphocyte apoptosis in 2 separate assays
2.	Somatic or germline pathogenic mutation in FAS, FASLG, or CASP10
Secondary	
3.	Elevated plasma sFASL levels (> 200 pg/mL), plasma IL-10 levels (>20 pg/mL), serum or plasma vitamin B12 levels (>1500 ng/L) or plasma IL-18 levels >500 pg/mL
4.	Typical immunohistologic findings as reviewed by a hematopathologist
5.	Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) with elevated IgG levels (polyclonal hypergammaglobulinemia)
6.	Family history of a non malignant/noninfectious lymphoproliferatio with or without autoimmunity
Definitive diagnosis	
Both required criteria plus one primary accessory criterion.	
Probable diagnosis	
Both required criteria plus one secondary accessory criterion.	

From: Rao VK, Oliveira JB. How I treat autoimmune lymphoproliferative syndrome. Blood. 2011 Nov 24;118(22):5741-51

It is possible that patients initially diagnosed with primary AIN over time develops other autoimmune symptoms that lead to a subsequent, late diagnosis of ALPS. Sometimes other autoimmune associations may be subtle or remain smoldering. For this reason if spontaneous recovery from AIN exceeds 3 years from initial diagnosis or if autoimmune symptoms appear, the diagnostic work-up for ALPS is mandatory. The risk of developing Hodgkin lymphoma is estimated at 50 times that of the general populations whereas that of NHL is increased 14-fold. Median age at lymphoma diagnosis in the NIH cohort was 17 years (range, 5-50 years).[17] Infection prophile related to neutropenia in ALPS has not been deeply investigated. If diagnosis is established and the patient is not under immunosuppressive treatment it seems reasonable to propose an approach similar to that of more classical AIN. Fundamental treatment of lymphoproliferation is based on a flow chart considering in front line steroids and i.v. Immunoglobulins and in case of non response Micofenolate, Sirolimus, Rituximab and splenectomy in sequence.[17]

Neutropenia of CVID

CVID is the most common and clinically relevant primary immunodeficiency of adults with most patients diagnosed between 20 to 40 years,. About 20% of diagnoses occur in childhood (>2 years) and adolescence. The key diagnostic elements include low IgG (2 SD below mean of age) along with low IgA and/or IgM. CVID is a highly heterogeneous disorders with varying complexity and different associated symptoms that overlap ALPS and other autoimmune syndromes. Immune cytopenia is a clinically relevant association of CVID. Thrombocytopenia (14%) is the most frequent, followed by autoimmune haemolitic anemia (7%) and AIN (about 1%).[18] Although rare, the association of CVID with AIN justifies the inclusion in the first line investigations of the determination of immunoglobulin serum level (Fig 1). CIVD has to be considered as a diagnostic alternative in primary AIN lasting more than 3 years or if new cytopenias/other autoimmune signs appear. Anti infection approach and cytopenia treatment are similar to that of ALPS.

Levamisole-related neutropenia in cocaine abusers

Levamisole, is an anti helmintic medication used in autoimmune diseases but also as an additive contaminating cocaine. It has been estimated that about 75% of cocaine abusers in the US are exposed to levamisole. Only a minority of them present autoimmune-mediated neutropenia, cutaneous vascular complications, and/or leukoencephalopathy. However levamisole exposure should be considered in the diagnostic itinerary of patients presenting in the setting of cocaine abuse with neutropenia, vasculopathy and/or neurological involvement. Neutropenia usually resolves rapidly with withdrawal of the harmful compound and therefore G-CSF therapy may not be essential.[19]

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AUTOIMMUNE HEMOLYTIC ANEMIA

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Autoimmune haemolytic anaemia (AIHA) is a relatively uncommon disorder, with an estimated incidence of 1-3 per 105/year, a prevalence of 17:100,000 and a mortality rate of 11%. It can be idiopathic or secondary to lymphoproliferative syndromes, infections, immunodeficiency and tumors. AIHA is classified as warm, cold (which includes cold haemagglutinin disease and paroxysmal cold haemoglobinuria) or mixed, according to the thermal range of the autoantibody. The diagnosis is usually simple, although atypical cases of difficult diagnostic classification are reported with increasing frequency. AIHA may develop gradually, with concomitant physiologic compensation, or may have a fulminant presentation with rapid onset of profound, life-threatening anemia. Clinical features in AIHA are determined by the presence/absence of underlying diseases, and by the rate and type of hemolysis which mainly depend on the autoantibody's characteristics. The treatment of this disease is still not evidence-based as there are no randomized studies and only a few prospective phase 2 trials. We will briefly considere the main diagnostic, clinical and therapeutic aspects of AIHA with a focus on atypical cases of difficult diagnosis and on treatment options for patients refractory to traditional therapy.

Diagnostic aspects of AIHA

The diagnosis of AIHA is based on the presence of hemolytic anemia and serologic evidence of anti-erythrocyte autoantibodies, detected by the direct antiglobulin test (DAT), and identified in the serum and/or eluate obtained from the patient's red blood cells (RBCs) [1]. DAT-tube, the traditional agglutination technique, is usually performed with broad-spectrum Coombs' reagents. Clinically, it is important to perform the test with monospecific antisera, to distinguish "warm" (WAIHA), "cold" (Cold Haemoagglutinin Disease, CHD), and "mixed" forms. The former, which represent approximately 70% of all cases, are due to IgG which generally react at 37°C, are usually directed against epitopes of the Rh system, and mainly determine extravascular hemolysis. Cold forms (roughly 20% of all cases) are due to IgM, which are able to fix complement more efficiently than other isotypes, have an optimal temperature of reaction at 4°C, are directed against the I/i system, and prevalently cause intravascular hemolysis. The presence of cold IgM autoantibodies can easily be revealed by the spontaneous agglutination of RBCs at 20°C. It is worth underlying that the amount of RBC destruction by intravascular hemolysis has been calculated in 200 mL of RBCs in one hour, whereas by extravascular hemolysis it is 10-fold lesser.[1] It is important to remind that DAT with polyspecyfic or anti-IgG and anti-C antisera may yield false-negative results due to the presence of IgA, low-affinity autoantibodies, or numbers of RBC-bound IgG molecules below the threshold of the test (400 molecules per RBC). For the former two conditions, the use of monospecific antisera against IgA and low ionic strength solutions (LISS) or cold washings can overcome the DAT negativity; small amounts of RBC-bound IgG can be detected employing more sensitive but less specific techniques such as microcolumn and solid-phase antiglobulin tests that are suitable for automation, or other more sophisticated techniques such as the complement-fixation antibody consumption test, the enzyme-linked and radiolabeled tests, and the flow-cytometry, that are not routine in the majority of laboratories. The latter deserves a particular mention because of its high sensitivity, being able to detect up to 30-40 molecules of anti-RBC autoantibodies. In case of DAT positivity with polyspecific antisera and clinical features of cold-induced hemolysis it is recommended to perform the Donath-Landsteiner test. Finally, the mitogen-stimulated (MS)-DAT has been proposed as a functional and quantitative method for the detection of anti-RBC antibodies in mitogen-stimulated whole-blood cultures, which may facilitate the diagnosis of some DAT-negative AIHA cases.[2] Despite the numerous tests available for the detection of antibodies against RBCs, and the development of additional more sensitive techniques, a portion of AIHA remains DAT negative, and the diagnosis is made after exclusion of other causes of hemolysis and on the basis of the clinical response to therapy. These forms, reported with increasing frequency in both adults and children, represent a critical diagnostic problem and may cause delays in therapy. According to Petz & Garratty,[1] DAT-negative AIHA represent11.3% of warm forms (8% of total AIHA), with a maximum reported frequency up to 21%. Kamesaki *et al*[3] compared the clinical characterisics of DAT-negative and DAT-positive AIHA and found that the former generally suffered slightly milder anemia and hemolysis but there were no significance differences between the two groups with respect to the survival rate at 1 year following diagnosis and to the effectiveness of steroid treatment. There are rare cases of warm AIHA caused by IgM "warm" autoantibodies. Patients with IgM warm AIHA often have more severe hemolysis and more fatalities than patients with other types of AIHA. Arndt *et al*[4] reported 11 fatalities in a series of 49 IgM warm AIHA patients. These forms are characterized by spontaneous RBCs agglutination due to crosslinking of IgM autoantibodies and are difficultt to diagnose because routine serologic data are not always informative: most cases appear to have only C3 on RBCs by routine DAT, so that the diagnosis is sometimes confused with cold agglutinin disease or (if IgG is also present) with "mixed"-type AIHA, and some cases may also appear DAT-negative at routine DAT. A serologic work up by a specialistic reference laboratory can help with the diagnosis. RBC-bound IgM warm antibodies can be identified by DDAT (Dual Direct Antiglobulin Test).[5] A diagnostic flow-chart for AIHA is reported in Fig. 1. It is worth reminding that DAT positivity may be due not only to autoantibodies, but also to alloantibodies, possibly present in transfused subjets and in multiparous females; moreover, the coexistence of auto- and alloantibodies has been reported in 1/3 of AIHA patients. The presence of alloantibodies is often masked by autoantibodies in AIHA, and may cause severe hemolytic reactions in case of RBC transfusion. Thus, it is important to differentiate the allo- and autoantibody by immunoabsorbance techniques and extended RBC genotyping.

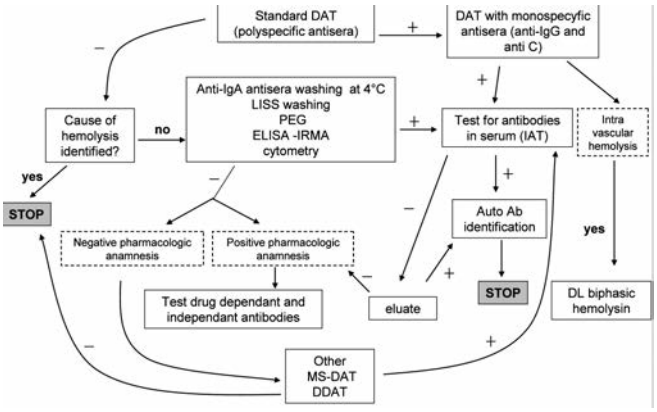

Figure 1. Diagnostic flow-chart for autoimmune haemolytic anaemia (AIHA): DAT, direct antiglobulin test; LISS, low ionic strenght solutions; PEG, polyethylene glycol; ELISA, enzyme-linked immunoassay; IRMA, immunoradiometric assay; IAT. indirect antiglobulin test; DL, Donath-Landsteiner; MS-DAT, mitogen-stimulated-DAT; DDAT, dual DAT.

Clinical aspects

The extent of hemolysis, which is greatly variable from fully compensated to fulminant, depends on the autoantibody pathogenicity, whose major determinants are the Ig class, subclass, thermal amplitude, specificity, affinity, efficiency in activating complement and ability to react with tissue macrophages. Moreover, it is worth mentioning that the degree of anemia also depends on the activity of the reticuloendothelial system and on the efficacy of the erythroblastic response. In particular, it is apparent that both warm and cold autoantibodies may cause not only peripheral destruction of mature red cells but also intramedullary death of red cell precursors, so that in these cases an anti-erythroblast reactivity may be hypothesized.[1] Liesveld *et al*[6] analysing the variability of the erythrocyte response in a large goup of AIHA, found that twenty per cent of patients displayed reticulocytopenia in the presence of marrow erythroid hyperplasia and concluded that reticulocytopenia was due to ineffective erythropoiesis in most cases, although apoptosis may also contribute to the occurrence of this complication in AIHA. Moreover, a large French national observational study[7] reported that 39% of children with AIHA had reticulocytopenia of variable duration. It is important to bear in mind that AIHA with reticulocytopenia may often represent a clinical emergency with extremely high transfusion needs. Recently, we reviewed one-hundred fifty seven AIHA patients (Table 1) referred to our institution in the last 15 years. They had been followed-up for a median of more than 2 years, and 50% were still in follow-up. As regards the thermal characteristics, 40% of cases were WAIHA, 32% CHD, 19% mixed forms and 9% atypical (12 DAT negative and 1 DAT positive for IgA only). Considering the severity of anemia at onset, 2/3 of cases had Hb levels <8 g/dL, and were mainly mixed (18/30, 60%) and atypical (6/13, 46%) forms, whereas only a small fraction of CHD was characterized by a severe onset (8/51, 16%). Inadequate bone marrow compensation (reticulocytes <100.000 μL) was more frequently observed in cases with severe onset (14/52, 27%), possibly contributing to the clinical picture. Eleven patients experienced an acute event at the onset of hemolysis and the majority of them (7/11, 63%) were WAIHA; we recorded 4 deep venous thrombosis (with 2 subsequent pulmonary embolisms), 1 disseminated intravascular coagulation, 3 cardiac ischemic events, 2 acute renal failures and 4 acute infections (3 pneumonias and 1 sepsis); 18 patients died because of AIHA during the follow up. In conclusion, our data showed that AIHAs are clinically heterogeneous: 1/3 of cases, mostly mixed or atypical forms, displayed a severe onset, and 7% of patients presented with life threatening complications. It is worth mentioning that AIHA is very rare in infancy and childhood, so that current knowledge on clinical aspects of this disease is mainly based on reports of adult patients. A large French national observational study of 265 children has recently given new insights into childhood AIHA.[7] In particular, they foud that it was primary in 37%, post-infectious in 10%, and associated to immune disorders in 53% of cases; moreover, Evans syndrome was diagnosed in 37% of patients, and first degree familial immunological diseases were present in 15% of cases. After a median follow-up of 3 years, 4% of children had died (10% mortality in the AIHA/Evans syndrome subset), 28% were still treatment-dependent and 39% were in continuous complete remission.

Table 1. Clinical and laboratory data from 157 AIHA patients.

Male/Female, number of patients (%)	61 (39) / 96 (61)
Median age at onset, years (range)	57 (5-95)
Median follow up, days (range)	782 (30-5470)
Type of AIHA, number of patients (%)	
WAIHA	63 (40)
CHD	51 (32)
Mixed AIHA	30 (19)
Atypical AIHA	13 (9)
Severity of anemia at onset, number of patients (%)	
Hb <6 g/dL	52 (33)
Hb 6-8 g/dL	54 (34)
Hb 8-10g/dL	28 (18)
Hb >10 g/dL	23 (15)
Lines of therapy, number of patients (%)	
Corticosteroids	132 (84)
Splenectomy	20 (13)
Immunosuppressors	29 (18)
Rituximab	33 (21)

Treatment

The most common treatment options for AIHA are listed in Table 2. Conventional therapy of warm AIHA includes administration of corticosteroids as first line therapy, which is expected to provide a response in 70-85% of patients; however, only one third of them remains in long-term remission once the drug is discontinued, a further 50% requires maintenance doses, and approximately 20-30% needs additional second-line therapies. In case of refractoriness to the initial corticosteroid treatment, a diagnostic reevaluation should be made for a possible underlying disease, since patients with malignant tumors, benign ovarian teratomas, or with warm IgM antibodies are often steroid-refractory. It is not known how many adult patients are possibly cured by steroids alone, but it is estimated that this occurs in less than 20% of patients.[8] Second line therapy includes splenectomy and immunosuppressive agents. Splenectomy should be considered in patients who had an unsatisfactory response to initial corticosteroids, relapse after having responded, or require the equivalent of more than 10 to 15 mg prednisone per day to maintain acceptable hemoglobin levels; although its efficacy has never been compared to that of other second-line therapies and no high-quality data on long-term success are available, splenectomy is probably the most effective second-line treatment for warm AIHA as there is a good evidence that a substantial number of patients will remain in remission without need of medical intervention for years.[8] Splenectomy has an initial response rate of approximately 50%, but it may be associated with surgical and infective complications (Table 2). Conventional treatment options for patients who fail to respond or are not suitable for splenectomy are limited: cytotoxic and immunosuppressive drugs (such as azathioprine, cyclophosphamide, cyclosporine) provide a 40-60% response rate but their use may be associated with serious side effects. The effectiveness of other options, such as high-dose intravenous immunoglobulins, plasmapheresis and danazol, is controversial and mostly anecdotal. Conventional treatment has failed to demonstrate a convincing effect in AIHA due to cold autoantibodies, particularly cold haemagglutinin disease. A small proportion of warm AIHA cases (5-

Table 2. Most common treatment options for AIHA.

	Treatment	Approximate response rate	Toxicities	Duration of sustained response
First line therapy	**Corticosteroids** (i.e. prednisone 1 mg/kg/day p.o.)	~75-80% [1]	Vary with length of administration: mood swings, weight gain, anxiety, insomnia, Cushingoid faces, diabetes, osteoporosis, hypertension, gastro-intestinal distress and ulcers, immunosuppression, psychosis, cataracts. Tolerability decreases with repeated dosing. Possibly lower rate of adverse events when used as short-term bolus therapy	30% once the drug is discontinued, a further 50% require maintenance doses, and approximately 20-30% needs additional second-line therapies which include splenectomy and immunosuppressive agents[3]
Further lines of therapy	**Splenectomy**	~50%[1,2]	Surgical complications (pulmonary embolism, intra-abdominal bleeding, abdominal abscess, abdominal wall haematoma), and infective complications, such as gram negative sepsis, particularly in patients over 65 years	20-60% after a 4-7 years follow-up 3,4
	immunosuppressive drugs (i.e. azathioprine, cyclophosphamide, cyclosporine)	~40-60%[4]	Side effects vary with the drug (i.e. severe neutropenia with infection, renal or liver impairment, tremor, acute deep venous thrombosis)	N.A.
	Rituximab standard dose (375 mg/m^2/wk x 4 wk) low dose (100 mg/wk x 4 wk)	~60% [5] ~80-90% [6] (100% WAIHA, 60% CHD)	Mild to moderate infusion-related side effects (hypotension, fever and chills and upper airway oedema); grade 4 neutropenia (roughly 2%) and infectious events (roughly 7%).	>3 years in some cases ~70%at 2 and 3ys (similar for WAIHA and CHD)[7]

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10%) is refractory to standard therapies and may represent a medical emergency, particularly when reticulocytopenia is present or hemolysis is due to "warm" IgM. Rituximab has been shown to be effective in refractory AIHA, although the comparison of response rates in various studies is difficult in the absence of common response criteria. In recent reviews[9] Rituximab has been shown to be effective both in patients with idiopathic and secondary AIHA, including those associated with autoimmune and lymphoproliferative disorders, and bone marrow transplant. Responses to treatment were observed in monotherapy or in combination with corticosteroids, immunosuppressant drugs and interferon- , and regardless of prior therapy. The time to response varies considerably, with some patients responding very quickly and others taking weeks or even months to achieve their maximum response. In two recent studies the median time to response was 3 and 6 weeks, respectively (range 1-16), but late responses between 6 and 8 weeks were also found. Finally, rituximab re-treatment may be effective for both warm AIHA and cold haemagglutinin disease, and some patients responded to re-treatment more than once. Rituximab treatment for AIHA was well tolerated and no adverse events were reported for most patients, excluding mild to moderate infusion-related side effects, such as hypotension, fever and chills and upper airway oedema. Few patients have experienced grade 4 neutropenia (roughly 2%) and infectious events that were possibly related to rituximab (roughly 7%). It is noteworthy that rituximab was the only treatment able to induce a complete response in CHD with an overall response of 60% (10% complete, and 50% partial), compared with 16% (all partial responses) of the next best treatment regimen with alkylating agents with or without corticosteroids. Finally, fludarabine-rituximab combination therapy was reported very effective, resulting in 75% response rate, complete remissions in about 20%, and more than 66 months estimated response duration. In attempt to minimize side effects and reduce costs, low dose rituximab (100 mg fixed dose x 4 weeks) was reported effective in patients with AIHA who failed to respond to conventional treatment.[10] In particular, low dose rituximab associated with standard oral prednisone (1mg/kg/day for 30 days, followed by quick tapering) as first- or second-line therapy in 32 patients with primary AIHA was able to induce 89% overall response (complete response 67%) and 68% relapse-free survival at 36 months; the relapse risk was greater in CHD than WAIHA, although not significantly. It is nothworthy that almost all relapses occurred within month 13, so that the relapse risk at 2 and 3 years was equivalent, suggesting that patients who maintain a sustained response beyond 1 year may be considered as long-lasting responders/recovered.[11] Alemtuzumab, a humanized anti-CD52 monoclonal antibody, has been shown to be effective in both idiopatic and secondary AIHA, with a complete remission rate of 13/16 and 11/12 respectively, including 3 pediatric cases; however, because of the high toxicity, it is considered a treatment of last resort in severe idiopathic AIHA not responsive to none of the known drugs.[8] In small series of patients with CLL and warm AIHA (refractory to corticosteroid, splenectomy and rituximab), alemtuzumab induced a complete or partial responses in all patients, suggesting that it may be highly effective in warm AIHA accompanied by progressive CLL.[12] Data on the use of mycophenolate mofetil in patients with refractory warm AIHA are limited. Most of the treated patients have been reported to achieve partial responses, and continuous treatment was required to maintain the response. Mycophenolate mofetil has been shown to be particularly effective in the treatment of the refractory AIHA in children with the autoimmune lymphoproliferative syndrome, of whom 12/13 responded with reduction or cessation of the need for other immunosuppressive drugs. The cumulative data may therefore suggest a potential place for mycophenolate mofetil in the treatment arsenal of refractory immune cytopenias.[12] Other treatments are anecdotally reported in the literature. High-dose cyclophosphamide was effective in 4/5 patients, all of whom were previously treated with corticosteroids. Bortezomib, an inhibitor of 26S proteasome, was able to induce a response in two patients with refractory CHD.[13] Eculizumab, a monoclonal antibody that targets protein complement C5, has been shown effective in a transfusion-dependent patient with refractory CHD: continuous therapy with eculizumab resulted in sustained reduction of hemolysis and elimination of transfusion requirements.[14] It is worth mentioning that C1-esterase inhibitor has potential as safe therapy to control complement-induced RBC destruction in AIHA patients.[15] Moreover, the administration of EPO was successfully used in patients with therapy-refractory AIHA, particularly in the presence of reticulocytopenia.[16] Finally, both autologous and allogenic bone marrow transplantation were reported, in some 52 immune cytopenias, including severe AIHA and Evans syndrome.[17] As regards our 157 AIHA patients, we found that 45% of cases (mostly WAIHA) were treated with steroids only, 23% with an additional line of therapy, and 16 % with 3 or more lines. Splenectomy was performed in 20 cases, mostly mixed and severe forms; 23 patients were treated with various cytotoxic drugs, and 33 with rituximab (6 warm, 15 cold, 10 mixed and 2 atypical AIHA); 16% of cases have never been treated, mostly CHD with mild anemia. Transfusions were required in 65 cases, plasma-exchange in 3 (all with Hb<6g/dL), and erythropoietin administered in 6. Of note, the presence of an Hb value lower than 6 g/dL at onset was a risk factor for the requirement of 3 or more lines of therapy. Response rate to steroid therapy was similar in warm, mixed and atypical AIHAs (~70%); rituximab was equally effective in cold and warm AIHA forms (~70%). Splenectomy was effective in 5/8 WAIHA and 8/10 mixed and atypical cases, whereas it was unsuccessful in the 2 CHD who had undergone surgery. These results showed that cases with a severe onset, mostly mixed or atypical forms, are frequently refractory to different therapies. In conclusion, the therapeutic arsenal now available for steroid-refractoryAIHA is certainly broader than in the past. Rituximab is certainly the best option for patients who are not elegible for or who refuse splenectomy; although published evidence supporting the use of rituximab is more extensive than splenectomy, is current opinion that the sequence of second-line treatment in primary warm AIHA should be splenectomy, rituximab, and thereafter any of the immunesuppressive drugs [8]. However, in clinical practice rituximab is used with increasing frequency before splenectomy, particularly in most severe cases and children before the age of 5-6 years. In any case, the choice of second-line therapies depends on patients, values and preferences, and local circumstances [18]. Randomized clinical studies are needed to identify the best sequence in the administration of the available drugs and their most appropriate dosing.

