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Bergamo, July 3-6, 2005

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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.

40° Congress of the Italian Society of Hematology, Bergamo, July 3-6, 2005

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40° Congress of the Italian Society of Hematology

Bergamo, July 3-6, 2005

ABSTRACT BOOK

Educational Program

Acute Lymphocytic Leukemia

IMPLICATIONS OF BASIC RESEARCH IN CHRONIC LYMPHOCYTIC LEUKEMIA.

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Chronic lymphocytic leukemia (CLL) is a B-cell lymphoproliferative disorder showing heterogeneous clinicobiological features. It may derive from the transformation of a CD5+ B cell, which has encountered the antigen in a T-cell dependent response or, alternatively, which has probably undergone antigenic stimulation outside the germinal centre. In the former case somatic hypermutation of the variable portion of the immunoglobulin gene are present in the tumor DNA, which reflect a physiologic immune response occurring within the germinal centre ("mutated" CLL), whereas in the latter case the enzymes involved in the activation and control of the mutational process have not been activated by antigenic challenge ("unmutated" CLL). Over the last few years, a wealth of information deriving from the application basic research techniques dramatically changed our views on the pathogenesis of CLL. Salient novel findings include: 1) preferential usage of VDJ families by the neoplastic lymphocyte; 2) ongoing antigenic stimulation in some cases; 3) a significant and measurable cell turnover; 4) telomere shortening and enhanced telomerase activity, especially in the more aggressive cases; 5) elaboration of angiogenic factors which may be more pronounced in those patients with active disease; 6) a complex network of interactions with the microenvironment; 7) variable expression of activation markers such as CD38 and ZAP-70; 8) possible involvement of the TCL1 gene in the molecular pathogenesis of the disease; 9) down-regulation of microRna genes (miR15 and miR16) in patients with 13q14 deletion; 10) identification of specific recurrent chromosome defects (deletions involving 17p13/p53; 11q22/ATM, 6q21; 13q14; trisomy 12q13, translocation 14q32/IgH), playing an important role in the pathogenesis of the disease, where they may identify specific disease entities. New abnormalities observed in CLL and related disorders include the t(1;6)(p36;p21); partial trisomy at 2p24/MYCN and the translocation t(X;11)(q13;q23) which disrupts two novel genes. Some cytogenetic subgroups in CLL showed a preferential response to specific drugs. Cells with trisomy 12 proved to be partially resistant to alkylating agents, the presence of a 17p deletion predicted for refractoriness to purine analogs, which was overcome by the anti CD52 monoclonal antibody; the 11q- chromosome was associated with resistant disease even after autologous bone marrow transplantation. A correlation was found between response to the anti CD20 monoclonal antibody, used as a single agent, and specific cytogenetic lesions. The study of gene expression profiles showed that the lymphocyte transcriptome (including traditional genes and microRna genes) recalls that of a memory B-cell in mutated and unmutated CLL with some remarkable differences, possibly identifying disease subsets associated with specific chromosome defects.

According to these novel findings, the pathogenesis of CLL can be viewed as a dynamic process, initiated by antigenically-driven oligoclonal expansion of lymphocytes subsets bearing a restricted set of VDJ families. Depending on the type of antigen involved, the hypermutation machinery may be or may not be activated. Repeated cell cycling may induce telomere shortening which, in turn, may favour genetic instability with the development of an as yet unknown primary event, followed by the emergence of specific molecular cytogenetic defects. The consequent alteration of gene expression profiles may influence in many ways the lymphocyte turnover rate, its interaction with the microenvironment and drug sensitivity, ultimately leading to the heterogeneous clinicopathological manifestations of the disease

CHRONIC LYMPHOCYTIC LEUKEMIA: PROGNOSTIC STRATIFICATION BY BIOLOGICAL PARAMETERS AND NEW THERAPEUTIC STRATEGIES

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Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in the Western hemisphere representing 30% of all adult leukemias in Europe and North America. The median age at presentation is about 65 years, but 20% of patients present under the age of 55. Although the median survival is around 10 years, CLL patients have an extremely variable clinical course and prognosis. Thus, some patients will have an excellent prognosis and never require treatment, while in other patients the clinical course is more aggressive and the life span very short. The remarkable progress witnessed over the last few years in the field of prognosis and therapy of CLL, in the light of the dramatic improvement of the biologic age of populations and of the increase of median life expectancy, has profoundly changed the conservative management which for many years has been the normal approach to this disease. In fact, after decades of stagnation the therapeutic armamentarium in CLL has broadened dramatically and today the availability of newer and potentially more efficacy drugs, as purine analogs and monoclonal antibodies, and the extension of auto and allografting procedures, allow to achieve higher response rates, including molecular remissions. Furthermore, taking into account the biological improvements recently achieved, on the basis of a number of laboratory parameters it is now possible to effectively stratify prognostically CLL patients at presentation and particularly to identify early patients with a poor likeli-

hood. In patients with adverse prognostic features it needs to be clarified if an early and aggressive treatment may impact on life expectancy. All these issues indicate that in the future we could offer all CLL patients a more targeted treatment algorithm based on the clinical and biological characteristics of the disease.

CHRONIC LYMPHOCYTIC LEUKEMIA THERAPY: AN UPDATE

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Many CLL patients survive for decades withouth requiring treatment, whereas others die from disease-related complications within a few months of diagnosis, despite therapy. Until now, the National Cancer Institute (NCI) criteria for initiation of treatment include presence of non-autoimmune cytopenias (Rai stage III and IV), symptomatic lymphadenopathy or hepatosplenomegaly, disease-related B symptoms (fever, night sweats, weight loss, fatigue), autoimmune haemolytic anemia or thrombocytopenia not controlled with steroids. These guidelines were based on data from several trials that had shown no improvement in overall survival for asymptomatic early-stage CLL patients undergoing early therapeutic intervention with alkylating agents. However patients included in those trials were not stratified according to biological prognostic markers. At present new biological prognostic factors (e.g. cytogenetic abnormalities, IgVH genes mutational status, ZAP 70 expression) should be considered when evaluating patients.

In the past, the initial treatment of patients with symptomatic CLL had often involved therapy with chlorambucil ± prednisone at variable dosage. Chlorambucil seems to slow disease progression, but does not increase survival. The treatment of the patients in advanced-stage with combination chemotherapy regimens such as CAP (cyclophosphamide, doxorubicin, prednisone) or CHOP (CAP+vincristine), produced higher response rates than that with alkilating agents, but not longer survival rates.

The purine analogs such as fludarabine, particularly in combination with other drugs (e.g. cyclophosphamide, mitoxantrone), have shown higher complete response rates, prolongation of progression-free survival, and doubled remission duration, as compared to chlorambucil or the combination of cyclophosphamide, doxorubicin, and prednisone. Nevertheless, no-one of these combination chemotherapy has shown a survival advantage and relapse is predictable in all patients.

The use of both the anti-CD20 chimeric monoclonal antibody rituximab and the humanized anti- CD52 monoclonal antibody alemtuzumab has opened new horizons for the up-front treatment of CLL. The addition of rituximab to fludarabine-based therapies in previously untreated patients with CLL, has greatly increased the likelihood of achieving CR. Alemtuzumab has been successfully used in patients with CLL previously treated with alkylating agents and having fludarabine-refractory disease.

Therapy for CLL can further exacerbate preesisting anemia, especially in ederly patients. Randomized double-blind studies have demonstrated that recombinant erythropoietin may ameliorate anemia and symptoms associated with it in patients with CLL.

Patients with CLL often have baseline neutropenia that can be further accentuated by chemotherapy. The administration of granulocytes colony stimulating factor (G-CSF) can decrease the risk of neutropenia and the frequency of serious pulmonary infections in high-risk patients who are receiving fludarabine based therapy.

The use of both erythropoietin and granulocyte colony stimulating factors has now been considered part of the treatment regimens for patients at high risk for anemia and/or febrile neutropenia.

A great number of patients have been transplanted with autologous stem cell transplantation. (SCT). Currently, autologous SCT is thought to be not curative but seems to prolong remission duration. Some CLL patients appear to stay in a long-term disease-free state after allogeneic SCT. The young CLL patients with adverse prognostic factors (e.g. unfavourable genomic aberrations such as 17p-, 11q–, unmutated IGVH genes, high expression of ZAP 70), are candidates to be enrolled in transplantation clinical trials.

The development of non-ablative transplant has extended the eligibility for allogeneic SCT also to elderly patients.

An increased number of options are now available for patients in all stages of CLL and the most adequate therapeutic strategy should be defined in each patient on the basis of biological prognostic factors. Further therapeutic possibilities include the use of vaccines and cellular therapy.

Hodgkin's Lymphoma

ADVANCED-STAGE HODGKIN'S DISEASE: WHICH IS THE BEST TREATMENT?

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The treatment of patients with Hodgkin's disease (HD) has improved dramatically in recent decades. Today, more than 90% of patients with early favourable stages and more than 80% of those in early unfavourable and advanced stage disease achieve long term remission. The allocation to treatment is based on clinical stage and a number of risk factors. Patients in stages III and IV as well as patients in clinical stage IIB with risk factors are allocated to the advanced-stage risk group.

For patients with advanced-stage disease, nitrogen mustard, vincristine, procarbazine, prednisone (MOPP) or MOPP/ABVD had been the mainstay of treatment. Since ABVD alone gives at least equally good results and is associated with lower toxicity, this regimen is now being regarded standard for most patients with advanced stages. In order to improve the long term outcome associated with ABVD, new regimens such as Stanford V or BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) were developed. BEACOPP given in baseline and escalated dose was compared to eight cycles of COPP/ABVD in the HD9 study of the GHSG. This study demonstrated that escalated BEACOPP is superior with 5-year freedom from treatment failure of 87% compared to 69% after COPP/ABVD. On the other hand, as expected, escalated BEACOPP was associated with grater hematological toxicity including a higher number of red blood cell and platelet transfusions; second malignancies, including acute myeloid leukemia possibly related to etoposide were reported.

The breathtaking progress of clinical research made in the last 30 years in HD with accumulating tumorfree survival rates at 10 years of up to 80% has initiated the question how much overtreatment and how much long term negative effects do we allow to balance such high success rates at the beginning of the carreer of our patients. These strategies (ABVD vs BEACOPP) are realized at the moment in an international global prospective trial under the leadership of the EORTC with paricipation of most of the large cooperative study groups in Europe, Canada and Australia.

ROLE OF POSITRON-EMITTING TOMOGRAPHY

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It is well known that many neoplastic forms demonstrate increased uptake of the glucose analog ^{18}F-FDG. The detection of areas of increased accumulation of FDG is the basis of PET usefulness in patients with haematological tumours. Adult and pediatric lymphomas are diseases for which FDG-PET is utilized with very successful results. In recent years, FDG-PET has proved itself as a valuable tool for clinicians by providing important information with direct impact on patient management. Several indications for FDG-PET have been suggested in patients with malignant lymphoma. A useful role has been established for staging, for evaluating early response to chemotherapy, for assessing end response to therapy, for radiation therapy planning and during follow up.

Apart from staging, the effectiveness of this modality is due to its capability of identifying active disease. After therapy completion, FDG-PET affects patient management by differentiating patients with residual lymphoma (non-responders or partial responders) from those without viable tumours (complete responders). Similarly, in cases of relapse, early identification of disease recurrence may influence the success rate of therapy by allowing for earlier treatment.

WHEN AND WHICH ALLOGRAFTING IN HODGKIN'S LYMPHOMA?

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Allografting (AlloSCT) is an effective treatment for a broad range of hematological malignancies but only few patients with Hodgkin's Lymphoma (HL) were included. The theoretical advantages of allografting in HL are the avoidance of tumor cell infusion and a possible graft-versus-HL both of which might reduce the probability of relapse after AlloSCT. Analyses of patients who underwent myeloablative AlloSCT revealed a lower relapse rate and a higher TRM compared to autografted patients. Since there is no evidence that the reinfusion of autologous tumor cells in HL leads to the higher relapse rate among autologous recipients, graft versus HL following AlloSCT is the most logical explanation for the lower relapse rate in allografted patients. The recent goal of ongoing research was to improve the efficacy, whereas reducing the morbidity of allografting procedures. The use of less toxic, non-myeloablative preparative regimens (RICT) have allowed the engraftment and the generation of graft versus HL effects; moreover, this kind of transplant can be extended to older patients and those with comorbid conditions. After about 6 years from the RICT introduction, the data seem promising and during the presentation will be discussed the most recent results with particular emphasis on when and which allografting HL patients should receive.

Hemostasis and Thrombosis

HEMOSTATIC DRUGS

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When an underlying defect of a specific component of the hemostatic system, either congenital or acquired, can be identified in a bleeding patient, substitutive treatment is the most appropriate choice. However, there are situations in which bleeding can either occur without a recognizable specific cause (e.g. in primary menorrhagia or some cases of recurrent epistaxis) or be favoured by anatomical abnormalities (like subarachnoid hemorrhage after rupture of an intracranial aneurysm or, possibly, in primary intracerebral hemorrhage). In other instances, bleeding can complicate surgery or occur in patients with severe traumas due to a complex coagulopathy induced by massive transfusion, shock, hemodilution, hypothermia, tissue anoxia, drugs. In all these situations, the use of prohemostatic agents can be effective. In some surgical procedures like orthotopic liver transplantation or cardiac surgery, important bleeding is expected and the main goal of avoiding allogeneic transfusion can be achieved by using hemostatic agents. Various drugs have been suggested to be able to reduce bleeding, but for only a few of them there is sufficient evidence from clinical trials to suggest an appropriate use in specific situations.

Synthetic antifibrinolytic lysine analogues, like aminocaproic acid (EACA) and tranexamic acid (TA), which inhibit plasmin generation from plasminogen and subsequent fibrin degradation by competing with the lysine binding site of the substrate, have proved to be effective in most mucosal bleedings, including primary menorrhagia, or in patients with refractory immune or non-immune thrombocytopenia. In practice, only TA is used at 15-25 mg/kg e.v. every 8 hrs or at higher dosage per os. TA is effective in reducing bleeding after orthopedic surgery and orthotopic liver transplantation, whereas its efficacy is not proved in trauma patients. No increase in thrombosis is reported. TA has been suggested as transfusion-sparing agent in cardiac surgery when aprotinin is not available or indicated. Patients in oral anticoagulation can safely undergo tooth extraction using mouthwash (1 g TA every 6 hrs).

Aprotinin, a 58 residue peptide isolated from bovine lung in 1930, initially proposed for the treatment of pancreatitis for its capacity to inactivate pancreatic trypsin, has been shown to be a major inhibitor, at therapeutic doses, of both kallikrein and plasmin, thus inhibiting the initiation of coagulation and fibrinolysis. This agent, not available in Italy due to the theoretical risk of transmitting the new variant of Creutzfeld-Jacob disease, is the only FDA approved drug as transfusion-sparing agent in cardiac surgery. In this setting it is the first choice agent without significant side effects (apart from anaphylaxis in about 5% of those already exposed in the last 6 months), associated with a reduction also of mortality and hospital stay length.

Desmopressin has also been tried in several acquired conditions without proved benefit and some increase of thrombosis, especially in the setting of cardiac surgery. It can be used to control bleeding in uremia or some drug-induced thrombocytopathies.

Introduced some 10 years ago for the treatment of hemorrhage in patients with hemophilia A and B and inhibitors against FVIII or FIX, recombinant activated FVII (rFVIIa) has been increasingly used outside the approved indication, mainly to control bleeding in a variety of complex situations, including refractory thrombocytopenia, warfarin intoxication, massive traumas, complicated surgery like liver orthotopic transplantation or partial hepatectomy, or during cardiac surgery. Hundreds of anecdotal reports have described the life-saving capacity of this agent after the failure of all standard treatments. A publication bias of favourable only results cannot be excluded. Some prospective controlled trials have been published, two showing no effect of the agent in controlling bleeding in patients with hepatic disease or in major hepatic resection and two with favourable results in retropubic prostatectomy and in acute intracerebral hemorrhage. Increasing evidence is accumulating that the agent could be life-saving in massive trauma. However, the thrombotic risk, although apparently minimal, is not negligible and not yet fully assessable in patients with concomitant risk factors. The prohibitively high cost, the lack of controlled trials in many specific situations, the inherent thrombotic risk suggest that rFVIIa should be used outside the approved setting only as life- or organ-saving agent, after the failure of all standard treatments. At the moment, rFVIIa should not be used as transfusion-sparing agent in complex surgery. Institutional guidelines should help the clinician to individualize its use.

DIFFERENTIAL DIAGNOSIS AND THERAPY OF VON WILLEBRAND'S DISEASE

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Von Willebrand disease (VWD) is the most frequent inherited bleeding disorder and is due to quantitative (type 1 and 3) or qualitative (type 2) defects of von Willebrand factor (VWF). VWD is inherited by autosomal dominant or recessive patterns, but women with milder VWD forms are apparently more symptomatic. Type 3 VWD is rare (1-3 cases per million), inherited by a recessive pattern and marked by the total absence or only trace amounts of VWF in plasma and platelets. Type 2A and 2B VWD are marked by the absence of high molecular weight VWF multimers in plasma; in type 2B, there is increased affinity for platelet GpIbα and thrombocytopenia can be found in most patients. Type 2M is marked by decreased platelet-dependent function with relatively normal multimers and type 2N by reduced VWF binding to Factor VIII (FVIII). Type 1 is characterized by partially reduced levels (20-40 U/dL) of normal VWF: these mild VWD forms are under-misdiagnosed and molecular defects should be searched for within the entire VWF gene, including promoter. An acquired von Willebrand syndrome (AVWS) with laboratory findings similar to those for congenital VWD has been report-

ed mainly in lympho-myeloproliferative, disorders. Unlike the congenital form, AVWS usually occurs in individuals with no personal or family history of bleeding. Diagnosis of AVWS is based on assays measuring ristocetin cofactor activity, which are usually abnormally low, while FVIII is sometimes normal. FVIII/VWF inhibiting activities are found in only a minority of cases. The aim of treatment of VWD is to correct the dual defect of hemostasis, i.e. the abnormal platelet adhesion due to reduced and/or dysfunctional VWF and the abnormal coagulation expressed by low FVIII. Desmopressin (DDAVP) is the treatment of choice for type 1 VWD because it can induce release of normal VWF from cellular compartments and transiently correct the FVIII/VWF levels in most of these patients. Prospective studies on biological response versus clinical efficacy of DDAVP in VWD type 1 and 2 are in progress to further explore its benefits and limits as therapeutic option. In type 3 and in severe forms of type 1 and 2 VWD, DDAVP is not effective and for these patients plasma virally-inactivated concentrates containing FVIII and VWF are the mainstay of treatment. Several intermediate- and high-purity FVIII/VWF concentrates are available and have been shown to be effective in clinical practice (bleeding and surgery). New products specifically devoted to VWD could be useful but should be validated by current methodologies before their introduction in clinical practice. Dosage and timing of FVIII/VWF administrations should be planned to keep FVIII levels between 50 and 150 U/dL. Appropriate dosage and timing in repeated infusions are also very important in patients exposed to long term prophylaxis for recurrent bleedings. Other therapeutic options such as recombinant FVIII in rare congenital VWD type cases with allo-antibodies as well as high dose immunoglobulin or recombinant activated factor VII in cases with auto-antibodies (AVWS) against VWF can be also useful.

BLEEDING AND THROMBOSIS IN ACUTE LEUKEMIA

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Patients with acute leukaemias are at increased risk of haemorrhage and thrombosis. Bleeding in untreated patients with acute leukemia is mostly due to thrombocytopenia, almost always a direct result of bone marrow invasion by leukaemic cells. After intensive remission induction chemotherapy and consolidation, a further contributory factor for the development of hemorrhagic complications is the myelosuppressive effect of most active drugs. The probability of developing life-threatening haemorrhages varies according to the types of acute leukemia and the type of therapy. While most if not all of the patients with acute leukaemia may have mild mucocutaneous bleeding that is responsive to platelet transfusion and institution of the appropriate chemotherapy, instances in which bleeding complications are life-threatening can develop and deserve a separate analysis. In this setting, an important role is played by the occurrence of disseminated intravascular clotting activation, which is mostly associated with acute promyelocytic leukaemia, and leads to the consumption of coagulation factors and platelets, with resulting severe bleeding diathesis. The correct evaluation of the risk of life-threatening bleeding is critically important, since proper therapy can prevent from death. Thrombosis of large vessels is not rarely seen in acute myeloblastic leukemia, but is an emerging and yet unresolved problem, during treatment, in acute lymphoblastic leukemia both in adults and children. Pathogenetic mechanisms involve the capacity of leukaemic blasts to interact and activate in several ways the haemostatic system. However an important role is also played by infections and anti-leukaemic treatments.

Iron

GENES AND PROTEINS OF IRON METABOLISM

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Iron is essential in a large number of fundamental biological processes, but is also potentially toxic due to its capacity to catalyse free radical production via Fenton-like reaction. Thus, both deficiency and excess are harmful, and organisms developed sophisticated mechanisms to maintain the level of active iron within very restricted levels. Most iron is transported in the body by transferrin and is taken up by transferrin receptor 1 (TfR1), with a mechanism that has been clarified. This pathway is essential for erythroid cells, but other cell types can acquire iron with mechanisms that are more complex and not yet fully clarified. The transporter DMT1 mediates iron entry into the cytoplasm. Iron is secreted by cells via Ferroportin, a molecule that is crucial for haemoglobin iron recycling by macrophages and for duodenal iron absorption. Within the cell iron can either be taken up by ferritins to be stored until needed, or be addressed to the mitochondria for the synthesis of heme of iron-sulphur complexes (ISC) to be used by the various enzymes. Although most proteins involved in the processing of ISC have been described, little is known on the regulation of mitochondrial iron trafficking. Frataxin, which is responsible of Freidreich'a ataxia, and ABC7, which is responsible of ataxia with sideroblastic anaemia, are both proteins involved in the ISC synthesis. A specific mitochondrial ferritin has been described, and this is highly expressed in sideroblastic anaemia, apparently to protect the organelle from iron excess. A mechanism for the coordinated regulation of cellular iron homeostasis has been characterized. It is ubiquitous and involves the interaction between Iron Regulatory Proteins (IRP1 and IRP2) with Iron Responsive Elements (IREs) on the transcripts of proteins for iron storage, transport and utilization. IRP1 is a Fe/S protein, and its activity is likely to be dependent on mitochondrial functionality. IRP2 is readily degraded in conditions of iron excess, but the mechanism has not been fully clarified.

The study of hemochromatosis and animal models has lead to the identification of other proteins involved in the regulation of systemic iron. The most important one is hepcidin, a small secretory peptide that acts as an iron-hormone. Its synthesis in the liver is induced by iron and inflammation, and it acts as an inhibitor of iron absorption and recycling. Recent data show that its activity is mostly based on a specific interaction with ferroportin, followed by a fast degradation. Hepcidin mutations cause rare forms of juvenile and severe types of hemochromatosis. HFE, which is responsible for most cases of adult-onset hereditary hemochromatosis, seems to be involved in the hepcidin pathway, probably upstream. Also mutations of TfR2 cause hemochromatosis, accompanied by a reduction of hepcidin expression. The latest gene to be identified is hemojuvelin, and is responsible for most cases of juvenile hemochromatosis. It encodes a GPI-anchor protein of unknown functions. In conclusion, most proteins involved in the regulation of cellular and systemic iron homeostasis are now known. It needs to be clarified how they interact and regulate cellular and systemic iron trafficking.

GENETIC DISORDERS OF IRON METABOLISM

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Iron is an essential component for human life and its availability is tightly regulated through duodenal absorption and macrophage release of recycled iron from red cell catabolism. Recent studies of molecular genetics have clarified the molecular basis of several inherited disorders of iron metabolism leading to the identification of proteins with a key role in regulating iron availability such as che hepcidin and ferroportin 1. An important contribution to our present knowledge of iron absorption, transport and regulation was also derived from the study of animal models of human genetic disorders. Hemochromatosis may result from inactivation of different genes (HFE, TFR2, Hepcidin, Hemojuvelin) encoding proteins that are likely components of the hepcidin pathway. The study of dominant hemochromatosis has led to the identification of ferroportin that is now considered the hepcidin putative receptor. The variable clinical features observed in patients with different mutations may be partly explained with the distinct roles of these proteins in the hepcidin pathway

All these advances have revolutionized the diagnosis of hemochromatosis, have implemented our knowledge of regulation of iron homeostasis, but have also contributed to better define new genetic disorders, at the edge between iron deficiency and overload, such as ferroportin disease, aceruloplasminemia, atransferrinemia and DMT1-related anemia. The advances achieved studying genetic disorders may be relevant also in secondary iron overload and anemia of inflammation. Hepcidin peptide is produced by the hepatocytes following not only iron excess but also cytokine signals in inflammation/infection and is switched off in conditions of hypoxya, iron deficiency and anemia. In anemia of chronic disorders the elevated hepcidin production is part of the inflammatory response and a defense mechanism to limit iron availability through the interruption of duodenal and macrophage iron release.

IRON METABOLISM AND ERYTHROPOIESIS: MOLECULAR RELATIONSHIPS AND THEIR CLINICAL RELEVANCE

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In the last few years, several novel genes of iron metabolism have been identified. Among these genes, a crucial role is likely played by hepcidin and hemojuvelin, which appear to control both intestinal iron absorption and reticuloendothelial iron release. Hepcidin is produced by the hepatocyte. In a rare forms of juvenile genetic hemochromatosis, inactivation of the hepcidin gene results in abnormally increased iron absorption and excessive iron release

from the reticuloendothelial cell. In inflammatory states, in the contrary, the excessive hepcidin production results in decreased iron absorption and in reticuloendothelial iron block, which in turn contribute to the pathogenesis of anemia of chronic disease. With respect to juvenile genetic hemochromatosis, recent studies have shown that the most common type is not due to mutations in the hepcidin gene, but rather to mutations in the gene encoding hemojuvelin. The fact that inactivation of the hemojuvelin gene involves absent production of hepcidin suggests that this novel protein, mainly present in hepatocytes, behaves as a chaperon of hepcidin. Both hepcidin and hemojuvelin might play an important role in the so called iron loading anemias. In these disorders, the expansion on the erythroid marrow is associated with increased intestinal iron absorption and increased iron release by the reticuloendothelial cells. Investigations on hepcidin and hemojuvelin expression in these disorders might provide interesting information about the so called erythroid regulator of iron absorption. A particular type of iron loading anemia is represented by sideroblastic anemia, which is characterized by ring sideroblasts in the bone marrow, by iron accumulation in mitochondria of these erythroid cells, and by ineffective erythropoiesis. Our collaborative research group has identified a novel protein of iron metabolism defined as mitochondrial ferritin. We have recently found that most of the iron deposited in perinuclear mitochondria of ring sideroblasts is present in the form of mitochondrial ferritin and that this latter might be a specific marker of sideroblastic anemia.