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CHRONIC MYELOPROLIFERATIVE NEOPLASMS: "NON-TYPICAL" FORMS

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In addition to the "classic", chromosome Philadelphia-negative myeloproliferative neoplasms (MPN), the 2008 WHO organization enlists additional "non-classic" MPN diseases (listed in Table 1).[1] Some of these (disorders of eosinophils and mastocytosis) are covered in other presentations within this Educational Symposium; therefore, according to the "non-canonical" philosophy reflected by the title of this presentation, I will focus on atypical chronic myeloid leukemia and chronic neutrophilic leukemia, two rare disorders for which recent molecular insights provided important information with potential therapeutic relevance, and on the "non-typical" myeloproliferative disorder(s) comprised within the still much debated form of "prefibrotic" myelofibrosis. Atypical chronic myeloid leukemia (aCML) and chronic neutrophilic leukemia (CNL) are two clonal disorders characterized by marked and almost selective expansion of the myeloid lineage. Less than two hundreds of patients with CNL have been reported to date, while the estimated incidence of aCML is less than 1-2 every hundred cases of BCR/ABL1-positive CML. Mature neutrophils predominate in the peripheral blood (where they represent more than 80% of the leukocytes) in CNL while in aCML greater than 10% of the cells are immature myeloid precursors that, as well as the mature neutrophils, present clear signs of dysplasia. Accordingly, CNL is classified within the Myeloproliferative Neoplasms category while aCML is comprised within the Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN).[2] At presentation aCML has many features of classic CML such as leukocytosis with prevalence of granulocytic cells, circulating immature myeloid cells and splenomegaly, but it lacks the BCR/ABL1 rearrangement and the typical basophilia, while at variance with CML it displays moderate to severe dysgranulopoiesis. Granulocytes may show nuclear hypolobation, chromatin clumping, pseudo-Pelger-Huet changes, and cytoplasmic hypogranularity.

Table 1. The 2008 World Health Organization Classification for Chronic Myeloid Neoplasms.[2]

Myeloproliferative neoplasms (MPN)
3.1 Chronic myelogenous leukemia (CML)
3.2 Polycythemia vera (PV)
3.3 Essential thrombocythemia (ET)
3.4 Primary myelofibrosis (PMF)
3.5 Chronic neutrophilic leukemia (CNL)
3.6 Chronic eosinophilic leukemia (CEL), not otherwise classified
3.7 Hypereosinophilic syndrome (HES)
3.8 Mast cell disease (MCD)
3.9 MPN, unclassifiable (MPN-u)
Myelodysplastic/Myeloproliferative neoplasms (MDS/MPN)
4.1 Chronic myelomonocytic leukemia (CMML)
4.2 Juvenile myelomonocytic leukemia (JMML)
4.3 Atypical chronic myeloid leukemia
4.4 MDS/MPN, unclassifiable
Myeloid neoplasms associated with eosinophilia and abnormalities of PDGFRA, PDGFRB, or FGFR1
5.1 Myeloid neoplasms associated with PDGFRA rearrangement
5.2 Myeloid neoplasms associated with PDGFRB rearrangement
5.3 Myeloid neoplasms associated with PGFR1 rearrangement (8p11 myeloproliferative syndrome)

The bone marrow is hypercellular, with myeloid to erythroid ratio of up to 10:1; dysplastic features are mainly associated with the myeloid lineage, although small/dwarf dysplastic megakaryocytes can be seen. CD34+ blasts may be increased, and reticulin fibers are also eventually increased. Chromosomal abnormalities have been reported, in small series, in up to 80% of the subjects with aCML, preferentially +8 and del20(q). The clinical course is more aggressive than BCR/ABL1 CML with median survival inferior to 2 years with up to 40% of the patients transforming to acute leukemia. The diagnostic criteria for CNL include >25x10^9/L leukocytes of which more than 80% are mature neutrophils with banded forms; less than 10% are represented by by immature granulocyte precursors and other leukocyte types are typically not increased. Bone marrow histology shows marked expansion of the granulocytic lineage without signs of dysplasia and less than 5% myeloblasts (must be <1% in the peripheral blood; othere siers are not affected, although hypolubulated megakaryocytes can be occasionally detected. There is no increase of reticulin fibrosis. Typical BCR/ABL1 positive CML, reactive neutrophilia, classic MPN and MDS, and overlapping MPN/MDS need to be excluded. Patients with CNL suffer from hepatomegaly, splenomegaly, constitutional symptoms; leukocyte alkaline phosphatase is typically increased as are the vitamin B12 levels. The disease course is unpredictable, median survival is only 2 years (range 3-106 months, according to the largest series available that included 40 patients). Progression is marked by refractory neutrophilia, progressive increase of spleno/hepatomegaly, worsening of general status and eventually transformation to acute leukemia. Conventional treatment of a CML and CNL consists of hydroxyurea, other chemotherapeutics, and interferon; splenectomy can be used for massive symptomatic spleen enlargement, but might be associated with disease acceleration. Experience with stem cell transplant is limited but evidence of cure has been produced anecdotally, especially in patients with early stage disease. Until the recent discovery of mutations in CSF3R and SETBP1, there was no major and recurrent cytogenetic or molecular marker of these disorders.[3] The

JAK2V617F mutation was reported to occur at low frequency in both diseases. By using deep sequencing of 1,862 genes representing all known kinases, phosphatases, no-kinase growth factor or cytokine receptors, and selected adapter genes, Maxon et al. found mutations in two distinct regions of the gene encoding for the receptor for colony stimulating factor 3 (CSF3R; GCSFR) in 59% of 27 patients with CNL or aCML3. Mutations preferentially clustered in the extracellular domain and in the cytoplasmic tail, and all resulted in enhanced intracellular signaling preferentially mediated by the SCR family kinase and the tyrosine kinase non-receptor 2 (TNK2), both inhibited by dasatinib, and the JAK family kinase, inhibited by ruxolitinib, in case of truncating mutations and membrane proximal mutations, respectively. Mutations occurring in the extracellular domain are point-mutations while those in the cytoplasmic tail were protein truncating nonsense or frame-shifts mutations; the T618I is the most frequent. A CNL patient with the latter mutation showed prompt and durable response to ruxolitinib. In a subsequent study, CSF3R mutations were reported in 83% of 12 CNL patients, in none of 9 aCML, none of 170 patients with primary myelofibrosis (PMF) and none of 94 chronic myelomonocytic leukemia (CMML).[4] These very early observations would suggest that analysis of CSF3R mutations not only has diagnostic relevance because of its high specificity for CNL, but also might help to predict clinico-hematologic response to different TKI, based on type and location of mutations, although this remains to be demonstrated. Somatic truncating mutations in CSFR3 have been reported, on the background of germline mutations in ELANE (elastase), HAX1 (a protein that is a substrate of Scr tyrosine kinases) and G6PC3 (glucose-6-phosphatase 3), also in about one third of patients with severe congenital neutropenia, and were usually associated with progression to myelodysplasia or acute leukemia. As the result of exome sequencing in eight cases of aCML, recurrent mutations in SETBP1 were detected in 24.3% of 70 aCML patients by the group of Gambacorti-Passerini;[5] these mutations are located in a short stretch of aminoacids corresponding to those involved in germline mutations described in the Schinzel-Giedon syndrome, a rare congenital disease characterized by mental retardation, malformations and a high risk of epithelial tumors. Mutations in this hotspot cause the disruption of a functional binding site for ubiquitination and degradation of SETBP1, overall resulting in increased SETBP1 expression levels. SETBP1 (SET Binding Protein) protects SET, a negative regulator of the tumor suppressor protein phosphatase 2A (PP2A), from protein cleavage. Therefore, mutations finally result in lowered PP2A activity and enhanced cell proliferation, as shown by effects of mutant SETBP1 transduction in mouse myeloid progenitors.[6] A low-frequency of SETBP1 mutations have been found in CMML (4-15%) and secondary acute myeloid leukemia (15%) but are virtually absent from de-novo AML, CLL and ALL. Therefore, SETBP1 mutations represent a reliable, although not absolute, marker of aCML. However, mutations of CSF3R and SETBP1 are not mutually exclusive: 21% of 29 CNL patients showed both mutations with 31% having CSF3R only and 7% SETBP1 mutations only.[3] Pre-fibrotic myelofibrosis. First, does it exist as a separate diagnostic entity or just a disease phase within the continuum of a single disease entity?[7] Second, does it have clinical relevance? The distinction of patients with MPN-associated thrombocytosis between a condition of "true ET" and "early/prefibrotic MF" relies on both clinical, yet subtle, differences and specific histological BM patterns. The latter refer to the overall BM cellularity (normal/reduced in true ET, increased in prefibrotic MF with left shifting of myelopoiesis), proliferation of the erythroid lineage (normal in ET, usually reduced in prefibrotic MF) and the characteristic abnormalities of the megakaryocyte lineage. In ET, increased proliferation of the megakaryocyte lineage is made up of large to giant well-maturing megakaryocytes with hyperlobulated nuclei, scattered over the BM spaces with little tendency to form loose clusters. In prefibrotic MF, on the other hand, the hyperproliferating megakaryocytes show a preferential endosteal/paratrabecular location, form both dense and loose clusters, and are highly variable in size with small to giant forms; in addition, the nuclei are markedly abnormal with hypolobulation, irregular folding, resulting in a bulbous/cloudy-like/balloon-shaped appearance. The number of bare nuclei is markedly increased. In both ET and prefibrotic MF, reticulin fibers may be only modestly increased. Notwithstanding these very carefully described morphological features, debates about the reproducibility of these findings and their validity for patient classification have been raised, and are still ongoing (please refer to Ref.7 for a deeper discussion). In a study comparing 132 subjects with prefibrotic MF with 551 receiving a diagnosis of typical, fibrotic PMF, Barosi *et al*. showed some clinical features marking pre-fibrotic MF (female dominance, younger age, higher hemoglobin and platelet count, lower leukocytes, smaller spleen, and a high incidence of splanchnic vein thrombosis), and concluded that pre-fibrotic MF may be a presentation mode of PMF characterized by a very indolent disease course and presentation.[8] Although no specific molecular asset has been highlighted yet that could eventually help in differentiating true ET from prefibrotic MF, it is of interest that subcellular localization of the transcription factor NFE2 has been shown to discriminate between the two entities;[9] the proportion of erythroid cells showing preferential/exclusive staining of NFE2 at the nuclear level was double in PMF (33.7%) as compared to ET (16.3%; P<0.001), although it remains to be evaluated prospectively how these observations can be transferred to the clinical practice. Distinguishing between these two entities may have clinical relevance. The progression to overt myelofibrosis and the transformation to acute leukemia were both increased, and overall survival was conversely decreased, in prefibrotic MF compared with ET.[10] There was no difference in the rate of arterial thrombosis, while the rate of hemorrhagic complications was statistically superior in prefibrotic MF than in ET, with an annual rate of 1.39% and 0.79% patient-years, respectively.[11] Owing the diagnostic and prognostic relevance of distinguishing true ET from prefibrotic MF, the conclusion of a recent perspective paper that " a good solution of the still ongoing controversy regarding these issues could be to launch a scientific project, including the community of pathologists and hematologists for providing scientifically sound, objective, repeatable quantitative criteria for the prefibrotic variant of PMF followed by a corresponding prospective clinico-pathological study" seems to be the most sound and productive approach.

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SYSTEMIC MASTOCYTOSIS

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The term mastocytosis encompasses a heterogeneous group of clonal diseases characterized by proliferation and accumulation of mast cells (MC) in different tissues, mainly skin and bone marrow (BM).[1] Cutaneous mastocytosis (CM), typical in the childhood, is the most frequent form of mastocytosis, while systemic disease (SM) mainly affects adults and involves one or more extra-cutaneous organs (BM, gastrointestinal tract, lymph nods and spleen), with or without skin involvement. The majority of cases of SM show a somatic 'autoactivating' point mutation at codon 816 of kit-receptor gene.[2] Mastocytosis has a wide variety of clinical manifestations due to the inappropriate release of mediators by MCs (*e.g.* pruritus, urticaria, angioedema, flushing, nausea, vomiting, abdominal pain, diarrhea, episodic anaphylactoid attacks, osteopenia/osteoporosis) and to skin disease (urticaria pigmentosa); in the rare cases of aggressive disease, the clinical features are related to organ dysfunction from MC tissue infiltration (*e.g.* hypersplenism, pathologic bone fractures, ascites, malabsorption, cytopenias).[3]

Epidemiology

There are few epidemiogical data about SM in the general population. The prevalence of SM in European adults is estimated 0.7-1.3 per 100,000. Mastocytosis can affect any age and reported male/female ratio varies from 0.8 to 1.5. In the majority of cases mastocytosis is sporadic but rare familial clusters are also described.

Biology

MCs are myeloid cells predominantly found in the perivascular spaces of almost all tissues and easily recognizable by the cytoplasmic contents in metachromatic granules when stained with Giemsa or Toluidine blue. These granules contain numerous vasoactive and pro-inflammatory mediators, which are released after MC activation via the receptor binding of IgE by allergens or other factors. MCs derive from BM multipotent hematopoietic precursors, which migrate via peripheral blood into tissues and differentiate into mature MCs under the influence of various interleukins. In particular, the Stem Cell Factor (SCF), which is the ligand of c-kit (CD117), a tyrosine kinase (TK) receptor constitutively expressed on MCs and their precursors, has a crucial role in MC development. The most common mutation of the KIT gene leading to mastocytosis is an adenine by timine substitution at 2447 position determining the D816V aminoacid substitution and auto-activation of the c-kit receptor, independently from SCF binding. This mutation is found in BM MCs from more than 90% of adults with SM.[2] The same mutation has also been detected in skin lesions of 42% infants with mastocytosis. Infant CM may be also associated to other KIT mutations (D816F, D816Y, V560L, V560G, del419D, K509I, M541L, E839K).[4] In most cases with poor-prognosis SM, as well as in a smaller proportion of indolent SM (ISM), KIT mutation was detected in MCs and also in the CD34 hematopoietic progenitor cells or other hematopoietic cell compartments (multilineage mutation), supporting the origin of SM from a pluripotent hematopoietic precursor.[2]

Diagnostic criteria and classification

The diagnosis and classification of mastocytosis are based on the identification of neoplastic MC by their morphological, immunophenotypic, and molecular characteristics using well established WHO criteria (Table 1).[1]

Classification

The WHO classification defines 7 variants of mastocytosis: CM, ISM, SM with an associated clonal hematologic non-MC-lineage disease (SM-AHNMD), aggressive SM (ASM), MC leukemia (MCL), MC sarcoma and extracutaneous mastocytoma. To subclassify SM, criteria related to MC-burden and multilineage-involvement (B-findings) or aggressiveness of disease (C-findings), are applied (Table 1). The term "Monoclonal MC Activation Syndrome" (MMAS) has been proposed to identify the subjects with unexplained or recurrent anaphylaxis without skin lesions who do not fulfil sufficient criteria for SM, but with documented MC clonality markers.[4]

Table 1. WHO Diagnostic Criteria for Systemic Mastocytosis (SM).

The Diagnosis of SM is established when at least one major and one minor or at least three minor criteria are present	
Major	Multifocal dense infiltrates of MC in bone marrow sec tions or other extracutaneous organ(s) (>15 MCs in aggregate)
Minor	a.MC in bone marrow or other extracutaneous organ(s) show an abnormal (spindle-shaped) morphology (>25%)
	b.KIT mutation at codon 816 in extracutaneous organ(s). In the majority of cases the mutation is D816V
	c.MC in bone marrow express CD2 and/or CD25
	d.Serum total tryptase >20 ng/ml (does not count in patients who have AHNMD-type disease)
"B" findings	A.BM biopsy showing >30% infiltration by MC (focal, dense aggregates) and/or serum total tryptase level >200 ng/mL
	B.Signs of dysplasia or myeloproliferation, in non-MC lin eage(s), but insufficient criteria for definitive diagnosis of a hematopoietic neoplasm (AHNMD), with normal or slightly abnormal blood counts.
	C.Hepatomegaly without impairment of liver function, and/or palpable splenomegaly without hypersplenism, and/or lymphadenopathy on palpation or imaging.
"C" findings	1.Bone marrow dysfunction manifested by one or more cytopenia(s) (ANC <1.0x10^9/L, Hgb <10 g/dL, or platelets <100x10^9/L), but no obvious non-mast cell hematopoietic malignancy.
	2.Palpable hepatomegaly with impairment of liver func tion, ascites and/or portal hypertension.
	3.Skeletal involvement with large osteolytic lesions and/or pathological fractures.
	4.Palpable splenomegaly with hypersplenism.
	5.Malabsorption with weight loss due to gastrointestinal mast cell infiltrates.

Diagnosis of: a) Indolent SM (ISM): meets criteria for SM. No "C"findings. No evidence of AHNMD. b) Smouldering SM (SSM): as ISM, but with 2 or more "B" findings, and no "C" findings. c) Isolated Bone Marrow Mastocytosis (BMM): as ISM with bone marrow involvement, but without skin involvement. d) Aggressive SM (ASM): meets criteria for SM. One or more "C" findings. No evidence of mast cell leukemia. e) Mast cell leukemia (MCL): meets criteria for SM. Bone marrow biopsy shows a diffuse infiltration, usually compact, by atypical, immature mast cells. BM aspirate smears show ≥20% mastcells. In typical MCL, mast cells account for ≥10% of peripheral blood white cells.

Abbreviations: MC, mast cell(s); AHNMD, associated clonal hematologic non-mast cell lineage disease; ANC, absolute neutrophil count; Hgb, hemoglobin.

Cutaneous Mastocytosis

Although typical skin lesions are present in 70-80% of mastocytosis, the definition of CM should be limited to cases with exclusive skin involvement. CM is rather restricted to childhood; in recent adult series, when sensitive and modern diagnostic tools were used, less than 10% of patients were found with true CM. In infants the age of onset is between birth and 2 years in 85% of cases and between 2 and 15 years in the remaining cases. In the majority of infants with CM, skin lesions spontaneously regress by the adolescence.The WHO classification includes three variants of CM: Urticaria pigmentosa (UP)/Maculopapular cutaneous mastocytosis (MPCM), Diffuse cutaneous mastocytosis (DCM), Solitary mastocytoma of skin.[1] MPCM is the most frequent form in adults: it presents as symmetrical distributed macular, papular or plaque red-brown lesions with highest density on the trunk, whereas the palms, soles, face and head are often spared. Mechanical irritation may cause reddening and urticarial swelling of the lesions, via a release of MC mediators, the so-called Darier's sign, which is pathognomonic for CM. DCM is a rare (1-2% of cases) and severe variant, which occurs

exclusively in children with onset under the age of 3 years. Solitary mastocytoma of the skin affects almost exclusively the newborns usually in the first 3 months of life; in rare cases it can be multiple.

Systemic Mastocytosis

In the 2008 WHO classification SM is included among the chronic myeloproliferative neoplasms (MPN). The frequency of the variant forms of SM is very different in published series, reflecting in part the differences in referring centres (Table 2). ISM is by far the most frequent variant of mastocytosis. Two provisional subvariants are recognized by WHO: smoldering SM (SSM) and isolated BM mastocytosis (BMM). SSM is characterized by a higher burden of MC, as defined by the presence of 2 "B-findings". BMM patients do not present skin lesions, have low serum tryptase and MC burden, and very frequently suffer from mediator-related symptoms, including anaphylaxis. Due to the heterogeneous clinical presentation, the diagnosis of BMM is considerably more challenging than the other SM variants and it is certainly an underestimated disease.[5] Recurrent episodes of anaphylaxis, flushing, osteoporosis, gastrointestinal ulcerative disease, or chronic abdominal cramping may lead to the suspicion of BMM. Hymenoptera sting is the most frequent eliciting factor for anaphylaxis in mastocytosis, particularly in patients without skin involvement. A recent report shows that almost 7% of patients with systemic reactions to hymenoptera sting suffer from SM or MMAS.[8] ASM is a rare form of disease, characterized by the presence of at least one "C-finding". In SM-AHNMD the associated haematological disorders involve the myeloid lineage in about 80 to 90% of cases (e.g MPN, myelodysplastic syndromes, chronic myelomonocytic leukemia, acute leukemias, chronic eosinophilic leukemia - CEL) and the lymphoid compartment in the remaining 10-20% of cases (*i.e.* lymphoma or myeloma). MCL is very rare and characterized by an extremely poor responsiveness to treatment and prognosis. An unusual form of SM with skin involvement, not yet recognized by the WHO classification, is the "Well-Differentiated SM" (WDM).[4] In these form MCs present a rounded morphology and a densely granulated cytoplasm. At variance with the other SM forms, in WDM MCs lack the expression of CD25 and/or CD2, and in most cases a wild type KIT or non-D816V mutation are documented. This form has been shown to be sensitive to Imatinib therapy.

Table 2. Frequency of different variants of Systemic Mastocytosis.

Systemic Mastocytosis (WHO classification)	Horny (2004) n (%)	Lim (2009) n (%)	Wang (2013) n (%)	Sanchez (2011) n (%)	Wimazal (2012) n (%)	GISM* unpub n (%)
ISM	35	159	31	93	81	265
	(54.7)	(46.5)	(45.0)	(82.3)	(82.6)	(95.3)
isolated BM mastocytosis	nr	36 (10.5)	nr	16 (14.1)	nr	126 (45.3)
SSM	nr	22 (6.4)	nr	nr	7 (7.1)	3 (1.0)
ASM	7	41	5	11	5	3
	(10.9)	(12.0)	(7.2)	(9.7)	(5.1)	(1.1)
SM-AHNMD	20	138	29	6	11	9
	(31.3)	(40.4)	(42.0)	(5.3)	(11.2)	(3.2)
MCL	2	4	4	2	1	1
	(3.1)	(1.2)	(5.8)	(1.8)	(1.0)	(0.4)
total	64	342	69	113	98	278

Abbreviations: ISM: indolent systemic mastocytosis, SSM: smouldering systemic mastocytosis; BM: bone marrow; ASM: aggressive systemic mastocytosis, AHNMD, associated clonal hematologic non-mast cell lineage disease; MCL: mast cell leukemia.

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Diagnosis

Morphological evaluation

In the SM BM smears, stained with May-Grunwald Giemsa or Toluidine blue, MCs show variable cellular atypia. Type I atypical MC, the more frequent ones, are characterized by two or three of the following characteristics: cytoplasm spindle-shape and/or with protrusions, eccentric oval nucleus and hypogranulated cytoplasm with focal accumulation of granules or without fusion of the granules. In some cases it is possible to find atypical type II MC, characteristic of aggressive diseases, with monocytoid bi-or multi-lobed nuclei, or round mature MCs, as in WDM.[3]

Histology

Histological diagnosis of CM in skin biopsies requires the demonstration of monomorphic MC infiltrate that either consists of large aggregates of tryptase-positive MCs (>15 cells/cluster) or scattered MCs exceeding 20 cells per microscopic high power field. Diagnosis of SM is based primarily on the recognition of compact and multifocal infiltrates of MCs (at least 15) in the BM or other extracutaneous organs (major criterion). The recognition of atypical MCs within these aggregates allows satisfying sufficient criteria for the diagnosis of SM. The aggregates often contain scattered eosinophils and lymphocytes, or they are adjacent to reactive lymphoid nodules. The diagnosis can be confirmed by immunohistochemical techniques, in particular the expression of MC tryptase, c-kit or CD117 and CD25. Not infrequently the major histological criterion is not met, as only small clusters or isolated scattered MCs are detectable in the BM. In such cases, other sensitive tests are needed to meet the criteria for SM, such as the flow cytometry (FC) and the search for mutation in codon 816 of the KIT gene.[7]

MC immunophenotyping on BM aspirate

MCs can be easily identified through a multi-parametric FC study with a minimum panel of markers. Normal MCs are characterized by strong expression of CD117, expression of CD45 and negativity for CD34. Pathological MCs are distinguishable for the aberrant expression of CD25, as well as CD2. Expression of CD2, assessed by either FC or immunostaining, is quite variable in SM, and, consequently, CD25 expression may be considered a more reliable marker for neoplastic MCs. The number of pathological MCs present in the BM of ISM patients is generally <2% of CD45-positive cells, ranging from 0.002 to 1.7%. In our experience FC proved to be the most sensitive technique for identifying abnormal MCs, especially in cases with low MC burden.[7]

Molecular studies

Since the neoplastic BM MC burden is frequently very low, specially in ISM patients without skin involvement where also normal MCs may coexist, it could be sometimes difficult to analyze the mutational status of KIT. Direct sequencing by Sanger method was considered as the 'gold standard' for sequence analysis but is not enough sensitive. Recent guidelines admit the use of Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) with Restriction Fragment Length Polymorphism, Peptide Nucleic Acid PCR and allele-specific PCR.[3] In cases with persistent eosinophilia the research of the FIP1L1-PDGFRα fusion gene is warranted in order to document an associated CEL. In cases with clear myeloproliferative features the research of the JAK2 V617F mutation is indicated, although it is not commonly observed in SM patients.[3,4]

Serum tryptase level

Tryptase is the main serine protease contained in the secretory granules of human MCs and has a trypsin-like activity. It is produced almost exclusively by MCs, with the exception of a small amount produced by basophils, thus it is considered the marker of the MC diseases. The determination of serum tryptase (sT) is based on an immunometric method. In the majority of laboratory the upper normal value is 11.4 ng/mL. Patients with CM have normal or only mildly elevated sT. In the early stages of SM sT may be normal, increases over a period of months or years and further remains stable in the absence of progression to a more aggressive form of the disease. sT correlates with the MC burden, with the exception of cases that develop diffuse bone sclerosis, that is associated with a great increase in sT not related to MC load.[8,9] Elevated sT levels are not exclusive of mastocytosis. Transiently elevated sT is documented in severe anaphylaxis with hypotension, with a half-life of 1.5-2 hours. Raised sT could be also found in the course of many hematologic malignancies, particularly of myeloid origin, and in non-neoplastic condition as end-stage chronic renal failure, onchocercosis and chronic urticaria.

Prognosis

The prognosis is strictly related to the WHO variant, as recently confirmed by Lim *et al.*: ASM, SM-AHNMD and MCL are associated with a poor prognosis, with a reported median survival of 48, 24 and 2

months respectively.[10] In particular, in SM-AHMND the prognosis is dependent on the prognosis of the associate disease. Other prognostic factors associated with poor survival are advanced age, weight loss, anemia, thrombocytopenia and excess of blasts. ISM is associated with a good prognosis and a (near) normal life expectancy in the great majority of patients. The risk of ISM progression to a more aggressive form is estimated of 8.4 % at 20 years;[8] raised plasma concentration of beta-2-microglobulin and multilineage hematopoietic KIT mutation are reported as independent predictors of progression. Since evaluation of multilineage hematopoietic KIT mutation requires considerable technical expertise, and consequently is not routinely available, various parameters have been reported to correlate to its presence, as raised beta2microglobulin associated with reduced lactate dehydrogenase serum levels and an immature BM MC phenotype (CD251/FceRIlow/FSClow/SSClow/CD45low). In ISM patients, age at diagnosis >60 years, increased serum alkaline phosphatase, increased plasma IL-2Rα/CD25 levels and development of an AHNMD are reported to correlate with poor overall survival. The prognostic role of additional mutations detectable in patients with mastocytosis, including mutations of RAS, TET2, IgE receptor genes, and the expression of CD30 on neoplastic MC (mainly identified in SM-AHNMD, ASM and MCL), are currently unclear.[4]

Therapy

A rational approach to the patient with SM should include an appropriate counselling, comprising detailed information about the disease addressed to patients, relatives and doctors involved in care. Patients should be instructed how to avoid the triggers of acute release of mediators, and should be also educated on the use of self-injectable adrenaline. Medical therapy with anti-H1 antihistamines is effective in reducing itching and episodes of flushing in most patients, while the use of H2 blockers (cimetidine, ranitidine) or proton pump inhibitors is indicated in patients with gastrointestinal symptoms. The combined use of anti-H1 and anti-H2 and/or of sodium cromoglycate is recommended for the prophylaxis of episodes of hypotension or anaphylactic reactions. The cromolyn sodium is effective in reducing gastrointestinal symptoms (abdominal pain, nausea, vomiting and diarrhoea). The use of corticosteroids is controversial. Phototherapy is indicated in patients with symptomatic skin lesions, which had not benefit from anti-mediators therapy, even though such a strategy confers a transitory improvement. In patients with systemic reaction to hymenoptera sting the use of specific immunotherapy (SIT) is safe and recommended, providing effective protection to severe allergic reactions following subsequent bites. It is also recommended the lifelong administration of SIT, since cases of fatal reactions to Hymenoptera sting after its discontinuation have been reported. Besides anaphylaxis, the most severe complications related to mediator release in ISM patients are the vertebral fractures secondary to osteoporosis, which mainly occur in males. In our experience 21% of ISM patients show moderate or severe vertebral fractures at diagnosis.[9] Correction of vitamin D deficiency, X-ray of column at diagnosis and annual bone densitometry are recommended. Oral bisphosphonates are indicated in cases of mild osteoporosis, while intravenously bisphosphonates are to be used in cases with severe osteoporosis complicated with fractures, applying the appropriate security measures, such as for myeloma.[3] A cytoreductive therapy is indicated in patients with MCL, ASM, SSM in progression, while in ISM it is recommended only in the rare cases where severe and persistant symptoms are not manageable with anti-mediators treatment. In cases of SM-AHNMD, treatment is generally directed to the more severe form of the two associated diseases. The treatment of choice in slow progressing SM includes interferon-α and Cladribine (2-CdA), always associated with steroids and anti-mediators therapy; both are able to induce remission in only 40 - 50% of patients. In cases with rapid progression, a multi-drug chemotherapeutic regimen and, in young patients, BM transplant are indicated. Hydroxyurea can be also useful to control the disease. Among the TK inhibitors, Imatinib has proven effective in the rare forms with non-mutated KIT or with KIT mutations different from those involving codon 816; Imatinib is also active in the forms of MS associated with CEL. The *in vitro* anti-kitD816V activity of Dasatinib has not translated into significant therapeutic activity in most SM patients. In contrast, recently updated data confirms that Midostaurin (PKC412) is effective in patients with advanced SM and might produce a 50% decrease in BM MC burden in about half of the patients. However, it is currently not clear which SM patients are more likely to benefit from such treatment and further studies are needed to clarify the advantage of PKC412 over "standard" treatment with interferon-α or cladribine.

Experience of the multisciplinary outpatients clinics of Verona, Italy

Due to the extreme heterogeneity of symptoms at onset and the rarity of the disease, a multidisciplinary diagnostic approach to SM is mandatory. Since 2005 in Multisciplinary Outpatients Clinics for Mastocytosis of the Azienda Ospedaliera Universitaria Integrata of Verona several specialists (Dermatologists, Allergists, Hematologists, Rheumatologists) work together in the evaluation, diagnosis and follow-up of patients suspected for or diagnosed with mastocytosis. Moreover, they closely collaborate with Pathologists, Molecular biologists and specialists in Laboratory medicine and within a network of colleagues in Veneto and surrounding regions. From January 2005 to June 2013, 421 adult patients were referred, 265 sent by Allergists (63%), 46 by Dermatologists (11%), 20 by Rheumatologists (5%), 55 by Hematologists/Oncologists (13%) and 35 by specialists in Internal Medicine (8%). A total of 315 adult patients were found to have clonal MC disease. Heighty-four of the patients were resident in the Verona province. It is conceivable that almost all adults with mastocytosis residents in Verona and its province are referred to our Outpatient clinic. Height had diagnosis of CM (10%), 68 of ISM (82%), 1 of ASM (1%), 2 of SM-AHNMD (2%), 5 of MMAS (6%) (Figure 1). SM without skin involvement (*i.e.* BMM) presents a frequency (36%) comparable to that with cutaneous manifestations (46%), and much higher than described in other institutions.[3] It can be estimated on the basis of data from 2008-2011 a mean incidence of 1:100,000 new diagnosis/year in adults inhabitants (range 0,6-1,3) and a prevalence of 1:10,000/adults residents in the Verona province. Both these epidemiological data are about ten times higher than incidence of mastocytosis in adults in the years 2002-2005 (0.12/100,000/year) and the prevalence from 1996 to 2005 (0.65 cases / 100,000/year), reported by the Cancer Registry of Veneto.

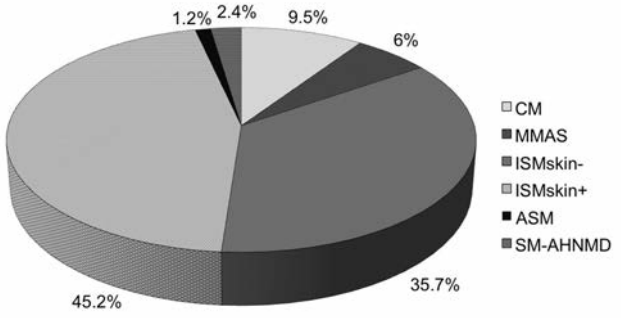

Figure 1.

Conclusion

Mastocytosis is a rare neoplasm with heterogeneous and sometimes misleading clinical presentation. A comprehensive clinical evaluation and sensitive laboratory techniques are needed to correctly diagnose and classify the disease subvariants. Patients with CM and ISM have a quite normal life expectancy, but may require chronic symptomatic treatment and lifelong immunotherapy in cases presenting with severe reactions after Hymenoptera sting. A long-term follow-up is required to prevent osteoporosis and skeletal complications. The uncommon cases presenting with an aggressive course need cytoreductive treatment ranging from single agent chemotherapy or TK inhibitors to BM transplant, and have a considerably less favourable prognosis.

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REGULATION OF SELF-RENEWAL IN CANCER STEM CELLS

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We showed that extended self-renewal of leukemia stem-cells (LSCs) is the consequence of the constitutive activation of the cell-cycle inhibitor p21. The pool of LSCs is markedly reduced in p21-/- APLs, and the residual LSCs hyperproliferate and accumulate massive DNA-damage (DD). Notably, p21 is indispensable to maintain the pool of LSCs in PML-RAR leukaemias (PML-RAR – p21-/- leukaemias are not transplantable in syngenic mice). It is not known whether p21 extends the self-renewal of LSCs by activating DD repair, restricting the cell-cycle or regulating their survival. Preliminary results from our lab showed that p21-/- APLs re-acquire the ability to initiate leukemogenesis when transplanted into immunodeficient mice (RAG) or syngeneic mice after γ-irradiation. In these conditions, the growing leukemias are indistinguishable from primary WT APLs and can be re-transplanted into immunodeficient, but not syngeneic mice, suggesting that expansion of p21-/- LSCs is controlled by the host immune-system. Further experiments suggested that p21-/- LSCs are cleared by T-cells since p21-/- APLs: i) initiate leukemias when transplanted into RAG1-/- mice (which lack B, T and NKT cells), but not in JHT mice (lacking only B-cells), and ii) do not grow in NOD/SCID mice when the recipients are injected with a T cell population primed with p21-/- blasts (*e.g.* obtained from the spleen of WT mice injected with p21-/- APLs), but not with non-primed T cells or T cells primed with WT blasts. T-cells primed with p21-/- blasts are also able to suppress the *in vivo* growth of wt APLs, but not of T-ALLs. We hypothesize that the immune-surveillance contributes to the elimination of DNA-damaged SCs, and that DNA-damaged LSCs have developed evasion strategies based on p21 activation.

THE "SIXTH SENSE" OF HEMATOPOIETIC STEM CELLS: HOW THE HEMATOPOIETIC STEM CELL COMPARTMENT SENSES INFLAMMATION AND DANGER SIGNALS

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Hematopoietic stem cell research has recently blended into the field of immunology, as new findings contributed to show how immunological signals regulate the hematopoietic stem cell (HSC) compartment. Inflammatory cytokines (such as IFN-alpha, IFN-gamma or TNF-alpha), pathogen-related molecules, and general mediators of cell stress and damage have all been demonstrated to affect stem cell functions. Among the molecules that over the past decade have emerged to have immunomodulatory functions are extracellular nucleotides. Widely known for their key role in metabolic functions, nucleotides have recently been the focus of a growing number of investigations unambiguously unveiling their role as extracellular mediators of cell-to-cell communication. Similarly to other endogenous danger signals (named alarmins), nucleotides can be rapidly released into the extracellular fluids upon necrotic cell death or through specialized efflux pathways. Once released, nucleotides can bind to a dedicated family of membrane receptors (collectively named P2-receptors or P2Rs) and modulate a variety of cell functions in target cells, including migration, chemotaxis, innate and adaptive immune responses, cells proliferation and tissue recovery after injury. Beside their role as mediators of inflammation and immune responses in terminally differentiated effector cells, purinergic signals have now been shown to extend their regulatory activity to the compartment of BM-derived stem cells. In human hematopoietic progenitor and stem cells (HSPCs), extracellular nucleotides not only promote proliferation, but also enhance CXCL12-driven migration and improve BM engraftment. In addition, purinergic signaling acts indirectly on HSPCs by regulating the BM microenvironment: extracellular nucleotides can modulate differentiation patterns in BM-derived human mesenchymal stromal cells and can affect the release of proinflammatory cytokines within the BM stem cell niche. Interestingly, purinergic signals appear to play a role within the tumor microenvironment associated to acute myeloid leukemia (AML). AML cells express several functional P2XRs and P2YRs and, conversely to HSCs from healthy donors, stimulation with ATP inhibits AML cell proliferation and reduces their engraftment potential when transplanted into immunodeficient mice. These findings contribute to depict purinergic signalling as an important trait d'union between inflammation and HSC activation, and illustrate a new picture of defence mechanisms altogether: along with specialized immune cells, primitive progenitors and stem cells also take part in mounting defence mechanisms, helping protect the organism from danger and re-establish homeostatic conditions.

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GENETIC MODIFICATIONS OF T CELLS FOR ADOPTIVE IMMUNOTHERAPY IN CANCER PATIENTS: FROM BENCH TO THE BEDSIDE

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The transfer of chimeric antigen receptor (CAR) genes that combine the antigen binding property of monoclonal antibodies with the cytolytic capacity of T-lymphocytes, represents one means of preparing T cells with a known antitumor specificity. There has been interest in generating T cells expressing CARs targeting antigens expressed in a broad array of hematological malignancies and solid tumors. Antitumor activity of redirected T cells both *in vitro* and *in vivo* are independent of MHC restriction and can be increased by co-expression of different costimulatory molecules within the CAR or by expressing CARs on T cells with a well defined antigen specificity, such as Epstein-Barr specific cytotoxic T cells (CTLs) or CMV-specific CTLs. Dr Dotti will summarize the steps that lead to clinical translation of this approach focusing on CARs relevant for hematological malignancies as a model. We will also review the procedures of gene transfer, generation of T cells for clinical use, T-cell subsets used in clinical trials, preconditioning of the host and finally safety issues directly associated with this technology.

DIAGNOSTIC PREDICTIVITY OF MORPHOLOGY

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Cytomorphology including cytochemistry (*i.e.* MPO, non-specific Esterase and Iron Staining) is still the backbone in the diagnosis of malignant diseases in hematology. This is especially true for acute leukemias, myeloproliferative neoplasms and also myelodysplastic syndromes. The parallel investigation of peripheral blood smears, bone marrow smears and histology of bone marrow trephines could not only lead to the diagnosis of the respective disease in many cases but also is mandatory as a gatekeeper for the respective algorithms to initiate other diagnostic methods. As the portfolio of other methods is still growing (immunophenotyping, cytogenetics, FISH, molecular genetics including next-generation sequencing), morphology in parallel increases with respect to its importance. However training of morphology is cumbersome and needs a lot of time and experience. This is true especially for the diagnosis of MDS meaning validation of dysplasia in granulopoiesis, erythropoiesis and megakaryopoiesis as well as percentage of blasts and ring sideroblasts. As reproducibility of these important aspects is far away from 100%, intra-laboratory ring trials as well as inter-laboratory ring trials are mandatory. This is not only true for routine laboratories but is absolutely warranted within accreditation. In addition to cytomorphology at least immunophenotyping and if possible cytogenetic and molecular studies need to be implemented in a complete diagnostic setting today. As more and more targeted treatment is available (*i.e.* CML) the morphological aspects should not be underestimated. However for follow-up studies and minimal residual disease (MRD) immunophenotyping and especially molecular techniques such as PCR demonstrate much more importance. Therefore morphology of peripheral blood and bone marrow smears for benign diseases such as EBV-infection or iron deficiency (only blood) as well as malignant diseases such as leukemias, MDS and lymphomas should still have a very high priority in a diagnostic setting in hematology.

INTERACTIVE DIAGNOSTIC MORPHOLOGY: DISCUSSION ON MAJOR DISAGREEMENTS OF THE LONG DISTANCE TRAINING SIE ECM PILOT PROJECT

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Abstract

The current strict connections between morphology, immunology, cytogenetics, molecular biology, chemotherapy, transplant, surgery and bioethics are crucial for a correct, top quality and timely treatment of hematological patients. As a result, professionals in hematology are required to have continuous medical education (CME) and long-life training on various topics and aspects in this area. Morphological evaluation of peripheral blood (PB) and bone marrow (BM) cells through microscopic examination of properly stained smears remains a cornerstone in hematological diagnosis. Many factors contribute to a lack of standardization of this diagnostic tool, such as differences in bone marrow processing procedures, staining, degree of skill in interpretation and terminology used. Starting from the French-American-British (FAB) morphological approach[1] till the new WHO classification[2] it is evident the relevance of all morphological aspects, quantitative as well as qualitative, for the recognition and classification of disease entities and for the most appropriate stratification of patients with hematological neoplasms, in particular myeloid neoplasms and, above all, myelodysplastic syndromes (MDS). Therefore the microscope still remains a robust basis in the integrated diagnostic process of hematological diseases. Several studies are reported in the literature,[3,4,5] in which experienced morphologists have reviewed slides from different Institutions: the rate of concordance is highly variable especially when the threshold is low, such as 3% of blasts [6,7] or 10% of single lineage dysplasitic cells.[2,8] Traditional education, training and accreditation systems are usually face-to-face: the onset of traditional and on-line courses, represents an effort to satisfy this peculiar need. The current information and communication technology (ICT) era provides the opportunity of exchanging, via internet, images and information without geographic limitation, saving time and resources. Computerized images, standardized according to the significant content and technological aspects such as resolution, weight and compression, provide us today with the highest excellence in terms of accreditation, training, and information exchange. These systems and devices work with freezed images selected by the microphotographers: within this approach, the European Leukemianet (ELN) Morphology Faculty WP10[9] works having as its major goals of morphological consensus, concordance and uniformity of diagnostic features: the outcome of this study is freely available on-line through both ELN and EHA (European Hematology Association) websites. The development of a new technology, the Zeiss VSlide System, a Medial Microscopy (MM) based on the scan of the whole smear, adds new and realistic opportunities: by making available on the web the scans of PB and BMsmears, all the virtual community involved in this knowledge process can be trained adopting a diagnostic approach that closely reproduces the one adopted in the real life for the diagnosis at microscope of hematological patients: easy navigation, zooming and microscopic field identification trough a grid system do represent only some of the facilities in the use of this device. Figure 1 represents the screen of the MM: on the left of the image there is a field of the scanned slide, on the right the same field with a superimposed grid with coordinates, that allow unique cell identification, while at the bottom of the right side there is the whole scanned smear with a small white square exactly indicating the enlarged zone. Together with the SIE (Italian Society of Hematology) we have organized in the year 2013 a CME course on cell identification: starting from January every two months a total of five cases have been and will be consecutively uploaded into the web: for each case, a maximum of 100 users, identified by the system through a unique ID, can navigate through the scan of the BM and/or PB and can perform a myelogram and/or a PB differential as many times as they wish for a personalized as well globalized training with geographic independency, great convenience comfort and flexibility: finally, 18 questions with multiple choices answers are presented for each case, as an online testing with an automatic record-keeping system: users can repeat the test, within two months, for a self-assessment training. The possibility to mark the cell with coordinates is a very strong and easy tool for discussion. This methodology, in conclusion, does represent the best available tool for distance learning, training and education in morphology, with the aim to reach an harmonized morphological diagnosis among different groups for a consensual patient stratification. Finally, major disagreements in cell identification and/or classification of the first three morphological cases submitted in the year 2013 of SIE CME Pilot Project will be discussed during the interactive session on morphology and final results will be published on the web.

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THE MANAGEMENT OF VENOUS THROMBOEMBOLIC DISEASE: CLASSIC STRATEGIES AND NEW PERSPECTIVES

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The primary objectives for the treatment of deep venous thrombosis (DVT) are to prevent pulmonary embolism (PE), reduce morbidity, and prevent or minimize the risk of developing the postthrombotic syndrome (PTS) and the chronic thromboembolic pulmonary hypertension CTPH). The mainstay of medical therapy has been anticoagulation since the introduction of heparin in the 1930s. Other anticoagulation drugs have subsequently been added to the treatment armamentarium over the years, such as vitamin K antagonists and low-molecular-weight heparin (LMWHs). Traditional anticoagulants, such as warfarin and acenocoumarol, unfractionated heparin and LMWHs, have several limitations, including need for laboratory monitoring, ongoing dose adjustment and parenteral administration, which may limit optimal patient care. Newer oral anticoagulants, such as direct thrombin inhibitors (*e.g.*, dabigatran etexilate) and direct factor Xa inhibitors (*e.g.*, rivaroxaban, apixaban, and edoxaban), have been developed to overcome these drawbacks, and thereby improve patient care. Several of these agents have been approved for use in the prevention and treatment of venous and/or systemic thromboembolism. Their introduction is likely to have a major impact in the years ahead. Many large clinical trials have been published in the past few years showing these agents are generally safe and effective in several clinical settings including acute venous thromboembolic disease, prophylaxis in the postoperative setting, prevention of thromboembolism in patients with atrial fibrillation, and in the management of acute coronary syndromes. Rapid onset and offset of action and predictable pharmacodynamics with relatively wide therapeutic window allowing for unmonitored drug use are the major predicted advantages.The relatively short half-life, rapid onset of action, and predictable pharmacokinetics should simplify periprocedural use of these agents. The objective of this presentatiuon is to provide an overview of the available clinical trial data for these new oral anticoagulants in the prevention and treatment of venous thromboembolism and a practical update for clinicians.

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SPLANCHNIC VEIN THROMBOSIS: FOCUS ON ANTITHROMBOTIC TREATMENT

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Splanchnic vein thrombosis (SVT) is a manifestation of unusual site venous thromboembolism (VTE). Veins draining from different abdominal organs may be involved, leading to portal vein thrombosis (PVT), mesenteric veins thrombosis (MVT), splenic vein thrombosis (SpVT) and Budd-Chiari syndrome (BCS). Pathophysiology, clinical presentation and prognosis vary according to the site of thrombosis, although showing some common features.

Epidemiology

The epidemiology of SVT is poorly defined and varies greatly depending on data sources. PVT is the most common manifestation in the spectrum of SVT, with a reported annual incidence of less than 4 per million people in hospital registry data in the 1980s and a population prevalence of approximately 1% in a recent large autopsy study1. Viceversa, BCS is the least frequent disease, with an incidence ranging from 0.1 to 0.4-0.8 per million people per year (in Japan and Western countries, respectively) and, inversely, a prevalence ranging from 1.4 to 2.4 per million people (in Western countries and Japan, respectively)1. SVT has also a non-negligible rate of asymptomatic incidental findings, in imaging studies performed for other indications, such as follow-up of patients with cancer or liver cirrhosis.