Minimal Residual Disease

MINIMAL RESIDUAL DISEASE: MATERIALS AND METHODS

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A multitude of factors influence treatment response in patients affected with haematological malignancies. Cell lineage, maturation, cytogenetic aberration and molecular alterations that regulate cell proliferation, differentiation and apoptosis are factors known to influence clinical response to therapy. Beside this, other important factors include size of tumour burden and its diffusion. All this parameters are actually included in staging system for the definition of relapse risk. Nevertheless, their predictive power is far from absolute and their use in treatment decision in individual patients is inherently limited. Measurements of response after treatment reflect the combined effect of clinical and cellular variables providing a direct information of effectiveness of treatment in each patients. Conventional morphological techniques have a very limited sensitivity and accuracy and when neoplastic population are detected, the relapsing disease is diagnosed at a substantially yet advanced disease. Methods to detect Minimal Residual Disease (MRD) include technologies designed to detect residual malignant cells behind the sensitivity of conventional approaches, like morphology and classic cytogenetic, in leukemia and lymphomas. A wide variety of techniques have been developed and the choice of the best method for a particular clinical situation depends on the biology of the individual malignancies and the type of specific markers which are used to revealed the neoplastic residual cells. Because of the variety of methods used an agreement should be reached between various laboratories and different multicenter studies on sample sources, method and necessity of cellular purification, nucleic acid extraction and primer and PCR standardisation have been performed. Detection of MRD is now becoming routinely implemented in treatment protocols and is increasingly used guiding therapy and for evaluation of new treatment modalities. Nevertheless, clinicians have to be aware that important differences about detection sensibility are present between different laboratories also when equivalent techniques and similar experimental conditions are used. Moreover, in some applications an inherently sensibility heterogeneity is present also between different samples due to the high variability of the marker used. Example of application of MRD in the field of acute leukemia and lymphomas will be examined and discussed.

MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIAS

Saglio G[1], Cilloni D[1], Gorrello P[2], Gottardi E[1], Guerrasio A[1], Bernard L[3], Diverio D[4], Cimino G[4], Elia L[4], Martinelli G[5], Pane F[6], Cazzaniga G[7], Biondi A[7], Pelicci PG[3], Falini B[2], Mecucci C[2], Lo Coco F[8]

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Monitoring of residual disease in patients with malignant hematologic disorders is now recognized as an important diagnostic tool for assessment of the response to treatment and the individual risk of relapse. The study of minimal residual disease has been fueled by the technologic advent of the polymerase chain reaction (PCR) and basic developments identifying the genetic lesions involved in human malignancies. In acute myeloid leukemias (AML), however, employment of very sensitive techniques permitting the identification of tumor cells within a 10^4-fold or greater excess of normal cells revealed that the presence and persistence of residual disease at this level does not necessarily imply inevitable relapse. In particular, persistence of leukemia-specific hybrid transcripts in the bone marrow and peripheral blood of patients with a t(8;21) or inv(16) AML at the end of the conventional therapy is normal and is apparently compatible with a durable hematological remission. Therefore, quantitative assessment of MRD rather than detection could likely be of clinical and prognostic value in AML and, indeed, studies performed by several groups including our own indicate that quantitative RT-PCR (RQ PCR) analysis is required in order to obtain from MRD detection prognostic information about the risk of relapse in AML. Unfortunately, the most common fusion transcripts are present only in about 40% of the AML cases and, to overcome this problem, we have recently tried to exploit a new molecular marker, the transcript of the WT1 gene (Wilms' tumor gene), as the RQ PCR methods clearly distinguish between the level of WT1 transcripts in normal and in leukemic cells. The WT1 levels were shown to strictly parallel the behaviour of the other molecular markers (fusion gene transcripts) used for the MRD monitoring and the increase of the WT1 levels could also precede the occurrence of the overt hematological relapse of some months. On the opposite, normal WT1 values have always been found associated with persisting remissions. Furthermore, the finding of extremely low and often undetectable WT1 levels in the peripheral blood of normal individuals and in leukaemia patients in CCR, suggests that PB could even more sensitive than bone marrow in revealing impending relapses. Very recently, a new important molecular marker has been identified in AML. Aberrant cytoplasmic location of NPM, a nucleo-cytoplasmic shuttling protein with nucleus-restricted expression, predicts NPM gene exon-12 mutations, and this defect appears to be the most frequent and specific molecular lesion associated with normal karyotype in AML. As a consequence, new RQ PCR approaches able to identify the presence and the amount of MRD in these patients are under development. Having almost completed the panel of makers suitale for MRD quantification in AML, the major tasks for the future will remain standardization of Q-PCR techniques, exact definition of threshold levels, and monitoring schedules in bone marrow (BM) and peripheral blood (PB), in order to allow RQ PCR to become a very robust and reliable tool for clinical decision making in AML therapy.

MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA

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Minimal residual disease (MRD) evaluation is critical in the evaluation of treatments aimed at maximal cytoreduction in multiple myeloma. Qualitative evaluation of MRD has now a ten-year long history, but remains a relatively sophisticated and poorly standardized procedure. Technical difficulties include effective sequencing of the immunoglobulin heavy chain rearrangement and appropriate choice of tumor-specific primers. Despite these problems some basic observations obtained by quantitative PCR approaches had a major impact on the current approach to MM treatment. Among these observations the most important has been the high rate of molecular remission obtained using allogeneic transplantation compared to any alternative procedures.

Only recently, quantitative PCR has become a useful approach for disease monitoring of MM patients. This result has been mostly achieved through the development of real-time PCR, which is the only technique that is robust, reproducible and accurate enough, to be effectively employed in a clinical setting. Real time PCR using the IgH rearrangement is a rapidly evolving field from the technical point of view. Since its first establishment in 2000, some major improvements have being obtained particularly in probe chemistry and assay simplification. Main results from the cognitive point of view include extensive evaluation of tumor load in stem cell harvests, and more preliminary although very intriguing results of minimal residual disease monitoring the post autologous and post-allogeneic BMT setting.

Future issues in the field focus on the strict monitoring of the graft versus myeloma effect in the post allogeneic BMT setting and the evaluation of the impact on residual tumor cells of new non-chemotherapeutic drugs such as thalidomide, immunomodulatory drugs and bortezomib.

Transfusion Medicine

CELL THERAPY: REALIZATION OF GRADE PROTOCOLS

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The renewed interest in cellular and gene therapy has attracted the attention of the regulatory agencies in the United States and Europe. They have responded by proposing that these products be regulated in a similar manner to pharmaceuticals. This poses a number of issues. Firstly, many of these products are at an early stage of development and are not intended for commercialization; and secondly, that the academic facilities in which these products are manufactured are generally unfamiliar with working under pharmaceutical manufacturing conditions.

There are three steps in translation of a pre-clinical concept into a clinical trial. The first is to validate a pre-clinical model and avoid spending many years of efforts on ever more complex approaches in animals that turn out to poorly to reflect behaviour in humans. The second is to demonstrate at least some evidence of clinical efficacy, and the third is to show that the approach can feasibly be exported to the wider medical community. These translation efforts are therefore initially going to be iterative - that is they are likely to be small scale, moving from laboratory to clinic and back to the laboratory. It is therefore important to have efficient and effective ways of developing clinical trials within institutions since pharmaceutical and biotechnology companies find such iterative studies difficult to develop. Newer and stricter regulations have been issued also in Italy and include different parameters, whose respect is mandatory: 1) to possess an adequate facility; 2) a well established and continuous monitoring of the quality (Quality Assurance and Quality Control); 3) an efficient and controlled tracking system to have a proper identification of the product from the entrance into the laboratory till the emission for clinical use; 4) a well-trained personnel who operates in the absolute respect of pre-established Standard Operating Procedures (SOP); 5) the role of the Technical Director who assumes the responsibility of the entire production process and releases a Certificate of Analysis, allowing the use of the cell product itself. In this presentation we will address all the issues related to the realization of clinical protocols of cell therapy under Good Tissue and Good Manufacturing Practices (GTP and GMP).

TRANSFUSION-RELATED ACUTE LUNG INJURY (TRALI)

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Transfusion-related acute lung injury (TRALI) was first identified as a distinct clinical entity in the 1980's, with the report of a series of 36 patients. In recent years, with more aggressive use of blood components, TRALI has become recognized as a more common clinical complication of transfusion. Its frequency, usually considered to be 1 in 5,000 transfusions, is likely underestimated because of both lack of recognition and underreporting. Prevalence rates of 1 in 2,000 transfusions for stored cellular components and 3 in 1,000 for platelets concentrates obtained from whole blood have recently been documented. In published series of TRALI platelet concentrates separated from whole blood have been the most commonly implicated blood component, followed by packed red cells, whole blood, fresh frozen plasma, apheresis platelets, granulocytes and cryoprecipitate. Two pathophysiologic mechanisms have been proposed for TRALI: the antibody hypotesis and, more recently, the neutrophil priming hypothesis or "two-event model". This model hypothesizes that biologically active compounds generated during stress (such as trauma, thermal injury, infection, recent surgery, induction phase of treatment for acute leukemia, cytokine therapy, cardiovascular disease) represent the first event, during which the recipient's pulmonary vascular endothelium becomes activated and recipient's PMNs become primed, change from a nonadhesive to an adhesive phenotype, and activate the pulmonary endothelium. This leads to increased surface expression of ICAM-1 and other adhesion molecules, and consequently to pulmonary sequestration of PMNs with maximal cytotoxic potential. A second event, the transfusion of priming agents as specific immunoglobulins, lipids, and cytokines contained in plasma fraction of stored components, activate the primed PMNs, resulting in endothelial damage, capillary leak, and pulmonary injury culminating in TRALI.

TRALI is a serious complication of hemotherapy that occurs within 6 hrs of transfusion, most cases presenting within 1-2 hrs. In the classic, severe form of TRALI, the clinical pattern is identical to that of the acute respiratory distress syndrome (ARDS), with the insidious onset of acute pulmonary distress temporally related to transfusion of blood or blood components. The clinical findings of TRALI consist of a constellation of symptoms, including fever, tachypnea, cyanosis, and dyspnea. Auscultation of the lungs reveals diffuse crackles and decreased breath sounds, especially in the dependent areas if the patient is in the decubitus or prone position. The physiological findings are consistent with hypoxemia and decreased pulmonary compliance despite normal cardiac function. Radiographic examination shows diffuse, fluffy infiltrates consistent with pulmonary edema, with progression of the alveolar and interstitial infiltrates such that the entire lung is obscured. Treatment consists of aggressive respiratory support, including supplemental oxygen and mechanical ventilation. Milder forms of TRALI have been described that require prompt delivery of supplemental oxygen alone. Unlike ARDS, TRALI is not associated with a high mortality rate: 80 to 90% of patients survive, with most recovering in the first 72 hr.

The differential diagnosis of patients who develop pulmonary insufficiency after transfusion must include circulatory overload, anaphylactic transfusion reactions, and transfusions of blood products contaminated with bacteria. Moreover, the diagnosis of TRALI requires the exclusion of other risk factors for ALI. The adherence to current guidelines to minimize the inappropriate use of blood will diminish TRALI. Other preventive measures include the temporary disqualification from donation of all implicated

donors until leucocyte antibody testing are completed, washing of cellular components for high risk surgical procedures and using fresher products in high risk patients. Current data do not seem to support the disqualification of multiparous female donors.

PLATELET TRANSFUSION: REFRACTORINESS AND OPTIMAL DOSE

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Platelet transfusion is a cornerstone of supportive therapy in a number of medical and surgical conditions. Although most patients seem to benefit from platelet transfusion from random donors, as indicated by adequate post-transfusion platelet count increments and prevention of clinically relevant hemorrhage, a proportion of recipients, mostly chronic recipients from the onco-hematology setting, develop a condition termed 'platelet refractoriness'. Refractoriness to random donor platelets is defined as the occurrence of 2-3 post-transfusion platelet count increments corrected for the patient's size and number of administered platelets (corrected count increment, CCI, the absolute increment multiplied by the patient's BSA and divided by the number of administered platelets) at 10-60 minutes and at 18-24 hours posttransfusion below 4,500-5,000 and 2,500 platelets per microliter respectively. In most cases refractoriness is associated to clinical and pharmacological causes. In the proportion of cases in which refractoriness is due to immune factors, anti-HLA antibodies are most frequently implicated. Immune and non immune factors can develop contemporarily or at different times in the same patient. Validated strategies to select effective platelets for alloimmunized refractory patients (about 15 and 5% of recipients of standard and leukoreduced blood components respectively) include the selection of HLA-matched platelet donors from HLA-typed donor registries and the use of manual or automated platelet cross-match. Both strategies, which require significant organizational and economical resources, can restore successful platelet support in about 2/3 of transfusions. The optimal dose of platelets to be administered in a single transfusion event is still a matter of debate. Although most guidelines and manuals indicate to transfuse one platelet concentrate obtained from 450 mL of blood (which may contain an average of 60-70 billion platelets) per 10 kg of recipient body weight (or an equivalent dose of platelets obtained by apheresis), until recently limited data was available to support the rationale for this dose. Despite this apparent limitation, the traditional dose has proven its effectiveness in preventing the occurrence of clinically relevant bleeding episodes during most thrombocytopenic patient days. In fact, some recent large investigations have shown that WHO grade >2 bleeding occurs in no more than 2-3% of thrombocytopenic in-hospital days of onco-hematology recipients treated with current therapy and requiring platelet support.

More recently, following the developments of apheresis technology, it has become possible to collect platelet doses exceeding 700 billion platelets per collection. This technological advancement opened two perspectives: to split one high-dose concentrate to transfuse two patients or to increase the standard dose with the aim of increasing the inter-transfusion interval. Mathematical models and some clinical studies have provided information which has been considered supportive of the 'high dose' policy by many but not all platelet transfusion experts.

The opposite possibility, i.e. to reduce the standard platelet dose towards a 'low dose' policy is currently under evaluation. This evaluation has been prompted by observations reported by Sherrill Slichter and coworkers, who showed that thrombocytopenic oncohematology patients do not seem to consume (and thus require) more than 7,000 platelets/uL (a small number, easily replaced with 'low-dose' transfusions) to repair their microvasculature.

It can be expected that in the next few years fresh information collected in prospective trials contrasting the high and the low doses will be available, thus allowing a conclusive definition of the optimal platelet transfusion dose.

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SIES Symposium

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF HEMATOPOIETIC STEM CELL COMPARTMENT

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The gene expression profile of hematopoietic stem cells (HSC) and the correlations between their phenotype and their biological properties are still poorly understood. To address this issue, we used the DNA microarray technology to compare the expression profiles of different peripheral blood hematopoietic stem/progenitor cell subsets, lineage negative (Lin-) CD34-, Lin-CD34+ and Lin+CD34+ cells. The analysis of gene categories differentially expressed shows that the expression of CD34 is associated with cell cycle entry and metabolic activation, such as DNA, RNA and protein synthesis. Moreover, the significant up-regulation in CD34- cells of pathways inhibiting HSC proliferation2 induces a strong differential expression of cyclins, CDKs (Cyclin Dependent Kinases), CDK inhibitors and growth-arrest genes. According to the expression of their receptors and transducers, interleukin (IL)-10 and IL-17 showed an inhibitory effect on the clonogenic activity of CD34- cells only. Conversely, CD34+ cells were sensitive to the mitogenic stimulus of thrombopoietin. Furthermore, CD34- cells express preferentially genes related to neural, epithelial, and muscle differentiation. The analysis of transcription factors expression shows that the CD34 induction results in the up-regulation of self-renewal- and lineage-commitment-related genes. The preferential expression in CD34+ cells of genes supporting HSC mobilization and homing to the bone marrow, such as chemokine receptors and integrins, gives the molecular basis for the higher engraftment capacity of CD34+ cells into immunodeficient mice. Thus, the different kinetic status of CD34- and CD34+ cells, detailed by molecular and functional analysis, significantly influences their biological behaviour.

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NEW INSIGHTS IN RED CELL MEMBRANE DISORDERS: THE LEAKY RED CELL SYNDROMES

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Leaky red cell syndromes (LRC) are genetic disorders of the red cell permeability to monovalent cations (for a recent exhaustive review, see : Current Opinion in Hematology 1999 : 6, 110-114). Hereditary stomatocytosis is the first case of LRC described. It was so coined due to the conspicously abnormal shapes of the red cells (Br J Haematol 1961: 7, 303-314). 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. 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 (suppressed would probably be more appropriate a designation). 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. A definite classification of LRC syndromes will ultimately come out from mapping and identifying the responsible genes. During the last four years we have largely contributed to the knowledge of these diseases by mapping on 16q23-24 the gene responsible for DHS1 (Am J

Hum Genet 1998 : 63, 810-816), FP 1 (Blood 1999 : 93, 3120-31123) and SFE syndrome (Blood 96: 2599-2605, 2000). In addition, we have demonstrated the presence of genetic heterogeneity first showing the absence of linkage of DHS2 to 16q23-24 (Haematologica 1999 : 84, 862-864). 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.

UNIVERSAL MARKERS OF MINIMAL RESIDUAL DISEASE

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Monitoring of acute leukemia patients during and after treatment for the presence of remaining leukemic cells (minimal residual disease) have been shown to give major insight into the effectiveness of treatment. However, so far applicability of this strategy has been limited to those leukaemia subsets characterized by genetic markers amenable to sensitive detection by PCR. Although PCR for immunoglobulin and T cell receptor gene rearrangement represents the gold standard for MRD detection in most cases of ALL lacking the availability of fusion gene transcripts as molecular markers, the situation in AML is more complicated because, at present, more than 50% of them lack any sort of clonality markers suitable for minimal residual disease (MRD) monitoring. Thus, a number of studies have been performed in the attempt to identify cytogenetic and molecular abnormalities associated with leukemic transformation.

The Wilms' tumour gene (WT1) is a tumour suppressor gene identified for its involvement in the pathogenesis of the Wilms tumour. WT1 is expressed at very low levels in normal BM and PB samples. By contrast WT1 is overexpressed in many types of haematological malignancies including AML, ALL, MDS, CML, and chronic myeloproliferative disorders. The significance of WT1 expression in hemotopoietic cells and hematopoietic malignancies is at present quite completely obscute. In spite of the the role of WT1 in the leukemic process, it is now commonly accepted that WT1 represents a sort of universal marker of acute myeloid leukaemia (AML) and the quantitative assessment of WT1 expression can be a useful tool for monitoring Minimal Residual Disease (MRD) AML patients. The WT1 levels were shown to strictly parallel the behaviour of the other molecular markers (fusion gene transcripts) used for the MRD monitoring. Furthermore, increased WT1 expression above the range found in normal BM and/or in normal PB samples during follow-up of AML patients was always found to be predictive of an impending hematological relapse even in AML patients lacking additional molecular markers. The increase of the WT1 levels could also precede the occurrence of the overt hematological relapse of some months, although the kinetics of the relapse appears highly variable. On the opposite, normal WT1 values have always been found associated with persisting remissions. Therefore, evaluation of WT1 expression could represent a sort of universal marker that can allow a rather sensitive evaluation of the MRD in all AML patients Furthermore, the finding of extremely low and often undetectable WT1 levels in the peripheral blood of normal individuals and in leukaemia patients in CCR, suggests that PB could even more sensitive than bone marrow in revealing impending relapses.

GITMO Symposium

REDUCED INTENSITY CONDITIONING FOLLOWED BY ALLOGENEIC TRANSPLANTATION: THE GITMO EXPERIENCE (YEARS 2000–2005)

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On behalf of Gruppo Italiano Trapianto di Midollo Osseo (GITMO)

During 1997, the first studies regarding the reduced intensity transplants (RIT) have been published. Most of the trials employed the fludarabine in combination with other alkylator agents or a low dose total body irradiation (TBI 2 Gy). In 1999, the RIT study group of the GITMO designed 7 protocols which were mainly disease oriented:

1. acute leukemias, chronic myeloid leukemia, myelofibrosis (thiotepa 10 mg/kg, cyclophosphamide 100 mg/kg);
2. high risk myelodysplastic syndromes and secondary leukemias (thiotepa 10 mg/kg, fludarabine 125 mg/ms);
3. multiple myeloma at diagnosis (auto BMT followed by TBI 2Gy);
4. multiple myeloma at relapse (melphalan 80 mg/ms, thiotepa 5 mg/kg, fludarabine 90 mg/ms);
5. chronic lymphocytic leukemia, lymphomas (thiotepa 10 mg/kg, fludarabine 60 mg/ms, cyclophosphamide 60 mg/kg);
6. patients between 60 and 70 years (fludarabine 150 mg/ms, TBI 2 Gy);
7. matched unrelated donors (alemtuzumab 80 mg, melphalan 30 mg/ms, fludarabine 90 mg/ms, TBI 2 Gy).

The protocols were designed for patients older than 45 years or not eligible to myeloablative conditioning for the presence of comorbidities. Primary endpoint of all protocols was a reduction of transplant related mortality. As to may 2005, 464 patients were transplanted from a sibling and 55 received a graft from a matched unrelated donor. Transplant related mortality from siblings ranged from 6% to 22%, for unrelated donors was 16%. All the protocols will be closed by the end of 2005.

UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY REGIMENS IN HIGH RISK PATIENTS FOR AGE OR DISEASE: RESULTS FROM TWO INDEPENDENT PROSPECTIVE GITMO STUDIES

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Allogeneic transplantation after reduced intensity conditioning (RIC) regimens is mainly indicated for patients who otherwise are not eligible for a standard myeloablative conditioning either because of advanced age or type of disease. Although most RIC programs were shown to be effective in promoting hematopoietic engraftment and immune reconstitution, the incidence and severity of graft versus host disease (GVHD) remains an open, unresolved issue particularly in the case of unrelated donors. This is why, in 2002 the *Gruppo Italiano Trapianto di Midollo Osseo* (GITMO) promoted 2 independent prospective RIC studies for high risk patients (age >55 or disease) undergoing allogeneic transplantation from marrow unrelated donors. The first program (Melphalan 30 mg/m^2 at day -8, Alemtuzumab 20 mg/die from -8 to -5, Fludarabine 30 mg/m^2 at days -4 to -2 and TBI 200 cGy single fraction, at day -1) was given to patients with the following underlying diseases: MM (8), NHL (14), HD (15), AML (7), B-CLL (4), MMF (1), CML (1), MDS (2), ALL (1), mycosis fungoides and Sezary' syndrome (2). Before conditioning, 72% of these patients had received 3 or more chemotherapy treatments and in 69% of cases an autologous or allogeneic transplantation had already been performed. Only 10 patients were in hematologic remission at time of transplantation. Median age was 47 (range 17-64). The stem cell source was the Peripheral Blood (PB) in 27 patients (with a median value of TNC and CD34$^+$ cells of 10x10^8/kg and 7x10^6/kg, respectively) and the bone marrow (BM) in 21 (with a median value of TNC and CD34$^+$ cells of 2.9x10^8/kg and 2.4x10^6/kg, respectively). Graft failure was observed in 12% of pts. Complications included aGVHD (I-III) in 16 cases (in 1 case after DLI), cGVHD in 4 cases, CMV reactivation in 24 patients. With a median follow-up of 429 days the overall survival is 56%. The transplant related mortality (TRM) at 100 days was 16%. A second program (based on thiotepa 10 mg/kg at day -5, cyclophosphamide 50 mg/kg and ATG 3,75 mg/kg at day -3 and -2) was given to 43 patients (AML 18, NHL 9, HD16). A sustaind hematologic engraftment was achieved in most cases, with a donor full chimerism demonstrated in 24 out of 30 evaluable patients. Six patients had a rejection/graft failure (14%) but all of them are still alive after autologous reconstitution. The incidence of severe aGVHD was low (grade III-IV, 9%). At 1 year the TRM is 20% while the disease related mortality rate is 22%. DLI was performed in 39% and at two years, the overall survival is 58%. These preliminary results show that, both RIC programs were well tolerated and gave comparable results in terms of overall survival, relapse rate and TRM. In search of a standard conditioning regimen for these high risk patients, a randomized trial may be appropriate to better evaluate the overall antitumor activity and the safety profile of these two strategies.

GRUPPO ITALIANO TRAPIANTO DI MIDOLLO OSSEO (GITMO): PROSPECTIVE CLINICAL TRIALS FOR PREVENTION AND TREATMENT OF GRAFT VERSUS HOST DISEASE (GVHD)

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Gruppo Italiano Trapianto di Midollo Osseo (GITMO)

GITMO has performed three prospective clinical trials for prevention and treatment of graft versus host disease (GvHD). The first trial asked the question whether a higher dose of corticosteroids would be more effective as first line therapy of acute GvHD (Blood 1998; 92, 2288): 95 recipients of an HLA-identical BMT were randomized to receive intravenous methylprednisolone (MPred) 2 mg/kg/day for 5 days or 10 mg/kg/day for 5 days as initial treatment of GvHD . The study showed that a higher dose

MPred did not improve response rate , survival, nor did it reduce transplant mortality (TRM). One additional observations was that patients receiving 5 days of MPred 2 mg/kg, who could taper their MPred dose on day +5 as per protocol, had a significantly lower TRM (23%) as compared to patients who could not taper their MPred dose (46%) : this observation led us to identify responders as patients who can follow the MPred tapering schedule. We then designed a second study which would keep the low dose MPred up front, and then test whether intensified second line treatment would reduce TRM: in this trial all patients receive 2 mg/kg /day for 5 days at diagnosis of acute GvHD (grade I-IV); responders were followed for events and survival, whereas non responders were randomized for second line MPred treatment with or without anti-thymocyte globulin (ATG); 237 entered this multicenter GITMO trial. The study confirmed that (a) large proportion of patients respond to first line therapy with MPred 2 mg/kg, (b) response on day +5 is predicted by GvHD severity at the time treatment is initiated , (c) response identifies patients with significantly lower risk of TRM and (d) intensified second line treatment of non responders with a combination of MPred and ATG does not improve outcome as compared to MPred alone (manuscript in preparation). These two trials show that intensified immune-suppression is not beneficial for the management of patients with acute GvHD. The third trial looked at prophylaxis of GvHD in the setting of unrelated donor transplantation: we could show that the addition of anti-thymocyte globulin (ATG) to a combination of cyclosporine-methotrexate can significantly reduce acute GvHD (Blood 2001; 98: 2942) but does not influence transplant mortality (TRM) and survival in the short term. We have now updated that study with a median follow up of nearly 6 years : we have shown that the addition of ATG pretransplant to cyclosporine-methotrexate provides significant protection against chronic GvHD and bronchiolitis obliterans organizing poneumonia (BOOP) syndrome, reduces late transplant mortality and improves quality of life, in patients undergoing unrelated donor transplants.

These three trials confirm that collaborative efforts can produce relevant clinical information, also in a difficult setting such as graft versus host disease.

HIGH-RISK FOLLICLE CENTER LYMPHOMA (FCL) AT DIAGNOSIS: AN UPDATE OF THE TWO GITMO ("GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO") PROSPECTIVE MULTICENTER TRIALS

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The intensive regimen i-HDS is an effective treatment for advanced-stage FCL patients at diagnosis (Tarella C et al, Leukemia 2000). Its feasibility has been already documented in the context of a multicenter trial, performed by 20 Italian Transplantation Units associated to the GITMO. Between 1997 and 1999, 92 untreated patients, aged 18–60 years, with stage III-IV FCL requiring treatment entered this study protocol. At presentation, most patients had some adverse prognostic features and an aaIPI ≥ 2 was observed in 37%. Overall, 87% of patients completed the planned treatment, with a CR rate of 88%, as previously reported (Ladetto M et al, Blood, 2002). The study has been recently updated at 42 months from the previous closing date. At the present analysis, late toxic effects included five myelodysplastic syndromes and/or secondary leukemias (MDS/2AL) occurring between 24 and 54 mos. since autograft. The projected overall survival (OS) at 8 years is 74%. Notably, following i-HDS, patients with age-adjusted (aa)IPI ≥2 had an outcome comparable to those with aaIPI 1 (p=NS). Indeed, the 7-yr OS of 81% recorded in the aaIPI 2-3 patients is definitely promising, compared to the life expectancy historically reported for high-risk FCL following conventional chemotherapy. However, it is now clear that anti-CD20 Rituximab monoclonal antibody has markedly improved treatment options for FCL patients In the mean time, Rituximab can be easily integrated into autografting-containing regimens. Thus intensified treatments should still be considered as effective therapeutic weapons worthwhile of being evaluated. To verify this hypothesis the GITMO has decided to launch at the end of 1999 a second multicenter trial comparing rituximab-supplemented i-HDS (R-HDS) vs. rituximab-supplemented CHOP (R-CHOP). Patients were considered eligible if they had FCL at diagnosis with an aaI.P.I. score ≥2 or ≥3 adverse parameters according to the Italian Lymphoma Intergroup (I.L.I.) score. The study has been closed in March 2005, 136 patients have been enrolled by 29 GITMO Centers, and 108 patients (54 patients in each treatment arm) are presently evaluable. The two arms appear so far well balanced in terms of age, sex, histological grade, and entry criteria. There were six fatal toxicities (2 in the R-CHOP and 4 in the R-HDS arms), resulting in an overall 5.5% TRM. Overall CR rate was 71%, PR rate was 5%, while 24% of patients had stable disease or disease progression. Response was significantly higher in R-HDS compared to R-CHOP treated patients, with 87% and 55% CR rates, respectively. This translated in a significantly better progression-free survival following R-HDS (61% at 3-yrs) vs. R-CHOP (41% at 3-yrs). The OS for the whole group is projected to 81% at 3 yrs., with no significant differences between the 2 treatment arms. In conclusion, the 2 GITMO studies indicate the following: i. the intensified approach with HDS-based programs is feasible at the multicenter level, with early and late toxicities analogous or even lower than those reported in the principal studies on intensified therapy for FCL so far published; ii. the OS recorded in high-risk FCL in both GITMO studies compare favorably with that achieved with Rituximab-free non-intensified therapy; iii. R-HDS proved to be superior to R-CHOP in terms of response rate and response durability; iv. a prolonged follow-up is still needed to verify whether the higher response rate of R-HDS vs. R-CHOP also translates in improvements of the overall life expectancy.

Presidential Symposium

CELLULAR AND GENE THERAPIES TO IMPROVE BONE MARROW TRANSPLANTATION

Introna M

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In the last several years a plethora of informations on the basic mechanisms of immune recognition and regulation have emerged. During the same years solid clinical observations have also been produced on the efficacy of cell mediated therapies, which successfully start to apply the knowledge obtained from basic immunlogy to the clinic, as exemplified by bone marrow and stem cell transplantation (HSCT) or donor leukocyte infusions (DLI).

Adoptive immunotherapy with DLI after allogeneic HSCT has provided an effective means of augmenting the graft versus leukemia (GVL) response, particularly for patients with CML or MM, although at the cost of inducing GVHD. In a recent review of the EBMT-95 survey and of the North American survey (USA-97) the complete remissions vary from 80% for CML patients in cytogenetic relapse to 36% for patients with CML in transformed phase. Moreover complete remissions have been observed in 26% patients with AML/MDS, 15% with ALL and 29% with MM. No clear cut explanation exists for the different GVL efficacies of allogeneic T cells in different tumor types. Many experimental approaches have suggested the possibility of separating GVHD and GVL but clinical application is still lacking. In an attempt to overcome this issue, other donor's populations can be used for the same purpose. As one example, cells activated and expanded in the presence of interferon-Á followed by anti-CD3 monoclonal antibody and IL-2 have been termed cytokine induced killer cells (CIK) and recently shown to be CD8+ T cells which display MHC unrestricted TCR-independent cytotoxicity against malignant targets. A central role for NKG2d mediated recognition has been suggested for these cells which coexpress CD56, produce TH1 type cytokines, and undergo rapid in vitro expansion up to 1000 fold. Our experience with CIK cells will be shown, including a discussion on the application of cellular therapies in the clinical reality. One limitation is certainly posed by the technical constraints and costs inherent to ex vivo manipulations, as well as the recently introduced requirements to perform these procedures under good manufacturing practice (GMP) conditions. Data on our attempts to organise and rule a GMP facility in the Hospital will be discussed.

UMBILICAL CORD BLOOD AS SOURCE OF HEMATOPOIETIC STEM CELLS FOR ALLOGENEIC TRANSPLANT IN ADULTS WITH HEMATOLOGICAL MALIGNANCY

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During the last 15 years, umbilical cord blood (CB) as source of hematopoietic stem cells (HSC) alternative to bone marrow (BM) has been increasingly used for unrelated transplant in children lacking an HLA identical sibling. Less stringent HLA matching criteria, lower incidence and severity of GvHD, lower risk of viral contamination and more immediate availability represent the main characteristics of CB, which is becoming a more attractive source of HSC than BM, particularly for patients who require a transplant on urgency. Despite the high proliferative potential of CB HSC, the low number of cells per unit with the consequent risk of graft failure and delayed engraftment has firstly limited the use of unrelated CB in adults. However, the expansion of CB banks worldwide, the promising results from several single center studies and the identification of better criteria in selecting patients and CB units for transplant have greatly increased the experience on CB transplantation in adult patients. As of March 2005, of 1984 CB transplants registered at EUROCORD, 1596 are from unrelated donors and 35% of them have been performed in adults. A recent retrospective analysis produced by the EUROCORD on 171 adult patients with high risk hematological malignancies, who received an unrelated and HLA mismatched CB transplant later than 1998, shows a 2-year cumulative incidence of engraftment, acute-GVHD, relapse and transplant related mortality (TRM) of 72±3%, 32±4%, 22±4% and 51±4%, respectively. The 2-year probability of leukemia-free-survival (LFS) is 41±9% for patients transplanted in early disease phase, 34±10% for patients in intermediate phase and only 18±4% for patients transplanted in more advanced phase. In multivariate analysis, advanced disease phase (p=0.03), female gender (p=0.05) and ABO major incompatibility (p=0.015) were identified as significant factors negatively affecting LFS. As performed in children, two independent studies, recently published in the N Engl J Med, have compared adult patients with leukemia transplanted with an unrelated CB with patients transplanted with BM from an unrelated donor. Despite some differences between the two analyses, in both studies the main outcomes (relapse, TRM, LFS) were similar between CB and BM transplanted patients. Finally, the limit of the low number of CB cells per unit should be overcome, if the favorable results in terms of engraftment and disease free survival recently reported by the use of 2 unrelated CB units in 23 adults would be confirmed. In conclusion, CB represents an effective source of HSC also for transplant in adults and a search for an unrelated donor should always simultaneously be addressed towards the International BM Donor Registries and Cord Blood Banks.

Selected Oral Communications

S001

LONG TERM FOLLOW UP OF TWO RANDOMIZED TRIALS ON ANTITHYMOCYTE GLOBULIN (ATG) FOR GVHD PROPHYLAXIS IN UNRELATED DONOR TRANSPLANTS: CHRONIC GVHD, BRONCHIOLITIS AND QUALITY OF LIFE.

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Background. We have reported that rabbit antithymocyte globulin (ATG Sangstat-Genzyme) prevents acute and chronic graft versus host disease (GvHD) in patients undergoing an unrelated donor transplant (Blood 2001; 98 (10): 2942-2947). Patients had entered two consecutive randomized trials: in trial-1, 54 patients were randomized to non-ATG (n=25) or 7.5 mg/kg rabbit ATG (n=29). In trial-2, 28 patients were randomized in the non-ATG arm and 27 in the ATG 15 mg/kg arm.

Aim of the study. to assess the risk of extensive chronic GvHD, bronchiolitis obliterans , quality of life, survival and transplant related mortality (TRM) 4 years later.

Patients. Seventy five patients survived 100 days after BMT, and were available for analysis: each patients was updated and assessed for survival, chronic GvHD, for bronchiolitis obliterans obliterating pneumonia (BOOP) syndrome, including forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and relapse of the original disease and quality of life.

Results. Results are given in percentage, in the order non-ATG vs ATG patients. At last follow up chronic GvHD (limited+extensive) was scored in 68% vs 31% respectively for non-ATG and ATG of patients (p=0.02) and extensive chronic GvHD was scored in 35% and 13% of patients (0.03).

Bronchiolitis: the actuarial risk of developing a BOOP syndrome was 65% vs 22% (p=0.001). Median timing of bronchiolitis was 1155 days. In the non-ATG group there was a significant decrease of FEV1 beyond 2 years (average delta of –23%, p=0.02) and the same was true for FVC (average delta of –20%, p=0.005). This was not the case for patients receiving ATG (DFEV1: -3%, p=0.2; and DFVC: +3%, p=0.3). Quality of life was assessed by looking at proportion of patients with Karnofski score of 100% at last follow up: the proportion was 57% vs 89% (p=0.03). Relapse related deaths were 16% in the non-ATG and 14% in the ATG group (p=0.5).

Survival. Actuarial 9 year survival for non-ATG and ATG patients alive at day +100 (n=75) is 43% vs 65% (p=0.1) , and TRM 45% vs 19% (p=0.2); for patients alive at 1 year (n=57), survival is 58% vs 85% (p=0.09) and TRM 36% vs 4% (p=0.03).

Conclusions. This updated analysis of two randomized GITMO trials, confirms with longer follow up, that ATG pre-transplant produces (1) a significant reduction of chronic GvHD and (2) a significant reduction of extensive chronic GvHD. As a consequence quality of life is significantly improved in ATG patients. This study also shows that ATG reduces the risk of chronic bronchiolitis and late TRM. These data give strong support for the inclusion of ATG in the conditioning regimen of unrelated transplants: dosing and timing should be considered and could be tested in a prospective trial.

This work was supported by Fondazione CARIGE, Genova

S002

DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS OF NPM1 MUTATIONS IN ADULT PRIMARY ACUTE MYELOID LEUKEMIA

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Nucleophosmin (NPM1, B23, numatrin), a nucleolar phosphoprotein which regulates the ARF-p53 tumor suppressor pathway, is the translocation partner of MLF1 (Myeloid Leukemia Factor 1) in AML with t(3;5), of RARA (Retinoic Acid Receptor Alpha) in acute promyelocytic leukaemia with t(5;17) and of ALK (Anaplastic Lymphoma Kinase) in large anaplastic cell lymphoma with t(2;5). We first identified NPM1 mutations as the genetic lesion underlying cytoplasmic delocalisation of NPM protein in 60% of adult AML (NPMc+AML) characterized by normal karyotype and absence of major fusion genes (BCR/ABL1, PML/RARA, AML1/ETO, CBFB/MYH11, DEK/CAN)[1]. Denaturing high performance liquid chromatography (dHPLC) is as highly sensitive and accurate as direct sequencing[2]. To assess dHPLC specificity in detecting NPM1 mutations, we analyzed DNA from 120 primary adult Acute Myeloid Leukemia (AML) and compared dHPLC results with immunohystochemical and sequencing results.

Materials and Methods. Patients. 120 adult AML from the GIMEMA cooperative group were selected on the basis of reaction to an anti-NPM monoclonal antibody (26 NPMc+; 94 NPMc-).

Mutation detection. Genomic DNA derived from blood/bone marrow cells of all patients was extracted from blood and/or bone marrow aspirate as previously described[3]. RNA extracted by TRIzol (Invitrogen Life Technologies, Inc., Paisley, UK) was retrotranscribed with use of the Thermoscript RT-PCR System (Invitrogen). Sequencing was done using primers NPM1_25F (5'GGTTGTTCTCTGGAGCAGCGTTC3') and NPM1_ 1112R (5'CCTGGACAACATTTATCAAACACGGTA3'). PCR amplification fragments of the NPM1 gene was amplified using the following oligonucleotide primers described NPM1-F (5'TTAACTCTCTGGTGGTAGAATGAA3') NPM1R (5'CAAGACTATTTGCCATTCCTAAC3'). The twelfth coding exon of the NPM1 gene was screened for mutations by DHPLC (Wave System, Transgenomic Inc., Omaha, Nebraska, USA). Analysis gradient and temperature were determined according to the PCR product nucleotide sequence using Wavemaker software and then optimized by studying alterations in sample elution pro-

files. Electrophoregrams from patients were compared with normal controls.

Results. 94 NPMc- AML gave a wild type chromatogram. In the 26 NPMc+ leukemias the elecrotrophoregram profiles were different to the wild type and all corresponded to NPM mutations according to sequencing. Seven different elution profiles were obtained considering specific time of retention between homo and hetero-duplex, shape and number of peaks. No changes in melting temperature and elution gradient were needed to unravel the 7 mutations. Sequencing categorized three in 9/26, 5/26, and 5/26 patients, as mutations A, B and D respectively (e)1. In the other 7 patients elution profiles identified four new variants.

Conclusions. In conclusion, we have developed and validated a DHPLC method that identifies NPM1 mutations. We showed that dHPLC is as efficacious as direct sequencing in specifically detecting NPM mutations, including new variants. As a rapid and cost-effective approach, dHPLC may serve in multi-centre clinical trials

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S003

SUPERIORITY OF THALIDOMIDE-DEXAMETHASONE OVER VINCRISTINE-DOXORUBICIN-DEXAMETHASONE AS PRIMARY THERAPY IN PREPARATION FOR AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA

Cavo M, Zamagni E, Tosi P, Tacchetti P, Cangini D, Cellini C, Testoni N, Nicci C, de Vivo A, Terragna C, Perrone G, Ceccolini M, Grafone T, Tura S, Baccarani M, Writing Committee of the "Bologna 2002" clinical trial.

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Thalidomide represents a new treatment paradigm for multiple myeloma (MM), because of its alternative mechanisms of action that include disruption of myeloma-bone marrow stromal cell interactions, inhibition of cytokine secretion and immunomodulatory effects. We and other groups have recently reported that first-line treatment with thalidomide and dexamethasone (Thal-Dex) yielded a rate of response similar, or even superior, to that expected with conventional chemotherapy and did not interfere with subsequent collection of peripheral blood stem cells (PBSC). Based on these data, Thal-Dex has been proposed, and is currently accepted at many centers, as a front-line treatment option for patients with symptomatic MM, particularly if it is planned to offer subsequent high-dose therapy with autologous transplantation. However, no comparative study of Thal-Dex with vincristine-doxorubicin-dexamethasone (VAD), the reference treatment used so far to reduce tumor cell mass before autologous transplantation, has been reported. To address this issue, we performed a retrospective matched case-control analysis of 200 patients with symptomatic MM who were primarily treated with Thal-Dex (n=100) or VAD (n=100) in preparation for autologous stem cell transplantation as part of two consecutive studies conducted from 1996 to 2004. Case-matching was performed with respect to age (within 2 years), clinical stage at diagnosis (same stage, according to the Durie and Salmon system) and serum beta2-microglobulin levels (within 1 mg/L). By design of both studies, Thal-Dex and VAD were administered for 4 months. Thal was given at the starting dose of 100 mg/d for 14 days, and then increased to the dose of 200 mg/d. Dex combined with Thal or with vincristine-doxorubicin was administered at the dose of 40 mg/d on days 1 to 4, 9 to 12 and 17 to 20 (odd cycles) and 40 mg/d for 4 days on even cycles, repeated monthly. In both studies patients who proceeded to PBSC collection received high-dose cyclophosphamide (HD-CTX) (7g/m^2) and granulocyte-colony stimulating factor (G-CSF) (5 µg/kg/d, starting 48 hours after HD-CTX infusion and continuing until completion of PBSC collection). Thal was discontinued the day before administration of HD-CTX. Objectives of the study were to compare i) the rate of response (at least partial response, PR) to Thal-Dex with that to VAD; ii) PBSC yields following Thal-Dex and VAD, and iii) the toxicity profiles of both these regimens. On an intent-to-treat basis and using stringent EBMT criteria, response was documented in 76 patients out of 100 who were treated with Thal-Dex; the corresponding figure among patients who received VAD was 52 out of 100 (p=0.0004). The percentage of patients who failed on VAD was twice that observed in the Thal-Dex group (48% versus 24%, respectively; p=0.0004). The magnitude of tumor reduction effected by Thal-Dex or VAD was also evaluated by comparing the pre-treatment and post-treatment levels of monoclonal immunoglobulins. In comparison with VAD, Thal-Dex was found to induce more profound reduction in tumor cell mass, as reflected by significantly lower levels of residual IgG (p=0.02) and IgA (p=0.03) M proteins. Toxicities registered during therapy with Thal-Dex or VAD were different. The major toxicity of VAD was hematologic, particularly granulocytopenia; it was severe (grade 3-4) in 12% of patients. Neurotoxicity and severe cardiovascular events, usually congestive heart failure, were found less frequently, in 7% and 3% of patients, respectively. Side effects with Thal-Dex did not require dose reduction or interruption in most of the cases. The most common toxicity was DVT (15% of all cases, including 26% among the first 19 patients who did not receive any prophylaxis and 12% among 81 patients who were subsequently treated with low-dose warfarin); other grade 3-4 toxicities included constipation (9%), fatigue (6%), infections (4%), neuropathy (4%) and skin rash (1%). In each of the two treatment groups, 91% of patients received HD-CTX and G-CSF in an attempt to mobilize and collect PBSC. The median interval between start of therapy with Thal-Dex or VAD and HD-CTX was 138 days and 142 days, respectively. The median number of collected CD 34$^+$ cells was 7.85 x 10^6/kg for patients with prior exposure to Thal-Dex and 10.5 x 10^6/kg for patients who received VAD (p=0.4). Considering 4x10^6 CD 34$^+$/kg as the minimum threeshold dose to safely perform double autologous transplantation, adequate cell yields were obtained in 83% of patients treated with Thal-Dex and in 88% of patients in the control group (p=0.3). In both treatment groups the median number of aphereses to col-

lect adequate cell yields was 2. In conclusion, results of this retrospective case-matched comparison of Thal-Dex with VAD as initial therapy in preparation for autologous stem cell transplantation for MM provided demonstration of the superiority of Thal-Dex over VAD in terms of response rate and extent of tumor reduction. Obviously, data should be cautiously interpreted since the study was not randomized, albeit well controlled. Given that high-dose Dex is the most active component of VAD, it is tempting to replace this complex, and cumbersome to administer, combination with an oral regimen like Thal-Dex that avoids the morbidity and risks associated with central venous access, as well as the discomfort of continuous infusion of cytotoxic drugs and possible patients' hospitalization. While in our study irreversible toxicities were not seen with limited exposure to Thal-Dex, the increased risk of DVT associated with the use of this regimen in previously untreated patients should be considered. Newer and safer analogues of thalidomide that exert similar or higher antitumor activity without teratogenic effects of thalidomide are currently under evaluation in phase 3 clinical trials and could hopefully replace thalidomide in clinical practice in the near future; until then, thalidomide will continue to play a major role in the treatment of MM.

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S004

A LONG-TERM FOLLOW-UP STUDY ON THE ROLE OF CA-125 AS PROGNOSTIC FACTOR IN 214 NEWLY DIAGNOSED PATIENTS WITH HODGKIN'S LYMPHOMA

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This study has explored the possible value of Ca125 as prognostic factor in Hodgkin's lymphoma (HL) . Ca-125 is a glycoprotein with a high molecular weight (about 200Kd), that is expressed in coelomic epithelium during embrionic development. Radioimmunometric assays have provided a useful marker for clinical diagnosis and for monitoring response to treatment in a majority of patients with epithelial ovarian cancers. However, detailed information on its biochemical and molecular nature is lacking and more information is needed to full explore its potential and understand its mechanism of action. In neoplastic diseases, almost 30% of patient with non-ovarian cancer have been reported to have elevated Ca 125 serum levels. Reports on Ca-125 in lymphomas are infrequent and usually deal with a small number of cases or a single case. In particular, there are no published data for Ca-125 in HL.

Our objectives were: 1) To define the distribution of sCa125 levels at diagnosis in a large consecutive series of patients with Hodgkin's lymphoma. 2) To evaluate the possible existence of a significant correlation between sCa125, presentation features of the disease and standard prognostic factors. 3) To describe the trend of sCa125 during the treatment. 4) To evaluate the predictive significance for complete response (CR) and survival (FFP and OS) of sCa125 levels at diagnosis, and sCa125 variations during treatment. 5) To examine whether the antigen Ca125 is expressed in lymphomatous cells of patients. From October 1992 to Mars 2005 sCa125 214 newly diagnosed adult patients with HL, treated at the Unit of Haematology of NCI in Naples entered in this study (Table 1). Clinical status was evaluated by physical examination, computerized tomography (CT) of the thorax and abdomen, and bone marrow biopsy in all patients. Ga67 scan and/or FDG-PET were also used in the majority of cases. Patients were classified according to Ann Arbor and the Cotswold's meeting criteria. All 214 pts but one were treated with combination chemotherapy (ABVD=109; MOPP/ABVD and MOPP related =77; other CT schedules = 27). 113 out of 213 pts also received IF RT. All patients, regularly controlled in an outpatient setting, were observable for response and survival, The mean and median follow up were 59 and 58 months, respectively. CR was obtained in 189 pts (88%) and, as the 28th Mars 2005, FFP and OS rates were 78% and 85%, respectively. sCa125 was measured at presentation in all 214 pts. Using the standard upper limit of 35 U/mL the distribution of patients was 125 (58%) with values lower than 35 U/mL and 89 (42%) with values greater than 35 U/mL. Complete response (CR) Freedom from progression (FFP) and overall survival (OS) rates were statistically different in the two group: 75% vs 98% for CR , 60% vs 92% for FFP, and 72% vs 94% for OS (Table 2 and Figure 1) To test the efficacy of this cut-off value against other possible values we re-elaborated the statistics for cut-off at the upper limits of the first and second tertile. However, little was gained compared with using the standard cut-off of 35 U/mL. (Table 2 and Figure 2).

Table 1. Presentation features of 214 HL patients.

n. pts	Male sex	Age >45 yrs	Early Stage	Adv. stage	E sites >1	Bulky	B sym	Stage IV	high LDH	ESR > 50	nodal Sites >3	Groin nodes	WBC >15 X10³	Ly <0.6 X10³	Alb < 4 g/dL	Hb <10.5 g/dL	IPS 3+
214	108	55	62	152	24	83	89	34	89	103	93	49	35	27	145	31	63
%	51%	26%	29%	71%	11%	39%	42%	16%	42%	48%	43%	23%	16%	13%	67%	15%	29%

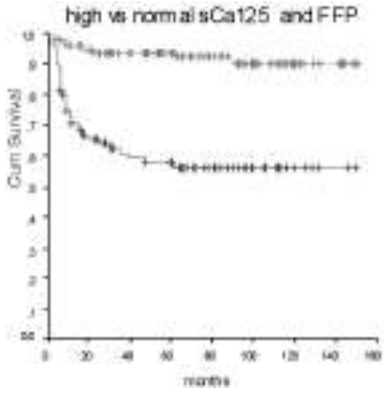

Figure 1.

Table 2. Prognostic factors predictive of CR, FFP and OS.

cance	N. of Pts	N. of Pts in CR	% of CR	exact test 2-sided	FFP rates	Log-rank signifiicance	OS rates	Log-rank Signifii-
total	214	189	88%		78%		85%	
sCa-125 (U/mL)								
1st tertile 3-18)	77	75	97%	0.001	90%	0.0001	95%	0.0001
2nd tertile (19-41)	67	64	96%		88%		88%	
3rd tertile (42-466)	70	50	71%		57%		70%	
>35 U/mL	89	67	75%	0.001	60%	0.0001	72%	0.0001
<35 U/mL	125	122	98%		92%		94%	
Advanced stage	153	130	85%	0.017	75%	0.0245	82%	ns
Early stage	61	59	97%		89%		92%	
Stage IV	34	23	68%	0.001	59%	0.0001	65%	0.0001
any other stage	180	166	92%		82%		88%	
E Sites 2+	24	15	62%	0.001	54%	0.0001	58%	0.0001
E sites 0-1	190	174	92%		82%		88%	
nodal sites > 3	121	113	82%	0.010	71%	0.0216	89%	ns
nodal sites < 3	93	76	93%		84%		78%	
Alb < 4 g/dL	145	123	85%	0.022	74%	0.0352	81%	ns
Alb > 4 g/dL	69	66	96%		87%		91%	
Lymph < 600 mmc	27	20	74%	0.023	63%	0.0201	67%	0.0073
Lymph > 600 mmc	187	169	90%		81%		87%	
inguinal node(s)	48	38	79%	0.039	68%	ns	73%	ns
no inguinal	166	151	91%		81%		88%	

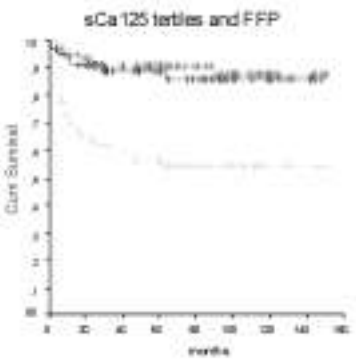

Figure 2.

A high pre-treatment sCa125 was statistically associated with advanced stage, bulky disease, stage IV, E sites>1, serum albumin level <4 g/dL, serum LDH ratio >1, Hgb <10.5 g/dL and B symptoms. At univariate analysis (Pearson Chi-square and Fisher's Exact Test) advanced stage, sCa125, Stage IV, albumin <4 g/dL, lymphocytopenia, nodal sites>3 and E sites>1 were the prognostic factors predictive of lower CR rate. In addition, only Ca125, stage IV, E sites>1, and lymphocytopenia were significantly predictive of lower survival rates (Table 2). At multivariate analysis (Stepwise Cox regression) sCa125 (loss chisquare=26 Sig. 0.0001) and E sites>1 (loss chisquare=5 Sig. 0.019) were the only independent prognostic factors that negatively influenced the outcomes for FFP, and OS. sCa125 was monitored during treatment, at the end of treatment, and during the follow-up, in 51 out of 89 pts with a pre-treatment value upper the limit of 35 mU/mL, and in 34 out of 125 pts with a pre-treatment level within 35 mU/mL. 13 out of 51 monitored pts, with sCa125>35 U/mL did not achieved a CR; all these 13 pts maintained an abnormal level of sCa125 during and at the end of the treatment; 36 out of 38 monitored pts with sCa125>35 U/mL who had a CR, normalized sCa125 at the end of treatment. The remaining 2 patients maintained higher level of sCa125 at the end of treatment and during the follow up; they both relapsed within the first year. 33 out of 34 monitored pts with sCa125 within the cut-off of 35 mU/mL maintained lower values of the marker during the follow-up . All these 33 pts were in continuous CR.The remaining patient relapsed at 20 months from diagnosis.