Risk factors

SVT may be associated with different underlying disorders, either local or systemic. Abdominal cancer (mainly in the pancreatic, hepatobiliary or gastrointestinal system) and liver cirrhosis are the most common risk factors for PVT, being present in 31% and 34% of patients in a recently published study.[2] The most common local risk factors for isolated MVT are cancer and abdominal inflammations or infections, each being present in about 20% of cases.[2] Isolated spVT was associated with underlying acute pancreatitis in nearly half of the patients, followed by cancer, cirrhosis and splenectomy.[2] Myeloproliferative neoplasms (MPNs) are the leading systemic cause of SVT, diagnosed in 40% of BCS patients and approximately 30% of patients with non-cirrhotic non-malignant PVT.3 Moreover, MPN subtypes showed different frequency according to the site of thrombosis: polycythemia vera and myelofibrosis were more prevalent in BCS than in PVT patients; while no difference has been reported in the prevalence of essential thrombocythemia and unclassifiable MPNs.[3] Moreover, the JAK2 V617F mutation, the main molecular marker of the Philadelphia-negative MPN, emerged as an independent factor for SVT.[4] Among inherited thrombophilias, higher prevalence of prothrombin G20120A mutation has been reported in patients with extra-hepatic PVT, while Factor V Leiden mutation was more frequent in BCS patients.[1] Common systemic risk factors for BCS are also hormonal stimuli, such as the use of oral contraceptives and pregnancy or puerperium.[1] Recently, an association between SVT and paroxysmal nocturnal hemoglobinuria has also been reported.[1] Overall, permanent or transient risk factors are identified in at least 80% of patients, thus leaving a minority of events classified as unprovoked SVT.[2]

Clinical presentation

The clinical presentation of SVT is heterogeneous and varies accord-

ing to the characteristics of the onset and the involved veins. Abdominal pain is the most frequent symptom, with a prevalence ranging from 40% in patients with PVT to more than 60% in patients with MVT.[2] Acute MVT is indeed associated with intestinal infarction in almost one-third of patients.[1]Gastroesophageal varices and gastrointestinal bleeding, triggered by portal hypertension, are reported in one-quarter of patients, mainly with PVT or SpVT, and represent a challenge for treatment decisions.[2] Chronic PVT is also associated with the finding of portal cavernoma, portal cholangiopathy and hepatic encephalopathy1. In the majority of BCS patients, ascites, hepatomegaly, splenomegaly and right upper abdominal pain are reported.[1] In a large cohort of 832 patients diagnosed with SVT over a 20-year period, 18% were asymptomatic.[2]

Antithrombotic treatment

The choice of the optimal treatment in patients with SVT is challenging. These patients have an increased risk of bleeding, due to the presence of oesophageal varices or thrombocytopenia, but in the meantime they also have a prothrombotic predisposition, resulting from the underlying cirrhosis or malignancy. In literature, there is a lack of randomized clinical trials to guide treatment decision, and contrasting evidence emerged from observational studies in the last decades. The majority of studies evaluated the antithrombotic treatment only in patients with non-malignant non-cirrhotic PVT. The retrospective study performed by Condat *et al.*[5] included 136 patients, with a median follow-up duration of 46 months. Anticoagulant treatment with heparin or vitamin K antagonists (VKAs) has been administered only in 84 patients, of whom 54 continued throughout the follow-up period and 30 discontinued the treatment before, but its duration was not reported. The anticoagulant treatment reduced the risk of recurrent thrombotic events in the portal venous system by two thirds (0.64/100 patient-years vs 1.87/100 patient-years, with and without anticoagulant therapy, respectively), without increasing the risk or severity of gastrointestinal bleeding. Indeed, the incidence of gastrointestinal bleeding in the overall cohort was high (12.5/100 patient-years) and the only independent predictor of bleeding was the presence of moderate or large esophagogastric varices without adequate prophylactic measures. Plessier *et al.*[6] evaluated the early initiation of heparin therapy, followed by oral anticoagulation, in 95 patients with acute PVT enrolled in a prospective European study. The anticoagulant treatment has been prescribed for at least 6 months, prolonged to long-term if mesenteric vein thrombosis or permanent prothrombotic disorder, for a median treatment duration of 234 days. At 1-year follow-up, recanalization was detected in one-third of PVT patients, and more than half of MVT and SpVT patients. Although major bleeding occurred in 5% of patients, no death resulted from haemorrhage. Opposite results emerged from the largest unselected cohort of SVT patients, diagnosed and followed up at a single institution, the Mayo Clinic, over a 20-year period.[2] This retrospective study enrolled 832 patients with thrombosis of different splanchnic veins (including hepatic, splenic, portal or mesenteric) and different aetiologies (particularly malignancy and cirrhosis). Warfarin has been provided to 235 patients (28%), of whom 175 lifelong, but no information is available on the use of alternative anticoagulant drugs such as heparins. After a mean follow up of 27 months, the incidence of recurrent venous thrombosis was 3.5/100 patient-years, but the recurrence-free survival was not improved by the anticoagulant treatment (0.89 vs 0.77, p=0.38). The overall incidence of major bleeding was 6.9/100 patient-years and these complications were significantly higher in patients receiving warfarin compared with not-anticoagulated patients (26.2% vs 18.9%, p<0.05). More recently, Spaander *et al.*[7] retrospectively collected information on 120 patients with non-malignant non-cirrhotic PVT. Only 66 patients (55%) were anticoagulated, with heparin or VKAs, for a median treatment duration of 1.9 years. The anticoagulant therapy showed a tendency to prevent recurrent venous thrombotic events (HR 0.2, p=0.1) but significantly increased the risk of gastrointestinal bleeding (HR 2.0, p<0.01). Indeed, 58 bleeding episodes happened in 66 patients on anticoagulant therapy vs 25 bleeding episodes in 54 patients without anticoagulant therapy. At multivariate analysis, independent predictors of bleeding included also gastrointestinal bleeding at baseline (HR 2.1, p<0.01) and ascites at baseline (HR 2.0, p=0.01). Again, these findings are not generalizable to the whole population of SVT patients, given the highly selected population included in this study. Currently available guidelines recommend, in the absence of major contraindications, to start the anticoagulant therapy in all patients presenting with acute symptomatic SVT,[8-9] with the aim to prevent the intestinal infarction and the long-term complications of chronic portal hypertension. After an initial period with either low-molecular weight heparin (LMWH) or unfractionated heparin, most of the patients are candidates to VKAs. However, LMWH should be considered for extended treatment, if there is active malignancy, liver cirrhosis or thrombocytopenia.[9] There is no consensus about the use of anticoagulant drugs in chronic SVT, which presents with variceal bleeding and hypersplenism but without signs of recent occlusion.[8] Moreover, current guidelines suggest not to treat patients with asymptomatic incidentally detected SVT, even though the level of evidence is low.[9] A recent prospective international registry evaluated the use of antithrombotic treatment for SVT patients in real life clinical practice.[10] This unselected cohort included 613 patients, with a non-negligible proportion of liver cirrhosis or solid cancer (27.8% and 22.3%, respectively). The most commonly site of thrombosis was PVT (76.3%), with or without the involvement of other venous segment, but there was also a minority of patients with isolated MVT (10.9%), BCS (8.3%) or SpVT (3.1%). During the acute phase, 136 patients (22.2%) remained untreated. Factors associated with the decision not to administer anticoagulant therapy were: incidental diagnosis, single vein thrombosis, gastrointestinal bleeding at onset, solid cancer, liver cirrhosis and thrombocytopenia. Excluding a minority of patients that underwent interventional procedures, parenteral or oral anticoagulation has been administered to 470 patients (76.7%), of whom 295 continued with VKAs. Factors associated with the decision to start VKA treatment were: younger age, symptomatic onset, multiple veins involvement, unprovoked thrombosis or SVT secondary to a persistent risk factor such as MPNs. Interestingly, 61.1% of incidentally detected SVT received anticoagulant treatment. Patients with chronic liver disease represent a particular subpopulation with a delicate haemostatic balance. Despite prolonged routine coagulation tests, they have actually a higher incidence of venous thromboembolic events, compared with the general population.[11] On the other hand, gastroesophageal varices are common in cirrhotic patients, but they do not necessarily represent a contraindication to anticoagulant therapy. Since it has been demonstrated that haemodynamic factors, such as variceal pressure, have higher impact on gastrointestinal bleeding than the anticoagulant treatment,[11] it is highly recommend to establish an appropriate prophylaxis with beta-blockers or with endoscopic treatment of oesophageal and gastric varices.[8] Patients with liver cirrhosis have been excluded from the majority of previous studies addressing SVT, but in the last decade they have been enrolled in specific management trials. Senzolo *et al.*[12] proposed a therapeutic algorithm to treat cirrhotic patients with PVT. The therapeutic regimen consisted of nadroparin 95 antiXa U/Kg twice daily, with a reduction of the dose by 40% if platelet count below 50000/mm^3. Patients with previous gastrointestinal bleeding or with high grade varices, underwent banding of oesophageal varices before starting the anticoagulant treatment. After complete repermeation, prophylactic dose of nadroparin (3800 anti-Xa U/daily) was continued for at least 6 months, or longer if underlying thrombophilic genetic defect. Transjugular intrahepatic portosystemic shunt was used when anticoagulation was contraindicated or failed to prevent thrombotic progression. This treatment algorithm achieved good rate of repermeation (36% complete plus 27% partial) and decreases the rate of thrombosis progression, thus reducing also portal hypertensive complications. Recently, patients with advanced liver cirrhosis have been included in a randomized trial evaluating the safety and efficacy of enoxaparin (4000 U/daily for 48 weeks), compared with no treatment, in the prevention of PVT13. During the active treatment period, enoxaparin effectively prevented the development of PVT (0% *vs* 16.6%, p=0.025, in treated and control patients, respectively), without increasing the risk of bleeding complications (p=0.521). Moreover, enoxaparin appeared also to delay the occurrence of hepatic decompensation (11.7% *vs* 59.4%, p<0.001, in treated and control patients, respectively) and to improve survival. Although the small number of patients included in this study impose caution in the interpretation, these results are indeed interesting because they suggest that, in cirrhotic patients, PVT prevention might be less harmful than SVT treatment. Patients with BCS represent another particular subpopulation, given the frequent need for second-line interventional procedures. In addition to medical management (anticoagulants and diuretics), therapeutic options for BCS include local thrombolysis, percutaneous transluminal angioplasty (PTA), surgical or transjugular intrahepatic porto-systemic shunting (TIPS) and orthotopic liver transplantation (OLT), which are recommended in case of worsening despite medical treatment.[8] Darwish-Murad *et al.*[14] prospectively followed 163 patients with BCS in a European multicenter study. Many patients were

safely managed with medical treatments (86% anticoagulation and 61% diuretics), but 51% required an invasive procedure (6% thrombolysis, 9% PTA, 34% TIPS, 2% surgical shunting, 12% OLT). With this combined management, the overall survival rate of BCS patients was 87% at 1 year and 82% at 2 years. Variceal bleeding occurred in 8% of the anticoagulated patients, without any fatality. The optimal duration of the anticoagulant treatment is still a matter of debate. SVT is a potentially life-threatening disease, since one fourth of recurrences occurred as hepatic, mesenteric or splenic venous infarction.[5] At the same time, gastrointestinal bleeding represents a frequent complication. Few studies provided information on potential risk factors associated with VTE recurrence. Underlying prothrombotic states (such as MPN, thrombophilic abnormalities or hormonal therapy) emerged as independent predictors of recurrence in some studies; while there was no agreement on the role of the anticoagulant treatment.[15] Current guidelines, derived from usual site VTE, suggest discontinuing anticoagulant therapy after 3 months if there is a reversible provoking factor (such as intraabdominal sepsis or recent surgery). *Viceversa*, extended anticoagulant treatment, with periodic re-assessment of bleeding risk, is advised for unprovoked SVT or for SVT secondary to a persistent risk factor (such as MPN).[9] Moreover, experts' opinions suggest indefinite anticoagulant treatment also for patients with BCS, considering the potential severity of a recurrent event.[16] In conclusion, the choice of the optimal treatment for SVT patients is challenging, given the delicate balance between thrombotic and bleeding risk. From recent evidences, the anticoagulant treatment appeared to be safe and effective for most SVT patients, but a careful evaluation of each patient risk-benefit ratio is needed. Future studies should aim at better assess the role of antithrombotic treatment in the acute and long-term management of SVT.

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WHAT'S NEW IN HAEMOSTASIS TESTING?

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In the past years the coagulation laboratory has developed along a traditional scientific "reductionist path", an approach to studying and understanding the nature of complex systems by reducing them to a set of simpler components. The translation of this scientific method to haemostasis has resulted in a practice where the coagulation system has been dissected into its individual components and the amount and/or activity of a few potentially relevant factors (procoagulant or anticoagulant) are measured to predict the coagulation potential. Recently, an increasing number of genetic factors that influence haemostasis have been identified, but the measurement of coagulation phenotype rather than the genotype might provide laboratory assessments that are more relevant for clinical aspects. The coagulation clinical laboratory has come as far as it can with this reductionist approach and all that can be achieved now is increasing accuracy and decreasing imprecision. Given the complexity of the coagulation system, which involves several interrelated procoagulant and anticoagulant pathways, measuring the plasma levels and/or activities of individual proteins might have little utility in evaluating the haemorrhagic or thrombotic risk and in the clinical management of hypo- and hypercoagulable states. On the haemorrhagic side, it is known that patients with the same coagulation factor deficiency (*e.g.* haemophilia A) may present with different bleeding tendencies. Indeed, factor assays are limited by their sensitivity at very low levels. Factor levels below 0.01 IU/mL have therefore not been traditionally quantified. In many patients with coagulation disorders, factor assays alone do not correlate well with clinical symptoms. It has been shown that plasma from some patients with severe haemophilia A has the ability to generate thrombin. The exact basis for this phenomenon is not well understood, but may be related to the balance of levels of different procoagulant and anticoagulant proteins in the blood. On the thrombotic side, carriers of a particular defect (*e.g.* factor V Leiden) often experience different thrombosis risks, depending on additional genetic and environmental modulators that can increase the risk or can be protective. Moreover, acquired conditions such as pregnancy and oral contraceptive use modify the levels of several coagulation factors and inhibitors at the same time. A different approach allowing the global measurement of coagulation potential instead of single components may be of more clinical utility being the likelihood that the test result will lead to an improved health outcome. It is possible that tests that assess global haemostasis may be better reflective of the clinical features. Hence the need for global assays evaluating the overall coagulation function in order to reliably estimate the bleeding/thrombosis risk in single patients. The process of thrombin generation and fibrin clot formation can be captured with greater sensitivity and completeness by tests that measure global haemostasis. These include the thrombin generation assay and thromboelastography, which were originally described several decades ago. Recently, several studies have investigated the correlation between the results of these global assays and the risk of bleeding or thrombosis. It has been shown that global assays are able to discriminate between mild and severe bleeders among haemophilic patients with undetectable factor VIII or IX levels. In addition, they can be useful in monitoring haemophilia treatment with inhibitor bypassing agents. Thrombin generation was found to be elevated in carriers of all inherited thrombophilic defects, as well as in acquired conditions that predispose to venous thrombosis (*i.e.* pregnancy and oral contraceptive use). For this reason, it is not surprising that several retrospective and prospective studies have found a correlation between elevated thrombin generation and the risk of the first venous thrombosis, as well as of recurrence after a first event. These findings suggest that global assays

might provide useful information to guide clinical decisions in the prevention and treatment of coagulation disorders in individual patients. Currently, there are no widely available global tests that can quantitatively assess the overall haemostastic potential of blood and several standardisation problems still prevent their widespread application in clinical settings. However, it has been recently shown that the use of standardized protocols and reagents can help to minimize these problems, making possible their implementation in clinical coagulation laboratories in the future.

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FIL: ITALIAN LYMPHOMA FOUNDATION

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The Italian Lymphoma Foundation is an NPO which coordinates the activities carried out in Italy in the field of lymphoma by more than 144 centers located throughout the country. The aim is improving the centers skills in terms of research and assistance. FIL conducts scientific research activities in the field of lymphoma and, as a non-profit organization, its purposes only concern social solidarity. The Italian Lymphoma Foundation has been the natural evolution of the Italian Lymphoma Intergroup, which was founded in 1993 with the first meeting held in Florence as a group of spontaneous cooperation between clinicians and Italian researchers involved in the study and treatment of lymphoma. Its existence and its assets were later formalized in July 2004 with a notary deed that led to the creation of a foundation with legal status and to the enrolment in the register of NPOs. The Italian Lymphoma Foundation was born with the aim of make active groups in the study of lymphoma cooperate; afterwards groups have merged and the IIL has decided to become a reference point for their cooperation. FIL Onlus was born in Alessandria on September 30, 2010 with a notarial deed that has marked the transformation of the statute, with the fusion of all groups within one large organization. FIL encourages prospective and retrospective studies in order to answer questions that require a large case report. Therefore it intends to foster partnerships with international organizations, to which it is configured as a partner of choice. FIL also seeks to organize and improve services for the diagnosis and treatment of other lymphoproliferative disorders and promotes the formation of the Italian Registry of lymphoma (RIL). FIL Onlus develops projects for the diffusion of information on lymphoma, in order to raise the awareness of the problem and help patients and relatives; coordinate research groups in the fight against lymphoma; constitute the scientific, organizational and legal framework for managing clinical trials on lymphoma; coordinate the efforts of researchers to create one large Italian cooperative group and collaborate with European groups as for international studies on lymphoma.

GIMEMA: MULTIPLE MYELOMA WORKING PARTY

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The GIMEMA (Italian Group for Hematological Diseases in Adults) is a no-profit foundation, that began in 1982, with a small groups of Italian hematology centers. They realized that only by joining forces and comparing studies, they could achieve results of any significance, which they would not otherwise have been able to do. Over the years it was created network to which almost all Italian hematology centers belong. Membership requires only two strict undertakings: the rigorous observation of clinical trials and the proper comparison of results. In February 2004, a new organizational model was set up, the Working parties (WPs), each focused on a different pathotology or a specific approach. They are scientifically autonomous but agree to operate in accordance with the good clinical practice guidelines. These groups represent the real scientific "soul" of GIMEMA. They are made up of the top Italian experts in each field and they meet regularly to share scientific opinions and ideas, suggest changes, propose new therapeutic strategies, evaluate the use of new drugs and new ways of collaborating with European and International cooperative groups. To date 8 WPs have been set up and 1 is focused on multiple myeloma (MM). The MM WP includes 3 life members, the chairman, the co-chairman and the secretary and the elected board, that includes 9 members elected every 3 years by all Italian hematology centers. Overall the WP-MM includes 132 Italian centers all over the country, of these about 50% has got a transplant unit. The enrollment capability is about 650 newly diagnosed patients per year and 400 relapsed or refractory patients per year. At diagnosis two third of patients are younger than 65 years and eligible for high dose treatment with autologous stem cell support while one third is older than 65 years and not eligible for high dose treatment. On the contrary, at relapse, one third of patients is younger than 65 years and two third are older. In the last 10 years, WP-MM was involved in 21 sponsored trials, the most of them for relapsed/refractory patients and in 28 investigator sponsored protocols, fifty per cent for newly diagnosed patients and fifty per cent for relapsed or refractory patients. In 2008, 5 territorial districts were set up, such as north western Italy, north eastern Italy, central Italy and south Italy. Each district includes few near centers that can stronger collaborate, assuring uniform data. The main objectives of districts were managing small phase I/II trials with about 50 patients, testing new compounds or rare disease or specific subset of patients. Update about WP-MM activities will be presented at the meeting.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM ALTERNATIVE DONOR: IS A TRANSPLANT DOABLE FOR EVERY PATIENT?

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Introduction

Allogeneic stem cell transplantation (ASCT) represents a potentially curative treatment for several haematological disorders. However, its feasibility has been hampered by the limited availability (~30%) of a suitable, HLA-matched familiar donor. Over the last decades, many factors have contributed to extend the ASCT feasibility including the better prophylaxis and treatment of post-transplant complications, the improving supportive care and the introduction of conditioning regimens at reduced intensity. Moreover, its applicability has largely been increased due to the evolution in the strategy of donor selection. The advances in Cord Blood (CB)[1] such as in Haploidentical Transplant[2] have achieved results comparable to those obtained in ASCT from volunteer unrelated donor (VUD), allowing to consider all these stem cell sources as valid alternatives for patients lacking an HLA-identical sibling. Therefore, the chance of patients to receive an ASCT primary depends on the transplant centre policy in defining the strategy of alternative donor search and selection. This widespread approach of searching for a suitable alternative donor overcomes the "genetic randomization" based on the "donor versus no donor" concept by the availability of an HLA identical sibling.

Volunteer Unrelated Donor Identification

Over the past 20 years, the International Donor Registries have progressively grown and more than 20 millions of VUD are presently available worldwide. Overall, about 60% of patients of Northwest European origin may identify a 10/10 allele-matched VUD, but this percentage is greatly reduced when the patients express an unusual allelic linkage, an uncommon haplotype or belong to a minor ethnicity. The probability of finding VUD within 6 months from the starting of search has been estimated high, intermediate or low , including in the last category patients with rare allele or unusual B-Cw, DRB1-B3, DRB1-DQB1 association, also if a frequent haplotype occurred on the other chromosome.[3] The NMDP confirmed this analysis and estimated as high, intermediate or low, respectively, the probability of >95%, 50% or <5% of identifying a 10/10 HLA allele matched VUD. The probability and timing of donor identification is greatly affected by the lack of high resolution 4-digit HLA typing of most registered donors, as only 50% of the serologically HLA compatible donors remains 10/10 allele matched after high resolution confirmatory typing. Duration and success rate of VUD identification for Caucasian patients has been also related to the HLA-DRB1 allele and DRB1-DQB1 haplotype frequencies.[4] Despite most donors in the Registries are not typed for DQB1 locus, the DRB1-DQB1 haplotype can be predicted with high confidence because of the strong linkage between the two HLA class II loci. According to the DRB1 allele frequency and DRQB1 association, the probability for a successful donor search was classified as high (58%), intermediate (28%), low (10%) and very low (3.3%). In this study, the overall identification of high resolution 10/10 matched VUD in the groups with high, medium, low and very low probability of search success was 78.3%, 49.7%, 17.9% and 5.6% in a median time of 20 (range, 7-330), 27 (range, 8-539), 45 (range, 7-1225) and 477 (range, 2-2879) days, respectively. More recently, of 1602 HLA-A/B/DRB1 broad haplotypes, assigned by the inheritance methodology to 402 families, 50 common-4-digit haplotypes were identified with the principal involvement of HLA DRB1 and DQB1 loci and a hierarchy of HLA-C >HLA-A or –B distinguished common from less common haplotypes.[5] Deleting the less useful intermediate group, the probability of finding an HLA 10/10 matched VUD was high (98%) in the presence of the following findings: 1) at least 1 common 4-digit haplotype and ≤2 rare alleles/unusual B/C or DRB1-DQB1 association on the 2nd haplotype; 2) no common 4-digit haplotype and ≤1 rare allele or unusual B/C or DRB1-DQB1 association on the 2nd haplotype. The probability was considered low (26%) for all the remaining patients. Furthermore, in the high probability group the presence or absence of at least one common haplotype resulted, respectively, in 94% and 61% probability of finding a 10/10 HLA matched VUD. A more precise estimate of the probability of identifying a HLA matched VUD at the preliminary phase of the search, allowing a prompt classification of patients in low or high category, may improve the general therapeutic strategy. BMDW is able to provide this important information through a specific "matching program".

Volunteer Unrelated Donor Selection

Many factors contribute to the choice of a VUD such as the CMV status, sex, AB0 compatibility, age and weight of the donor. However, the main criteria for selection is represented by HLA compatibility. The current gold standard of an HLA matched donor/recipient pairs is considered the high-resolution allelic matching at HLA-A, B, C, DRB1, and DQB1 loci. However, a VUD matched at 10/10 HLA alleles is not frequently available, so that an HLA compatibility of ≥8/10 alleles is currently acceptable. From a large retrospective analysis of NMDP including 1874 donor/patient pairs the following conclusions have been drawn: 1) mismatches for HLA-A,-B, -C, and –DRB1 are similarly associated with increased risk of GVHD and mortality, suggesting the important role of C locus in the strategy of donor selection; 2) high resolution mismatching, particularly involving HLA-A and –DRB1 loci, is related to increased mortality; 3) TRM is higher in donor/recipient mismatching determined by only low resolution typing ; 4) the risks of GVHD, rejection and TRM raise with increasing numbers of HLA mismatches.[6] Overall, from these results the high resolution typing of the donor/recipient pairs is mandatory. Furthermore, HLA-DQA1 and DPA1 differences do not seem to influence the transplant outcome, while the role of HLA-DPB1 and DQB1 loci is still controversial. In particular, the HLA-DPB1 alleles have been classified at high, intermediate and low risk according to their immunological expression.[7] The combinations based on the DPB1 mismatching allow to define the donor/recipient pairs as permissive or non-permissive in GvH or HvG direction. Despite the high allelic polymorphism of HLA-DPB1, it is advisable to consider it when several donors are available. The crucial role of Cw and DPB1 alleles in supporting GVL/GVHD effect has been recently confirmed by a retrospective analysis including 4643 patiens which identifies 10 mismatch combinations (4Cw and 6 DPB1) significantly related with a decreasing risk of relapse (p<.003, p<.002, respectively).[8] Although secondary to the HLA compatibility, an other important criteria for VUD strategy selection is represented by the cytomegalovirus (CMV) serologic status of the donor/recipient pairs. The CMV-seronegative status in both donor and recipient is related to the lowest risk for developing CMV infection, while the highest risk for CMV reactivation is represented by the combination of CMV-negative donor to CMV-positive recipient. As to regard VUD age and gender, a younger male donor is preferred. Several studies have shown that donor age >45 years is related to an increased risk of TRM and relapse, while female donors are generally at risk for developing immunization due to previous pregnancies and/or miscarries. As the minor histocompatibility antigens encoded by the Y chromosome are targeting by female donor T cells, the female donor/male recipient pairs is associated with increased cGVHD occurrence (66%vs38%; p=.02) and a reduced relapse rate (6%vs23%; p=.046), so that it is advisable to choice male donor or nulliparous donor or female donor with the lowest number of pregnancies. Whenever possible, AB0 matched pairs should be selected, otherwise the minor ABO incompatibility over the major one. Finally, the donor closest to the country of origin of patient should be preferred in order to reduce total time and costs for search and procurements .

Cord Blood Unit Identification

The number of unrelated allogeneic Cord Blood Transplants (CBT) has rapidly grown over the last decade with a wide use in both pediatric and adult patients. The number of Cord Blood Units (CBU) available in BMDW database has increased more than 10 fold from 1998 and, to date, is >600.000. The main advantages of CBT are represented by 1) the easy and quick procurement of the stored CBU fully HLA typed and assessed for cell dose and infectious contamination; 2) the presence of "naïve" T cells in the graft allowing a greater degree of donor/recipient HLA disparity without increasing the GVHD incidence; 3) a higher potential access of ethnic minorities to ASCT due to the reduced stringency of HLA compatibility required for CBT. On the other hand, the

main disadvantage consists of the low cell dose contained in the CBU with a consequent risk of graft failure or engraftment delay. The CBU selection is mainly done according to pre-freezing cell dose and HLA donor/recipient compatibility. Eurocord recommends to select CBU ≥4/6 HLA loci matched with the recipient and containing ≥2.5x10^7/kg nucleated cells and/or ≥1x10^5/kg CD34+ cells of the recipient body weight. Thus, the preliminary CBU search is based on a report that lists the CBUs according to the best compatibility degree with the patient and the highest pre-freezing total nucleated cell dose (TNC). Usually, CBU are typed serologically for class I HLA antigens and at allele level for DRB1. The CBU with high resolution DRB1 typing go head in the list. In a study including 525 patients of whom 35% non-Europeans, VUD and CBU availability has been compared for patients undergoing simultaneous searches according to European versus non European ancestry, CB significantly extended ASCT access, especially to recipients of non-European origin but also to other racial and ethnic minorities. A recent study assessing the need of a CBU national inventory has calculated that a CBB including 50000 units provides at least 1 donor to 98%, 80% and 34% of North Western European patients with 4, 5, and 6 out of 6 HLA matched loci, respectively. Extending the CBB inventory to 150000 units the probability of finding a donor slightly increased for this ethny, but , the probability for non–North Western European patients of finding at least 1 donor 4, 5, and 6 out of 6 HLA matched loci increased from 96 to 99%, from 49.7% to 77% and from 8.9 to 17%, respectively (9)

Cord Blood Unit Selection

Nowadays, the selection of a suitable CBU for stem cell transplant is mainly based on the pre-freezing cell dose and HLA matching. In CBT setting, several analysis have reported a direct correlation either between pre-freezing NC and CD34+ cell dose and neutrophil and platelet recovery or between CBU cell content and survival. In particular, the number of ≥2.5x10^7 TNC/kg and ≥1.7x10^5 CD34+cells/Kg of recipient body weight are the recommended minimum cell doses to assure engraftment in both adult and pediatric transplants. According to the Eurocord recommendations, CBU are required to be matched with the recipient at least for 4/6 HLA loci serologically typed for class I and at high resolution for DRB1 antigens. Until now, DQ matching seems to be not relevant, while the role of C locus disparity is still controversial.[10] In terms of CBT outcome, 0-1 HLA mismatches are better than 2 and HLA class I mismatches is preferred to class II incompatibility. However, 1-2 HLA differences has been correlated with a reduced risk of post-transplant relapse, which, similarly to what observed in T-cell depleted haploidentical tranplants, further seems to depend on the NK donor cell alloreactivity . In case of double CBT, each CBU should contain a minimum cell dose of 1.5X10^7 TNC/kg and the 2 units should share at least 4/6 A, B, DR HLA loci each other and with the patient. Despite the high coefficient of variation among laboratories, the content of Colony Forming Cell (CFC) either pre-freezing or at infusion represents a significant factor for hematopoietic engraftment and a cut-off of >1x10^4/kg of post-thawing CFU-GM has been identified as a prognostic factor for TRM and survival. *in vitro* cell cultures from the thawed segment accompanying the CBU is very useful in order to minimize the risk of graft failure secondary to undetected loss of clonogenic efficiency. As few data are available on the viability of cord blood cells and transplant outcome,[11] prior to be included in the current pre-releasing check as criteria for CBU selection, the viability test on the thawed CBU fragment needs further investigations. ABO donor/recipient incompatibility may lead to either delayed engraftment or chronic hemolysis after transplant, so that in cases of two available CBU, with similar cell dose and HLA matching, the ABO matched CBU should be chosen. Finally, according to the Fact/Netcord standards CBU with positive microbiological cultures should be discarded and infectious disease markers must be negative. Although CMV testing is optional, a potential CMV infection during pregnancy may occur so that the transplant center policy should include the maternal antibody IgM and, in the positive cases, the CMV PCR checked on the maternal plasma and on whole blood samples stored with the bags.

Haploidentical Related Donor Selection

In the last years, the results of transplants from haploidentical related donor have been particularly encouraging and, at least in some malignant diseases, are well comparable with those obtained through VUD or CB transplants. For several years the practice of haploidentical transplant was strongly linked to that of T-cell depletion of the graft in order to avoid lifethreating GVHD. However, more recently, the use of particularly effective regimens for *in vivo* GVHD prophylaxis have provided that haploidentical transplants could be successfully performed employing non T-cell depleted bone marrow or peripheral blood.[12,13] These kinds of transplant, avoiding the sophisticated, expensive and time consuming manipulations required by T-cell depleted procedures are becoming progressively widespread among the transplant Centers. Therefore, as virtually all of us have at least one HLA-partially matched family member (parent, sibling or son), who is immediately available to serve as a donor, for patients lacking an HLA identical sibling all more close family members should be HLA typed and their clinical records acquired. This policy integrated in the plan for VUD and CBU search provides a complete overview on the potential availabilities of an alternative donor and highly contributes to drive the donor search process. Degree of HLA mismatching, NK alloreactivity, familiar relantionship with preference for the mother and CMV donor status should in this order to be followed for selecting the best mismatched related donor.

Conclusions: a paradigm of search for an alternative donor

In order to identify a suitable donor for allogeneic transplant in adequate timing, the search policy of Rome Transplant Network (RTN) considers all the potential sources of hematopoietic stem cells (HLA identical sibling, VUD, CB and haploidentical relative).In the figures, the algorithm of the search for an alternative donor and our transplant polcy are reported: The main selection criteria for VUD consisted of high resolution ≥8/8 HLA loci matching for both class I and II, while the selection of a single CB unit was based on cell dose (TNC ≥3x10^7/kg and CD34+ cells >1x10^5/kg) and ≥ 4/6 HLA antigen compatibility. From April 2006, the option of an haplodentical donor was simultaneously considered. The time to transplant from the start of the donor search was fixed at three months, in particular for patients affected by acute leukemia. Following this policy of donor search, the intention to treat (ITT) analysis showed that an allogeneic transplant can be offered to a large majority of patients: a suitable donor was identified for 618 of 680 (91%) patients eligible for transplant, which could be definitively applied in 83% of them (232 HLA identical sibling; 149 VUD; 64 CB; 118 HRD; 4 scheduled). In summary, following the policy of widespread donor search, all patients with hematological malignancy for whom an allogeneic transplant is included in their therapeutic program can proceed towards a well-timed ASCT. In designing future prospective trials, the concept of "donor versus no donor" should presently be changed according to the concept of "transplant versus no transplant".

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EVALUATION OF THE RISK RELATED TO ALLOGENEIC STEM CELL TRANSPLANTATION: IS EVERYTHING CLEAR OR JUST THE BEGINNING OF A PATH?

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Allogeneic hematopoietic stem cell transplantation (HSCT) is an established therapy for a variety of hematological disorders but, although in the last years changes in practice have improved the outcomes, it still carries a significant risk for treatment-related mortality (TRM) that compromises its curative potential and may even determine a reduced survival in some patients. In recent years novel approaches such as the reduced intensity conditioning regimens have expanded the use of HSCT also to elderly patients and to patients otherwise ineligible for conventional transplants. Moreover the increased availability of a donor (sibling donor, unrelated donor, cord blood, haploidentical donor) has contributed to enhance the number of patients who could approach the transplant. The increase in the number of candidates to the transplant and the variety of the possible approaches combined with the growing trend to transplant patients earlier in their disease course, makes even more necessary a careful assessment of risks and benefits before transplantation. To this aim it is important to assess the most significant parameters that could predict TRM. Last but not least this issue remains essential for patient counseling. The risk of transplant depends both on transplant procedure (type of donor, source of stem cells, conditioning regimen, GVHD prophylaxis, etc.) and on patient's characteristics (age, performance status, organ functions, comorbidities, psycho-social status, etc.). Whereas for the former there are relatively well-established data, for the latter new studies have begun to be developed only recently. Standard factors such as age, performance status, lung and heart functions, if considered alone, are not sufficient to predict TRM, and the studies aimed to test their prognostic usefulness have reported conflicting results. Therefore in recent years multiparameter scoring systems have been proposed in order to gain more information that could be useful to predict the transplant risk. Among these scoring systems the PAM, the HCT-CI and the EBMT risk score are the most used. Each of them has different characteristics and applicability. All these systems are derived from retrospective analysis; a recent GITMO study has prospectively validated the HCT-CI. Although these scoring systems are useful, they are still imperfect tools. In the future they should be improved by incorporating biochemical and biological markers (and perhaps also the clinical and therapeutical history of the patient), to achieve the aim of a more informative, accurate and tailored estimate of the transplant risk. This individualized transplant risk should then be compared with the disease risk in order to allow us to tailor the patient's risk/benefit ratio and consequently evaluate whether it will be or not advantageous to proceed to the transplant, and, if yes, which kind of transplant is better to use. The multiparameter evaluation of the transplant risk should also lead to the identification of possible correlations between a specific risk parameter and a specific post-transplant complication to ideally allow us to apply a specific preventive strategy. We are just at the beginning of the path.

CLINICAL AND INSTRUMENTAL DIAGNOSIS OF PLASMA CELL PROLIFERATIVE DISORDERS

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The plasma cell (PC) proliferative disorders are characterized by the proliferation of a single clone of PC in the bone marrow and by the production of monoclonal immune globulins (Ig). These disorders may range from a phenotypically benign entity, monoclonal gammapathy of undertermined significance (MGUS), to symptomatic multiple myeloma (MM) with bone destruction, renal insufficiency and suppression of bone marrow function. Criteria for the diagnosis of PC proliferative disorders are shown in Table 1. Initial investigation of a patient with suspected PC proliferative disorder should include the tests shown in Table 2.

Table 1. Diagnostic criteria of plasma cell disorders (adapted from Bianchi G et al, Hemat Onc Clin North Am 2012; 26: 383-393).

	M spike		BM	CRAB §	Comments
MGUS	< 3g/dL	AND	< 10%	absent	Diagnosis require exclusion of other lymphoproliferative diseases
SMM	> 3g/dL	OR	≥ 10%	absent	
Solitary Plasmacytoma	generally absent *	AND	Absent	absent	Defined by a single site of abnormal PC proliferation in the bone (osseus) or soft tissue (extraosseus)
MM	any concentration	AND	Any % or presence of plasmacytoma	present	Truly non secretory MM has no M spike on SPEP, UPEP or FLC
PC leukemia	absent/present	AND	Any %	absent/present	Defined by the presence of peripheral blood circulating PC > 2×10^9/L or 20% of leukocytes

* a small M spike can be occasionally seen

§ CRAB criteria are the following: hypercalcemia defined by serum calcium higher than 11.5 mg/dL, renal insufficiency defined as serum creatinine exceeding 2 mg/dL or estimated glomerular filtration less than 40 ml/min, anemia defined as haemoglobin less than 10 g/dL or less than 2 g/dL the normal reference value, bone lesions include lytic lesions, pathologic fractures or severely osteopenic bone disease.

Abbreviations: M, monoclonal; BM bone marrow, CRAB, hypercalcemia, renal failure, anemia, bone lesions; MGUS, monoclonal gammapathy of undertermined significance; SMM smoldering multiple myeloma; MM, multiple myeloma; PC, plasma cell; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis; FLC, free light chain.

Table 2. Laboratory test for monoclonal gammapathies.

Test	aim
History and physical examination	Screening of monoclonal gammapathy
Complete blood count and differential, peripheral blood smear	Screening of monoclonal gammapathy
Chemistry screen, including calcium and creatinine	Screening of monoclonal gammapathy
Serum electrophoresis, immunofixation	Screening of monoclonal gammapathy
Nephelometric quantification of serum immunoglobulin	Screening of monoclonal gammapathy
Routine urinalysis, 24-hour urine collection for electrophoresis and immunofixation	Screening of monoclonal gammapathy
Bone marrow aspirate and/or biopsy	Diagnosis of MM
Marrow flow cytometry	Prognosis of MGUS and SMM, evaluation of MRD in treated MM
FISH cytogenetics	Prognosis of MM
Radiologic skeletal survey	Diagnosis of MM
Serum beta 2-microglobulin	Prognosis of MM
Lactate dehydrogenase	Prognosis of MM
Measurement of serum free light chain	Diagnosis of non secretory MM, light chain MM and solitary plasmacytoma, evaluation of CR in treated MM, prognosis of MGUS and SMM
Spine and pelvis MRI	Diagnosis of solitary plasmacytoma, in certain circumstances in active MM
Total body PET-CT	Diagnosis of solitary plasmacytoma, in certain circumstances in active MM

Abbreviations: MM, multiple myeloma; MGUS, monoclonal gammapathy of undertermined significance; SMM, smoldering multiple myeloma; MRD, minimal residual disease; FISH, fluorescent in situ hybridization; MRI, magnetic resonance imaging; CR complete remission; CT-PET-CT, positron emission tomography integrated with computed tomography.

Agarose gel electrophoresis or capillary zone electrophoresis of serum and urine is preferred to screen for the presence of monoclonal gammapathy (MG). Serum immunofixation is the the "gold standard" method to confirm the presence of a MG and to distinguish its heavy and light chain. Moreover, serum MG should be quantified by nephelometry, while urine MG should be measured by electrophoresis of an adequately concentrated 24-hour urine specimen. Complete blood count and differential and chemistry including calcium and creatinine are useful to recognize organ damage. In patients with an established new diagnosis of MM the quantification of serum albumin, beta 2-microglobulin and lactate dehydrogenase are important to establish the prognosis of the disease. Serum beta 2-microglobulin, which reflects tumor burden, and serum albumin, with probably reflects effects on the liver by interleukin-

6 produced by the microenvironment of myeloma cells, form the basis for the three-stage International Staging System. Serum lactate dehydrogenase has an independent prognostic significance in several studies. Free light chain (FLC) assay is recommended in patients with nonsecretory myeloma (with negative serum and urine immunofixation), in those with oligosecretory myeloma (with secretion of small amount of MG in serum or urine) and in light chain myeloma.[1] Serum FLC can not substituted the 24-hour urine collection. Moreover, serum FLC is indicated in patients with solitary plasmacitoma to exclude multiple localizations and in patients with smoldering MM and MGUS, since an abnormal k/lambda ratio is associated with higher risk of progression to active disease. A patient with suspected MM should undergone a unilateral bone marrow aspirate and/or biopsy and the diagnosis is confirmed when more than 10% clonal PCs are detected. A biopsy should be performed at diagnosis to provide a reliable assessment of PC infiltration. Immunophenotyping by multiparametric flow cytometry is performed by some centers, but it is not standardized for clinical general use. PC can be identified and enumerated by the expression of CD38, CD138 and CD45 antigenes. The clonality of marrow PC can be assessed using a panel of antibodies directed at least towards CD19 and CD56 and possibly extended also towards CD20, CD117, CD27 and CD28.[2] Quantification of phenotypically abnormal PC is useful 1) for diagnosis , to confirm results of histologic and morphologic bone marrow examination 2) for prognosis of MGUS and asymptomatic myeloma, since relative proportion of abnormal and normal PCs correlates with risk of progression to active disease 3) for quantitative evaluation of minimal residual disease levels in patients with symptomatic MM treated with chemotherapy. Standard methaphase cytogenetics has a very low sensibility and it is informative in less than 20% of the patients. Patients with an hyperdiploid karyotype have a good prognosis, while hypodiploid karyotype and chromosome 13 deletion are associated with an adverse outcome. Patients with suspected MM should routinely undergo fluorescent in situ hybridization (FISH), after sorting of PC probes that include chromosome 17p13, t(4;14) and t(14;16) (1). Evidence of prognostic negative impact of other chromosomal alterations, such as gain in chromosome 1 q and deletion of chromosome 1 p, has emerged in recent studies. Identification of high-risk patients by gene expression profile is still investigational. The skeletal survey including skull, spine, humeri, femora, pelvis and chest remains the standard method for imaging screening at diagnosis due to the low cost, the ability to assess large areas of the skeleton and to identify lesions at risk of fracture. However, this technique is rather insensitive for the detection of osteolytic lesions, because it requires at least 30% cortical bone destruction. Magnetic resonance imaging (MRI) and F-18-fluorodeoxyglucose positron emission tomography integrated with computed tomography (PET/CT) are indicated in certain circumstances.[3,4] An MRI of the spine and the pelvis can precociously identify bone lesions; therefore, it is recommended in the diagnostic work-up of a presumed solitary plasmacytoma and should be considered in patient with smoldering myeloma. Moreover, MRI provides information about bone marrow involvement and its pattern (focal, diffused or mixed) and about soft tissue disease. MRI is recommended in patients with symptomatic MM in case of suspicion of cord compression by vertebral plasmacytoma or in case of new collapsed vertebra to differentiate osteoporosis and myelomatous involvement or in patients with painful areas in spine and pelvis and negative plain radiographs. Moreover, pattern of bone marrow involvement and its modification after high-dose therapy may have prognostic significance. The role of PET-CT is still under investigation. Some studies have demonstrated that PET-CT at the onset of MM can detect active sites of the disease, both medullary and extramedullary, with a higher accuracy in comparison with skeletal survey. In particular, it is helpful for the detection of extraosseus soft tissue masses, which can represent true extramedullary disease, not contiguous to bone, or breakout (paramedullary) lesions, arising from bone lesions and growing in the surrounding soft tissues.[4] Moreover, the entity of PET-CT involvement at diagnosis, as reflected by the number of lesions, the intensity of tumour metabolism represented by standard uptake value (SUV) and the presence at baseline of extramedullary disease, emerged in some studies as strong predictor of unfavourable clinical outcome in patients treated with high-dose therapy.[5] However, standardization of criteria for PET-CT imaging definition is warranted before this technique can widely used in clinic as a diagnostic and prognostic tool. Specific tests may be required during the initial assessment of a patient with a MG. In fact, in some cases, end-organ damage is not related to CRAB criteria and it is independent on size and proliferation of PC clone, but it is caused by the structural characteristics of the MG. These disorders, caused by "dangerous small B-cell clones" include cryoglobulinemia, hyperviscosity syndrome, POEMS (polineuropathy, organomegaly, endocrinopathy, monoclonal gammapathy and skin changes), light-chain deposition disease and AL amyloidosis.[6] AL amyloidosis is the most important of these disorders and should be suspected in presence of nonselective proteinuria, symptoms and sign of peripheral or autonomic neuropathy, unexplained hepatomegaly or restrictive cardiopathy. If staining of subcutaneous fat aspirate and bone marrow are negative, biopsy of a suspected organ may be necessary. However, the demonstration of amyloid deposit in a patient with MG is not conclusive evidence of AL amyloidosis, since demonstration of the light-chain origin of amyloid deposit in tissues should be achieved by proteomic characterization of the amyloid deposits or by immunoelectron microscopy. Focus on specific PC proliferative disorders.

Monoclonal gammapathy of undertermined significance (MGUS)

If the MG is below 3 g/dL on electrophoresis and is not associated with symptoms and signs of organ damage such as hypercalcemia, renal failure, anemia and bone lesions (CRAB criteria) or other abnormal laboratory findings (reduction of serum concentration of uninvolved Ig or urine MG), it is likely to be diagnosed as MGUS. Bone survey and bone marrow aspirate or biopsy are not mandatory part of the work–up of patients with MGUS. The risk for progression to MM or related malignancy (AL amyloidosis, Waldestrom's macroglobulinemia) in MGUS patients is 1% per-year, but it is yet unremitting and lifelong. MGUS patients can be stratified in groups at different risk of progression on the basis of 2 risk model. A risk model proposed by the Mayo Clinic is based on the presence of non-IgG subtypes, MG equal or higher than 1,5 g/dL and an abnormal serum FLC ratio (k to lambda ratio lower than 0.26 or higher than 1.65). Patients with 3, 2, 1 or no risk factor have a likelihood of MM progression of 58%, 37%, 21% and 5% over a period of 20 years, respectively.[7] The Spanish group has proposed a second risk progression model based on the preponderance of aberrant monoclonal PC in the BM, evaluated by multiparametric flow cytometry. A percentage of aberrant PC equal or exceeding 95% of the total bone marrow PC population and the presence of DNA aneuploidy were established as risk factors for progression to symptomatic MM, ranging between 2% to 46% at 5 years.[8] Follow-up is recommended in MGUS patients with the aim of promptly identifying transformation to MM and avoiding complications. Risk progression models are helpful for defining the interval of clinical and laboratory examinations and the need to complete the work-up with bone survey and bone marrow biopsy. Complete work-up and laboratory exams should be performed yearly in patients with high risk features, while patients with low risk MGUS and stable clinical conditions may repeat exams every 2-3 years.

Smoldering multiple myeloma (SMM)

SMM is diagnosed by the presence of an M spike of 3 g/dL of higher and/or bone marrow invasion by malignant PC of 10% or more, in absence of CRAB criteria. Bone involvement has to be excluded with routine skeletal survey. However, spine and pelvis MRI and total body PET-CT should be considered in the initial work-up of SMM patients, due to their ability to detect bone lesions more precociously than standard X-Ray and consequently change the clinical approach from observation to anti myeloma therapy. Patients with SMM have a risk of progression to active MM or related PC dyscrasia of 10%/year in the first 5 years, 3%/year in the following 5 years and 1 %/year thereafter, with a cumulative probability of progression over 70% at 15 years. Researchers at Mayo Clinic have identified bone marrow involvement by 10% or more MM cells, M spike equal or greater than 3g/dL and an abnormal FLC ratio (equal or less than 0.125, or equal or exceeding 8) as risk factors for progression to active disease.[9]

Solitary plasmacytoma

Solitary plasmacytoma is a variant within PC dyscrasia and occurs in around 3-5% of the cases. It is characterized by a single area of monoclonal PC proliferation either within the bone (osseous plasmacytoma) or in the soft tissue (extraosseous plasmacytoma), typically of the upper respiratory or gastrointestinal tract, in the absence of systemic disease and BM involvement. These patients can occasionally present a small MG. Since solitary plasmacyoma can be eradicated with local radioteraphy without need of systemic chemotherapy, it is mandatory to differ-

entiate true solitary plasmacytoma from MM and exclude multiple lesions. Besides skeletal survey, it is recommended to exclude multiple bone lesions with spine and pelvis MRI and total body CT-PET.