In 15 cases, of this series of newly diagnosed HL patients, the Ca 125 expression was studied in paraffin embedded lymph node biopsies; no traces of the Ca125 antigen was detected in lymphomatous cells. Since the close relationships between high sCa125 and presence of pleural effusions and/or ascite, it has been suggested to look towards the role of reactive mesothelium in the secretion of CA 125. In our series, the only 9 patients who had , at diagnosis, ascite and/or pleural effusion showed very high sCa125 levels. In addition 52/83 pts (63%) with bulky disease (almost all in the mediastinum) and 17/24 (71%) with more than one extranodal site of disease showed , at diagnosis, high sCa125 levels. This may suggests that an elevated Ca 125 serum level at presentation may reflect an occult involvement of serous membranes which determines a reactivity of mesothelium and consequently a CA 125 secretion. However in many other cases of this series abnormal sCa125 levels were apparently independent from presentation features.In conclusion in this series of 214 newly diagnosed HL patients:

1. sCA 125 was significantly elevated in over 40% of newly diagnosed HL pts.
2. Patients with High sCa125 levels had a statistically significant reduction of CR; failure of normalization of sCA125 during the treatment was indicator of incomplete response.
3. Advanced stage, E sites>1, stage IV, serum albumin <4 g/dL, lymphocytopenia <600 mmc., nodal sites>3, inguinal involvement were the other factors predictive of a lower CR rate.
4. High sCa125, Stage IV, Extranodal site>1, lymphocytopenia <600 mmc were the only prognostic factors strongly predictive of lower FFP and OS rates.
5. sCa125 and extranodal disease were the independent factors that negatively influenced the outcomes for FFP, and OS.
6. Ca125 antigen seems not expressed in lymphoma cells.

These results induce us to consider sCa125 a simple, reliable, costless and reproducible tool useful to refine the existing prognostic score systems. The mechanisms of sCa125 secretion remain unclear.

S005

CD8+ CD57+ T CELLS WITH SPECIFIC T CELL RECEPTOR SEQUENCES MAY BE IMPLICATED IN THE PATHOGENESIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of the hematopoietic stem cell (HSC) characterized by a somatic mutation in the X-linked PIG-A gene: this results in complete or partial deficiency of all proteins anchored by the glycosylphosphatidylinositol (GPI) on the cell membrane of the mutated HSC and its progeny. Clinical features of PNH are intravascular hemolysis, venous thrombosis, and variable degrees of bone marrow failure. The close association between PNH and idiopathic aplastic anemia (IAA), and other lines of evidence, support the hypothesis that auto-reactive T cells might be responsible for the expansion of hematopoietic PNH clone(s), which is required to cause clinical PNH. Specifically, these T cells might damage selectively normal HSC, whereas PNH HSC survive and expand because they escape the attack. Our observation of a unique patient with PNH and with a large granular lymphocyte leukemia (*Karadimitris et al, Br J Haematol 2001, 115:1010*) has strongly suggested that this clonal expansion of $CD8^+$ $CD57^+$ T cells (NKT) could be responsible for the damage to normal HSC in this patient. Thus, we have measured the percentage of NKT cells in the peripheral blood of 18 PNH patients and 21 healthy controls. The proportion of this cell population was quite variable and similar in patients (7.0±6.1; range: 0.8–22.3%) and in controls (6.7±4.7; range: 0.9–21.2. $p>0.5$). However, the analysis of the size distribution of the complementarity-determining region 3 (CDR3) of the TCR-beta chain genes in sorted NKT cells has shown a difference. In 8 controls we observed a normally distributed ladder of bands of different sizes. By contrast, in 17 out of 18 PNH patients we observed a non-random (*oligoclonal*) pattern; and in each patient some clones were predominant. In 6 patients followed-up longitudinally over 6-18 months the *oligoclonal* pattern was consistent and persistent. In each of 11 patients in whom we carried out systematic sequencing of the TCR-beta CDR3 of sorted NKT cells we have observed an average of 25 (range: 17 to 39) different CDR3 sequences out of an average of 80 total sequences obtained: but only one or two sequences were predominant. Interestingly, an identical or quasi-identical (single amino acid difference) sequence was found in 4 patients; and in two of these the sequence belonged to one of the predominant clones. The presence of clones bearing this same CDR3 sequence has been demonstrated in follow up samples from the same patients, but not in samples from 3 healthy controls. In addition, in 5 cases a sequence found in one patient was subsequently found also in another patient. Our data are similar to the findings reported in patients with IAA (*Risitano et al, Lancet 364:355, 2004*): however, in IAA patients no identical sequences were found. It is possible that specific T cells clones may be responsible for damage to normal HSCs in both PNH and IAA patients. However, it is possible that in IAA there is a large variety of antigens that T cells may recognized on HSCs in each patients; whereas in PNH the number of potential target antigens is more restricted, because they must be present on normal HSC but not on PNH HSCs, thus enabling them to escape the auto immune attack and to expand. These data support the hypothesis that NKT cells include a subset of cells that may be directly implicated in the pathogenesis of PNH.

S006

SIGNIFICANCE OF JAK2 VAL617PHE MUTATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Most cases of essential thrombocythemia (ET) have traditionally represented a coumbersome diagnostic task for the clinician because of the lack of specific markers of disease. The recent description of a Val617Phe mutation in the exon 12 of JAK2 gene may represent the first reliable molecular marker of ET although, unlike in polycythemia vera where 74-97% of patients studied presented the mutation, only 32-50% of patients with ET were Val617Phepositive, a figure comparable to the finding in 35-57% of patients with idiopathic myelofibrosis.

The aim of the study was to evaluate the incidence of JAK2 mutation in patients with ET and its association with clinical characteristics and with the results of some putative biomarkers of disease. Eighty-four pts (median age 49, range 16-89 years) with a diagnosis of ET based on WHO criteria were studied; there were 7 males and 77 females. Pts were either newly diagnosed or established cases in follow-up. Forty-two pts had presented thrombotic signs, either or both major arterial and/or venous thromboembolism and microvascular symptoms, while major thrombosis were documented in 20 (24%). JAK2 mutation was evaluated on granulocyte DNA using an allele-specific PCR, as originally reported by Baxter *et al.* (*Lancet 2005; 365:1054*). Clonality status of hematopoiesis, evaluated with the HUMARA assay, was available in 77 patients. Endogenous erythroid colonies (EEC) growth from peripheral blood was determined in methylcellulose in 65 patients. Platelet c-Mpl content was assayed by a Western blot methodology as described (*Vannucchi AM et al, BJH, 2004; 127:214*) in 60 patients. The expression of PRV1 was quantified by Real Time RT-PCR on granulocyte mRNA in 81 patients. Forty-three patients (51%) had the JAK2 Val617Phe mutation (JAK2positive). There was no correlation between JAK2 mutational status and a number of clinical characteristics, including platelets or white blood cell count at the diagnosis or splenomegaly; on the other hand, there was a positive correlation between JAK2positivity and higher hematocrit level ($p<0.01$). There was also no meaningful correlation between JAK2 mutational status and the different cellular and molecular assays employed, as detailed in the Table. Furthermore, while

clonality status maintained its significant correlation with thrombotic events (all and major only), as reported previously, the presence of JAK2 mutation had no impact on thrombosis events. These data indicate that the 50% of ET patients bearing the JAK2 mutation do not cluster in a well-defined subgroup of pts, based on clinical and laboratoristic parameters; phenotypic heterogeneity still persists. Furthermore, the presence of subjects with clonal myelopoiesis but negativity for the JAK2 mutation suggests that other still unknown clonal molecular abnormality(ies) must be searched for.

Table 1.

	N. pts evaluable	N positive pts (% of evaluable)	JAK2^{positive} (n=43) All pt	JAK2^{pos}	JAK2^{negative} (n=41) All pt	JAK2^{neg}
JAK2 **Val617Phe mutation**	84	43 (51%)	51%	-----	49%	-----
CLONAL HEMATOP.	77	50 (**60%**)	**46%**	57%	**54%**	73%
ABNORMAL PRV1	81	29 (36%)	**62%**	44%	**38%**	27%
EEC^{positive}	65	31 (48%)	**52%**	50%	**48%**	45%
REDUCED c-MPL	60	44 (73%)	**43%**	68%	**57%**	78%
THROMBOSIS	84	42 (50%)	**45%**	44%	**55%**	23%
MAJOR THOMBOSIS	84	20 (24%)	**45%**	21%	**55%**	27%

S007

A PROSPECTIVE RANDOMIZED TRIAL OF ORAL MELPHALAN, PREDNISONE, THALIDOMIDE VS ORAL MELPHALAN, PREDNISONE: AN INTERIM ANALYSIS

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In multiple myeloma (MM) untreated patients, the combination melphalan, prednisone and thalidomide (MPT) induces a fast tumor response with a high complete remission rate. We compare the efficacy and toxicity of oral MPT and MP in a prospective multicenter randomized study. The end points of the trial were: response, EFS, OS and toxicity.

Between January 2002 and December 2004, 250 patients (median age 72, range 56-85) with newly diagnosed symptomatic multiple myeloma were randomized to receive either 6 courses of oral MP (melphalan 4 mg/sqm and prednisone 40 mg/sqm for 7 days) or 6 courses of oral MPT (melphalan 4 mg/sqm and prednisone 40 mg/sqm for 7 days every month plus thalidomide 100 mg/day continuously). The dose of thalidomide was reduced to 50% if grade II toxicity occurred, and suspended for any grade III or any sign of progressive disease or relapse. On December 2003, the protocol was amended and enoxaparin prophilaxys was added, because of high rate of thromboembolism. At present, 177 patients were evaluated for toxicity and response. All results were evaluated on an intent-to-treat basis. Response were evaluated according to the EBMT/IBMTR criteria. The response rate after MPT was: 22.2% immunofixation negative CR (CR), 5.5% immunofixation positive near CR (nCR), 49.4% partial remission (PR) (M-protein reduction 50-99%), 14.5% stable disease (SD) (M-protein reduction 0-49%) and 8.4% progressive disease (PD). The response rate after MP was 4.2% CR, 1.2% nCR, 41.3% PR, 25.3% SD and 28% PD. Response was followed by significant improvement of performance status, skeletal pain, anemia and transfusion requirement. After a median follow up of 18 months, 38 patients relapsed: 11 (29%) after MPT and 27 (71%) after MP. The median EFS was 25.2 months after MPT and 13.7 after MP ($p<0.001$). The median OS has not been reached.

Treatment-related mortality was 5% after MPT (1 septicemia, 1 pulmonary thrombo-embolism, 1 renal failure and 1 heart failure), and 5% after MP (1 myocardial infarction, 1 heart failure, 1 septicemia and 1 disease progression). The major adverse events of MPT vs MP were: deep-vein thrombosis (19% vs 4%), grade III-IV infections (10% vs 1%), grade I-II neurotoxicity (32% vs 11%), grade III-IV hematologic toxicity (18% vs 25%). Thalidomide discontinuation was required in 33.8% of patients (8 thrombo-embolic events, 4 neurotoxicities, 4 constipations, 2 infections, 3 miscellaneous); dose-reduction in 24.2% of patients (8 neurotoxicities, 3 constipations, 4 miscellaneous). In conclusion MPT significantly improves response rate and EFS in elderly myeloma patients with a median age of 72 years. An update of these data will be presented.

S008

CLINICAL SIGNIFICANCE OF SPONTANEOUS APOPTOSIS IS INDEPENDENT FROM IMMATURITY AND PROLIFERATION IN ACUTE MYELOID LEUKEMIA

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Multidrug resistance and recurrent disease are central themes in the variable response of acute myeloid leukemia (AML) patients to treatment. Biological heterogeneity lies at the bottom of these key problems: cytogenetics, P-glycoprotein, apoptosis resistance and autonomous proliferation in vitro have been associated in turn with a poor prognosis. Higher levels of anti-apoptosis related proteins bcl-2, bcl-xl, Mcl-1 were reported in CD34^{+} AMLs presenting a poor outcome (Del Poeta G, *et al*, 2003). On the other hand, expression profiling studies in blasts from relapsed AML showed mRNA expression changes in genes correlated with increased cellular proliferation and indicated by the overexpression of the transferrin receptor CD71 (Staber PB, *et al*, 2004). Therefore, in order to verify whether the immaturity and/or the proliferative profiles may explain apoptosis levels and prognosis in AML, from 1995 to 2004, a large series of 325 patients, affected by de novo AML, except FAB M3, median age 55 years, treated with intensive chemotherapy regimens (EORTC/GIMEMA AML10, AML12 and AML13) were studied. The aims of our research were: 1) to correlate bax/bcl-2 ratio, representing a measure of spontaneous apoptosis, with progenitor markers (CD34, CD117 and CD133) and the proliferative rate levels (CD71) and 2) to demonstrate that the clinical significance of spontaneous apoptosis is independent either from immaturity or proliferation. Bcl-2 and bax proteins, CD34, CD117, CD133 and CD71 were determined by multicolor flow cytometry. The thresholds of positivity

were set at >20% for CD34, >10% for CD133 and >375 for CD117 (evaluated both as percentage and mean fluorescence intensity [MFI]. CD71 was evaluated as MFI and the threshold was set at >5. Bax/bcl-2 was obtained by dividing MFI bax/MFI bcl-2 and the threshold was set at >0.3 (median value). One hundred-seventy five patients were bax/bcl-2 ratio positive (175/325; 53.8%). There were close correlations between bax/bcl-2 ratio and CD34 (p<0.00001) or CD117 (p<0.00001) or CD133 (p=0.010). On the other hand, no significant correlation was found between bax/bcl-2 and CD71, confirming that different apoptosis profiles may have variable proliferation levels. With regard to clinical outcome, a significant lower complete remission (CR) rate was found in patients with lower bax/bcl-2 ratio (43% vs 72%, p<0.00001). Overall survival (OS) was significantly shorter in patients with lower bax/bcl-2 ratio (1% vs 15% at 4 years; p=0.00001) and a longer disease-free survival (DFS) was observed in patients with higher bax/bcl-2 ratio (21% vs 0% at 2,5 years; p=0.001). In order to confirm the independent prognostic value of bax/bcl-2 ratio either from immaturity or proliferation, we enucleated and then investigated the following AML subsets: $CD34^+$ (187 patients), $CD117^+$ (137 patients), $CD133^+$ (80 cases), CD71+ (204 patients) and CD71- (120 cases). As a matter of fact, a lower CR rate was found in patients with lower bax/bcl-2 ratio either within $CD34^+$ (41% vs 74%, p=0.00008) or $CD117^+$ (32% vs 64%, p=0.0007) or $CD133^+$ (41% vs 71%, p=0.010) or $CD71^+$ (34% vs 61%, p=0.0009) or CD71- (60% vs 90%, p=0.0006) subgroups. Also a lower bax/bcl-2 ratio was associated with shorter OS and DFS in $CD34^+$ (0% vs 22% at 3,5 years, p<0.00001; 0% vs 27% at 2,7 years, p=0.0027, respectively), in $CD117^+$ (0% vs 10% at 3,2 years, p=0.017; 0% vs 16% at 2,7 years, P=0.025, respectively), in $CD133^+$ (0% vs 24% at 2,5 years, p=0.004; 0% vs 27% at 1,2 years, p=0.034, respectively) and finally in $CD71^+$ (2% vs 10% at 4 years, p=0.008; 0% vs 6% at 2,5 years, p=0.05, respectively) and, more significantly, in $CD71^-$ (0% vs 24% at 3,5 years, p=0.00008; 0% vs 30% at 2,7 years, p=0.003, respectively) patients. The independent prognostic value of bax/bcl-2 ratio was confirmed in multivariate analysis with regard to CR (p=0.00007) and OS (p=0.00003). From a biological point of view, our results confirm that in AML apoptosis and proliferation are independent events, whereas apoptosis and maturation pathways are significanltly correlated. From a clinical point of view, the capacity of bax/bcl-2 ratio of clearly identifying patients at different prognosis within *immature* and *proliferative* or *non-proliferative* subsets implies that apoptosis has an intrinsic more relevant clinical significance in AML. Therefore, our study confirms the superior prognostic role of mitochondrial apoptotic proteins over maturation and proliferation tools. That has to be taken in account when therapeutic strategies are to be planned in order to resolve chemoresistance and relapse in AML.

S009

A PREVIOUSLY FAILED AUTOGRAFT SIGNIFICANTLY INFLUENCE NON-RELAPSE MORTALITY AND OVERALL SURVIVAL IN PATIENTS OVER 55 YEARS RECEIVING REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC TRANSPLANTATION

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Allogeneic stem cell transplantation (SCT) represents a potentially curative treatment for several hematologic malignancies, but is associated with a relevant nonrelapse mortality (NRM); in particular, older age and a previously failed autograft are considered risk factors associated with a NMR ranging between 50% and 80%. We report the results of a prospective multicenter study investigating the impact of age and previously failed autograft on the NRM of 150 patients affected by hematological neoplasms. Patients received the same reduced intensity conditioning (RIC) (Thiotepa 10 mg/Kg, Fludarabine 60 mg/ms and Cyclophosphamide 60 mg/kg and) and GVHD prophylaxis (cyclosporin 2 mg/kg and short course methotrexate). They were divided in two cohorts according to the age: 90 patients were younger and 60 older than 55 years. Pretransplant characteristics were fairly balanced between the two cohorts. At a median follow-up of 927 days, the estimated 5-year OS was 66% for younger and 61% for older patients (p=0.25); the estimated 5-year NRM was 13% for younger and 19% for the older cohort (p=0.1). On univariate analysis, a statistically significant association was found between a previously failed autograft and higher NRM in the elderly cohort: in fact estimated 5-year NRM was 11% for patients not having failed a previous autograft versus 37% (p=0.01). Also older patients with refractory disease had a significantly higher risk of NRM as compared to chemosensitive patients (31% vs 8%, p=0.03). In multivariate analysis, a statistically significant interaction was found between classes of age and both disease status (p=0.03) and previous autografting (p=0.02), indicating that older patients with refractory disease or a failed autograft had a NRM rate higher than younger ones. Moreover the association of elderly age and previously failed autograft had a significantly negative impact on OS (p=0.002) if compared with younger patients. A significantly higher NRM was observed in patients developing grade III-IV aGVHD in both cohorts. When the effect of chronic GVHD on NRM was evaluated, we found a difference between limited and extensive cGVHD: it was statistically significant for younger patients (NRM rate of 0% versus 28%, p=0.03) while there was a trend in the elderly (NRM rate of 0% versus 31%, p=0.05). On univariate analysis the onset of grade III-IV aGVHD represented a significantly negative predictor for OS: 5-year OS was 42% and 21% for young and older patients with grade III-IV aGVHD instead of 60% (p=0.05) and 85% (p<0.001) for patients experiencing grade I-II aGVHD, respectively. Also chronic GVHD had an impact on survival: in fact in the young group OS was

82% for patients with limited cGVHD compared to 45% for pts with extensive cGVHD (p=0.2); in the elderly OS was 100% vs 33% (p=0.007) respectively for patients with limited and extensive cGVHD. Our results indicate that RIC transplants show a rather low NRM, age above 55 years per se cannot be considered a risk factor anymore. Timing of transplant and novel strategies for GVHD prevention could further improve the patients' outcome.

S010

THE INHIBITION OF IKK KINASE INDUCES GROWTH ARREST AND APOPTOSIS IN AML BLAST CELLS

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The therapeutic results for many patients affected by acute myeloid leukemia (AML) are at present largely unsatisfactory. The overall failure of current treatments is even more disappointing in older patients who cannot be enrolled in clinical trials with conventional chemotherapy. It is therefore of great interest to identify specific molecular targets to design new therapeutic approaches. An increased NF-kB activity has been demonstrated in blast cells from AML patients. PS1145 (Millennium) is a compound which acts as an inhibitor of NF-kB through the inhibition of the IKK kinase. The aim of the study was to evaluate the in vitro effects of PS1145 in AML blast cells and cell lines collected from AML patients at diagnosis. BM cells collected form 15 AML patients had been studied. Purified blast cells were obtained from BM samples by MACS separation in 8 out of 15 cases, in the remaining 7 cases unfracrtioned BM cells had been analyzed. Moreover HL60 and U937 cell lines had been analyzed. As control we analyzed 5 BM samples from healthy volunteers. Cells were incubated with 20 microM PS1145 for 24-48 and 72 hrs. The inhibition of NF-kB DNA binding activity was evaluated using an ELISA method. Immunofluorescence technique with an antibody against NF-kB for the detection of nuclear-cytosol localization has been performed. The inhibition of IKB phosphorilation has been demonstrated by Western Blot. The proliferation rate was evaluated by MTT assay and the percentage of apoptotic cells by the detection of annexin V positive cells. After incubation, colony growth inhibition has been evaluated. In HL60 and U937 the incubations with PS1145 resulted in an inhibition of NF-kB binding activity of 70% and 85%. The proliferation rate was reduced of 63% and 72% respectively and the apoptotic cells increased to a value of 55% and 58%. In all these experiments, western blot detected the absence of the phosphorilated form of IKBalpha. Similar results were obtained in BM MNC cells and sorted blast cells from AML patients. After incubation western blot assay demonstrated the block of IKB phosphorilation, and immunofluorescensce analysis was able to detect the localization of NF-kB at the cytoplasmatic levels. ELISA assay performed after incubation with PS1145 20 microM was able to detect a reduction of the NF-kB DNA binding activity of 85% as a mean value (range 70-92%). After 48 hrs of incubation with PS1145we detected a decrease of proliferation of 62% (range 35-75%) and an increased percentage of apoptosis to a mean value of 75%.(range 54-89%). In addition, colony growth was suppressed of 62% as a mean value (range 46%-70%). BY contrast no significant effects were noted in normal samples, neither in terms of proliferation nor of apoptosis or colony growth inhibition. These data demonstrated that the in vitro treatment with the NF-kB inhibitors PS1145 is able to block the proliferation and to induce apoptosis in AML blast cells. IKK may therefore be considered an attractive target for a molecular therapy in AML patients not candidate for conventional chemotherapy.

S011

THE KINETIC OF REDUCTION OF MINIMAL RESIDUAL DISEASE IMPACTS ON DURATION OF SURVIVAL AND RESPONSE OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Multiparametric flow-cytometry (MPFC) is frequently used to assess the levels of minimal residual disease (MRD) in acute myeloid leukemia (AML) patients achieving complete remission after intensive chemotherapy. In our previous experience, MRD negativity after consolidation therapy, as defined by a level of bone marrow residual leukemic cells (BMRLC) > $3.5x10^{-4}$, was associated with a significantly longer disease free survival (DFS) and overall survival (OS). The present analysis was designed to confirm the prognostic role of a delayed MRD determination (at consolidation check-point) and to analyze the kinetic of MRD reduction (at induction and consolidation checkpoint) in a large series of 100 AML patients receiving intensive chemotherapy. The patients were entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61yrs) or AML13/AML15 (age >61 yrs), consisting in intensive induction and consolidation cycles and allogeneic or autologous stem cell transplantation for patients aged < 61 years. Median age was 52 years (range 18-78), all FAB subtypes were represented with the exception of APL cases which were not included. The analysis of the quartile distribution of MRD levels identified a threshold of $2x10^{-4}$ BMRLC corresponding to the lower quartile, both after induction and consolidation. After induction course, of 100 patients, 30 (30%) had <$2.0x10^{-4}$ BMRLC and were considered MRD–, the remaining 70 were MRD+ (>$2.0x10^{-4}$ BMRLC). Ninety-two patients proceeded to the consolidation phase (8/100 had an early relapse after induction) and were suitable for the analysis. After consolidation, 26 patients (28%) tested MRD–. The remaining 66 (72%) were MRD+ since the measured levels were >$2.0x10^{-4}$ BMRLC. However, among these MRD+ patients in 11 the level or BMRLC, although still above the value of $2.0x10^{-4}$, was reduced by at least 1-log as compared to the post-induction assessment. These 11 patients were considered as having a *chemosensitive MRD*, the remaining 55 MRD+

patients did not show any significant modification in the amount of BMRLC between induction and consolidation check-point and their condition was called *chemoresistant MRD*. Therefore, we selected 3 discrete categories of patients: 1) 26 patients MRD$^-$ at the end of consolidation therapy (BMRLC <2.0x10^{-4}); 2) 11 patients MRD+ at the end of consolidation (BMRLC >2.0x10^{-4}) but with *chemosensitive MRD*; 3) 55 patients MRD$^+$ at the end of consolidation therapy (BMRLC >2.0x10^{-4}) but with *chemoresistant MRD*. These 3 groups differed significantly in terms of relapse rate (23% vs 45% vs 81%, respectively) both in univariate and multivariate analysis (p<0.001). Accordingly, 5-years OS (74%, 50% and 15%, respectively) and DFS (67%, 49% and 17%, respectively) duration also differed (p<0.001). The multivariate analysis confirmed the independent prognostic role of MRD status at the end of consolidation both for OS and DFS (p=0.039 and .008, respectively). In conclusion, 1) post-consolidation MRD evaluation provides the best predictive information on patients' outcome, independently from the MRD status at the post-induction check-point; 2) the quantitative determination of MRD at specific time-points may allow the identification of MRD$^+$ patients with variable prognosis (chemosensitive vs chemoresistant MRD$^+$ patients).

S012

GENE EXPRESSION PROFILING AND FLT3 ABERRATION IN ACUTE PROMYELOCYTIC LEUKEMIA

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Acute Promyelocytic Leukemia (APL) represents approximately 10-15% of Acute Myeloid Leukemias (AMLs) and is considered an unique disease clearly distinguishable from the other types of AMLs for its clinical, haematological and molecular characteristics. Nevertheless, there are some different aspects that are variable among APL patients: first, beside the classical morphologic type, known as hypergranular form (M3), a hypogranular or microgranular APL variant form (M3v), that account the 15-20% of cases, was identified. Beside this, other aspects appear variably present among APL patients: peripheral white cells count at diagnosis is increased in only a minority of patients, mostly related to the M3v subtype; due to the variable breakpoint on the PML gene involved in the t(15;17) translocation, a long and a short form of the PML/RARα chimeric transcript have been recognized and, more relevant, although the great majority of APL patients experiences a long term remission, a significant number of them, equal to 20-30%, dies for early hemorrhagic complication or for leukemia relapse. Despite the fact that APL cells invariably express aberrant fusion proteins involving the RARα gene, mainly joined with the PML gene, data deriving from PML/RARα transgenic mice experiments suggest that additional mutations are required to develop APL. A candidate gene to play such a role is FLT3, a receptor tyrosine kinase (RTK) belonging to type III family. Mutations, leading to a constitutively activation of the FLT3 receptor kinase, are found in about one quarter of all AMLs and are considered the most common genetic alteration in human acute leukemia. In particular, internal tandem duplications (ITDs) or point mutations of FLT3 seem to be present at even a higher frequency (35-40%) in human APL cases. Association between FLT3 mutations and clinical or molecular characteristics of APL has been found, and in particular high white blood cell count, M3v morphology and the presence of the short PML/RARα isoform were reported to be related to FLT3-ITDs. We assessed the FLT3-mutation status in 29 adult patients with APL at the time of diagnosis and in 3 relapsing patients. 12 APL patients (41.3%) carried a FLT3 mutation (ITD and/or Asp835 mutation), whereas 17 APL patients (58.6%) showed a FLT3 gene in a wild-type configuration (FLT3-WT). As expected, FLT3-ITD group was significantly associated with higher white blood cell count (p=0.009) at presentation as well as higher circulating blast cell percentage (p=0.021). Moreover, the presence of ITD in FLT3 gene was related to M3v (p<0.0001) and short-type PML-RAR-alfa isoform (p=0.042). 18 APL patients, selected from our series for the high quality of total RNA obtained, were hybridized on microarray containing approximately 20,000 human genes synthesized by inkjet technology. FLT3-ITD and FLT3 D835 mutation were present in 7 and 3 cases respectively, one patient had both FLT3 alterations and the remaining 7 cases evidenced a wild-type configuration of FLT3 gene (FLT3-WT). Resultant gene expression profiles were first analysed using an unsupervised, agglomerative hierarchical clustering with average link as heuristic criteria and correlation with mean subtraction as similarity metric. Beside a homogeneous expression profile pattern, subtle differences in gene expression had the strength to distinguish three clusters of APL patients (designed I, II, III). Comparisons between clusters patients clearly revealed a preferential distribution of FLT3 gene mutated cases in cluster I. In particular, all FLT3-ITD patients clustered tightly in the same leftmost side (group I) and all but one (case 19) FLT3-WT patients clustered in the right side of the dendrogram (group II and III) (p=0.007). 3 of 4 FLT3-APL cases localized on the right branch of the dendrogram more closed to FLT3-WT cluster. Again, cluster I was related to M3v (p=0.035) and short-type PML-RARα isoform (p=0.044). In particular, 71.4% (n=5) of cluster II evidenced a classic APL morphology in contrast with only 11.1% of patients in cluster I. Moreover, most t(15,17) breakpoint cluster region 3 belonged to cluster I (80% of all bcr3 cases), whereas 80% of bcr1 belonged to cluster II. Furthermore, cluster I was significantly associated with higher circulating blast cell percentage (p=0.030) at presentation as well as hyperleukocytosis (p=0.009). 147 genes were significantly differently expressed among classes. Among them, 92 genes were up-regulated in FLT3-ITD class and 55 were down-regulated. When the 147-gene list was applied to hierarchical clustering of the 14 FLT3-ITD and –WT specimens, all samples except one were correctly clustered. In conclusion, ITD-FLT3 is the characteristic that better discriminate into two subgroups APL cases. Differentially expressed genes were identified that were involved in blood coagulation, cell adhesion and proliferation as well as in cytoskeleton organization, cell migration and maturation.

Oral Communications

Acute and Chronic Lymphoproliferative Disorders

C001

HAIRY CELL LEUKEMIAS CARRY SOMATICALLY MUTATED AND UNMUTATED VH AND VL GENES WITH BIASED V-GENE USAGE AND SECONDARY LIGHT CHAIN REARRANGMENT

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Immunoglobulin (Ig) gene analysis delineates critical features of the clonal history of a B-cell tumor. After antigen interaction, mature B-cells undergo somatic mutation of the V-genes in the germinal center (GC), and isotype switch events also occur there. From small series of cases we observed that most hairy cell leukemias (HCL) carry mutated VH genes, with low levels of intraclonal heterogeneity, while a minor subset have unmutated VH genes. Both subsets commonly have ongoing Ig isotype switch events. However they lack germinal center markers CD27 and CD38. HCL also typically lack CD23, a chemokine essential for lymph node entry.

In order to further probe the differentiation status of the cell of origin and the events post-transformation, the full sequences of the expressed tumor VH and VL genes were evaluated for somatic mutation events in an expanded series of HCL (37 cases). The VH3 family was used most commonly (24/37, 65%), with significant preference of the VH3-30 member in 9/37 cases (24%), while JH4b segment was used in 50% cases. Most HCL (34/37) cases carried variable tiers of mutations in the VH genes (87.0-98.6% homology to germline), with low levels of intraclonal heterogeneity also documented in cases with 98%< homology <100% to germline. However, 3/37 (8%) HCL displayed completely unmutated (homology 100%) VH genes. Thirteen of 37 HCL were evaluated for the tumor VL genes. The lambda light chain was preferentially used (9/13, 69%). All (9/9) lambda cases used Jlambda3, and 3/9 (33%) used Vlambda3h segment. Consistently with the VH genes, 11/13 cases carried mutated VL genes (94,75%-98.1%) with low levels of intraclonal heterogeneity, while 2 cases carried unmutated VL genes. Interestingly, functional secondary VL chain rearrangement was observed in the tumor cells of 2/13 cases. In both cases, the first VL gene was mutated, while the second rearrangement carried unmutated VL genes, suggesting receptor revision in the light chain of the tumor clone. N-glycosylation sites, that are commonly introduced in the B-cell receptor of tumors of germinal center derivation by somatic mutation, were not introduced either in the VH or in the VL functional genes. These data confirm heterogeneity in the cell of origin in terms of mutational status, with a minor subset with unmutated VH genes. Low levels of ongoing mutations, restricted V-gene segment usage and the new observation of secondary light chain rearrangement indicate that selection by antigen could be a promoting factor in HCL development. Lack of novel glycosylation sites is in favour of interaction with antigenic stimuli that have occurred at extrafollicular sites.

C002

EARLY NEGATIVITY OF MINIMAL RESIDUAL DISEASE PREDICTS AN EXCELLENT OUTCOME WITH CHEMOTHERAPY ONLY IN UNSELECTED ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Introduction. Clinical prognostic models for adult ALL do not predict with great accuracy the risk of recurrence, which implies, for several patients and within a risk-adapted strategy, under- or over-exposure to a given therapy, including transplants. A study was initiated to investigate MRD as *best* predictive factor for relapse and decisional aid to choose among available options. This report describes the outcome of patients achieving MRD negativity, for whom chemotherapy only was planned.

Methods. Adult patients with ALL underwent homogeneous induction-consolidation consisting of IDR-VCR-PDN+/-ASP/CY (cycles 1-3, 5, 6, 8) and HD-MTX/Ara-C (cycles 4, 7) plus CNS chemo-radioprophylaxis (Phase A). Three marrow samples (S) obtained before cycle no. 4, 6, 8 were analyzed for MRD by PCR/RQ-PCR analysis of fusion genes or Ig-H/TCR rearrangements. MRD negativity was defined by a negative/low-positive ($<10^{-4}$) S-2 and a totally negative S-3. All patients with MRD- results were considered for maintenance therapy only (Phase B: 1st year 6MP/MTX alternating monthly with VCR/PDN or CY; 2nd year 6MP/MTX). MRD+ cases were instead considered for intensification with more high-dose therapy or allogeneic SCT. The only exception to this design concerned patients with Ph+/t(4;11)+ ALL, who were eligible to SCT as early as possible and regardless MRD study results.

Results. Adult ALL NILG (Northern Italy Leukaemia

Group) trial 09/00 was activated V/2000, enrolling so far 215 ALLs and 15 lymphoblastic lymphomas (LL) (last analysis IX/2004): age 16-66 (median 37) years, B-lineage 182, T-lineage 48, Ph^+ 49, t(4;11)+ 11. CR rate was 84%. One-hundred nine patients completed Phase A and were thus eligible to MRD-oriented Phase B. The MRD study was informative in 80 of them (80%), whereas it was not in 29 (14 lack of molecular probes, 15 inadequate sampling). Altogether, 36/80 evaluable patients were MRD- (45%), with some variations in different ALL subgroups: B-lineage low-risk (N=29) MRD- 38%, B-lineage Ph- high-risk (N=27) 44%, Ph^+ (N=8) 25%, T-ALL (N=11) 54.5%, LL (n=5) 60%. Most interestingly, all of these cases were found to be MRD- or very low positive ($<10^{-4}$) at S-1 (10th week of Phase A), maintaining their status through S-2/-3. Only 6 MRD- cases relapsed (17%), 1 with a different ALL clone and 4 with single molecular probe and/or suboptimal sensitivity. Thus, Kaplan-Meier DFS estimate in CR1 was 76% at 4 years for the 36 MRD- patients. To further evidentiate the independency of MRD from clinical risk features, projected 4-year DFS remained as high as 81% (n=25) when all clinical low-risk cases were removed from analysis.

Conclusion. MRD-oriented therapy is feasible in adult ALL and represents an invaluable prognostic tool when a risk-specific therapy is sought for. This multicentric experience documents the importance of an early achievement of the molecular remission, regardless of patient age and clinical presentation profile. MRD may be a universal prognostic marker, upon which to develop patient-specific programs and comparative therapeutic trials.

C003

MOLECULAR MONITORING OF COMPLETE HAEMATOLOGICAL AND CYTOGENETIC REMISSION IN PH^+ ALL PATIENTS

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Detection of Bcr-Abl mRNA by reverse trascriptase-polymerase chain reaction (RT-PCR) is generally used to assess minimal residual disease in patient with Philadelphia chromosome positive (Ph^+) acute leukaemia. Many studies have shown that qualitative and/or quantitative analysis of Bcr-Abl levels after allogeneic stem cell transplantation, chemotherapy and, more recently, imatinib has a strong predictive value for relapse. However, some patients without detectable level of MRD eventualy relapse and MRD positive patients with long term clinical remission have been reported. In order to better clarify the clinical significance of MRD, 9 patients with Ph^+ in complete haematological and cytogenetic remission (7 after BMT and 2 after induction with conventional chemotherapy and imatinib) were investigated with the simultaneous use of qualitative/quantitative Rt –PCR for p190 and p210 Bcr-Abl expression, JH gene rearrangement and WT1 gene expression. MRD assessment was performed every three months after BMT or during maintainance/consolidation therapy. After a median follow up of 24 months, 7 patients are alive in first complete remission (CR), 2 patients died in CR. At the last follow up, Bcr-Abl and JH rearrangement were not detectable in 3 out of 9 cases studied. All these patients had been treated with BMT. In 6 out of 9 patients, despite the absence of detectable levels of Bcr/Abl, residual neoplastic cells could be detected by JH rearrangement analysis. In this scenario we tested the significance of WT1 gene expression. This marker is still under investigation but might reflect a *functional state* of the staminal leukemic compartment thus indicating the biological activity of clonal cells. In all the nine patients normal expression levels of WT1 were detected in every studied sample. The kind of molecular response (Bcr-Abl and WT1 negativity and JH positivity) may suggest some biological considerations on the molecular pathogenesis of Ph^+ ALL. The first oncogenic event might lead to the growth of a clone that is detectable by JH rearrangement. The instability of such a clone may favour the acquisition of further genic events among which the acquisition of Bcr-Abl rearrangement, for its nature, might strongly increase the proliferative activity of the ALL clone. Alternatively JH rearrangement might identify a *silent* pre/leukemic clone, carrying the t(9;22), with a low bcr-abl expression, under the level of PCR detection. In conclusion, our data show that a pre/leukemic clone migth survive after BMT / Imatinib therapy during complete haematologic and cytogenetic remission but persistence of MRD by JH evaluation cannot be considered a marker of impending relapse. Further studies on larger cohorts of patients are needed to better clarify the clinical significance of these different markers of clonality. The definition of a molecular status associated with prolonged DFS or, alternatively, with high risk of disease recurrence might allow us to tailor further therapy according to the patient risk.

C004

ABL1 AMPLIFICATION AND P16 DELETION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-CELL ALL)

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Gene amplification is detected in 1-10% of patients with acute leukaemia. On conventional cytogenetic (CC) studies it is usually seen either intrachromosomally as homogeneously staining regions (HSRs) or extrachromosomally as double minute chromosomes (dmins). Fluorescence in Situ Hybridization (FISH) has demonstrated that AML1 and MLL are the gene most commonly amplified in ALL. Recently, various reports have discovered an amplification of the ABL1 gene in 19/366 T-cell ALL patients (5.2%) either pediatric or adult and in 3/22 T-ALL cell lines. It has been observed that such an amplification is caused by a cryptic translocation between ABL1 and NUP214 occurring at the episomal level. All these studies has stressed that ABL1 amplification might identify patients with a generally poor event-free survival (EFS). However, *in vitro* studies have shown that this genetic lesion is initially imatinib mesylate sensitive, but may become rersistant after the

development of additional mutation. The present study was aimed at determining the incidence and clinical significance of ABL1 amplification in a series of 31 consecutive adult T-ALL patients. All of them had been submitted to routine FISH screening for BCR/ABL and TEL/AML1 fusions and for MLL amplification. ABL1 amplification was discovered in two patients (6.4%). Both of them were males. Their white blood cell count was 31.8 and 21.8x10^9/L respectively; their blast cells exhibited a T-ALL immunophenotype and a L2 morphology; their lactic dehydrogenase level was elevated. Conventional cytogenetic studies did not yield analysable metaphases in one patient, while it showed a mixture of abnormal/normal karyotypes in the other: 46,XY,t(1;3) (p36;p21), del(6)(q23)/46,XY. FISH with a painting probe specific for chromosome 9 detected an occult trisomy in the former patient and a normal pattern in the other one. In both patients the number of ABL1 signals varied from cell to cell and the observer was always unable to count them properly. Metaphase FISH demonstrated that ABL1 additional copies were not localized on chromosome 9 but were scattered all over the chromosomes and revealed that amplification was extrachromosomal in nature even if no dmins were visualized. In addition interphase FISH with the LSI p16 (9p21)/CEP 9 dual color probe (the red signal hybridizes to 9p21, while the green spot to chromosome 9 centromeric region), showed only one red spot in the patient without analysable metaphases and no red spot in the other. In order to check whether the ABL1 gene was really over-expressed we performed a quantitative RT PCR (Q RT PCR) assay using the β-2-microglobulin as reference gene and total RNA from a normal subject for calibration. Quantification was made using the DDCt method. By this way we found that on clinical diagnosis the two patients expressed the ABL1 gene nine and twelve times more than the control. One patient achieved a complete remission (CR) of fifteen month duration and relapsed while still on maintenance treatment, the other did not respond to chemotherapy. In the former patient ABL1 expression was normal in CR but increased again on disease recurrence. In conclusion i) FISH is the only technique which promptly identifies T-cell ALL patients with ABL1 amplification; ii) quick identification with FISH is fundamental in the clinic since this T-cell ALL subset is imatinib sensitive but may become resistant due to the development of additional mutations, iii) ABL1 quantitative RT PCR may be easily applied to monitor minimal residual disease.

C005

RELATIONSHIP BETWEEN ZAP-70 AND CD38 EXPRESSION, AND MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION IN PREDICTING TREATMENT FREE SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Since chronic lymphocytic leukemia (CLL) is not curable with current therapy, treatment should be delayed until indicated. Recent advances in the diagnosis and molecular characterization of CLL permit improved prediction of disease prognosis, which could result in better management. In an effort to gain insight into the role of biological prognostic indicators, we measured CD38 and ZAP-70 expression together with immunoglobulin heavy-chain variable region (VH) mutational status in circulating leukemic cells and correlated them with patient clinical outcome. To determine the approximate percentage of ZAP-70 positive cells in a sample, we mixed different concentrations of purified T cells with mononuclear cells from the same healthy blood donors depleted of T cells and NK cells. The areas of the band corresponding to ZAP-70 in the western blot were measured and compared to that of B-CLL cells. The CLL samples were divided into three groups of ZAP70 expression: strong (100%-40% positive cells), weak <40%-20%) and negative (<20%). For the purpose of this study, samples showing a negative and weak ZAP-70 patterns were collectively analysed. Also, samples in which <2% of base pairs differed from those of the consensus sequence were considered unmutated. Furthermore, CD38 was considered as binary variable for a cut-off value of 30%. With a median follow up of 37 months (range: 0-310), 53 out of 117 CLL patients (45.3%) required first line chemotherapy. One hundred and five, 85 and 71 CLL cases were available for testing respectively the prognostic impact of CD38 and Zap-70 expression and VH mutational status on clinical outcome. Patients with CD38 <30% (69 cases) experienced a significantly longer time to treatment as compared with cases showing a higher CD38 percentage (301 versus 31 months, p<0.00001). In detail, 82% and 37% of patients with a CD38<30% and >30%, respectively, were still untreated at 3 years. Moreover, the median time to therapy for mutated VH cases was 124 months while only 18 months were projected for those with unmutated VH status (p<0.00001); at 3 years, treatment became necessary in 21% and 76% of CLL patients with mutated and unmutated VH status, respectively. Finally, the actuarial risk of treatment requirement was significantly higher among cases expressing a strong pattern of Zap-70 (35 cases) as compared with the combined group of negative and weak Zap-70 cases (301 months versus 27

months, p=0.0004); the 3 year therapy-free survival was 37% for those cases showing a Zap-70 strong pattern and 80% for the remaining patients. In order to concurrently evaluate the prognostic significance of CD38 and Zap-70 expression together with VH mutational status, we designed a score system considering the number of adverse prognostic factors accounted in each single case. The devised score system allowed us to split the patient cohort into 4 groups. Specifically, the median time to treatment for the 23 cases scoring 0 was 301 months, 1 (10 cases) 61 months, 2 (7 cases) 25 months and 3 (22 cases) 16 months (p=0.0003). In particular, the 3 year therapy-free survival was 79%, 60%, 33% and 10% for cases scoring 0, 1, 2 and 3, respectively. In conclusion, each single prognostic marker herein evaluated, as well as a combination of them, could allow physicians to offer CLL individual patients a good projection of the time in which therapy is to be administrated.

C006

CLINICAL SIGNIFICANCE OF ZAP-70 PROTEIN WITHIN INTERPHASE CYTOGENETIC GROUPS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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The considerable disease heterogeneity in B-cell chronic lymphocytic leukemia (B-CLL) implies that different biological parameters need to be added to the clinical staging systems to predict an indolent or aggressive outcome. Patients (pts) with B-CLL cells that express non-mutated genes have an aggressive clinical course when compared with B-CLL that express IgVH genes with somatic mutations (Damle,1999; Hamblin,1999). B-CLL cells that use non-mutated IgVH genes express ZAP-70 RNA, which encodes ZAP-70, a 70-kDa protein tyrosine kinase, associated both with an enhanced B cell receptor signaling and with an early disease-progression risk in B-CLL (Crespo, 2003). Moreover, with the development of interphase FISH techniques, it became possible to detect selected chromosome abnormalities in non-dividing cells (13q-, 11q- more common and +12 less common). In 325 pts with CLL, multivariate analysis identified 17p- and 11q- abnormalities as variables associated with a shorter overall survival (Dohner, 2000). The primary aims of our study were: 1) to determine progression-free survival (PFS) and overall survival (OS) upon cytogenetic groups and ZAP-70 expression; 2) whether ZAP-70 could predict varied outcome within interphase cytogenetic groups; and finally 3) whether ZAP-70 and/or interphase cytogenetic groups were independent prognostic factors. Therefore we investigated 249 pts, median age 64 years (range 37-84), 126 males and 123 females. With regard to modified Rai stages, 82 pts had a low stage, 159 an intermediate stage and 8 a high stage. To date, we have completed analysis of interphase cytogenetics in 149 pts, and ZAP-70 was quantified in 249 pts by a multicolor flow cytometric method using a cut-off value of 20%. With regard to cytogenetic groups, 81 (54.4%) pts had a normal karyotype and 38 (25.5%) pts had 13q- . Thirty (20.1%) pts with trisomy 12, 17p- and 11q- were pooled together and defined as *poor-risk* cytogenetic subset. ZAP-70+ pts were 86/249 (34.5%) and there was a significant correlation between high or low ZAP-70 expression and IgV gene mutational status (p<0.00001) in 97 examined CLL pts. Furthermore, we found significant associations either between higher ZAP-70 and trisomy 12, 17p-, 11q- or lower ZAP-70 and normal karyotype (p=0.00015). With regard to clinical outcome, a shorter PFS was observed in ZAP-70+ pts (0% vs 59% at 13 years; p<0.00001) and in "poor risk" cytogenetic pts vs normal karyotype pts (9% vs 38% at 12 years; p=0.002). The 13q- pts showed an intermediate outcome (23% at 12 years). ZAP-70+ pts showed also a significant shorter OS (33% vs 93% at 14 years; p<0.00001). To further explore the clinical impact of ZAP-70 among different cytogenetic groups, we investigated its expression within the normal karyotype subset and within the "poor risk" combined with the 13q- subset because of the small number of cases. As a matter of fact, ZAP-70 positivity was associated both with a shorter DFS and OS within normal karyotype (14% vs 66% at 10 years, p=0.00005 and 74% vs 92% at 10 years, p=0.020, respectively) and within "poor risk" pts pooled together with 13q- pts (0% vs 38% at 13 years, p=0.008 and 29% vs 100% at 14 years, p=0.009, respectively). In multivariate analysis of PFS, in which age, Rai modified stage, cytogenetics, CD38, soluble CD23 (sCD23) and lymphocyte doubling time entered, only ZAP-70 (hazard ratio=11.7, p=0.0006) and sCD23 (hazard ratio=5.3, p=0.02) resulted to be independent prognostic factors. Therefore, ZAP-70 expression predicts significantly both DFS and OS, and varies by interphase cytogenetic group. Within normal karyotype and poor risk cytogenetic subsets, where progression is heterogeneous, ZAP-70 positivity is able to distinguish pts who have early PFS and short OS. In conclusion, ZAP-70 adds prognostic information to cytogenetic data and, considering its superior clinical significance in multivariate analysis, will assist us in planning therapeutic decisions for B-CLL pts.

NON-HODGKIN'S LYMPHOMA AND HODGKIN'S DISEASE

C007

PREDICTIVE VALUE OF 18FDG-PET SCAN PERFORMED AFTER TWO COURSES OF ABVD ON TREATMENT OUTCOME IN ADVANCED-STAGE HODKIN'S DISEASE

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Background. The prognostic models in Hodgkin Disease (HD) show scarce reproducibility and unsatisfactory predictive value. The prognostic role of early evaluation of treatment response by TC or Gallium scan has been proved in the past. We report here, on behalf of Intergruppo Italiano Linfomi (IIL), the preliminary results of a clinical trial on the prognostic role of FDG-PET scan performed very early during treatment in advanced stage HD patients (pts), treated by conventional, standard-dose, chemotherapy (ChT).

Materials and methods. Starting from January 2002, 64 new HD pts admitted in eight Italian hematological Institutions were consecutively enrolled into the trial and are now valuable for the analysis. Pts characteristics were: mean age 31.1 years (14-79), male to female ratio 26/38; advanced disease (stages IIB-IVB) in 46, and stage IIA with adverse prognostic factor (> 3 nodal sites involved, subdiaphragmatic presentation, bulky disease and ESR > 40) in 18. Bulky and extra-nodal disease were recorded in 20 and 18 pts, respectively. Histopathology was: NS in 51, LP in 3, MC in 7 and LD in 3. All pts were staged at baseline with CT scan, bone marrow trephine biopsy and FDG PET scan (PET-0); they were re-staged after 2 ChT courses and at the end of the treatment, including radiotherapy, by CT scan and FDG-PET scan (CT-2, PET-2 and CT-6, PET-6, respectively). Standardized Uptake Value (SUV) was calculated in all PET scans. The end-point of the study was the correlation between PET-2 results and one-year and three-year freedom from treatment failure (FFTF). ChT was ABVD x 6 courses in 61 pts, and COPP/EBV/CAD x 6 in 3. In 31/64 additional radiotherapy was given. The mean interval between the end of the 2nd ChT course and PET-2 was 11.5 days (2-32); the interval between the end of the therapy (either ChT or radiotherapy) and PET-6 was never shorter than 50 days.

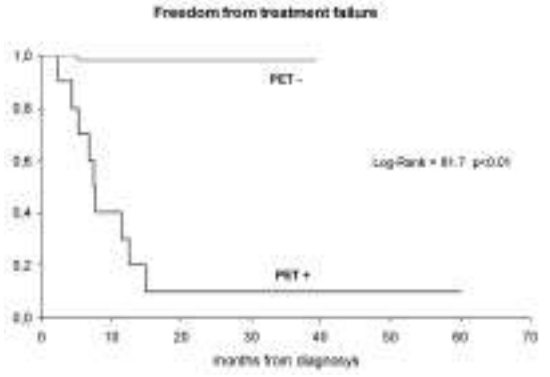

Figure 1.

Results. The mean follow-up from the diagnosis and from final restaging were 561 days (73-1804) and 311 days (1-903), respectively. At the end of the program 54 pts were in Complete Remission (CR) and 10 in progression; two pts relapsed 12 and 15 months after CR entry, respectively. CT-2 (performed in 55 pts.) showed Partial Remission (PR) in 53 pts and CR in 2. By contrast, PET-2 was positive in 10 pts: 7 progressed during therapy and underwent salvage therapy with high-dose chemotherapy and ASCT; 2 relapsed 12 and 15 months after CR, and one, showing a progressive reduction of SUV in subsequent PET scans, up to a complete negativity, remained in CR. The median International Prognostic Score value of these 10 pts. was higher than the one of the entire series of pts: 2.6 vs. 1.0, respectively ($p<0.01$). By contrast, 53/54 (98%) pts with a negative PET-2 showed a PET-6 persistently negative and remained in CR; one progressed during ChT. Thus, the Predictive Positive Value (PPV) of a PET-2 was 90% and the Predictive Negative Value (PNV) was 98%. The sensitivity of PET-2 was 90%, the specificity was 98% and the overall accuracy 97%. The 3-year FFTF probability for PET-2 negative and for PET-2 positive patients were 98% and 10%, respectively (log Rank test =64.9, $p<0.01$: see Figure 1).

Conclusions. the FDG-PET scan performed very early during therapy predicts the treatment outcome in most pts. (62/64: 97%), with a PPV value of 90% and a PNV value of 98%. If these results are confirmed after a longer follow-up and in a larger cohort of cases, standard ABVD regimen seems to be the proper therapy for most patients, with a small fraction of them (15-20%), identified early by a positive PET scan, requiring early aggressive chemotherapy intensification.

C008

PEGFILGRASTIM SUPPORTED DOSE-DENSE R-CHOP14 IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: A STUDY OF FEASIBILITY AND TOXICITY

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Background. CHOP chemotherapy (CT) in a dose-dense setting (CHOP14) or with the addition of rituximab (R-CHOP) may improve response rate and survival of patients with diffuse large B-cell lymphoma (DLBCL). Besides, in dose-dense regimens, G-CSF proved instrumental to deliver on time the planned CT dose.