Multiple myeloma

Diagnosis of multiple myeloma is established in presence of CRAB criteria and a MG identified by serum and/or urine electrophoresis or by immunofixation or by FLC assay associated with bone marrow infiltration by monoclonal PC and/or presence of plasmacytoma. Serum albumin and beta2 microglobulin levels allow to stratify patients in 3 stages with median overall survival ranging between 29 and 62 months. FISH karyotype can identify patients resistant to some anti myeloma drugs and with unfavorable outcome. Bones can be routinely studied with skeletal survey, but spine and pelvis MRI and total body CT-PET have been recently applied, with the advantage of recognizing soft tissue localizations and bone marrow involvement. Moreover, MRI is useful for studying spine involvement and recognizing vertebral plasmacytomas, which can cause cord compression or fracture.

Plasma cell leukemia

Plasma cell leukemia is defined by the presence of peripheral blood circulating PC exceeding $2x10^9$/L or 20% of leukocytes and can be either primary (occurring de novo) or the leukemic transformation of preexisting MM (secondary). Around 60% of PC leukemia cases are primary and are often associated with sign and symptoms of high PC burden, such as cytopenia, renal failure and high LDH serum level.

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THE CONTRIBUTION OF THE LABORATORY IN THE STUDY OF PLASMA CELL MORPHOLOGY BETWEEN REACTIVITY AND PROLIFERATION

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Introduction

Plasma cells (PCs) are long-lived and non-proliferating cells, migrated into bone marrow and generated through regulation of transcription factors that control the differentiation of memory B cells into plasmablasts, which unlike PCs, are short-lived proliferating cells. The differentiation of B cells occurs in the secondary lymphoid organs, in close contact with dendritic cells as part of the humoral immune response to infections and consequently to antigen activation.[1] Since they are differentiated tissue cells, circulating plasma cells are not detected in normal peripheral blood, whereas plasmablasts represent less than 0.1% of peripheral blood mononuclear cells. Plasma cell myeloma and plasma cell neoplasms are bone marrow based, multifocal neoplasms, and PCs are found in a small number on peripheral blood smears and only in approximately 15% of cases. Blood plasmacytosis (BP) is most commonly seen in the advanced stages of multiple myeloma or plasma cell leukemia. BP is also reported in rare cutaneous and systemic reactive lymphoplasmacytic disorders of unknown origin. Non-malignant, reactive blood plasmacytosis (RP) is a rare condition, associated with a variety of diseases, such as tumors, autoimmune disorders and infectious diseases which include: sepsis, dengue, acute respiratory infections, parvovirus B19 infection, rubella, hepatitis A, and primary infection or reactivation of the Epstein–Barr virus.[2] In contrast to myeloma, RP is characterized by a transient increased amount of polyclonal and highly proliferative PCs in the circulation, due to the differentiation of plasmablasts or PCs precursors under the control of growth factors particularly interleukin 63. RP is known to be most pronounced during the first weeks of the disease, disappearing completely when the underlying disease is controlled. However, some cases of RP with atypical clinical features can pose significant diagnostic challenges.

Automated haematological analyzers

Currently, many automated blood cell counters provide a differential count (LDC) of leukocytes including leukocytes, red cells and platelets cell blood counts (CBC), aiding laboratories in facing up to the increased request for hematologic tests in the shortest possible turnaround time. The latest technological improvements to automated LDC make cell counting more precise and accurate than ever before. New technologies for cell identification, together with refined analysis of the signals using sophisticated software, have lead to the discovery of quantitative abnormalities. Indeed, the up-to-date technologies allow a quantification of the various leukocyte populations, as well as the so-called extended differential count (EDC) that consists of the quantification of cells usually not present in peripheral blood, such as nucleated red blood cells. Furthermore, some analyzers quantify immature granulocytes, hemopoietic progenitor cells and in some cases, atypical lymphocytes.[4] Analyzers are able to provide specific scatterplots or cytograms, derived from multi-parametric analysis of cellular properties suitable to identify normal or abnormal leukocyte populations and qualitative information about pathological cells, using specific flagging functions. In particular, automated Sysmex analyzers (XE 2100, XE 5000 and XN 100) quantify a specific area, known as the HFL-count region that identifies high fluorescence antibody-synthesizing or secreting cells (ASC) *i.e.* plasma cells, activated B-lymphocytes and lymphoplasmocitoid cells. By their fluorescent RNA-stain, ASC are clustered into abnormal cell populations above the monocyte populations5thus making it possible to flag atypical lymphocytes. On the basis of cell properties, abnormal blood cells are clearly observable on a leukocyte cytogram. Generally, the specifically generated flag suggests a blood smear that should be performed, whereas the evaluation of a cytogram can provide useful information for the examination of the blood smear when looking for specific pathological cells.

Through the microscope

Blood leukocyte morphology and evaluation of differential counts are basic for hematological disease as well as significant in a wide range of clinical conditions.[6] Usually, mature plasma cells seen in bone marrow are easily recognizable. They are somewhat similar in size, but usually the average diameter is of about 15 micrometers, ranging between 9 and 20 micrometers. The nucleus is typicaly oval shaped, eccentric and practically polar. The cytoplasm varies in size and is deeply basophilic with a characteristic centrosome. This centrosome is a distinct perinuclear zone, adjacent to the nucleus that appears as clear in the basophilic cytoplasm and contains the enlarged Golgi zone that shapes the nuclear polarity. Plasma cell nuclear chromatin, appears as dark blocks on a reddish-purple background and is clumped in coarse chromocenters, with polygonal contours. Chromocenters are in the periphery of the nuclear membrane so that nuclear heterochromatin is radially distributed and the classical morphological description of the plasma cell nucleus is that of a "cart-wheel nucleus"that looks somewhat like the spokes of a wheel. Usually, no nucleoli are visible. The cytoplasm has a very developed rough endoplasmic reticulum (RER) with a large number of ribosomes, and stains deep blue due to its intense affinity for cationic dyes. Typical mature plasma cells are often called Marschalko-type. Normal plasma cells can sometimes show binuclearity.[7] Neoplastic or reactive plasma cells may resemble mature plasma cells. The characteristic abnormal features are an immature chromatin pattern, nucleoli and minimal cell differentiation from minimal to severe grades. Different cytoplasmic or nuclear inclusions have been described and do not usually show

pathognomonic features of malignancy. In May-Grunwald-Giemsa stained smears, Russel bodies are empty spherules, due to accumulations of mucopolysaccharides and immunoglobulins in RER. When many Russel bodies fill the plasma cells, they assume a particular type of globular form and are called Mott cells.[8] Dutcher bodies can be present in the cytoplasm as well as in the nucleus. They are cytoplasmic inclusions that can appear to be intranuclear because they are invaginated into or overly the nucleus.[9] The cytoplasmic inclusions are aspects of the same phenomenon with no essential differences between Dutcher bodies, single or multiple Russell bodies, and the inclusions of Mott cells. Plasma cell myeloma shows variation from mature to immature plasma cells. The mature cell types are Marschalko-like, whereas immaturity determines loose reticular chromatin, prominent visible nucleoli and a higher nuclear-cytoplasmic ratio, in comparison to mature type cells. When plasma cells are pleomorphic and multinucleated, they are indicated as "anaplastic" plasma cells. Nuclear immaturity and pleomorphism are considered reliable morphological features of malignancy.

Phenotypic markers

At present, flow cytometric immunophenotyping has a wide consensus because of its ability in distinguishing between normal, reactive, and malignant PCs, providing prognostic information in the progression risk in monoclonal gammopathy of undetermined significance (MGUS) and asymptomatic multiple myeloma (MM), in detecting minimal residual disease (MRD), and in identifying new therapeutic targets.[10] Plasma cell myelomas typically have monotypic cytoplasmic Ig, whereas they lack those of surface. Bone marrow percentage of clonal PCs distinguishes monoclonal gammopathy of undetermined significance (MGUS) from asymptomatic smoldering myeloma or symptomatic PCs myeloma. The most common approach is based on CD38, CD138 and/or CD45 expressions. The CD 138 marker, an integral heparan sulfate proteoglycan transmembrane protein (Syndecan–1), mediates cell adhesion and growth factors. CD138 is a marker of plasmacytic differentiation and is present in normal, malignant or reactive plasma cells. CD38 is a multifunctional ecto-enzyme involved in signal transduction, cell adhesion and calcium signaling. CD38 is predominantly expressed by bone marrow precursor cells and terminally differentiated plasma cells. Myeloma PCs show moderate to high expression levels of CD38. A gating strategy using combined CD38, CD138, CD45 and light scatter characteristics provides an efficacious detection of plasma cell populations. The first gate is set using an expression of CD38 versus CD138, the second gate uses CD38 brightly positive versus CD45 positive and negative cells and the last gate uses light scatter characteristics (high FSC and low SSC). These intersected gates make it possible to obtain a PCs population in multidimensional space. The combined use of CD38, CD138, and CD45 in a single tube is ideal for gating PCs. Demonstration of PCs clonality and aberration on the immunophenotype represent a crucial aid in the identification of malignant cells, and their distinction from reactive plasma cells. The most common aberrant phenotypes are the expression of CD56, a membrane glycoprotein belonging to the immunoglobulin superfamily, and CD20, an activated-glycosylated phosphoprotein not present on either early pro-B cells, plasmablasts and plasma cells or expression of CD117. Other aberrant phenotype is lack in the expression of CD19 or CD45. To identify malignant PCs the European Myeloma Network has defined as "essential" the evaluation of CD19 and CD56, whereas it recommends CD117, CD20, CD28, and CD27 and only suggests CD81, a cell-surface protein that mediates signal transduction and CD200 a type-1 membrane glycoprotein, commonly expressed on cells originating from the hematopoietic cells implicated as a prognostic factor in MM. The antigens most commonly found to be aberrantly expressed in patients with MM, in decreasing order of frequency, were CD19 (96.3%), CD45 (84.1%), CD56 (82.2%), CD117 (50.5%), CD52 (32.7%), and CD20 (10.3%). Neoplastic plasma cells (CD56+or-/CD19-/CD138+/CD45-) are recognized and separated from reactive plasma cells (CD56-/CD19+/CD138+/CD45+) in patients, including those with multiple myeloma. Using CD138+ versus side scatter the identification of neoplastic plasma cells (CD56+/CD19-/CD138+/CD45-) compared to reactive plasma cells (CD56-/CD19+/CD138+/CD45+) can be improved.[11] However, flow cytometry could be an important diagnostic tool in plasma cell neoplasm if used jointly with the evaluation bone marrow morphology. Methodological problems, such as the choice of the best gating strategies, the use of different monoclonal antibody clones, and different fluorochromes should be standardized. At the present, in evaluating PCs of bone marrow specimens quantitative information can be unreliable, because of pre-analytical problems, such as sample hemodilution with peripheral blood or physical loss of PCs.

Conclusions

In peripheral blood, difficulties can occur in distinguishing PCs neoplasms from reactive plasmacytosis, above without bone marrow examination. The diagnosis must therefore be based on correlation of clinical, biochemical, radiological, cytological and histological features. Laboratory findings such as low eGFR, proteinuria, high calcemia or anemia strongly suggest organ damage. CRAB symptoms, *i.e.* hypercalcemia, renal insufficiency, anemia and bone lesions are criteria for plasma cell neoplasms, with or without the presence of serum or urine monoclonal or light chain protein. The presence of plasmablasts, marked plasma cell dysplasia, immature chromatin, prominent nucleoli are morphological features of PCs neoplasm. Instead, the presence of circulating mature plasma cells should be evaluated in a full context to allow discrimination between reactive or malignant condition. In particular, in the peripheral blood, rouleaux formation or increased background staining on the blood smear point out the presence of a monoclonal protein. Conversely, polyclonal hypergammaglobulinemia is a typical feature of RP, circulating PCs in RP do not have a clonal pattern or aberrant phenotype in flow cytometry analysis.

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NEW APPROACHES IN CHILDHOOD REFRACTORY AND RELAPSED ACUTE MYELOID LEUKAEMIA

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Introduction

Acute Myeloid Leukaemia (AML) in childhood is a rare and heterogeneous disease, with an estimated incidence of about 7-8 cases per million of children aged 0–14. In the last 2 decades several national and international co-operative study groups (such as AIEOP, BFM, COG, DCOG, LAME, UK MRC, NOPHO, St. Jude) have conducted a number of randomized clinical trials aimed to improve overall end results by optimizing the schedule of standard AML cytotoxic drugs and by refining indications for haematopoietic stem cell transplantation (HSCT).[1] Further improvements in terms of long term outcome for children with AML may now mainly derive from biology-driven new drug development programs and their introduction into current therapeutic strategies through appropriately designed clinical trials. The main aims of this article are thus to outline 1. The most recent treatment approaches for both de novo and relapsed AML in children; 2. The availability of new drugs as single and combined agents; 3. The most innovative strategies in designing modern clinical trials. Drug developments regarding Acute Promyelocytic Leukaemia -APL, secondary AML –sAML and Down Syn-

drome AML -DS AML, were not included in this article.

Current front line and relapsed treatment options for childhood AML

Front-line treatment approaches are quite similar worldwide and are traditionally based on an intensive use of cytarabine or other nucleoside analogues and anthracyclines. Etoposide is frequently used in paediatric induction regimens, although there is no definitive evidence of its added benefit in adult studies and when combined with cytarabine and daunorubicin[2]. The differences among protocols mainly consist of the choice and dosing schedules of anthracyclines, the inclusion of particular disease sub-groups (for example, DS AML, sAML and APL), the number of consolidation courses and the indications for HSCT. Results of recently published study group trials for paediatric de novo AML treatment are presented in Table 1. Another important reason of the improved results in AML treatment strategy evolution has been the adoption of better supportive care guidelines which has led to a progressive reduction of mortality rates in induction and complete remission (CR). Five-year event free (EFS) and overall survival (OS) rates for children and adolescents diagnosed with de novo AML under 18 years of age is now around 55-60% and 65-70%, respectively.[3] Despite the improvements achieved, AML in children and adolescents remains an important clinical challenge for two main reasons: 1. The era of progress obtained through treatment intensification is coming to an end and the decrease of the mortality rates has clearly slowed down. In addition the vast majority of children invariably present with severe and life-threatening drug-related side effects. Chemotherapy drugs such as etoposide and anthracyclines bear an increased risk of secondary malignancy and cardio toxicity.[2] Such late effects are of particular concern in childhood because treatment is delivered during periods of growth and development, and the duration of survival is much greater than adults. 2. Although modern induction chemotherapy regimens will induce CR in approximately 90% of AML children, the disease will eventually recur in 30–40% of them; unfortunately refractory and relapsed AMLs are still associated with a poor prognosis, high morbidity and mortality rates. In such cases HSCT is generally regarded as the most effective anti-leukaemic therapy available. Kaspers et al. have recently reported the best outcome obtained so far for paediatric relapsed AML, with a 4 year OS rate of 38%, in the multi-national randomised controlled trial I-BFM-SG Relapsed AML 2001/01.[4] This study was able to demonstrate that high recruitment rates to an international randomized (FLA, *i.e.* Fludarabine and Cytarabine, ± DNX, *i.e.* Liposomal Daunorubicin) trial for relapsed AML are achievable in different countries and did produce a series of important results, *e.g.* allowed the identification of subgroups of patients with a particularly poor outcome such as those relapsing within 12 months of initial diagnosis and those having insufficient response after induction treatment delivery. Childhood AMLs resistant to currently available treatment can be mainly identified as those displaying poor response to chemotherapy thus including primary refractoriness after induction therapy (roughly representing 5-10% of de-novo AMLs), the early relapses (*i.e.* those occurring within 12 months of diagnosis and accounting for approximately 50% of all relapses) and those failing to achieve a second CR; to a lesser extent can also be included those bearing adverse chromosomal abnormalities, gene mutations or aberrantly expressed genes (including FLT3-ITD, WT1 mutations, subgroups of MLL rearranged, 5qdel, inv 3, -7). However, treatment failures occur across all sub-groups, including those with apparently normal cytogenetic profiles. For this reason new drugs for AML should ideally allow a broader application across different sub-groups, thus possibly including those diseases harbouring well-defined 'druggable' targets. Unfortunately there have been no new drugs registered in childhood AML in the last two decades. Notwithstanding, some "old" associations of drugs, *e.g.* asparaginase in combination with methotrexate, traditionally used in ALL, can still be exploited since respectable short and long term responses, with an excellent toxicity profile, have been reported in patients with relapsed or refractory childhood AML.[5] However, to obtain a further improvement of survival rates in children with AML there is an urgent need for the availability of new drugs, especially biology-driven. Since AML is considerably more common in adults than in children, the extrapolation of clinical data from adult AML trials is an important resource for paediatric drug development. The prognosis in children may be considerably different when different age groups are considered, being this partly attributable to the higher frequency of adverse cytogenetic profile of AML with increasing age and with less adverse cytogenetic profiles observed in the younger age group. In addition it is increasingly recognized that the biology of AML does differ between children and adults. For example the 'epigenetic' mutations of DNMT3A, IDH1/2, TET2 and NPM1 are all much less frequent in children than in adults.[2] Several novel classes of therapeutics are being investigated in childhood AML and among these the small molecule kinase inhibitors and immunomodulatory therapies. Table 2 reports the lists of the drugs currently under clinical evaluation in paediatric AML.

Table 1. Results of recently published study group trials for paediatric de novo AML (modified from [1]).

Study	Years	Patients	% Early death	% CR rate	% EFS	% OS
MRC-AML 12	1994 – 2002	504	4	92	54 (10 years)	63 (10 years)
AIEOP LAM 02/01[3]	2002 - 2010	482	3	87	55 (8 years)	68 (8 years)
AML – BFM 2004	2004 -2010	566	3.9	88	64 (5 years)	72 (5 years)
SJCRH AML02	2002 – 2008	230	1	94	63 (3 years)	71 (3 years)
COG AAML03P1	2003 – 2005	350	2.6	83	53 (3 years)	66 (3 years)
NOPHO 2004	2004 – 2009	151	1.3	92	57 (3 years)	69 (3 years)

Table 2. Lists of the drugs currently under clinical evaluation in paediatric AML (modified from [2]).

Class	Drugs	Target
NUCLEOSIDE ANALOGUES	*Clofarabine*	RIBONUCLEOTIDE REDUCTASE
MONOCLONAL ANTIBODIES	*Gemtuzumab Ozogamicin*	ANTI-CD33
FTL3 INHIBITORS	*Lestaurtinib, midostaurin, quizartinib*	*FTL3*-ITD
TYROSINE KINASE INHIBITORS	*Sorafenib*	MULTI KINASE INHIBITOR
FARNESYL TRANSFERASE INHIBITORS	*Tipifarnib*	RAS
PROTEASOME INHIBITORS	*Bortezomib*	PROTEASOME
HISTONE DEACETYLASE INHIBITORS (HDACI)	*Valproic acid, vorinostat and panobinostat*	HISTONE DEACETYLASE
DNA METHYL-TRANSFERASE INHIBITORS (DNMTI)	*5-azacytidine and 5-aza-2'deoxycytidine*	DNA METHYL TRANSFERASE
AURORA KINASE INHIBITORS (AKI)	*AT9283, AZD1152 – barasertib* *MLN8237- alisertib*	MULTI KINASE INHIBITOR SELECTIVE AURORA A INHIBITOR
CXCR4 ANTAGONIST	*Plerixafor*	CXCR4

Clofarabine

Clofarabine is a structural hybrid of cladribine and fludarabine, designed to have improved efficacy, through reduced deamination by adenosine deaminase, and improved stability. Clofarabine is approved to treat childhood ALL refractory to at least 2 regimens of chemotherapy. It is used also in combination with other drugs, especially cytarabine, due to its potent ribonucleotide reductase activity which increases deoxycytidine kinase activity.[6] Several clinical trials have been opened in recent years in adults and children and some of these have shown promising response rates in AML especially when clofarabine is used in combination with other nucleoside analogues and DNA damaging agents such as cytarabine, cyclophosphamide, daunorubicin and etoposide. The best use of clofarabine in both upfront and second-line AML treatment regimens has still to be defined since its ability to add efficacy to known regimens is still to be determined. Efforts to achieve this are on-going; one such effort is an on-going randomized phase II study conducted at St Jude comparing children treated with clofarabine and cytarabine to conventional ADE therapy (cytarabine, daunorubicin, etoposide) in terms of end-of-induction minimal residual disease.[2]

Gemtuzumab ozogamicin (GO)

GO, a humanized monoclonal antibody directed against CD33 and conjugated with the antitumor antibiotic agent calicheamicin, has already been widely evaluated in adults with AML. The vast majority of AML cases express CD33 thus representing an appealing therapeutic target. High CD33 expression in childhood AML may be associated with adverse disease characteristics such as FLT3-ITD and independently predicts poor outcome.[2] The "life" of GO has been quite challeng-

ing in recent years: initially the drug was associated with increased responses and improved outcome, later it was withdrawn from the market by the American FDA. This was due to concerns over the increased induction mortality and lack of therapeutic benefit in adults, with a fearsome liver toxicity observed when higher dosages were given in patients with previous HSCT.[7] More recently additional randomized studies have again focused on the efficacy of this drug given at lower dosages, with certain subgroups of AML with standard or intermediate risk cytogenetic features showing better results. Two different trials have assessed the safety of GO in children in association with conventional chemotherapy, NOPHO-AML 2004 and COG AAML 03 P1.[2] However there is still no clear evidence of its efficacy in the treatment of childhood AML in first CR. The new trial for childhood relapsed/refractory AML run by the International BFM Study Group will evaluate in a randomized fashion the effects of adding GO to the conventional FLA/DNX.

FTL3 ITD inhibitors

Internal tandem duplication (ITD) or tyrosine kinase domain (TKD) mutations of the fms-like tyrosine kinase 3 (FLT3) gene occur frequently in AML, resulting in constitutive FLT3 signalling and stimulation of leukaemic proliferation. FLT3-ITD is detected in de novo childhood AML in about one tenth of the cases. The presence of FLT3-ITD has consistently been associated with increased relapse rates and decreased outcome rates. CEP-701 (lestaurtinib) and PKC412 (midostaurin), were the first FLT3 inhibitors to be studied and put forward in early clinical development. So-called 'second-generation' FLT3 inhibitors such as AC220 (quizartinib), designed with increased potency and selectivity for FLT3, are currently being evaluated in early-phase clinical trials. Preliminary results of recently concluded or still on-going trials with FLT3 inhibitors show encouraging results in single-agent trials; however, it appears that these compounds seem to produce better results when associated with conventional chemotherapy. Sorafenib, a potent multi tyrosine kinases inhibitor that has shown encouraging preliminary safety data and proved efficacy in terms of CR when used in association with cytarabine or clofarabine,[8] is now included in the treatment for patients with FTL3-ITD positive in the St Jude AML 08 and COG AAML 103 protocols USA. In Italy sorafenib will be used during induction in the upcoming national front-line AIEOP AML for patients with FLT3-ITD.

Farnesyl transferase inhibitors (FTI)

Tipifarnib is a FTI developed to target malignancies with activated RAS, including leukaemia. Kaspers et al tested 52 paediatric AML samples for *in vitro* sensitivity to tipifarnib using a total cell-kill assay and compared these results to those obtained with normal bone marrow. AML samples were significantly more sensitive to tipifarnib compared to normal bone marrow samples. Within AML, French-American-British (FAB) M5 samples were the most sensitive to tipifarnib. There was a marked correlation between tipifarnib resistance and daunorubicin or etoposide resistance, but not to cytarabine or 6-thioguanine. RAS mutations were present in 32% of AML but there was no correlation between RAS mutational status and sensitivity to tipifarnib.[9] A randomized study of tipifarnib in the post-allogeneic HSCT setting is being planned within COG.