Aims. This phase II study was designed to evaluate feasibility, toxicity and efficacy of a dose-dense CHOP regimen, with the addition of rituximab (R-CHOP14), supported by pegfilgrastim as first-line therapy in patients affected with DLBCL.

Methods. Eligibility included patients with DLBCL in stage II-IV, aged 18-70. The CHOP regimen was delivered every 14 days, preceded on day 1 by rituximab (375 mg/m^2) and followed on day 3 by pegfilgrastim (6 mg per cycle). Fifty patients were enrolled; 41 have completed the program and 35 are evaluable for response to therapy, so far. Ten patients received adjuvant RT on sites of prior bulky disease or residual disease after CT. Median age was 55 yrs (range 22-70) and M/F ratio 0.92. Half of the patients were in stage IV (16% with bone marrow involvement) and 40% had bulky disease. Age-adjusted IPI score 0-1 accounted for 52%, IPI score 2 for 38% and IPI score 3 for 10% of total patients. Toxicity was calculated over a total 253 cycles of therapy; feasibility was calculated over 203 cycles, not considering the cycles 1.

Results. Cycles were delivered on time in 189/203 instances (93%). Delays occurred in 14 occasions; 3 cycles (1.5%) were postponed for grade 2 neutropenia and 11 for non-hematological toxicity. Average relative dose intensity was 95% for doxorubicin and cyclophosphamide, and 91% for vincristine. R-CHOP14 produced a complete response (CR) in 27 of 35 evaluable patients (77%); four patients (11%) proved resistant to this approach. The ANC nadir occurred on day 10, with a median value of 1.5 x10^9/L (range 0.01-23.4). Grade 3 and 4 neutropenia occurred in 33% and 18% of 253 cycles, respectively, with a mean duration of grade 4 neutropenia of 2±1.3 days. Neutropenic fever developed in 8 instances (3% of cycles), with a median duration of 5 days (range 2-10). Febrile episodes occurred in 16 additional cases with ANC above 1 x 10^9/L. Fourteen severe adverse events (SAE) were registered (4 of them developed in patients off-therapy) and consisted of interstitial pneumonia in 8 cases (in 3 Pneumocystis carinii was documented), bacterial pneumonia in 2, septic shock and GI hemorrhage in one case, each. Eight patients died so far; two of them during the program (both over 60 with advanced disease).

Conclusions. These results indicate that a single dose per cycle of pegfilgrastim successfully supports dose dense R-CHOP14 in DLBCL, allowing on-time delivery of therapy in 93% of cycles, with optimal average dose intensity. The overall program produced CR in 77% of patients, with low incidence of febrile neutropenia. Incidence of febrile episodes unrelated to grade 3-4 neutropenia was relevant and was mostly due to interstitial pneumonia. The occurrence of Pneumocystis carinii pneumonia was unexpectedly high and cotrimoxazole prophylaxis is mandatory in this setting of patients.

C009

ABERRANT HYPERMUTATION OF PIM1, PAX5, RHOH/TTF AND CMYC IN NODULAR LYMPHOCYTE PREDOMINANCE HODGKIN'S DISEASE

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Nodular lymphocyte predominance Hodgkin's disease (NLPHD) is a distinct clinico-pathological entity. The morphological features and Bcl-6 positivity of neoplastic L&H cells, the detection of Bcl-6 rearrangements in L&H cells, the overlapping morphology of NLPHD with T-cell-rich B-cell lymphoma, and the occasional transformation of NLPHD into diffuse large cell lymphoma B (DLCL-B), suggest that NLPHD might be related to DLCL-B.

The somatic hypermutation (SHM) process, normally targeting the IgVH and BCL6 gens in germinal center B-cells, functions aberrantly in >50% DLCL-B, leading to multiple somatic mutations in the 5' region of known proto-oncogenes (PIM1, PAX5, RhoH/TTF and cMYC) (Pasqualucci L. *et al.,* Nature 412:341, 2001). To assess whether NLPHD and DLCL-B share common genetic features, we investigated L&H cells from 10 NHLPD for mutations in the 5' sequences of these four proto-oncogenes, and IgVH genes as control.

Methods. The analysis was performed on laser-microdissected tumor cells and normal T cells (control) using a multiplex seminested PCR, followed by direct sequencing.

Results. Mutations in 1 or more genes were detected in 8 of 10 (80%) NLPHD, with 5 of 10 (50%) cases carrying mutations in 2 or more genes. The most frequently involved proto-oncogenes were PAX5 and cMYC, each mutated in 5 of 9 analyzed cases, followed by PIM1, mutated in 3/8 cases, and RhoH/TTF in 1/9 cases. A total of 28 mutations were detected in 8 NLPHD. The average frequency of mutations in the mutated cases ranged from 0.07 per 100 bp (cMYC exon-1) to 0.19 per 100 bp (PAX-5). Mutations were of somatic origin, since were absent in control T cells. Similarly to DLCL-B, mutations were main-

ly single nucleotide substitutions (n=25) with occasional deletions (n=3), and displayed features of the SHM process, including predominance of transitions vs transversions (ratio:1.5; expected 0.5) and hotspot (RGYW) targeting motif.

Conclusions. Aberrant SHM in NLPHD are comparable in features and frequencies to those of DLCL-B, suggesting a common genetic mechanism between these two diseases. NLPHD may represent a unique DLCL-B subtype characterized by a low number of tumor cells and nodular pattern of growth.

C010

IMPACT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON HIV-ASSOCIATED LYMPHOMA'S OUTCOME. TWENTY YEARS EXPERIENCE OF A SINGLE CENTER

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The introduction of HAART, by restoring the immune function of HIV positive individuals, has dramatically changed the natural history of HIV infection. In this study we evaluate the impact of HAART on the frequency, clinical course and outcome of HIV-associated lymphoma in our community based hospital, over the last 20 years. Since the onset of HIV epidemics to December 2004, 200 HIV positive patients (pts) with lymphoma have been seen at our hospital; thirteen were referred from other institutions, while 134 aggressive systemic non Hodgkin lymphoma (NHL), 17 primary central nervous system lymphoma (PCNSL), 6 low-grade NHL, and 35 Hodgkin disease (HD) were consecutively diagnosed and treated among our single center cohort of HIV positive subjects. When the two periods 1986-1996 (pre-HAART period) and 1997-2004 (HAART period) were compared, an increase was evident in the mean number of HD observed each year, from 1.0 in the pre-HAART period to 2.4 in the HAART period (p=0.05), and a marked decrease was seen for PSNCL (no more PSNCL were seen in the last 3 years); the number of NHL/year remains stable (from 7.6 to 6.2; p=0.7) and they represent the great majority of HIV-related lymphoma and are the object of the following analysis. After the introduction of HAART we were able to treat with full dose chemotherapy a significant greater proportion of HIV positive pts with NHL, compared with the pre HAART period (83% of all diagnosed pts versus 66%; p=0.04) and the median OS of the entire series of pts was 7 months in the pre HAART period and 47 months for the pts diagnosed after the introduction of HAART (p=0.002), with a median follow-up of respectively 119 (range 2-171) and 35 months (range 2-88). However, the response to treatment was similar in the two groups, with 56% of complete remission (CR) in the pre-HAART period and 53% in the post-HAART period. In spite of that, the median overall survival (OS) of treated pts was significantly higher after the introduction of HAART (47 versus 10 months; p=0.02), with a projected OS at 5 years of 49.5%(See Figure).

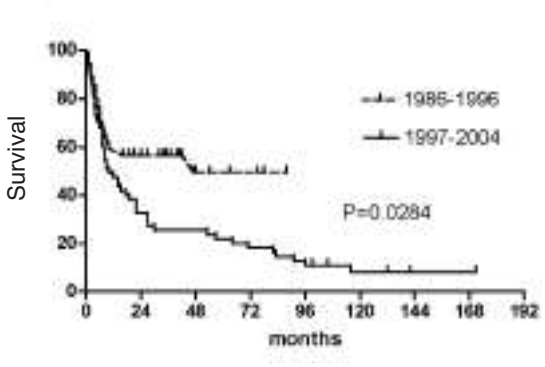

Figure 1. Overall survival of HIV-associated NHL treated with full dose chemotherapy before and after the introduction of HAART.

Indeed, 19/25 (83%) of the pts achieving CR in the pre-HAART period subsequently died for AIDS-related complications. This suggests that the restoration of immune function and the consequent decrease in AIDS-related complications could be the main factor determining the better outcome of these patients, as further documented by the recently demonstrated feasibility of high dose chemotherapy with stem cell rescue in pts receiving HAART. In conclusion, the use of HAART has changed the natural history of HIV-related lymphoma, increasing the proportion of patients that could receive adequate treatment and improving the long term outcome. In this new scenery, it should no longer be precluded to HIV positive subjects the opportunity to receive by hematologists and oncologists the intensive treatment they usually offer to HIV negative patients.

C011

THE MODIFIED INTERNATIONAL PROGNOSTIC INDEX PREDICTS THE CLINICAL OUTCOME OF 416 PATIENTS WITH PRIMARY EXTRANODAL HEAD AND NECK NON-HODGKIN'S LYMPHOMA

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Background. Head and Neck (HN) is the second most common site of localised extranodal presentation of non-Hodgkin's lymphoma and it is at high risk of CNS recurrence.

Aims. To evaluate the clinical outcome, prognostic factors and the rate of CNS recurrence in patients with HN lymphoma .

Methods. From December 1990 to June 2004, 416 patients (median age 60, range18-85) were referred to 11 international centers. The most common sites were Waldeyer's ring (65%), parotid and salivary glands (12%) and nose and paranasal sinuses (8%)). The prevailing histology subgroups were DLBCL (74%) and MALT (10%).

Adverse prognostic features included: stage II (65%), elevated LDH (15%), elevated beta 2-microglobulin (14%), bulky disease (10%), No of extranodal sites>1 (9%), B symptoms (8%), ECOG-PS>1 (6%), and stage modified IPI (MIPI)>1 (48%). Two hundred fifteen patients were treated with chemotherapy (n=160), surgery (n=15), or radiotherapy (n=40) alone, while 157 received CHOP or CHOP like regimens + IFRT. Only 34/348 (10%) patients received CNS prophylaxis (Methotrexate 12 mg i.t.; median cycles 3, range1-6).

Results. three hundred fifty-six patients (87%) achieved a complete remission, 22 a partial remission and 31 were resistant to therapy. Acute toxicity (G2-3) mostly consisting in xerostomia was 6.5 and treatment-related mortality 0.5%, respectively. Among 356 responders, 91 (26%) eventually relapsed, 40% in the same site, 54% in other sites and 6% in both. Only 1/234 patients (0.4%), who did not receive prophylaxis, relapsed in CNS. After a median follow-up of 41 months (range 6-220), 5-year estimate of OS, EFS, DFS was 72%, 54% and 69%, respectively. EFS at 5 years varied according to the site of presentation (66% in oral cavity vs 30% in tyroid) and the hystology (60% in DLBCL vs 40% in MALT). Combined treatment was superior to single therapy (5-yr EFS, 64% vs 45%; p=0.0000). By Cox multivariate analysis, a risk factor>1 according to MIPI predicted a poor EFS.

Conclusions. The present study showed that MIPI influenced the outcome of patients with primary HN lymphoma. Moreover, in the present series a very low rate of CNS recurrence occurred in high risk patients, who did not receive adequate prophylaxis, suggesting that CNS prophylaxis could not be mandatory in HN patients. This should be confirmed by prospective studies of clinical outcome.

C012

QUANTITATIVE PCR OF BONE MARROW BCL2/IGH+ CELLS AT DIAGNOSIS PREDICTS TREATMENT RESPONSE AND LONG TERM OUTCOME IN FOLLICULAR NON-HODGKIN LYMPHOMA

Carlotti E,[1] Oldani E,[1] Della Starza I,[2] Baccarani M,[3] Cortelazzo S,[1] Lauria F,[4] Arcaini L,[5] Morra E,[6] Pulsoni A,[2] Rigacci L,[7] Rupolo M,[8] Zaja F,[9] Zinzani PL,[3] Barbui T,[1] Foa R,[2] Rambaldi A[1]

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The sequential or simultaneous administration of chemotherapy and Rituximab can induce molecular remission in the bone marrow (BM) or peripheral blood (PB) of more than 60% of newly diagnosed follicular non Hodgkin's lymphoma (FL-NHL) patients. Despite this promising result, no data are available to demonstrate that PCR negativity achieved by chemo-immunotherapy bears the same long term clinical improvement observed after high dose chemotherapy and autologous transpantation. To investigate the possible relationship between tumor burden at diagnosis (BM and PB), Real Time Quantitative PCR reaction (RQ-PCR) was performed in 86 FL-NHL patients treated with the sequential administration of CHOP and Rituximab. At diagnosis, the amount of BCL2/IgH$^+$ cells in the BM was low (1 BCL2/IgH+ cell in 1000/100000 normal cells) in 43% of patients, intermediate (1 in 100/1000 normal cells) in 34% or high (>1 in 100 normal cells) in 23%. A 2 log decrease of BCL2/IgH$^+$ cells was achieved after CHOP and an additional 2 log reduction following Rituximab. By multivariate analysis, a low level of BCL2/IgH+ cells in the BM at diagnosis was the best predictor for the achievement of a complete clinical and molecular response. With a median follow-up of 56 months (range, 40-75 months) the Overall Survival (OS) of these patients is 87%. At 5 years, the Event Free Survival of patients with a low/intermediate or high tumor infiltration in the BM is 59% and 32% respectively; when analysis is done on results obtained by RQ-PCR performed on PB no significant different is recorded. The Freedom From Recurrence of patients who achieved a molecular response in the BM, no matter whether after CHOP alone or CHOP and Rituximab, is 64% as compared to 32%, for patients who did not (p<0.006). This study confirms that achievement of a molecular response in the BM correlates with a better clinical outcome. Moreover, RQ-PCR performed on BM samples predicts treatment response and long term outcome in Follicular Lymphoma patients.

TRANSPLANTATION AND CELL THERAPY

C013

NEURAL DIFFERENTIATION POTENTIAL OF MESENCHYMAL STEM CELLS AND NEUROTROPHIN PRODUCTION

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Mesenchymal stem cells (MSCs) are self-renewable multipotent cells able to support hematopoiesis and to differentiate into various cell types under environmental influences. Recent studies have demonstrated that transplanted bone marrow stem cells can generate neurons and glia (Brazelton *et al.,* 2000, Mezey *et al.,* 2000, Weimann *et al.,* 2003) and give rise to Purkinje neurons in the mouse brain through cell fusion (Weimann *et al.,* 2003, Alvarez-Dolado *et al.,* 2003). In the present study, we have explored the MSCs differentiation potential towards neural phenotype.

MSCs were obtained by plastic adherence from iliac crest bone marrow of healthy donors for allogeneic transplantation. For the *in vitro* studies, MSCs were plated on laminin-coated dishes in a B27 Neurobasal medium supplemented with 3% to 10% FBS and cultured for 3 weeks. Only a low percentage of cells (11%) expressed beta-tubulin III and GFAP. The data were confirmed by RT-PCR. Addition of exogenous neurotrophins to cultures did not improve neural differentiation.

For the *in vivo* studies, 50.000 cells labeled with a fluorescent dye (PKH26) were injected into the right parietal cortex of adult and newborn Balb/C (4 and 7 days old) and nude mice. Seven and 45 days later, brains were frozen in liquid-nitrogen-cooled isopentane and cut on a cryostat into 7-mm serial sections. Immunocytochemistry and RT-PCR were performed using the following neural specific markers: neurofilament-M, NSE, GFAP, beta-tubulin III, MAP-2ab, nestin, Gal-C, anti TrkC, antiTrkA and p75NFGR. Brain sections were also analyzed by FISH using both Cy-3 labeled human Pan Centromeric and FITC labeled mouse Pan Centromeric probes. To investigate human neurotrophins release by MSCs, ELISA analysis for the detection of NT3, NT4, BDGF and NGF was performed. In 7 out of 52 mice analyzed, fluorescent cells were detected 7 or 30 days post-injection. These data were confirmed by RT-PCR for the presence of human GAPDH. Fluorescent cells were also detected away from the site of injection indicating a migration of the cells throughout the brain. Thirty days after transplantation, in 1 out of 45 mice analyzed, cells expressing MAP2 neurofilament and GFAP by immunocytochemistry and positive by FISH analysis were detected. Moreover, 7 and 45 days post-injection, a high percentage of cells were shown to express the TrkC and p75 receptor. ELISA analysis from these dissected areas showed the expression of human NT3/NT4 and NGF neurotrophins. Finally, several vessels expressing human Ve-cadherin were detected close to the injection site 45 days after transplantation. To investigate the brain microenvironment effect on MSCs, the cells were also cultured on brain sections and supernatant of the cultures analyzed by ELISA. In this culture condition, MSCs were shown to express p75 and TrkC receptors and to release soluble human NT3/NT4 and NGF.

In conclusion, our data show an *in vitro* and i*n vivo* capacity of MSCs to express neurotrophins under epigenetic stimuli rather than a real neural differentiation potential.

C014

SUPERIORITY OF DOUBLE OVER SINGLE AUTOLOGOUS TRANSPLANTATION AS FIRT-LINE THERAPY FOR MULTIPLE MYELOMA

Cavo M, Cellini C, Zamagni E, Tosi P, Cangini D, Tacchetti P, de Vivo A, Perrone G, Ceccolini M, Lemoli RM, Tura S, Baccarani M, writing committee of the "Bologna 96" clinical trial

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The "Bologna 96" clinical trial was designed in an attempt to prospectively compare a single autologous transplantation (Tx-1) versus double autologous transplantation (Tx-2) as part of first-line therapy for younger patients with less than 60 years of age and a confirmed diagnosis of symptomatic multiple myeloma (MM). Tx-1 was given to support high-dose melphalan, 200 mg/m^2 (MEL-200); Tx-2 was given to support a first course of MEL-200 followed, within 3 to 6 months, by melphalan, 120 mg/m^2, and busulfan, 12 mg/kg. In both arms of the study autologous transplantation was preceded by 4 courses of VAD and subsequent collection of peripheral blood stem cells with high-dose cyclophosphamide, 7 g/m^2. An analysis was performed using an intent-to-treat approach on 228 patients who were randomly assigned to Tx-1 (115 patients; median follow-up: 53 months for all patients and 66 months for living patients) or Tx-2 (113 patients; median follow-up: 57 months for all patients and 73 months for living patients). In comparison with Tx-1, Tx-2 prolonged event-free survival (EFS) of 13 months (median: 22 vs. 35 months; p=0.002) and time to progression (TTP) of 16 months (median: 24 vs. 40 months; p=0.0001). Median overall survival (OS) was 73 months for patients assigned to Tx-1 and 59 months for patients assigned to Tx-2; the difference between the two groups did not reach the level of statistical significance. The probability of attaining at least near complete remission (nCR, as defined by the absence of M protein at routine electrophoresis, but positive immunofixation) was 35% for Tx-1 and 48% for Tx-2; the sample size was not powered to detect a statistically significant difference between the two groups. Among patients randomized to Tx-1, anytime attainment of at least nCR was an essential prerequisite for extended OS (p=0.0001), EFS (p=0.000002) and TTP (p=0.000007). At the opposite, the benefits of double autologous transplantation were the greatest among patients who ever failed to attain at least nCR. In particular, patients who failed at least nCR after the first autologous transplantation and, by

study randomization, received a second transplantation had a significantly longer duration of OS (p=0.01), EFS (p=0.000006) and TTP (p=0.000001) than patients who had the same response status but were assigned to receive a single autologous transplantation. Compared to Tx-1, Tx-2 significantly extended OS (p=0.04), EFS (p=0.000006) and TTP (p=0.000001) also among patients who failed at least nCR after receiving the entire treatment program to whom they were assigned (Tx-1 or Tx-2). At the opposite, for patients who were in CR or nCR after the first transplantation, there was no significant benefit from receiving a second autologous transplantation. In conclusion, data from the present analysis show that in comparison with a single autologous transplantation, i) double transplantation significantly prolonged EFS and TTP among younger patients (with less than 60 years of age) with previously untreated MM; ii) double autologous transplantation was of particular clinical benefit for patients who failed at least nCR. Mature data derived from the final analysis of the study must be awaited before definite conclusions can be given concerning the impact of double autologous transplantation on the ultimate outcome of patients with MM.

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C015

T-CELL-DEPLETED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANT IN HIGH-RISK ACUTE LEUKEMIA. A 10-YEAR SINGLE CENTRE EXPERIENCE

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Transplantation from a one-haplotype mismatched family member (haploidentical transplantation) offers an immediate source of haematopoietic stem cells (HSCs) to almost all leukemia patients who urgently need allogeneic transplantation because they are at high risk of leukemia relapse, when they do not have a matched, related or unrelated, donor. Over the past decade, our group has shown the two major obstacles to mismatched transplants i.e. severe acute GvHD in T-cell-replete transplants and graft rejection in T-cell-depleted transplants, can be overcome by infusing a megadose of extensively T-cell-depleted HSCs after an immuno-myelo-ablative conditioning regimen. Since our first reports (Aversa et al. Blood 1994; 84: 3948 and NEJM 1998;339:1186), our approach has been modified as follows: a) fludarabine replaced cyclophosphamide in our TBI-based conditioning regimen in October 1995; b) CD34$^+$ cells were positively selected from peripheral blood, initially with the Ceprate device and since January 1999, with the Clinimacs instrument which ensures a 4.5 log T-cell depletion in a one-step procedure with no E-rosetting; c) post-transplant G-CSF administration was stopped in the 134 patients transplanted since January 1999 so as to improve immune recovery. Here, we present outcomes in 214 patients with acute leukemia at high-risk of relapse who were transplanted from haploidentical donors between March 1993 and January 2005. The cohort included 121 AML and 93 ALL, median age 28 years (range 2-62), 46 (21.4%) in bad-risk CR I, 71 (33.1%) in second or later CR and 97 (45.3%) in relapse at transplant. Primary full-donor engraftment was achieved in 194 of the 210 who could be evaluated (92.3%); 12/16 patients who rejected, engrafted after second transplants. Overall full-donor engraftment was achieved in 206/210 patients (98%). Without any post-transplant immunosuppressive prophylaxis, grade II-IV acute GvHD occurred in 11/192 evaluable patients (6/32 after lectin-E rosetting graft processing, 5/160 receiving CD34$^+$ cell grafts) and 6/168 developed chronic GvHD. Disease status was the major risk factor for outcome. Leukemia relapse occurred respectively in 13/51 ALL and 10/66 AML transplanted in any CR and in 23/42 ALL and 18/55 AML transplanted in relapse. Infection was the main cause of non-leukaemic deaths: 36/93 ALL (21/51 in any CR, 15/42 in relapse) and 54/121 AML (26/66 in any CR, 28/55 in relapse). Patients transplanted in ALL and AML relapse have, respectively, a 6% and 13% probability of surviving. For those transplanted in remission, EFS is respectively 27% and 52% for 20 ALL and 26 AML patients in first CR at transplant; 30% and 35% for 31 ALL and 40 AML transplanted in second or greater CR. These results indicate the mismatched transplant should be offered to high-risk acute leukemia patients without a HLA-identical donor not as a last resort, but as a viable option in the early stages of the disease.

C016

RIC- ALLOGENEIC STEM CELL TRANSPLANTATION FOR RELAPSED/ REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA AND FOLLICULAR LYMPHOMAS: MOLECULAR REMISSION CORRELATES WITH A BETTER DISEASE FREE SURVIVAL

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RIC regimens decreased conventional transplant-related morbidity/mortality and made allogeneic stem cell transplantation (allo-SCT) a relatively safe option for patients (pts) with low-grade lymphomas. Aim of this phase II trial was to investigate whether allo-SCT after RIC could induce durable clinical and molecular remissions (MR) in pts with relapsed/refractory CLL or FCL. Forty-six pts (25CLL and 21FCL) were enrolled in the study, median age was 54 years (32-69 years). All pts had been pre-treated with at least 1 chemotherapy regimen, 25% pts had failed an autologous SCT. Before transplant: 14pts (32%) were chemorefractory; only 10pts (22%) were in complete remission (CR). The RIC regimen included thiotepa, fludarabine and cyclophosphamide. All pts had a HLA

matched sibling donor. Bcl-2 or immunoglobulin heavy chain rearrangements were used as molecular marker. After allo-SCT, serial BM samples were analyzed for minimal residual disease by nested- PCR. All 46 pts engrafted and at a median follow up of 20 months 2 patients died for treatment related mortality. Forty-one of 46 pts attained CR after transplant. Twenty seven of the 41 patients who attained CR had a molecular disease marker (bcl-2=11, IgH=16). Twenty-seven of 46 pts (59%) developed aGVHD (grade III-IV: 17%). cGVHD was observed in 22 of 44 evaluable patients (49%). There was no significant difference in the incidence of GVHD between PCR-positive and PCR-negative pts. Overall, at a median follow-up of 20 months, 9 pts (20%) experienced a progression. Seven of them never developed GVHD. At a median follow up of 20 months (5-62 months) 17 of 27 pts (63%) with an available molecular marker were alive in molecular remission (MR), 7 of them never attained MR (3 showed an intermittent pattern of PCR positivity after chemotherapy). Three of the persistently PCR positive pts (11%) relapsed after a median time of 9 months. These were all CLL pts and never developed GVHD before. The estimated-probability of DFS at 2 years for PCR-negative and PCR-positive patients was 100% and 57% respectively (p=0.01). This study suggests that: i) MR can be attained by RIC allo-SCT in a sizeable portion of relapsed CLL/FCL pts; ii) GVT can be rarely separated by GVHD; iii) PCR-positive patients without GVHD are at high risk of relapse.

C017

HB-EGF/HER -1 SIGNALLING IN BONE MARROW MESENCHYMAL STEM CELLS: INDUCING CELL EXPANSION AND PREVENTING REVERSIBLY MULTI-LINEAGE DIFFERENTIATION

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Epidermal growth factor receptor-1 (EGFR-1/HER-1/ErbB) regulates proliferation and cell fate during epidermal development. HER-1 is activated by several EGF-family ligands including heparin-binding epidermal growth factor-like growth factor (HB-EGF), a mitogenic and chemotactic molecule that participates to tissue repair, tumour growth and other tissue-modelling phenomena, such as angiogenesis and fibrogenesis. In many of the processes in which HB-EGF is often involved, mesenchymal stem cells (MSCs), the precursors of different mesenchymal tissues, have a role. We have studied whether the HB-EGF/HER -1 system is expressed in human MSCs and which role it plays in MSC biology. MSCs have been generated from bone marrow aspirates of healthy donors, recruited after informed consent, and expanded in complete DMEM medium (15% FCS). MSCs have been characterized by immunophenotype and *in vitro* multilineage differentiation. The expression of HB-EGF, HER-1 and HER-4 (the other receptor for HB-EGF) has been studied by flow-cytometry and RT-PCR. Then we have studied the short- and long-term effect of HB-EGF on MSC proliferation and multilineage differentiation by specific assays and differentiation-specific gene expression by quantitative RT-PCR. We have found that MSCs normally express HER-1, but not HB-EGF or HER-4. Under the effect of HB-EGF, MSCs proliferate more rapidly and persistently, without undergoing spontaneous differentiation. This effect occurs in a dose-dependent fashion, and is specific, direct, long-lasting, comparable to other growth factors such as bFGF, and HER-1-mediated, as it is inhibited by anti-HER-1 and anti-HB-EGF blocking antibodies. The effect is tighly controlled because surface HER-1 is down-regulated after interaction with HB-EGF: this occurs rapidly but reversibly, because HER-1 RNA is still synthetized and leads to the re-expression of surface HER-1 a few hours after HB-EGF removal from culture. By contrast, HER-1 expression is permanently lost during MSC differentiation into mesenchymal cell lineages. Moreover, HB-EGF reversibly prevents adipogenic, osteogenic and chondrogenic differentiation induced with specific media, by preserving MSC potential. This study provides the first evidence that HB-EGF/HER-1 signalling is mitogenic for MSCs and may prevent reversibly their differentiation, leading to self-renewing rather than differentiative cell divisions. The rapid *ex-vivo* MSC expansion and down-regulation of their sensitivity to physiological differentiation agents could represent a valid alternative to other factors, such as bFGF, with an advantage in terms of MSC differentiation potential, in situ recruitment and proliferation, and therefore of in vivo transplant efficiency. It has to be investigated whether the HB-EGF/HER-1 signalling may contribute in vivo to maintain a broad, proliferating pool of undifferentiated MSC, thus ensuring the regenerative process or the efficient angiogenesis to neoplastic growth. The use of HB-EGF inhibitors could have a role in these conditions.

C018

MOLECULAR ANALYSIS OF CIRCULATING T CELLS IN LONG-TERM SURVIVING PATIENTS TREATED WITH DONOR LYMPHOCYTES TRANSDUCED WITH A RETROVIRAL VECTOR EXPRESSING HSV-TK AND DELTALNGFR

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Donor lymphocytes play a crucial role in promoting immune reconstitution and anti-tumour activity in patients treated with allogeneic hematopoietic stem cell transplantation (HSCT). The efficacy of donor lymphocytes is, however, limited by the occurrence of graft-versus-host disease (GvHD). In three different clinical trials, we showed that the infusion of lymphocytes transduced by a retroviral vector expressing a suicide gene (HSV-TK) and a surface marker (deltaLNGFR) allows to efficiently controls GvHD while preserving anti-tumour and antiviral activities. In >40 patients treated with TK-cells in the context of HLA

identical and haplo-identical HSCT, we observed consistent expansion (up to 40% of circulating cells) and long-term persistence (>10 years) of transduced cells. No acute or chronic adverse or toxic effect due to the gene transfer procedure was observed in these patients, who were treated with a total of >10(e)11 cells generated by >60 independent transductions. Analysis of gene expression profiles showed that <1% of the 16,000 genes contained in an Affimetrix HG-U133A Gene Chip were differentially expressed in deltaLNGFR+ (transduced) vs. deltaLNGFR– (untransduced) T-cells *ex vivo*, suggesting the substantial biological identity of T-cell populations generated by HSCT and those administered after gene transfer. Administration of NGF to ?LNGFR+ T cells in culture caused no significant variation in their gene expression profile. Vector integration sites were analyzed by sequencing the vector-genome junctions amplified by LM-PCR from DNA of ?LNGFR+ lymphocytes obtained up to 10 years after treatment from 4 different patients. Over 85% of proviral integrations occurred within transcription units, with a preference for first introns (27%) and regions upstream of transcription start sites (24%). Over 75% of intragenic integrations occurred in genes active in T-cells at the time of transduction. Interestingly, distribution of integration sites and transcriptional orientation of integrated pro-viruses showed different patterns in T-cell obtained *ex vivo* from transplanted patients compared to cultured T-cells. Persistence of T-cell clones carrying specific vector integrations was followed in individual patients by quantitative PCR analysis of PBL-derived genomic DNA at different times after infusion. The effect of vector integration on the expression of hit genes was analyzed by micro-fluidic real-time PCR in RNA extracted from individual T-cell clones harbouring proviral integrations in the first intron or <10 kb upstream of the transcription start site of one or more genes. This analysis indicated that only a minority of the potentially "dangerous" integration events lead to detectable perturbation of gene expression. In conclusion, molecular analysis of circulating T cells expressing HSV-TK and deltaLNGFR in long-term survivors, confirms the safety of administration of retroviral transduced donor lymphocytes in the context of HSCT.

HEMOSTASIS AND THROMBOSIS

C019

CLINICAL SIGNIFICANCE OF IGG AND IGM ANTI-PHOSPHOLIPID, ANTI-BETA2-GLYCOPROTEIN I AND ANTI-PROTHROMBIN ANTIBODIES: RETROSPECTIVE AND PROSPECTIVE ANALYSIS OF THE WAPS STUDY

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Antiphospholipid antibodies include anticardiolipin (aCL), anti-beta2-glycoprotein I (aβ2GPI), and antiprothrombin (aPT) antibodies. Since their clinical significance in the antiphospholipid syndrome (APS) has not been clearly defined, we assessed 112 patients (24 males and 88 females, aged 23-81 and median 42 years) enrolled in the "Warfarin in the Anti-Phospholipid Syndrome" (WAPS) Study. Ninety-one subjects (81.3%) were lupus anticoagulants (LA)-positive. Thirty-two (28.6%) suffered from autoimmune disease, and 87 (77.7%) from APS. Eighty-one (72.3%) had a history of arterial and/or venous thrombosis, and 17 (19.3%) women had suffered from one or more abortions. During a median follow-up time of 4 years, 15 (13.4%) patients had arterial or venous thrombosis. IgG and IgM antibodies were measured by Asserachrom APA, Asserachrom Anti-b2GPI and Asserachrom Anti-Prothrombin (Diagnostica Stago). Values were expressed in units and grouped by tertiles. Odds Ratio and p values were calculated by logistic regression. The following statistically significant associations with clinical events occurring prior to registration were found: high vs low tertile of APA-G and APS (p=0.03); high vs low tertile of ab2GPI-G and APS (p=0.008), any thrombosis (p=0.01), and abortion (p=0.01); high vs low tertile of aPT-G and APS (p=0.02), any thrombosis (p=0.03), and venous thrombosis (p=0.04). No significant association was observed between tested variables and the M isotype, irrespective of the antibody. No significant association with thrombosis registered during follow-up was found, possibly because of the small number of events. In conclusion, these data suggest that measurement of IgG ab2GPI is useful in patients suspected of suffering from APS and raise the possibility that they may replace aCL in the diagnosis of APS. Finally, IgG aPT measurement also seems to play a role in APS.

C020

DENDRITIC CELLS OF IMMUNE THROMBOCYTOPENIC PURPURA SHOW INCREASED CAPACITY TO PRESENT APOPTOTIC PLATELETS TO T LYMPHOCYTES

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In the present study, we investigated whether platelet apoptosis and/or dendritic cells (DCs) may play a role in the stimulation of the immuno-mediated anti-platelet response in chronic immune thrombocytopenic purpura (ITP), since experimental evidences suggest that modifications of autoantigens during apoptosis may lead to the development of autoantibodies, and DCs also are likely to have a role in autoimmunity. Furthermore, it has recently been described that platelets may undergo apoptosis. Twenty patients with active ITP, either newly diagnosed (7 cases) or off treatment for at least 2 months (13 cases), have been enrolled into the study. The median platelet count at the time of the study was $25x10^9$/L (range 5-$83x10^9$/L). A control group (20 healthy adult volunteers) was also utilized. Normal or ITP fresh washed platelets and platelets aged in a plasma-free buffer for 3 days at 37°C were assessed by flow cytometry for phosphatidylserine exposure using annexin-V-FITC, caspase activation, and platelet activation markers (CD62P, PAC-1, CD40 ligand). In parallel experiments, normal or ITP CD14-derived DCs were characterized by immunophenotyping and ability of presenting fresh and aged platelets to autologous T lymphocytes. Our data demonstrate that ITP platelets, either fresh or *in vitro*-aged, show increased apoptosis (with low levels of activation) in comparison to their normal counterparts and that immature DCs readily ingest apoptotic platelets. DCs from ITP patients, pre-pulsed with autologous and allogeneic fresh and aged platelets, are highly efficient in stimulating autologous T-cell proliferation as compared with healthy donor-derived DCs. Taken together, these results indicate that in ITP two novel mechanisms are operative: increased platelet apoptosis and increased platelet-antigen presentation by DCs, which are characterized by a higher expression of CD86 costimulatory molecule. We suggest that these mechanisms may play a role in the stimulation of the immune system in ITP.

C021

INCIDENCE OF THROMBOTIC COMPLICATIONS ASSOCIATED WITH CENTRAL VENOUS CATHETERS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A PROSPECTIVE MULTICENTER SURVEY

Cortelezzi A,[1] Moia M,[2] Falanga A,[3] Pogliani EM,[4] Morra E,[5] Rizzoli V,[6] Boccadoro M,[7] Rodeghiero F,[8] Gallo E,[9] Pasquini MC,[1] Gussoni G,[10] Barbui T,[3] Mannucci PM[2] On Behalf Of The Cathem Study Group

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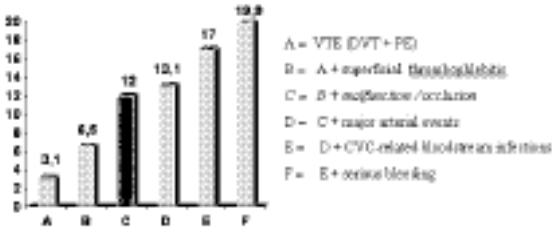
The aim of this prospective, observational, multicenter study was to assess the incidence of and risk factors for symptomatic venous thrombotic complications after central venous catheter (CVC) positioning in patients with hematological malignancies. Four hundred fifty-eight consecutive positionings of CVC were registered in 416 patients (81.2% of whom had severe thrombocytopenia). Over the observation period (3 months or up to catheter removal), the incidence of events was: CVC-related deep vein thrombosis (DVT), 1.5%; DVT of the lower limbs, 0.4%; pulmonary embolism (PE), 1.3% ; fatal PE, 0.6; CVC-related superficial thrombophlebitis, 3.9%; CVC-occlusion / malfunction of thrombotic origin, 6.1% ; major arterial events, 1.1%. Severe bleeding and CVC-related infections were observed in 3.5% and 4.6% of cases. A composite end-point (any venous thromboembolism or superficial thrombophlebitis or CVC occlusion/malfunction) was defined in order to consider venous thrombotic events with a significant impact on clinical practice. With this criterion, the overall incidence was 12.0% (2.54 cases/1000 catheter days). No factor helped to predict venous thrombotic complications, but thrombocytopenia was associated with a definite trend for a reduced risk (odds ratio 0.53; 95% C.I 0.27–1.01). No severe bleeding was observed in those patients who received antithrombotic prophylaxis. This study shows that the impact on clinical practice of symptomatic CVC-related thrombotic complications is not negligible in patients with hematological malignancies.



Composite incidence (%) of clinically relevant complications. Column C represents the clinical end point considered for univariate and multivariate analyses

C022

TROMBOCYTOPENIC TROMBOTIC PURPURA AND METALLOPROTEINASE. ROLE FOR A POSSIBLE PROCTETIVE EFFECT

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Background. Thrombocytopenic Trombotic purpura (TTP) is a rare disease. In TTP high concentration of multimeric factors cause platelet aggregations. The von willebrant factor (vwf) is a protein synthesized by endothelium and megakaryocytic is present in multimeric forms. Metalloproteinase are involved in cartilage proteolysis and participate in other processes of matrix cellular disruption.

Objective. To evaluate polymorphism state in MMP1 (Matrix metalloproteinase-1) promoter gene in heterozygous or homozygous conditions and clinical correspondenceto TTP.

Materials and methods. Forty-two consecutive cases with primary Thrombocytopenic trombotic purpura, median 42 year, range 43-82), 29 female, and 13 male were evaluated. DNA was collected from peripheral blood in mono nucleated cells. Saline method with small modification for DNA dried slides extraction was used. PCR method for transcript of a disintegrin and metalloprotease with thrombospondin type I domains 13(ADAMTS13) and beta actin controls were used. For study of MMP1 gene, polymorphism PCR method, and enzymatic ALU I digestions and agarose gel electrophoresis evaluation were used.

Results were compared (case control analysis) to 150 (ratio 1:3.5) Sardinian control, matched for age, geographic zone and sex.

Results. All transcripts for ADAMTS 13 were positive. The genotype analysis in patients groups (n=42) reveal 3/42 (7,1%) 2G/2G genotype, 25/42 (59.5%) 1G/2G , 14/42 (33,3%) 1G/1G. While in controls (n=150) were 50/150 (33.3%)2G/2G genotype, 66/150 (44.4%)1G/2G genotype, 33/150 (22.2%)1G/1G genotype respectively.

Then, from 42/42 cases evaluation was evinced significative (X=7.5808, p=0.005).

Respect control groups. On the basis of this observation the 2G/2G patient groups, we suppose a co-operative action between protheolitic activity of collagenase of MMP1 and reduced activity of ADAMTS 13. This could be originated from an increase of transcription activity of MMP1 with an increase of collagenase quantity. This 2G/2G genotype, could be s protective towards TTP disease. In addition and a prompt response to plasmapheresis did not present any relapse in 11,11, 8 years of continuous of follow-up respectively.

C023

F8 GENE MUTATION PROFILE IN ITALIAN HAEMOPHILIA A PATIENTS WITH INHIBITOR: LARGE DELETIONS CORRELATION WITH ITT UNRESPONSIVENESS

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Introduction. Immune tolerance therapy (ITT), consists of administration of daily high doses of factor VIII concentrates by intravenous infusions. It can induce tolerance to the exogenous protein and eradicate the antibody response to FVIII, which still represents the main complication of replacement therapy in haemophilia A (HA) severe cases.

Methods. We have investigated 71 HA patients with inhibitors (sixty-nine severe, one moderate and one mild cases). We screened the patients for the causative mutations in the F8 gene using Long Range PCR for the intron22 inversion, multiplex-PCR for intron1 inversion or conformation sensitive gel electrophoresis (CSGE) followed by DNA sequencing for other mutation types.

Results. Diverse genetic defects were detected in the severe cases, with a predominance of null mutations: F8 gene inversions, large deletions and nonsense mutations account for 68% of the mutations, whereas in the two non severe patients specific missense mutations were identified. ITT has been attempted in 16 HR patients of this cohort but failed in 5 cases.

Conclusion. We confirmed that the presence of inhibitors correlates well with the presence of null mutations, as reported by Schwaab *et al* .(Thromb Haemost 1995). Several predictive factors for ITT outcome have been so far taken into consideration but no correlation has been made between F8 gene defect and ITT response. In our cohort two large deletions, two intron 22 inversions and a nonsense mutations failed to respond. For the remaining 2 large deletions no ITT were attempted so no information are available. Large deletions appear to be a high risk genetic factor both for inhibitor development and for long term inhibitor persistence and ITT unresponsiveness. The identification of these patients at very high risk of inhibitor development and ITT unresponsiveness by mutation analysis should therefore be strongly recommended soon after the diagnosis, even because of the high cost of the therapy.

C024

RISK OF RECURRENT VENOUS THROMBOEMBOLISM DURING PREGNANCY AND PUERPERIUM

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The opportunity of administering antithrombotic prophylaxis to pregnant women with previous history of venous thromboembolism (VTE) is debated. We studied a retrospective cohort of 507 women with a first previous

VTE occurred during fertile age (15 to 45 years) and referred to our center for laboratory evaluation. The previous clinical history was taken at the admission blinded to the laboratory results. The median age at the first VTE event was 31 years. All the events were objectively diagnosed. Inherited thrombophilia was defined as the presence of deficiency of antithrombin, protein C, and protein S, factor V Leiden, prothrombin G20210A. Women were stratified according to the triggering circumstances of the first event: 178 had the first VTE during pregnancy and puerperium (PP), 95 during oral contraceptive (OC) use, 113 during transient risk circumstances (surgery, trauma, bed rest) (TR), and 121 spontaneously with no recognizable risk factor (SP). After the first VTE 107 women completed 149 pregnancies (in 38 cases receiving heparin prophylaxis and in 2 cases receiving aspirine); out of the overall 109 pregnancies not prophylaxed, 50 occurred in 30 women with thrombophilia and 59 in 39 women without thrombophilia. Twelve pregnancies (11.0% of deliveries) and 16 post-partum periods (14.6% of deliveries) without prophylaxis were complicated by VTE, in 13 cases in women with thrombophilia. Nineteen recurrences (68% of all the recurrences) occurred among the women of the PP group. In such group the recurrence rate in the subsequent unprophylaxed pregnancies (n=49) was 38.6%: 14.2% ante-partum and 24.4% post-partum, with a relative-risk 2.6-fold increased (95% CI 1.3-5.2) in comparison with the women of all the other groups (n pregnancies= 60). The risk for recurrence was quite similar in women with or without thrombophilia both overall (RR 1.0, 95% CI 0.5-1.9) and in the PP group (RR 0.7, 95% CI 0.3-1.6). The VTE rate during pregnancy and puerperium was 23.8% of deliveries in the SP group, 20% in the OC group, and 6.8% in the TR group. In conclusion the risk of recurrence among pregnant women with previous VTE is not negligible, particularly in those with previous VTE occurred during pregnancy and puerperium. The women having suffered from a first VTE during transient risk circumstances such as surgery, trauma, or bed rest have a relatively low risk, yet as high as 6%. In the women investigated the risk associated with the clinical history outweighed the risk associated with inherited thrombophilia. Antithrombotic prophylaxis is warranted through all the pregnancy and puerperium in all women with previous history of VTE.

ACUTE MYELOID LEUKEMIA AND MYELODISPLASTIC SYNDROMES

C025

MULTICENTER PHASE III TRIAL ON FLUDARABINE, ARA-C, AND I DARUBICINE (FLAI) VERSUS IDARUBICINE, ARA-C AND ETOPOSIDE (ICE) FOR INDUCTION TREATMENT OF YOUNGER NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA PATIENTS

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Background and objective. Combination of Fludarabine, Ara-C and Idarubicine (FLAI) was proved to be effective and safe for induction of newly diagnosed acute myeloid leukaemia (AML) patients. The aim of this phase III trial was to compare the activity and toxicity of FLAI versus ICE, a conventional induction regimen based on Idarubicine, Ara-C and Etoposide.

Design and methods. One-hundred-twenty two newly diagnosed AML patients younger than 60 years were randomized to receive FLAI (n=67) or ICE (n=55). Post-induction treatment consisted on high dose-Ara-C (HDAC). After HDAC, CR patients were addressed to allogeneic (allo) or to a third consolidation course (MEC) and autologous (auto)-SCT, according to the age, disease risk and donor availability.

Results. Complete remission (CR) rate was 72% in FLAI arm and 51% in ICE arm (p=0.02). Death during induction (DDI) rate was 3% and 9% in FLAI and ICE arm, respectively. Hematological and non-hematological toxicities were significantly lower in FLAI arm (p<0.05). CR rate in Pgp-positive patients was 68% (FLAI) and 20% (ICE) (p=0.02). In both arms, relapses were more frequent in patients who were not submitted to allo-SCT. After a median follow up of 17 months, 33% and 38% of the patients are in CR in FLAI and ICE arm, respectively.

Interpretation and conclusions. Our data prove the antileukemic effect and the low toxic profile of FLAI as induction treatment for patients who are eligible for intensification with SCT. Our observations confirm that allo-SCT must be considered one of the most important steps toward the cure of AML patients.

C026

FREQUENT ABERRANT METHYLATION OF THE DNA-REPAIR ENZYME BRCA1 IN THERAPY-RELATED ACUTE MYELOID LEUKEMIA

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BRCA1 is a tumor suppressor gene that encodes a 1863 amino acid protein, important for the regulation of transcription and cell proliferation, recombination processes related to the maintenance of genomic integrity, induction of apoptosis in damaged cells and repair of DNA damage, in particular due to agents crosslinking DNA strands or producing double-strand breaks, such as chemotherapy and ionizing radiation. During homologous recombination, BRCA1 cooperates with RAD51. Inactivation of the BRCA1 gene by promoter hypermethylation frequently occurs in breast and ovarian cancers. Our purpose was to determine whether the methylation status of BRCA1 plays a role in therapy-related acute myeloid leukaemia (t-AML). Using a methylation-specific PCR, we studied BRCA1 promoter hypermethylation in 133 patients with acute myeloid leukemia (AML). BRCA1 hypermethylation was related to patients' characteristics, BRCA1 expression and the RAD51-G135C polymorphism. Hypermethylation of BRCA1 was present in 39% (52) of AML samples. It was frequent in patients with karyotypic aberrations (p=0.067) and was significantly more frequent in AML secondary to therapy for other malignancies, as compared to de novo AML (16/21, 76%, versus 36/112, 32%, p=0.0004, OR=6.6, 95% C.I.2.2-19.4). BRCA1 hypermethylation was present in all patients who received radiotherapy as treatment for the primary tumor. Analyzing primary tumor samples of 8 patients with a secondary AML, 3 of 4 breast cancers were hypermethylated for BRCA-1, while in 5 patients with other tumors, hypermethylation was present in the t-AML sample only. BRCA1 expression, assessed by Real-Time PCR, was significantly reduced in AML samples, in particular in patients with BRCA1 hypermethylation, in comparison to normal bone marrow. The RAD51-G135C polymorphism was less frequent in patients with BRCA1 hypermethylation in comparison to patients without BRCA1 hypermethylation (5/43, 11.6%, versus 19/72, 26.4%, p=0.09). BRCA-1 hypermethylation could be one of the transformation pathways in AML, in particular in therapy-related forms, related to inefficient DNA repair.

C027

RESPONSE RATE AND SURVIVAL AFTER THALIDOMIDE BASED THERAPY IN 248 PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Thalidomide was chosen for clinical trials in MDS for its three important properties: the anti-TNF, anti-angiogenic and immune-modulatory effects. Apart from the response rate, an important question relates to the long-term effects of thalidomide (± other agents) on the survival of MDS patients, especially those who have responded to this therapy. Are thalidomide only providing short term palliation, or is there an improvement in the survival of responders ? A total of 248 MDS patients (pts) have received thalidomide (between 100 and 400 mg orally daily) either alone (83 pts) or in combination with other agents (165 pts) at the Rush Myelodysplastic Syndromes Center over a seven years period. All patients were symptomatic and/or transfusion dependent. The mean age on thalidomide protocols was 67±11 years and 160 pts were males. As per FAB groups: RA 89, RARS 41, CMML 13, RAEB 86, RAEB-t 19. According to International Prognostic Score System (IPSS): 40 low-risk, 126 Int-1, 53 Int-2 , 29 high risk. The data have been analyzed by intent-to treat analysis and responses were assessed using the International Working Group criteria (IWG). Overall, of 248 MDS pts, 52 (21%) obtained an hematologic response. When the patients received thalidomide as a single agent (83 MDS pts, 49/83 with RA/RARS, 56/83 low or Int-1 IPSS) we documented 16 (19%) responses (with 10 pts acquiring transfusion independence). In this study, the majority of responders belonged to the refractory anemia (RA) or RA with ringed sideroblasts (RARS) categories (14/16-88%). There were some bi-and tri-lineage responses but the most hematological responses were restricted to the erythroid series. Comparable results were obtained when thalidomide has been combined with ciprofloxacin and dexamethasone (66 MDS pts, 49/66 with RA/RARS, 54/66 low or Int-1 IPSS) with an overall responses of 11/66 (17%) cases. In other three studies thalidomide has been combined with other antineoplastic agents: thalidomide plus arsenic trioxide-ATO (28 MDS pts, 18/28 with RAEB/RAEB-t), thalidomide plus topotecan (45 MDS pts, 39/45 with RAEB/RAEB-t) and thalidomide plus etanercept (26 MDS pts, 3/26 with RAEB/RAEB-t). Overall in these three protocols we documented 25/99 (25%) responses both in the RAEB/RAEB-t (15/25) and in the RA/RARS (10/25) cases; however the duration of response in RAEB and RAEB-t pts was significantly shorter than in those with RA or RARS (P=0,02). The survival analysis of the whole population shows that the 52 patients who responded have a significantly better survival compared to 196 patients who did not respond to thalidomide based therapy (p=0.002, log-rank test). Furthermore when we compare the 52 patients who have responded to thalidomide with a matched population (52 MDS patients with the same characteristics of age, sex, race, duration of disease, IPSS, FAB type) treated, over the same period of time, only with a supportive therapy at the Division of Haematology of Udine (Italy), it appeared that the 52 patients responsive to the thalidomide have a significantly better survival compared to the matched population (p=0,034, log-rank test). To resume our data indicate that about 20-25% of MDS patients can achieve a response to thalidomide either alone or in combination with other agents. However only the patients with RA or RARS and a low IPSS are able to maintain this response while responsive patients with RAEB and RAEB-t have shorter response duration and no clear advantage on survival. Besides the

patients who responded to thalidomide have a significantly better survival compared not only to the unresponsive MDS cases but also to a matched control population receiving only a supportive therapy. These results and the news that are coming about MDS underline that the therapy can't be the same for all MDS patients and the chose of treatment strategy must take into account both the risk score of the disease and the presence of specific biologic targets for the drugs (combination of risk-based and molecular targeted-based therapy). In fact biological studies, made by Raza and coworkers, showed that arsenic trioxide and thalidomide combination produces multi-lineage hematological responses particularly in MDS pts with inv(3)(q21q26.2) and/or high pre-therapy EVI1 expression (5/7 pts who responded in ATO+thalidomide study had these characteristics). This suggest that perhaps some gene (s) (such as EVI1) on chromosome 3 may be directly interacting with ATO to inhibit clonal proliferation so that, in this setting of MDS, ATO should be the most suitable therapy. Besides, recently, more potent, specific and effective thalidomide analogs (such as CC5013-RevlimidTM) have been introduced into clinical trials with promising results (higher hematologic/cytogenetic responses and better tolerance compared to thalidomide) especially in the setting of patients with low and Int-1 risk MDS and in those with a del (5q) cytogenetic abnormality.

C028

BONE MARROW DEFECTIVE APOPTOSIS AND ANTI-ERYTHROBLAST AUTOIMMUNITY IN PATIENTS WITH EARLY MYELODYSPLASTIC SYNDROME

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It is known that early myelodysplastic syndrome (MDS) patients show alteration of apoptosis and autoimmune phenomena, particularly directed against RBC. We recently described a new method for the detection of anti-RBC antibodies in mitogen-stimulated whole blood cultures, named mitogen-stimulated-DAT (MS-DAT), which is able to disclose a latent anti-RBC autoimmunity in different diseases (autoimmune hemolytic anemia in clinical remission and in B-CLL).