Proteasome inhibitors

These drugs have been shown to induce apoptosis in several malignant cell types. Pre-clinical studies in primary AML specimens have evaluated the combination of the proteasome inhibitor bortezomib with idarubicin. Results have demonstrated rapid and extensive apoptosis of AML blasts both *in vitro* and *in vivo* whilst normal haematopoietic stem cells (HSCs) were not affected. When used as a single agent in a phase I COG study, bortezomib had, however, low activity. The current COG phase III trial (AAML1031) is randomising patients with de novo AML to receive conventional chemotherapy with or without bortezomib for all courses.

Histone Deacetylase inhibitors (HDACi) and DNA Methyl-Transferase inhibitors (DNMTi)

Deacetylation of histones and DNA methylation have relevant roles in leukaemogenesis through transcriptional silencing of critical genes. Histone Deacetylases (HDACs) are thus potential targets in the treatment of leukaemia, and, as a consequence, HDACi such as valproic acid, vorinostat and panobinostat are being studied for therapeutic purposes. HDACi promote or enhance several different anticancer mechanisms, such as apoptosis, cell cycle arrest, and cellular differentiation. Therefore HDACi are promising treatment options for children and adolescents with acute leukaemia, either as monotherapy or in association with other anticancer drugs. Genes regulating DNA methylation and demethylation such as DNMT3A, IDH1/2 and TET2 are frequently mutated in adult AML and have prognostic significance. Consequently, targeting such epigenetic processes has become an attractive therapeutic strategy in adult AML[2] and paediatric investigation plans are being developed to this purpose. Early phase clinical trials of low dose schedules of the DNMTi 5-azacytidine and 5-aza-2'-deoxycytidine in adult AML have shown their tolerability and clinical activity; paediatric data however are still too limited. Moreover, the randomised controlled trials in adult AML have compared single agent DNMTi against supportive care, often in elderly populations, whereas in children the main goal of this treatment would be to evaluate a DNMTi in combination strategies with the intention to cure.

Aurora Kinase inhibitors (AKi) and disruption of tumour-stroma interactions with CXCR4 antagonists

These two classes of agents represent additional promising pathways to be targeted due to both biological rationale and early clinical data emerging in AML. AKi (AT9283, AZD1152 - barasertib- and MLN8237 - alisertib) are agents capable of blocking critical checkpoints of mitosis and have shown mild toxicity profiles and encouraging activities in AML. Normal haematopoietic precursors, as well as leukaemia cells, interact with the bone marrow microenvironment through a variety of adhesion molecules and their corresponding ligands, such as the chemokine receptor CXCR4 and its ligand, CXCL12. It has been hypothesized that these interactions mediate drug resistance and that mobilization of leukaemic cells from the protective microenvironment may increase chemo sensitivity.[2] Plerixafor, a small molecule inhibitor of CXCR4, disrupts CXCR4-CXCL12 interactions, mobilizes AML cells, and increases sensitivity to conventional chemotherapy. A recent clinical trial demonstrated that plerixafor, given in combination with mitoxantrone, etoposide, and cytarabine, was safe and resulted in acceptable remission rates.[10]

Trial design strategies and future opportunities

Before starting paediatric studies with innovative agents, preliminary toxicity and efficacy profiles have to be established in adults. Although this is important from a patient-safety perspective it also slows down the process of paediatric drug development. In addition, until a decade ago, the pharmaceutical companies were not particularly interested in expanding their research programs with new agents to the paediatric age. So significant issues still exist for conducting early phase clinical trials in children. Accelerating drug development for childhood AML is of paramount importance. Often, patients may progress rapidly between screening and starting of a new agent; in addition heavy pre-treatment schedules often cause the onset of serious complications which could make children ineligible for such trials. The important question on how much can be extrapolated from adult biology and trial data remains difficult to answer, so defining the minimum dataset in children is an ongoing challenge. Although significant challenges remain for novel drug development in paediatric oncology, particularly AML, the encouraging results of some early phase paediatric trials together with the large number of new agents in the pipeline, is encouraging. Groups such as the Innovative Therapies for Children with Cancer (ITCC) European Consortium and the Therapeutic Advances in Childhood Leukaemia and Lymphoma (TACL) consortium have an existing focus on early-phase trials of novel agents for childhood leukaemia and there are on-going attempts to facilitate truly international early-phase studies. The design of clinical trials should define how to collect the critical information required to efficiently determine whether a drug should progress along the paediatric drug development pathway and accept that innovative methodologies should be used to enable the use and evaluation of new drugs in the context of small patient numbers. Important strategies will be: 1) Review carefully pre-clinical and clinical data available from adult trials to identify drugs to be prioritized. 2) Agree on which standard treatment backbones are adequate to promote the introduction of new drugs at an international level. 3) Include the more promising drugs on the agreed backbones, especially in relapsed/refractory diseases but also in a front line setting, either as window treatments or in a randomised fashion. 4) Foster cooperation between international groups to avoid studies duplications. 5) Establish an international reference network including individuals and centers capable to ensure a coordinated

approach aimed to avoid the overlook of new agents potentially useful for a paediatric patient population. A workshop gathering international experts in the field of childhood AML, clinical drug development and trial methodology, as a collaborative initiative between the academic consortia ITCC, I-BFM-SG and ENCCA, has been held to discuss how novel drug development programs can be integrated into future standard treatment protocols for childhood AML. Of course the key question is if and to which extent the regulatory authorities (EMA and FDA) will accept modern trial designs as providers of sufficient evidence in respect of more is conventional development and assessment strategies. Without their acceptance, appropriate drug development for rare diseases such as paediatric AML will continue to be inadequate.

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CHRONIC MYELOID LEUKAEMIA: WHAT ARE THE CURRENT CHALLENGES IN CHILDHOOD?

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Chronic myeloid leukemia (CML) in childhood is rare, representing only 3% of newly diagnosed pediatric leukemias. The diagnostic hallmark of CML is the Philadelphia (Ph) chromosome which results from the reciprocal chromosomal translocation t(9;22)(q34;q11). The annual incidence increases with age: from 0.7 per million in children less than 14 years of age to 1.2 per million in adolescents younger than 20. CML is even rarer in toddlers and infants.[1] In 2013, several questions are still open with regard to childhood CML. Within these: Are the biologic and clinical features of the disease the same in adults and children? What is the treatment of choice for pediatric patients? Which of the tyrosine kinase inhibitors (TKIs) are available for children with CML? What are the effective/optimal doses of imatinib (IM) in children and adolescents? What are the potential problems associated with TKI treatment in the pediatric age? Can IM be safely discontinued in responding children and adolescents with CML? How should disease outcome be monitored in pediatric patients? What is the role of allogeneic stem cell transplant (allo-SCT) in pediatric CML in the TKI era? How should pediatric CML forms resistant or intolerant to TKIs be managed? Hereby, the published data, reported experiences and personal opinions regarding these open questions will be discussed.

Are the biologic and clinical features of the disease the same in adults and children?

Pediatric and adult CML share the same genetic features: the balanced translocation t(9;22)(q34;q11), which leads to the fusion of the ABL1-oncogene located on chromosome 9 to the breakpoint cluster region (BCR) gene on chromosome 22, resulting in a constitutively dysregulated ABL1 tyrosine kinase, with either as 210 kDa or 190 kDa. Depending on the localization of the breakpoint site within the major BCR (M-BCR) region, the majority of CML patients exhibit transcripts with the b3a2 or b2a2 junction, or both. Other rare transcripts, like e1a2, can also occur on a breakpoint outside the M-BCR. A predominance of the b3a2 transcript type occurs in adults, while an equal distribution of the b3a2 and b2a2 transcripts has been described in children and adolescents in a large pediatric series.[2] Similarly to adult CML, the natural course of the disease in pediatric patients progresses from chronic phase (CP) to blast crisis (BC), through the frequently unrecognizable accelerated phase (AP). Approximately 95% of children will present in CP, with the remaining patients presenting in advanced phases, especially BC. In pediatric Ph+ acute leukemias (AL), the t(9;22) breakpoints occur in the known BCRs in the BCR gene on chromosome 22. It is thus difficult to discriminate a CML-BC from a Ph+ AL, though the 210 kDa TK is associated with CML whereas the 190 kDa protein is associated with B-lineage lymphoblastic AL (ALL).[1] Several studies have demonstrated that the BCR-ABL-1 alone is sufficient to induce a CML-CP and that the multistep leukemogenetic process requires many years to be completed.[3] However, some reports of infant CML suggest that transformation events may occur over a short period of time, leading to a disease development similar to that observed in adults.[1] No biologic differences in the activation pathway have been observed between the patients' populations. Ionizing radiations and other environmental agents have not been reported to be involved in childhood CML, although they are considered risk factors for the development of the disease in adults. There seems to be no ethnic or genetic predisposition. Concerning the hematological findings, children with CML-CP tend to present with a higher white blood cell count than in adults, frequently combined with thrombocytosis (60%) and splenomegaly (70%). Recently, a study on a large pediatric series has documented that in children, as in adults, specific BCR /ABL1 transcript types are associated with distinct hematological alterations, such as a high platelet count with the b3a2 transcript.[2] In addition, an Italian cooperative study has shown that a predominance of the b3a2 transcript type is associated with a platelet count significantly higher in girls than in boys (Table 1: Giona *et al*, 2010).

Table 1. . Results of imatinib (IM) treatment in larger studies of pediatric patients with CML.

Authors/References	Number of patients/ Disease phase	Response	Outcome	Comments
Champagne MA et al, *Blood 104:2655-60, 2004*	31 patients 14 CML-CP patients 7 patients with advanced myeloid diseases (1 AML; 6 CML-BP) 10 patients with advanced lymphoid diseases (9 ALL; 1 CML-BP)	CML-CP→ CHR=100%; CCyR = 83% Advanced myeloid diseases→CHR=33% Advanced lymphoid diseases →CHR=70%	13/14 CML-CP patients alive (93%) (1 patient died from TRM) Median servival of patients in advanced phases: 7 months (myeloid) and 15 months (lymphoid)	Phase I trial (COG). Aim: to determine the optimal dose of IM in pediatric CML. Adult doses of 400 mg and 600 mg equated to 260 and 340 mg/m^2/day, respectively, in children. Recommended starting dose for children was 340 mg/m^2/day.
Millot F et al, *Leukemia 20:187-92, 2006*	30 patients 22 CML-CP patients 5 CML-AP patients 3 CML-BC patients	CML-CP→ CHR = 80%; CCyR = 60% Advanced phase CML→CHR = 75%; CCyR =29%	Reduction of *BCR-ABL/ABL* ratio < 10^{-3} 50% in CP-CML children. Estimated 1-year OS: 95% for CML-CP patients and 75% for those in advanced phase.	Phase II study (8 European countries). Aim: to determine the efficacy of IM in pediatric CML in advanced phases, in relapse after SCT, or resistant to IFN.
Giona F et al, *Haematologica 95 (Suppl 2), abs 0826; EHA 2010*	29 CML-CP patients (5 resistant or intolerant to IFN)	CHR = 96%; CCyR = 96%; MMolR (<0.1% BCR-ABL /ABL) = 88%; CMR (<0.01% BCR-ABL/ABL) = 40%	8 MMR patients →IM at a same daily dosage for 3 weeks a month to reduce long-term side effects. 8 patients (7 responders to IM) →SCT. All patients alive at a median of 40 months	Multi-center Italian study. Aim: to evaluate the efficacy of IM (340 mg/ m^2/day) in CML-CP patients, previously untreated or resistant to IFN. 10% of the total cohort stopped IM because of toxicity.
Champagne MA et al, *Pediatric Blood Cancer 57 : 56-62, 2011*	51 CML-CP patients	CHR = 80%; CCyR = 72%; CMR (>3 log reduction) = 27%.	25 patients (13 responders to IM)→SCT (7 died of TRM) At 3 years: progression-free survival 72% ± 6%; OS 92% ± 3.9%.	Multi-center phase II study (COG). Aim: to determine the efficacy of IM (340 mg/m^2/day) in previously untreated CML-CP patients.
Millot F et al, *J Clin Oncol 29:2827-32, 2011*	44 CML-CP patients	CHR = 98%; CCyR = 77%; MMolR = 57%.	Estimated progression-free survival at 36 months =98%	French phase IV Trial. Aim: to evaluate the efficacy of IM (260 mg/m^2/day [max 400 mg/day] to 340 mg/m^2/day) in previously untreated CML-CP patients. 30% of patients discontinued IM, mainly due to unsatisfactory response.

CML-CP= chronic myeloid leukemia (CML) in first chronic phase; CML-AP= CML in accelerated phase (AP); CML-CB= CML in blastic crisis (BC); AML = acute myeloid; ALL= acute lymphoblastic leukemia; CHR= complete hematologic response; CCyR= complete cytogenetic response; TRM= transplanted related toxicity; OS= overall survival; IFN= alpha-interferon; CMR= complete molecular response; MMolR Major molecular response; SCT=stem cell transplantation.

What is the treatment of choice for pediatric CML?

The issue of cure versus long-term control is important when discussing treatment for pediatric CML. Prior to the availability of specific TKIs, allo-SCT was the first-line treatment with proven curative potential for children and adolescents with a suitable HLA-matched donor. However, the major hazard for allo-SCT is transplant related toxicity and mortality (TRM), and leukemic relapse that may also occur in patients who receive an allo-SCT in first CP. Prior of the use of IM, alpha-interferon (IFN), alone or in combination with cytosine-arabinoside, was the treatment of choice for children without an available donor. Results of IFN in small cohorts of children and adolescents were similar to those reported in adults, in terms of response rate (complete

hematologic response [CHR], 66.5% to 94.5%; major cytogenetic response [MCyR], 41.5-65%) and overall survival (OS) (63% at 8 years).[4] Most data on pediatric CML are available from retrospective collaborative studies of allo-SCT (5,6). For children and adolescents in first CP transplanted between 1982 and 2004, the 3- to 5-year event-free survival (EFS) ranged from 61 to 63%, with an OS between 66 and 87% for those transplanted with a matched sibling. For patients undergoing a transplant from a matched unrelated donor (MUD), the outcome was slightly worse, with an EFS ranging between 27 and 55% and an OS between 45 and 65%. Morbidity was significant, with grade >2 acute graft-versus-host disease (GVHD) observed in 28 to 37% and 52 to 80% of related donor and MUD transplants, respectively; chronic GVHD (cGVHD) was also common (38-44%). The TRM reported from the EBMT experience was as high as 20% in transplants from sibling donors and 31% in patients transplanted from a MUD. As for adults, children and adolescents transplanted in an advanced phase of the disease had a significantly worse outcome. In a prospective phase-I trial, aimed at transplanting children in first CML-CP from a matched family donor within 6 months from diagnosis or from a MUD within 12 months of diagnosis, after initial treatment with hydroxyurea + IFN, was stopped because of poor patients' accrual once IM had been fully licensed.[5] In the first phase-I study using IM in pediatric CML, a CHR was obtained in all children with CML-CP and also in some patients with more advanced disease; furthermore, a CCyR was recorded in 83% of evaluable CML-CP (Table1: Champagne *et al*, 2004). Further multi-center collaborative studies have reported results in children and adolescents treated with IM (Table 1: Millot *et al*, 2006 and 2011; Giona *et al*, 2010; Champagne et al, 2011) similar to those achieved in adults. In pediatric patients with CML-CP, IM is able to induce CHR and CCyR rates ranging from 80 to 98% and from 60 to 96%, respectively. In the French phase-IV trial, a major molecular response (MMolR) was achieved in 34% of children at 12 months, with an overall response rate during the subsequent follow-up of 57% (Table 1: Millot *et al*, 2011). Higher MMol rates were recorded in an Italian multi-center study including 29 patients with CML-CP newly diagnosed or previously treated with IFN (7 patients). A MMolR was achieved in 88% of the evaluable patients. Furthermore, 40% of patients, including 2 with a matched sibling donor, obtained a complete molecular response (CMR) (Table 1: Giona *et al*, 2010). In these reports, higher doses of IM resulted in both high response rates and better disease outcome with an estimated 3-year OS of 93-100%. So far, no study has directly compared the outcomes of children with CML in first CP treated with IM versus those submitted to allo-SCT (7). Up to now, few retrospective studies in small cohorts of children have been published. A single-center Japanese study reported a progression rate significantly lower in children treated with IM (n=12) than in those submitted to an allo-SCT (n=16) (0 vs. 28.6%, p = 0.006), underlining the dramatic improvement in survival of pediatric CML patients treated with IM.[7] More recently, an Iranian study, including patients with an age ranging between 1.5 and 29 years, showed no statistically significant differences in disease outcome between patients treated with IM and those receiving an allo-SCT.[7] Whether and how the introduction of IM or other TKIs affect allo-SCT outcomes in children and adolescents with CML remains uncertain. Recently, a retrospective review of 125 Japanese children with CML transplanted from a MUD revealed that patients previously treated with IM (n=17) had a 5-year OS of 82% compared to 50% for the whole group.[7] The use of IM as post-allo-SCT maintenance therapy in an attempt to prevent relapse is an unsettled problem in both children and adults. IM has been successfully employed to treat cGVHD, suggesting that its use may control cGVHD and prevent relapse. Many pediatric centers use IM after transplantation for up to 1 year. For patients experiencing a molecular, cytogenetic or hematologic relapse after an allo-SCT, the choice of treatment is still controversial: both donor lymphocyte infusions and TKIs, namely IM and more recently dasatinib, have demonstrated their potential in inducing a molecular remission.[7]

Which of the TKIs are available for children and adolescents with CML?

IM received approval for the treatment of children with CML in 2003. Generic preparations will soon be available world-wide. The second-generation TKIs, namely dasatinib and nilotinib, are not licensed for patients with BCR-ABL1+ leukemias younger than 18 years. In this patients' population, dasatinib is employed in an ongoing multi-center international phase-II study in relapsed/refractory Ph+ leukemias resistant/ intolerant to IM. Nilotinib is also being tested in an ongoing multi-center international phase-I trial in CP or AP CML resistant/intolerant to IM or in Ph+ ALL refractory to standard treatment.

What are the effective/optimal doses of IM in children and adolescents?

Adult doses of IM of 400 and 600 mg equated in a similar exposure in children when doses of 260 mg/m^2 and 340 mg/m^2, respectively, were used. The most commonly recommended starting dose for pediatric CML-CP is in the range of 340 mg/m^2/day, based on results from large pediatric studies (Table 1). In children younger than 4 years, the therapeutic dose is hard to define. Based on pharmacokinetics of other drugs, infants and toddlers, having an increased metabolism, may require higher doses of IM than older children/adolescents. The recommended pediatric dose for CML-AP and CML-BC are 400 mg/m^2 daily (maximum absolute dose 600 mg) and 500 mg/m^2 (maximum absolute dose 800 mg), respectively. The pediatric experience with second-generation TKIs is still very limited. In the first phase-I study, dasatinib proved effective in the dose range of 50-110 mg/m^2 twice daily. The ongoing phase II trial uses dasatinib at a dose of 60 mg/m2/day for CML-CP and 80 mg/m^2/day for more advanced phase. Information about the dose of nilotinib for children is also limited. In the compassionate use program, nilotinib was employed at a dose of 400 mg orally twice a day for patients >40 kg and at 300 mg twice a day for those <40 kg. In the ongoing phase I trial nilotinib is used at a dose of 230 mg/m^2 twice daily (to a maximum single dose of 400 mg).

What are the potential problems associated with TKI treatment in the pediatric age?

The absorption and metabolism of IM may be affected by other concomitant medications. Some drugs may increase or decrease IM plasma level (a comprehensive list is available at http://medicine.iupui.edu/clinpharm/ddis). Swallowing of TKIs as oral tablets or capsules is not easy for children, especially for the younger ones. Liquid formulations may be prepared and administered freshly to overcome the hurdle (Table 2). Short-term toxicity of IM is common, but the effects are generally mild to moderate and are manageable (Table 2). Grade >2 hematologic toxicity is observed mostly beyond the first months of IM treatment and may require short treatment interruptions. The most frequent extra-hematologic side effects include gastrointestinal tract toxicity, arthralgia/myalgia, oedema, weight gain and skin rashes. Myalgia and bone pain are observed especially in the first month after diagnosis and they tend to fade with longer therapy. Data on toxicity of dasatinib and nilotinib are available in adults, whereas they are lacking in children. Pleural effusions were recorded in 2 children treated with dasatinib and a prolonged QT syndrome was observed in patients treated with nilotinib. Growth delay, dysregulation of bone metabolism and pubertal development are the most common side effects of chronic exposure to IM in children, especially in those who started IM in the pre-pubertal age. The optimal management of children suffering from these side effects is still undefined. Attempts have been made by the Italian collaborative group in patients with persistent MMolR. In these patients, IM was administered at a same daily dose for 3 weeks a month (intermittent IM) (Table 1: Giona *et al*, 2010). Improvement of growth and bone metabolism was observed over the time in all cases. Patients who started intermittent IM in CMR, remained complete responders and later successfully stopped therapy.[8]

Table 2. Side effects of TKI and therapeutic options to overcome problems related to treatment.

TKI	Side effects	Therapeutic option to reduce side effects	Instructions to improve TKI treatment in younger children
Imatinib	Cytopenias Nausea Arthralgia/myalgia Fluid retention Skin rashes Long-term treatment toxicity	→short IM interruption → take IM with meals dissolved in apple juice → analgesic and anti-inflammatory agents →reduction of salt intake, diuretics →topical steroids →IM intermittent	Tablets may be dispersed in water or apple juice using 50 mL for 100-mg tablet or 200 mL for 400-mg tablet. The contents must be mixed until dissolved and used immediately.
Dasatinib	Cytopenias Diarrhea Pleural effusion *Pulmonary hypertension**		Tablets can be dissolved over 20 minutes at room temperature in 30 mL of lemonade, preservative-free apple or orange juices. After eating, rinse the residue of the glass with 15 mL of the juice and administer.
Nilotinib	Skin rashes and/or pruritus *Hematologic toxicity* *Hyperglicemia* *Dyslipidemia,* *Liver toxicity (increases in transaminases and bilirubin level)* *Anemia and thrombocytopenia**	→antihistamines	Capsules may be dispersed in 5 mL of applesauce and immediately ingested on an empty stomach, and abstain from eating for 1 hour afterward.

*Data in italic are based on results in adults.

Can IM be safely discontinued in responding children and adolescents with CML?

If and when IM can be safely stopped in responding patients is a pressing question, particularly for children facing different long-term side effects with potentially lifelong treatment. Results from the French STIM trial and from the Australian TWISTER study suggest that IM may be safely discontinued in selected adult CML patients with sustained undetectable minimal residual disease for at least 2 years. The probability of a stable treatment-free remission was 41% at 12 months in the STIM trial and 47.1% at 24 months in the TWISTER study. Most disease recurrences occurred within 4-6 months from stopping IM and no relapses were recorded beyond 27 months. Furthermore, all patients who had disease recurrence remained sensitive to the reintroduction of IM.[9,10] In children, sporadic data on IM discontinuation are available. Our encouraging data, firstly reported at the 22nd Annual I-BFM Meeting in May 2011 and recently updated at the 18th EHA Congress in June 2013, suggest that IM can be successfully discontinued in children with a deep CMR lasting >7 years. We first stopped IM in a patient with sustained CMR (<0.0032% BCR-ABLIS) lasting 75 months, who developed a pancreatitis associated to a Sijogren's Syndrome diagnosed at the time. Thereafter, we decided to discontinue IM in 2 more patients with a profound CMR (<0.001% BCR-ABLIS) lasting >8 years. A close (monthly) and regular monitoring was carried on in these 3 patients, who are currently in persistent deep CMR at 32, 33 and 50 months after IM discontinuation.[8] In December 2011, the CML Working Group of the I-BFM Consortium started an international collaborative study (STOPIMAPED) to interrupt IM in pediatric CML patients with CMR (<0.01% BCR-ABLIS) lasting >24 months. A close (at least every 4 weeks) molecular monitoring is recommended during and after discontinuation of IM. In case of molecular relapse, patients should resume IM or another TKI. This trial is open to patient accrual.

How should disease outcome be monitored in pediatric patients?

At diagnosis, qualitative molecular assessment is essential to establish the BRC-ABL1 transcript type. Monitoring response to treatment using an effective strategy is fundamental in an effort to offer optimal patient management and outcomes at all ages, including pediatric CML. The European LeukemiaNet (ELN) and the National Comprehensive Cancer Network (NCCN) have produced guidelines and recommendations to assess and monitor response to TKI, which include hematologic, cytogenetic and molecular monitoring (http://www.nccn.org/professionals/physician_gls/f_guidelines.asp# cml). In pediatric patients, evaluation of the HR should be performed at least weekly at the beginning of TKI treatment, and monthly as soon as HR has been achieved. Cytogenetic response should be carried out every 3 months during the first 2 years and assessed by analyzing at least 20 marrow metaphases. Molecular response should be evaluated by quantitative RT-PCR (qRT-PCR), on PB cells. The timing in pediatric patients is not well defined. At our Center, we closely monitor children and adolescents by qRT-PCR both in PB cells monthly (also in those who have stopped IM) and on BM cells when the cytogenetic evaluation is performed. The definition of molecular response and the standardization of the BCR-ABL1 transcript measurement remain an open issue. For this reason, the CML Working group of the ELN has recently proposed revised definitions of MolR, taking into account the sensitivity of molecular test.

What is the role of allo-SCT in pediatric CML in the TKI era?

IM has gradually replaced allo-SCT as first-line treatment. Children and adolescents with CML in first CP are still considered excellent candidates for this procedure, but the optimal timing is still a matter of ongoing debate. In the German trial CML-Paed II, treatment with allo-SCT was the recommended option after a 2-year period during which the leukemia cell burden is reduced with IM. However, none of the first 12 enrolled patients who had achieved at least a MMolR underwent an allo-SCT.[7] Currently, allo-SCT is the treatment of choice for children and adolescents who have progressed beyond first CP. Transplantation is also recommended for children who have failed to achieve a CyR or for those in cytogenetic relapse at 12 months from diagnosis and for those carrying T315I mutation.[7] An allo-SCT (also from an alternative stem cell source) is suggested for chidren and adolescents presenting or developing an AP or a BC. In this latter case, transplantation is recommended once a remission is achieved.[1]

How should pediatric CML forms resistant or intolerant to TKI be managed?

The most difficult clinical challenge is how to treat a child or adolescent who has clearly failed TKIs is lacking an allo-SCT donor. All forms of alternative stem cell source (mismatched cord blood or haploidentical procedures) can be considered in therapy-resistant patients. In addition, non-allo-SCT options can be considered, including IFN and new BCR-ABL1 targeting agents, already available in controlled trials for adult patients, obtained for compassionate use for individual pediatric cases. In conclusion, global registries and protocols for children and adolescents with CML are needed to overcome these issues and to optimally manage this patients population.

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MANAGEMENT OF ACUTE PROMYELOCYTIC LEUKEMIA IN 2013

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Acute Promyelocytic Leukemia (APL) is considered a relatively rare disease with estimated 100 to 150 newly-diagnosed cases per year in Italy. APL must be regarded as an emergency in which immediate diagnosis and initiation of supportive therapy and antileukemic therapy is essential to counteract the risk of early death and for optimal patient outcome. In the case of morphological and clinical suspicion of APL, therapy should be started promptly with retinoic acid (ATRA) even before a genetic diagnosis is available. Early death remains in fact the most relevant hurdle to final cure of this disease and its size is still very high approaching 20-30% of cases even in well developed countries.[1] It is estimated, in addition, that this early death fraction could be much higher in other parts of the world and particularly in developing countries and other areas with low resources. Actions to undertake to impact on this relevant issue should include educational programs at emergency doors and intensive care units with dissemination of basic notions about disease diagnosis and early management. Finally, more investigation on potential predictive factors of hemorrhagic early death is warranted.[1] First-line treatment recommendations will vary depending on the initial risk category as defined by Sanz *et al*.[2] Several large multicenter trials conducted in Europe, USA, Asia and Australia have reported that combined ATRA and anthracycline-based chemotherapy results in long-term remission and potential cure in >75% of patients with newly diagnosed APL3,4. In particular, the Italian group GIMEMA reported high rates of molecular remission in newly diagnosed and genetically confirmed APL

using a simultaneous Atra plus IDArubicin (AIDA) combination for induction treatment, followed by 3 courses of intensive chemotherapy as consolidation. This regimen, with minor modifications, was adopted by other groups including the Spanish PETHEMA cooperative group who reported similar antileukemic efficacy by omitting Ara-C and other non-intercalating agents from the original AIDA, with the advantage of sparing toxicity and increasing compliance to treatment. Two independent risk-adapted studies were subsequently designed by the PETHEMA and GIMEMA in which treatment intensification was based on the initial relapse risk. The results of both studies showed improved outcomes by adding ATRA for consolidation to the original AIDA scheme. In line with these findings, most studies include nowadays risk-adapted approaches in which treatment intensification is based on initial WBC counts.[4] Use of molecularly-driven protocols through detection of the PML/RARA fusion has proved invaluable to improve treatment outcome in APL. In fact, in addition to its diagnostic relevance, detection of the PML/RARA hybrid by sensitive RT-PCR techniques is relevant to assess response to therapy and for the monitoring of minimal residual disease (MRD) during follow-up.[5] As reported by several groups, the achievement of a PCR-negative status is associated with prolonged survival and higher probability of cure, whereas persistence of, or conversion to PCR-positivity in bone marrow after consolidation is invariably associated with subsequent hematological relapse. As a consequence, the achievement of molecular remission is nowadays universally considered as a therapeutic objective in this disease.[6] Despite the dramatic progress achieved in front-line therapy with the ATRA/chemotherapy combination, relapses still occur in approximately 20% of patients. Moreover, these regimens are associated with significant toxicity due to severe myelosuppression frequently resulting in life-threatening infections, and with serious, though infrequent late complications such as cardiomyopathy and the occurrence of secondary myelodysplastic syndromes and/or acute myeloid leukemias.[3,4] Several means are available to decrease toxicity in the treatment of newly diagnosed APL, including the availability of less toxic and highly effective agents such as arsenic trioxide (ATO) and the possibility of stringent MRD monitoring offered by RT-PCR. Following the demonstration of its striking activity in relapsed patients, arsenic trioxide (ATO) has been licensed in the USA and Europe for the treatment of relapsed and refractory APL.[7] Arsenic derivatives had been used since ancient times in Chinese medicine for the treatment of malignant and inflammatory diseases. The mechanism of action of ATO in APL is complex and not yet known in detail. At a high concentration (0.5 to 2.0 μmol/L) ATO induces apoptosis *in vitro*, through induction of caspases 2 and 3, while at lower concentrations (0.1 to 0.5 μmol/L) it induces partial differentiation of leukemic promyelocytes through PML/RARa degradation; furthermore, ATO is known to inhibit angiogenesis via down- regulation of vascular endothelial growth factor (VEGF).[7] As concerning its toxicity profile, ATO is usually well tolerated and its use is associated with a series of manageable adverse events including hyperleucocytosis, the APL differentiation syndrome, prolongation of the QT interval, peripheral neuropathy, mild myelosuppression, hyperglycemia and hypokalemia. Of these, QT prolongation and, particularly, the so called APL differentiation syndrome are the most serious ones as they can evolve into severe and potentially fatal ventricular arrhythmias (torsade de points) or respiratory failure, respectively (reviewed in ref[7]). According to original clinical trials reported in China, ATO was able to induce hematological CR in >85% patients who relapsed after front-line ATRA. These results were subsequently reproduced in the USA first in a pilot, then in an expanded multicenter trial for patients relapsed after ATRA. A CR rate of 86% was reported in the US multicenter study.[7] Significantly, unlike ATRA, ATO as a single agent was able to induce durable molecular remission after two cycles in the majority of patients treated for disease recurrence. Confirmation of the high efficacy of ATO for relapsed APL was provided successively by several trials conducted worldwide which reported CR rates >70% and 1-3 years survival rates in the range of 50-70%. In addition to trials in which ATO was used a single agent, some studies investigated its efficacy and toxicity profile in combination with other agents including ATRA. Synergism with ATRA and increased antileukemic efficacy in APL was demonstrated in a Chinese randomised study comparing ATO+ATRA vs. either ATO or ATRA used as single agents. No significant additional toxicity was reported in this or in other studies which analysed the effect of ATRA and ATO combination.[7] Following the experience in relapsed patients and based on the favourable toxicity profile, several investigators have more recently explored the effect of ATO in newly diagnosed APL patients and reported preliminary findings in front-line therapy. Results of studies from Shanghai, Houston, India and Iran conducted with ATO as single agent or combined to ATRA for newly diagnosed patients reported CR rates of 86-95%, molecular remission rates after two cycles of 76-100% and survival rates of 86-88%, with significantly better responses being obtained in patients with low and intermediate-risk disease as compared to high-risk patients (reviewed in ref.[7]). Although these data needed to be strengthened by studies in larger series and with more prolonged observation, they strongly suggested that at least non-high risk APL patients may be treated without chemotherapy. The possibility to cure APL without chemotherapy was actually tested in a randomised trial of the GIMEMA, AMLSG and SAL Cooperative groups which compared this approach with the current standard ATRA plus chemotherapy front-line therapy in patients with low-intermediate risk APL (Italian-German APL 0406 study). The recently reported results showed that ATO plus ATRA is at least as effective as AIDA-based therapy as first-line treatment in non-high risk APL patients.[8] Of 156 evaluable patients in this study, 77/77 (100% achieved remission in the ATRA-ATO group Vs. 75/79 in the ATRA and chemotherapy arm. After a median follow-up of 34 months, the 2-year EFS rates were 97% and 86% in the ATRA-ATO and ATRA-chemotherapy groups, respectively (P=0.02). The ATRA-ATO combination was associated with inferior hematologic toxicity and infectious complications although a higher frequency yet manageable hepatic toxicity and QT prolongation was seen in this group. Although a longer follow-up will be needed to draw firm conclusions, these data strongly suggest the possibility to eradicate APL without chemotherapy and through targeted agents only. Future challenges in this disease thus remain high-risk patients and particularly the problem of early death.

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HAPLOIDENTICAL TRANSPLANTS

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In the absence of a family matched, several alternative sources of stem cells are now available: unrelated volunteer donors (UD) selected through the international, network of Registries,[1] one or two unrelated cord blood (UCB) units,[2] or an HLA mismatched family donor, also referred to as haplo-mismatched (HAPLO).[3] As to the latter, T cell depletion (TCD) programs,[4] CD34 selection,[3] and more recently B cell depletion together with selective T cell depletion[5] have been used to prevent graft versus host disease (GvHD), with encouraging outcome, comparable to what can be obtained with UCB transplants.[6] Problems with TCD or CD34 selected HAPLO transplants, are associated with the technology and cost involved in graft manipulation, but mostly with slow immune recovery and a high incidence of infectious complications: as a consequence, transplant related mortality (TRM) has remained relatively high.[7] The introduction of unmanipulated HAPLO transplants some years ago, has overcome the issues of technology and costs, with encouraging early results,[8] recently confirmed.[9] Italy is currently the country with the highest rate of HAPLO transplants : as shown by the EBMT survey[10]

with over 35 HAPLOs /10^6 inhabitants, compared to 15/10^6 in Germany and less than 10/10^6 inhabitants in France.[10] In Italy there are currently 5 different platforms for HAPLO transplants: (A) CD34 selected G mobilized peripheral blood (PB) cells with or without add back of regulatory T cells (TREG), (B) negative selection of G mobilized PB cells for CD19 and alfa/beta CD3+ T cells, (C) G mobilized marrow , (D) G mobilized peripheral blood and (E) unmanipulated marrow with post transplant cyclophosphamide (PT-CY). Results from these different options will be presented and discussed.

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RED CELL MEMBRANE DEFECTS: OLDER CONDITIONS AND NEW INSIGHTS

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The red blood cell membrane is one of the major determinants of the ability of erythrocytes to cross repeatedly through the microcirculation. The skeleton that laminates the inner side of the red cell membrane plays an essential role in determining the shape and deformability of the red cell. Defects and deficiencies in the erythrocyte membrane protein structure have been described in several hereditary and acquired anemias, where they give rise to mechanically and /or thermally unstable erythrocytes with shortened life spans. Spectrin, the most abundant skeletal protein, consists of two chains, alpha and beta, intertwined in an antiparallel manner to form dimers. Self-association of spectrin dimers into tetramers is perhaps the best-characterised interaction of membrane proteins, allowing the erythrocyte to acquire its mechanical properties. Disruption of spectrin self-association has been shown to lead to hereditary elliptocytosis (HE) and pyropoikilocytosis (also known as hereditary poikilocytosis, HP) (tab.1). Defects of the COOH-terminal end of the beta-chain, *i.e.*, the "head" of the molecule, may lead to impaired spectrin self-association. Polymorphic variants of the SPTA1 gene could explain clinical severity of some cases. Hereditary spherocytosis (HS) is the most common hemolytic anemia due to a red cell membrane defect. It derives from alterations of these genes (ANK1, EPB3, ELB42, SPTA1 and SPTB). This condition is clinically, biochemically and genetically heterogenous. The osmotically fragile spherocytes are selectively trapped in the spleen and destroyed. An increased red blood cell destruction causes the three main clinical signs of HS: anemia, jaundice and splenomegaly (Tab.1). Regarding HS diagnosis there is a consensus on these points : (i.) Newly diagnosed patients with a family history of HS, typical clinical features and laboratory investigations (spherocytes, raised mean corpuscular haemoglobin concentration [MCHC], increase in reticulocytes) do not require any additional tests . (ii.) If the diagnosis is equivocal, a screening test with high predictive value for HS is helpful. The recommended screening tests are the cryohaemolysis test and EMA binding . (iii.) Gel electrophoresis analysis of erythrocyte membranes is the method of choice for diagnosis of atypical cases (Tab.1).

Table 1. Common Red Cell membrane Defects: biochemical and mole cular approaches.

Diseases	Biochemical Approach	Molecular Approach
Hereditary Spherocytosis	Osmotic fragility evaluations	alpha spectrin
	EMA test	beta-spectrin
	SDS-PAGE	ankyrin
		band 3
		Protein 4.2
Hereditary Elliptocytosis	Spectrin dimer evaluation	alpha spectrin
	SDS-PAGE	beta-spectrin
		protein 4.1
Hereditary Stomatocytosis	Osmotic fragility evaluations	band 3
	SDS-PAGE	Glut-1
	Ektacytometry	Piezo 1
		ABCB6
		RhAG

Molecular study is recommended in HS patients with normal parents. They could have a genuinely recessive pattern of inheritance (i. e., homozygosity or double heterozygosity for an a-spectrin mutation) or an apparently recessive one due to de novo mutational events affecting ankyrin or ß-spectrin genes . To discriminate between these possibilities SDS-PAGE followed by the evaluation of ankyrin or ß-spectrin gene expression, as reported in different paragraphs of this review, can be performed. Finally, a-spectrin alleles of patients with isolated spectrin deficiency and bi-allelic expression of ß-spectrin gene have to be investigated in order to find mutations (i. e., Spectrin a LEPRA) responsible for a recessive pattern of inheritance. Hereditary elliptocytosis (HE) is a relatively common, clinically and genetically heterogeneous, disorder characterized by the presence of a large number (>10%) of elliptical or oval erythrocytes on peripheral blood smears. The clinical severity varies continously from the absence of symptoms to fatal hydrops fetalis. In the most severe forms, spherocytes or bizarre-shaped poikilocytes predominate over elliptocytes in smears. Hereditary poikilocytosis (HP) is an example of the latter situation. Although HP was previously considered a separate entity, emerging biochemical and genetic information clearly indicates that it is related to HE, so that the two disorders will be considered together. (Tab.1). At least 20 mutations have been described in hereditary elliptocytosis in the spectrin □ gene (referred to as □ HE). These all occur round the site which codes for the self associ-

ation site of the spectrin dimer and the more remote mutations from this site have less effect on self association. Conditions associated with low expression of the spectrin□□lpha gene (referred to as □ LELY allele) have also been described. The allele has two mutations at position 1857 (CTA>GTA: Leu>Val; nt 7 of exon 40) and at position –12 of intron 45 (C >T), the second of which probably leads to skipping of exon 46. This allele is very common, being present in 20–30% of Europeans, Africans, Chinese, and Japanese. If □ HE mutations of □ spectrin occur with the low expression allele assembly of □ HE/□□ dimers is generally favoured. Such dimers cannot self associate and haemolysis results. Leaky red cell syndromes (LRC) are genetic disorders of the red cell permeability to monovalent cations (Tab.1). Hereditary stomatocytosis is the first case of LRC described. It was so coined due to the conspicously abnormal shapes of the red cells. The LCR syndromes are clinically and pathophysiologically heterogeneous including overhydrated HS (OHst), dehydrated Hst (DHst, hereditary xerocytosis), cryohydrocytosis and familial pseudohyperkalaemia (FP); and SFE which combines DHS1 with Foetal Edema and/or FP1. All LRC syndromes share a dominant pattern of inheritance (Table 1). Today, LRC syndromes are in the process of splitting into distinct entities, based on phenotypical features: osmotic parameters of the red cell, and cation flux rates. The SFE syndrome and DHS 2 are expressed as haemolytic anaemias. Nevertheless, the SFE syndrome is pleiotropic. Any manifestations may be present or absent independently from the others : the foetal oedema and/or the FP 1 type pseudohyperkalaemia are quite often missing; more rarely, the haematological picture is itself lacking. Familial pseudohyperkalaemia, types 1 and 2, FP 1 and FP 2, are symptomless genetic traits characterised by a major leak of monovalent cations when freshly collected blood is allowed to stand at room temperature. Mediterranean stomatocytosis or Mediterranean macrothrombocytopenia is a poorly understood hematological condition which combines stomatocytic hemolysis with the presence of very large platelets. Its genetic basis has never been understood. Very recently we demonstrated that Mediterranean stomatocytosis/macrothrombocytopenia is caused by an excess of phytosterols in the blood. Phytosterolemia, which does not respond to standard statin treatment, can be diagnosed via the distinctive hematology described here, even when the cholesterol is normal. Phytosterolemia should be considered in the differential diagnosis of all patients with large platelets. The platelet size should be measured in patients with hypercholesterolemia. Autosomal dominant dehydrated hereditary stomatocytosis (DHSt) usually presents as a compensated hemolytic anemia with macrocytosis and abnormally shaped red blood cells. DHSt is part of a pleiotropic syndrome that may also exhibits pseudohyperkalemia and perinatal edema. We identified PIEZO1 as the disease gene for pleiotropic DHSt in a large kindred by exome sequencing analysis within the previously mapped 16q23-q24 interval. In 26 affected individuals among seven multigenerational DHSt families with the pleiotropic syndrome, 11 heterozygous missense mutations cosegregating with the disease were identified in PIEZO1. PIEZO1 is expressed in the plasma membranes of red blood cells and its mRNA and protein levels increase during *in vitro* erythroid differentiation of CD34+ cells. PIEZO1 is also expressed in liver and bone marrow during human and mouse development. We suggest for the first time a correlation between a PIEZO1 mutation and perinatal edema. DHSt patient red cells with R2456H mutation exhibit increased ion channel activity. Functional studies of PIEZO1 mutant R2488Q expressed in Xenopus oocytes demonstrated changes in ion transport consistent with the altered ion content of DHSt patient. Our findings provide a first evidence that R2456H and R2488Q mutations in PIEZO1 alter mechanoreceptor regulation, leading to increased cation transport in erythroid cells. Isolated Familial Pseudohyperkalemia (FP) is a dominant red cell trait characterized by cold-induced slow 'passive leak' of red cell K+ into plasma, first described in a large Scottish family from Edinburgh. Although in freshly obtained blood samples plasma [K+] was normal, it was increased when measured in blood stored at or below room temperature. This trait was unaccompanied by clinical symptoms or signs except for mild abnormalities of red cell shape. FP Lille was later described in a large Flemish kindred with morphologically normal red cells. In this family, red cell K+ efflux measured in the presence of ouabain and bumetanide was normal at 37°C, but greatly increased at 22°C and 9°C. FP Lille mapped to 2q35-q36, whereas FP Edinburgh mapped to 16q23-qter. Subsequently, asymptomatic cases FP Chiswick and FP Falkirk with remarkable increased MCV were reported. 1) Functional gene mapping and sequencing analysis of the candidate genes within the 2q35-q36 critical interval in three multigenerational FP families with 20 affected individuals identified two novel heterozygous missense mutations in the ABCB6 gene that cosegregated with disease phenotype. The two genomic substitutions altered two adjacent nucleotides within codon 375 of ABCB6, a porphyrin transporter that in erythrocyte membranes bears the Langereis blood group antigen system. Structural modeling predicts subtle changes in protein structure associated with either mutation. 2)ABCB6 mRNA and protein levels increased during erythroid differentiation of CD34+ erythroid precursors (at 7 and 14 days of EPO induced differentiation), and of HEL and K562 erythroleukemia cells. However, the ABCB6 R375Q mutation altered neither levels of ABCB6 mRNA or protein, nor protein localization in mature erythrocytes or erythroid precursor cells. These data strongly suggest that missense mutations in residue 375 of the ABCB6 polypeptide either mediate the cold-induced K+ leak of chromosome 2-linked FP, or activate an independent, cold-induced cation permeability pathway of the red cell.

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AREER DEVELOPMENT AND EDUCATION: OPPORTUNITIES FOR

ITALIAN HAEMATOLOGISTS

Green A

European Hematology Association

Aims and Policy.One of EHA's goals is to promote basic, translational, clinical and experimental research in hematology by providing a Career Development Program to junior and advanced scientists.

The EHA fellowship program aims to help outstanding young trainees consolidate their research experience and develop into future leaders in their field. Since 2000 several different fellowships have been established by EHA or jointly with partner societies. In 2010 the EHA-ASH Translational Research Training in Hematology (TRTH) was launched which provides junior researchers with a unique, year-long training, mentoring and networking experience. This global program is focused on helping early-career scientists build successful careers in hematologic translational research.

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The Fellowship program offers the following opportunities:

EHA Clinical Research Fellowships provide financial support to clinicians with a PhD or equivalent qualification who wish to consolidate their research training or who are establishing their own independent research group. Award is €80 K per year for three years.

EHA Non-Clinical Junior Research Fellowships provide financial support to non-medical scientists with 1 – 4 years of postdoctoral experience. Award is €50 K per year for three years.

EHA Non-Clinical Advanced Research Fellowships provide financial support to non-medical scientists with ≥ 4 years of postdoctoral experience. Award is €80 K per year for three years

The EHA-ISTH*) Fellowship supports research specifically related to thrombosis and hemostasis. Award is €50 K per year for two years.

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All applications are reviewed by a panel of experts and selection is based on originality, clarity and feasibility of the research proposal, the CV of the applicant and the institute. So far over 75 hematologists have been awarded and the development of their career is monitored by EHA continuously.

EHA-ASH Translational Research Training in Hematology (TRTH)

The goal of the TRTH program is to offer researchers the tools, mentoring and access to resources for a successful career in translational research in hematology. Twenty early-career scientists are selected each year to participate in this rigorous training program. TRTH faculty is made up of international leaders in hematology and will focus on specific scientific methodology adapted to the needs of the participants as well as more general subjects with relevance for planning and pursuing a translational research project. Faculty will also serve as mentors during the yearlong training program. The program offers a unique opportunity to network and interact with key leaders in hematology in Europe and the USA and to build on global collaborations for the future.

For more information on the eligibility criteria and application to the EHA's Career Development program visit www.ehaweb.org.

*ISTH: International Society of Thrombosis and Hemostasis

**JSH: Japanese Society of Hematology

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