The aim of this study was to investigate apoptosis and MS-DAT positivity in bone marrow and peripheral blood cultures from 23 patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS), compared with 21 controls (miscellaneous haematological conditions). MS-DAT was performed by stimulating whole blood and bone marrow cultures with PMA, and anti-RBC or anti-erythroblast antibodies were detected by competitive solid phase ELISA. As apoptotic markers we evaluated NF-kB and Caspase-3 activity by ELISA and enzymatic assay, respectively. As shown in the table, the anti-apoptotic marker NF-kB was significantly increased in MDS versus controls, and consistently, caspase-3 activity decreased, although not significantly. The amount of anti-erythroblast antibodies was significantly greater in bone marrow cultures of MDS versus controls, and the test was strongly positive in 9/23 patients (not shown). On the contrary, peripheral blood cultures of MDS displayed no significant alterations of apoptotic and anti-apoptotic markers investigated and no MS-DAT positivity (not shown).

These findings suggest the existence of an anti-erythroblast autoimmunity in bone marrow of early MDS patients. This could be related to the observed defective apoptosis, which in turn determines survival of auto-reactive marrow effectors.

	MDS		Controls	
	medium	PMA	medium	PMA
NF-kB (OD units)	246±33*	310±52	92±25	121±48
Caspase-3 (OD units)	156±16	167±20	186±26	178±21
Anti-erythroblasts (pg/mL)	330±76*	342±72*	79±13	126±40

** denotes statistical significance versus controls. Values are the mean + SE*

C029

CONTINUOUS INFUSION IDARUBICIN AND ORAL BUSULPHAN AS CONDITIONING FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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One way for reducing the relapse rate after autologous stem cell transplantation (ASCT) in acute myeloid leukemia (AML) is the adoption of conditioning regimens specifically designed for the disease. We developed an original conditioning program, named idarubicin and oral busulphan (IBu), consisting of the combination of high dose idarubicin (IDA), given at 20mg/sqm as 3 days continuous infusion from day -13 to -11 and busulphan (Bu) at 4mg/kg from day -5 to -2, whose feasibility was previously demonstrated in a phase II study on 14 patients (Ferrara *et al*, THJ 2001). Here we report results from a series of 73 AML patients autografted in first or subsequent complete remission (CR) conditioned with IBu regimen. There were 45 males and 28 females with a median age of 54 years (16-77). Sixty-nine patients had non M3-AML autografted in first (n=65) or second (n=4) CR (karyotype evaluable in 65 cases, with favourable, intermediate and unfavourable cytogenetics in 5, 48 and 12 cases, respectively); four had M3-AML with t(15;17) in second (n=3) and fourth (n=1) molecular remission. All patients received peripheral blood stem cells (PBSC) collected after consolidation plus G-CSF. The median interval between CR achievement and ASCT was 3 months (1-8). The median number of $CD34^+$ cells infused was $6,3x10^6$/kg (2,1-29). In patients aged more than 60 years (n=19), IDA and Bu were reduced to two and three days, respectively. The median number of days with granulocytes <500/cmm and of platelets <20000/cmm was 10 (7-21) and 12 (6-168), respectively.

The median number of platelet and blood units transfused was 3 (1-8) and 2 (0-14), respectively. Extra-hematological toxicity mainly consisted of grade WHO III-IV stomatitis (59/73 or 81%) requiring in all cases total parenteral nutrition, while 2 patients had grade III hepatic toxicity and one experienced transient hallucinations. Furthermore, most patients had FUO, while 4 experienced documented infection. Median days of intravenous antibi-

otics, required in 69 cases, were 12 (4-50). One transplant related death occurred in a patient aged 55 years, due to septyc shock. LVEF examination post-ASCT did not reveal any cardiac toxicity. Finally, median time of hospitalization was 29 days (22-67). At the time of writing, 42 patients (58%) are in continuous CR, while 29 have relapsed at a median time from ASCT of 5 months (1-44), with only two patients relapsing after more than one year from ASCT. Two patients died in CR from late reactivation of pulmonary aspergillosis and gastric cancer, respectively. After a median follow-up for surviving patients of 23 months from ASCT, median overall and disease free survival have not yet been reached, as shown in the figure.

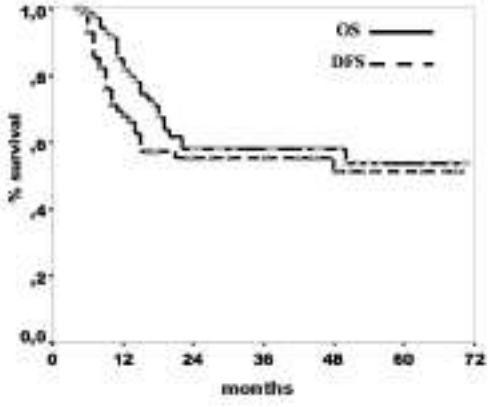

Figure 1.

Patients aged more than 60 years did not experience more complications than younger patients. In conclusion, our data demonstrate the efficacy of the IBu regimen in patients with AML, due to a substantial reduction of relapse rate. The most relevant toxicity of the regimen was severe mucositis requiring TPN. Accordingly, a phase II study with the keratinocyte growth factor palifermin has been planned in this setting.

C030

CHARACTERIZATION OF A NOVEL RECURRENT TRANSLOCATION T(2;3) (P15-22;Q26)OCCURRING IN ACUTE MYELOID LEUKAEMIA

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Six patients with de novo acute myeloid leukemia (AML) and a t(2;3)(p15-21;q26-27) were identified among over 1000 cases enrolled in two GIMEMA AML trials. The t(2;3) was the sole anomaly in 3 patients, in 3 cases monosomy 7, trisomy 15 and 22 and trisomy 14 were also found. No cryptic chromosome deletions at 5q, 7q, 12p, 20q were observed and no major AML-associated translocation was detected by routine RT-PCR screening. One patient carried a FLT3 D835 mutation, FLT3 ITD was not detected in 3 patients tested. Characterization of the translocation breakpoints using a 3q26 MDS1/EVI1 BAC conting showed that the breakpoints were located 5' to EVI1 as follows: between MDS1 exons 2 and 3 in three patients (Nos 1,2,4), in a slightly more centromeric position in one (No 5), within MDS1 intron 2 in one patient (No 6) and between MDS1 exon 1 and 2 in one (No. 3). A set of 2p16-21 BAC probes showed that the breakpoints on chromosome 2p were located within the BCL11A gene (cases 1,4) or distal to its the 3' portion (No. 2,5), within the thyroid adenoma-associated (THADA) gene at 2p21 (case 6) or in proximity of its distal portion (case 3). The orientation of BCL11A and THADA gene was not compatible with the formation of a fusion gene with EVI1. Regulatory elements (promoters, CpG islands) were present in proximity of these breakpoints, which were translocated 5' to EVI1. RACE-PCR studies did not reveal fusion transcripts, with the exception of case 3, which showed a fusion transcript between MDS1 exon 2 and a 125 bp sequence form chromosome 2, containing an in frame start codon supported by a consensus Kozak (ctgATGg). Quantitative PCR showed a 21-58 fold overexpression of the EVI gene in all cases, with overexpression of MDS/EVI1 in patient 3. FAB diagnosis was AML-M2 and AML-M4 in two cases each, with AML-M1 and AML-M5 in one case each. All the patients showed dysplasia of at least 2 myeloid cell lineages, with an immature $CD34^+$ $CD117^+$ immunophenotype, with occasional positivity for lymphoid markers. Elevated platelet count was not seen. Despite intensive chemotherapy and a median age of 43 years (range 36-59) only 2 patient attained a short lived response; 1 patient is alive with active disease at 12 months, 5 died at 4-14 months. We arrived at the following *Conclusions.* a) the t(2;3) is a recurrent translocation in de novo AML, having an approximate 0,5% incidence in our series; b) breakpoints involved the 5' region of EVI1 at 3q26, and the BCL11A, the THADA gene or other regions at at 2p16.1-21; c) the juxtaposition of regulatory elements normally located on chromosome 2 to the 5' region of EVI1 determined its consistent overexpression, with a chimeric EVI1-chr2 transcript occurring in one case; d) clinical outcome in these cases was severe.

CHRONIC MYELOID LEUKEMIA AND MYELOPROLIFERATIVE SYNDROMES

C031

THE RIMM: A NATIONWIDE PROSPECTIVE REGISTRY OF 1107 PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA

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Background. Myelofibrosis with myeloid metaplasia (MMM) is a rare chronic myeloproliferative disease. Knowledge about natural history, prognosis and therapy is burdened by selection bias and limited size.

Aims. The Italian Registry of Myelofibrosis (RIMM) is a nationwide network of 702 clinical and pathology units: its aims are to characterize the epidemiologic, clinical, and prognostic issues of myelofibrosis in a population-based prospective cohort.

Results. Since June 1999, 1107 patients were referred to RIMM: after clinical and pathologic revision, diagnosis was confirmed in 1042 patients (365 females), providing an estimated incidence rate of 0.34/100.000/year. Median age was 72 years (0.5% <30 years; 8% <50 years; 21% >80 years). Cluster of atypical megakaryocytes in bone marrow biopsies were reported in 699 out of 782 (89%) complete histopathological analyses. Bone marrow cellularity was usually high (median 80%), but in 22% of patients it was <50%. Diffuse bone marrow fibrosis was an originally necessary diagnostic criterion (Italian Consensus Conference on Diagnostic Criteria, 1999), however, 9 cases were a-posteriori classified as prefibrotic MMM according to WHO (2001). Out of 386 patients with a cytogenetic analysis performed on peripheral blood at diagnosis, 66 presented with cytogenetic abnormalities (17%): deletions (36 patients), especially in chromosomes 7, 13 and 20, and trisomies (23 patients), especially in chromosomes 8 and 9, were the most frequent abnormalities. In 817 patients with a complete clinical record, mean hemoglobin was 10.7 g/dL, but it was below 8 g/dL in 16% of patients. Mean WBC count was 13.4x10^9/L: 7% of patients had values >30x10^9/L and 14% <4x10^9/L. Mean platelet count was 351x10^9/L, but in 6% of patients the count was <50x10^9L and in 17% >600x10^9/L. Prognostic Lille score was *low* in 51% and "high" in 14% of patients. Mean spleen size was 6.8 cm from the costal arc (18 cm longitudinal US diameter; r=0.74): in 48% and 25% of patients, spleen was minimally enlarged at palpation (<5 cm from costal arc) and at US (10-15 cm), respectively. Overall, 172 (20%) patients reported a previous diagnosis of essential thrombocytemia (ET) or polycythemia vera (PV): they had a significantly lower age and lower (ET) or higher (PV) spleen size. Overall, 209 patients had circulating $CD34^+$ cells assessed at diagnosis: only 4% had counts <4/mL, and 17% <15/mL. Forty-four percent of patients were diagnosed at Internal Medicine or Oncology Units: they had an older age (73 vs 68 years; p<0.001), lower hemoglobin (10.2 vs 11.0 g/dL; p<0.001), lower platelet count (310 vs 390 x10^9/L; p=0.002) and higher Lille score (p<0.001) than those diagnosed at Hematology Units. After a median follow-up of 30 months, 18% of patients has died: the principal causes of death were cardiovascular, infective and hematologic (major bleedings, blastic transformation).

Conclisions. Data from the largest cohort of consecutive MMM patients support a shift to older age of the patient cohort. Clusters of atypical megacaryocytes in bone marrow biopsies and CD34+ count in peripheral blood are sensible diagnostic parameters.

C032

ABL MUTATIONS IN CP-CML PATIENTS RESISTANT TO IMATINIB ARE ASSOCIATED WITH SIGNIFICANTLY SHORTER TIME TO PROGRESSION AND SURVIVAL, WITH P-LOOP MUTATIONS CONFERRING A PARTICULARLY POOR PROGNOSIS

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Background. ABL kinase domain (KD) mutations have been associated with resistance to imatinib (IM) in chronic myeloid leukemia (CML) patients (pts), but their clinical and prognostic relevance is still controversial.

Aims. To shed further light on the clinical and prognostic significance of ABL KD mutations, we retrospectively analyzed an homogeneous cohort of chronic phase (CP) CML pts enrolled in the CML/002/STI571 multicenter clinical trial of the GIMEMA Working Party on CML, who showed either primary or secondary cytogenetic resistance to IM.

Methods. Using denaturing-high performance liquid chromatography and sequencing, we screened for ABL KD mutations 65 CP CML pts treated with IM, who did not reach a CCgR at 12 months (n=41) or who lost CCgR at any time while on IM therapy (n=24). All pts had failed previous alpha-IFN therapy (12 pts being intolerant of alpha-IFN, 18 pts showing hematologic resistance, 35 pts showing cytogenetic resistance). IM was administered at the dose of 400 mg/d until progression or grade 4 non-hematological toxicity. The median age at the time of IM start was 54 years (range, 29-73) and the median time from diagnosis to IM start was 3.6 years (range, 1-13.2). Median follow-up is 37.5 months (range, 10-51).

Results. KD mutations were found in 27/65 (41.5%) pts and mapped to 10 codons (Y253F/H, 5 pts; E255K/V, 4 pts; G250E, 4 pts; M351T, 4 pts; F359V, 2 pts; M244V, 2 pts; E355G, 2 pts; F317L, 2 pts; F311L, 1 pts; H396R, 1 pt). There were no significant differences between pts with and pts without mutations as far as sex (M/F: 15/12 vs. 17/21, respectively), median age at the time of IM start (53 vs. 55 years, respectively) and disease history (intolerance/hematologic resistance/cytogenetic resistance to alpha-IFN: 5/7/15 vs. 7/11/20 pts, respectively) were concerned. Median time from diagnosis to the start of IM therapy was sig-

nificantly longer for pts with mutations with respect to pts without mutations (4.8 vs. 2.8 years, Mann-Whitney U Test p=0.02). At 3 and 6 months, the CHR rate was 81% (22/27) and 89% (24/27), respectively, for pts with mutations, as compared to 82% (31/38) and 89% (34/38), respectively, for pts without mutations. However, presence of a KD mutation was significantly associated with a greater likelihood of subsequent progression to accelerated phase/blast crisis (Log-Rank p=0.0005) and shorter survival (Log-Rank p=0.006). Pts carrying P-loop mutations (codons 250, 253, 255; n=13 pts) showed a particularly poor outcome both in terms of time to progression (Log-Rank p=0.02) and in terms of survival (Log-Rank p=0.01).

Conclusions. These results in a homogeneous and relatively large cohort of IM-resistant CP CML pts support the concept of a gradual accumulation of a pool of BCR-ABL mutants over time, which expand under the selective pressure of IM therapy if favored by a lower affinity for the inhibitor. Moreover, they provide strong evidence that, irrespective of the hematologic response, regular monitoring for emerging mutations may help in identifying those CP pts with worse prognosis, for whom a revision of the therapeutic strategy should be considered.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L. grants.

C033

BCR-ABL RNA LEVELS AT THE TIME OF A COMPLETE CYTOGENETIC RESPONSE PREDICT THE DURATION OF CCR IN IMATINIB-TREATED CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. Most patients with chronic phase CML who receive imatinib achieve complete cytogenetic remission (CCR) and low levels of BCR-ABL transcripts. CCR is durable in the majority of patients, but relapse occurs in a subset. To determine the potential of quantitative RT-PCR (qPCR) of BCR-ABL to predict cytogenetic relapse, we serially monitored residual disease in 97 CML patients with an imatinib-induced CCR.

Methods and patients. mRNA was prepared from total nucleated cells from blood or bone marrow, and cDNA was synthesized using random hexamer primers. Relative BCR-ABL expression was measured by real-time quantitative PCR normalized for beta2 Microglobulin (beta2M). The lowest level of detectability of the method is 0,00001. Patients with late chronic phase CML after IFN-alpha failure were treated with imatinib 400 mg daily. During the imatinib follow-up time of 30 months (median), disease monitoring occurred by cytogenetics and qPCR. The CCR was achieved after 6 months (median) therapy of imatinib therapy.

Results. At the time of first achieving CCR, BCR-ABL RNA levels had decreased by a median of 2 logs below the median baseline level. During further follow-up, 19 patients (20%) experienced cytogenetic relapse (defined as any Ph-positive metaphase cell) at a median 18 months after CCR and a median 24 months after starting imatinib. There was no difference in the imatinib treatment time, the time to achieve CCR, or the post-CCR follow-up period between the patients with and without subsequent cytogenetic progression. The reduction of BCR-ABL transcript level at the time of first achieving CCR was significantly less in those patients with a subsequent cytogenetic relapse (median 1 log) compared to those with a sustained CCR (median 2 logs) (p=0,0051). In the 78 patients with a sustained CCR, the molecular response progressively improved over time to reach a median reduction of 3 logs at 30 months (median) after starting imatinib. Of the 19 patients achieving at least a 2-log reduction of BCR-ABL RNA at the time of first reaching CCR, only 3 (16%) had a subsequent cytogenetic relapse. In comparison, 12 of 37 patients (32%) with less than 2 log reduction at the time of achieving CCR had a subsequent cytogenetic relapse.

Conclusions. We conclude that, in the majority of imatinib-treated CML patients reaching CCR, the level of BCR-ABL RNA at the time that the CCR is first achieved is a sensitive predictor of the durability of the CCR.

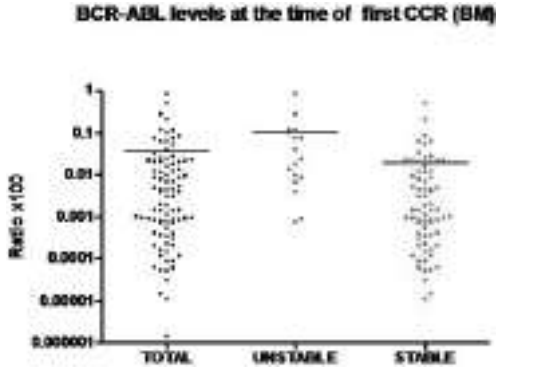

Figure 1.

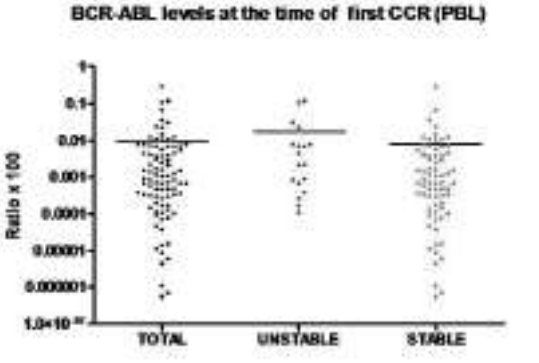

Figure 2.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L.

C034

DERIVATIVE CHROMOSOME 9 DELETIONS IN CHRONIC MYELOID LEUKEMIA: A MOLECULAR CYTOGENETIC STUDY ON 291 CASES AT DIAGNOSIS

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Introduction. Chronic myeloid leukaemia (CML) is characterized by a reciprocal translocation t(9;22)(q34;q11) that generates a BCR-ABL fusion gene on the derivative 22 called the Philadelphia (Ph) chromosome. Large deletions adjacent to the t(9;22) breakpoint on the derivative 9 chromosome have now been found, which result in genomic loss at both sides of the translocation breakpoint. We report an update of our FISH study on CML cases bearing deletions on der(9) chromosome.

Patients and Methods. FISH analysis with BAC/PAC clones specific for ABL1 and BCR genes (as previously reported) was performed on bone marrow cells of 291 CML patients at diagnosis. A set of BAC/PAC probes, belonging to chromosomes 9, 22 and to the third chromsome involved in variant rearrangements, was selected according to the University of Santa Cruz (UCSC) database and employed in FISH experiments. UCSC database was also queried for genes with known function mapping inside deleted regions.

Results. We have detected der(9) deletions in 51 (18%) CML cases. Deletions of chromosome 9 sequences on the der(9) were found in 37 (72%) cases; they were present in all Ph+ metaphases and ranged from 350 Kb to 41.6 Mb. A tumor suppressor gene (TSG) called prostaglandin E synthase (PTGES) was lost in 33 (89%) cases. Chromosome 22 deletions on der(9) were found in 36 (71%) of the analysed cases; the deleted chromosome 22 sequences were shorter than the deleted chromosome 9 sequences (ranging from 400 Kb to 12.7 Mb). Two TSGs mapping inside the deleted sequences of chromosome 22, SMARCB1 and GSTT1, were found deleted in 33 (92%) cases. Twenty-five (9%) CML patients showed a variant 9/22 rearrangement. Ten (40%) of them were deleted on der(9) chromosome; moreover, in 6 out of 10 cases genomic loss were detected on the third chromosome involved in the variant t(9;22) translocation.

Discussion. The observation that deletions on der(9) are associated with the loss of TSGs suggests their possible involvement in the CML outcome, mediated by a haploinsufficiency mechanism. Future work will aim to clarify whether in CML patients bearing TSGs loss and treated with Imatinib, the duration of the response to treatment is comparable to that of patients without deletions on der(9).

C035

OVEREXPRESSION OF THE P65 SUBUNIT OF NF-KB AND IKB MEDIATED NUCLEAR SEQUESTRATION OF P53 AS COMMON EVENTS IN PHILADELPHIA POSITIVE AND NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Chronic Myeloid Leukemia (CML) is characterized by the presence of Bcr-Abl fusion gene, which is the result of a reciprocal translocation between chromosome 9 and 22, cytogenetically visible as a shortened chromosome 22 (Philadelphia chromosome). Rare patients with a clinical presentation of CML are negative for the Ph chromosome. The pathogenesis of Philadelphia negative CML is poorly understood, although the activation of tyrosine kinases appears to be an essential feature.

Different reports have demonstrated that the transcription factor NF-kappaB is essential for Bcr-Abl mediated transformation. NF-kappaB is a transcription factor which is composed of two subunits (generally p65 and p50). NF-kappaB dimers are retained into the cytoplasm by the inhibitory protein IkappaB. Different stimuli trigger the Serine phosphorylation of IkappaB and its proteolitic degradation. Free NF-kappaB translocates into the nucleus where it mediates the transcription of different genes involved in cellular proliferation, transformation and in apoptosis resistance.

The aim of this work is to evaluate the role of NF-kappaB in primary chronic myeloid leukemia samples both positive and negative for the Philadelphia chromosome. Bone marrow samples of 10 chronic myeloproliferative disorders (6 Philadelphia positive CML and 4 Ph negative CML-like) have been collected at the diagnosis. As controls, we have collected two normal peripheral blood samples, two normal bone marrow samples, one CML blast phase and 3 Acute Myeloid Leukemia bone marrow samples.

Each sample has been lysed to obtain cytosolic and nuclear extracts. Western blot have been performed to evaluate the expression of the p65 subunit of NF-kappaB, the regulatory protein IkappaB and the antiapoptotic protein Bcl-2, whose expression may be regulated by NF-kappaB. Subsequently the DNA binding activity of NF-kappaB has been measured with an ELISA method (TransAM).

Our data show that in Ph^+ and in Ph^- CML samples p65 is over-expressed both in the cytosol and in the nucleus respect to normal peripheral blood and normal bone marrow samples. The antiapoptotic Bcl-2 protein is also detectable by western blot in all pathological samples. In normal samples IkB is detectable only in the cytosol and not in the nucleus while in Ph^+ and Ph^- CML samples it is expressed predominately in the nucleus. Interestingly DNA binding activity of NF-kappaB is not particularly increased in CML nuclear samples respect to normal bone

marrow samples. Only in CML blast phase and in Acute Myeloid Leukemia samples, the DNA binding activity of NF-kappaB is marked increased.

To further investigate the involvement of NF-kappaB in the pathogenesis of CML, immunoprecipitates of nuclear and cytosolic IkappaB have been performed. Interestingly, the pro-apoptotic p53 co-immunoprecipitates with IkappaB. Treatment with NF-kappaB inhibitors MG-132, Resveratrol and Bay11-7082 alone does not induce apoptosis of primary CML cells, but when inhibitors are associated with Doxorubicin we observed a marked increase of Doxorubicine-induced apoptosis. The association Doxorubicin and NF-kappaB inhibitors results in the disruption of the p53-IkappaB dimer. Our data suggest that in both Ph^+ and Ph^- CML, NF-kB signal transduction pathway is involved in the control of apoptosis not through its direct transcriptional activity but through the IkappaB-mediated nuclear and cytosolic sequestration of p53.

C036

HYDROXYUREA IN MYELOPROLIFERATIVE DISORDERS: A NEW TASK FOR AN OLD DRUG?

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Hydroxyurea (HU) is a chemotherapeutic agent used to treat myeloproliferative disorders (MPD) and suggested also to reduce the risk of thrombosis associated to MPD. The antithrombotic effect of HU could go beyond its capacity to reduce the platelet number. Actually, epidemiological studies did not find any correlation between incidence of thrombotic events and either platelet number or their in vivo or *ex vivo* activation status. Indeed, in MPD patients increased P-selectin expression on platelet surface as well as higher proportion of platelet-PMN aggregates were found. P-selectin is a physiologic agonist as inducer on PMN surface of tissue factor (TF) and CD11b expression, both markers of leukocyte activation and of a prothrombotic condition. We determined the effect of HU on markers of PMN response to P-selectin both in vivo and *in vitro*. CD11b up regulation, fibrinogen binding, total content and TF expression on PMN and platelet P-selectin expression, were determined (by two-colour flow cytometry) in 12 patients with MPD (6 TE and 6 PV) without any chemotherapy treatment and 6 patients under HU treatment (4 TE and 2PV). Both groups of patients were compared to a control group of 15 healthy donors.

The results obtained *in vivo* (table 1), expressed as the percentage of positive cells for each marker analyzed, showed an increase in P-selectin expression which was not modified by HU treatment. On the contrary, all markers of PMN activation were decreased in the group treated with HU. To better interpret the meaning of these observations, the effect of HU was tested in an *in vitro* model with washed platelets and PMN from healthy donors. PMN were incubated with increasing concentrations of HU (0-1.4 mM) for 15min at 37°C and washed twice. Washed platelets were stimulated by thrombin to induce full-expression of P-selectin, immediately fixed and then washed twice. 100,000 preactivated platelets/microL were coincubated under dynamic conditions at 37°C for 5 min with 5,000 autologous washed PMN/microL (treated or not with HU) to induce the formation of P-selectin dependent platelet-PMN aggregates. In some cases, PMN were treated with 1.4 mM of HU coincubated with increasing concentrations of platelets (from 200,000 to 1,600,000/microL). Tissue Factor expression on washed PMN (treated or not with HU) was also stimulated *in vitro* by purified P-selectin (5microg/mL).

Table 1.

	ND					
	YES					

Results (mean ± SE) are expressed as the proportion of positive cells in PMN or platelet cluster.

The results of *in vitro* formation of platelet-PMN aggregates as well as of TF expression induced by purified P-selectin are illustrated in table 2, showing that the treatment of PMN with HU prevents the formation of platelet-PMN aggregates in a concentration dependent manner: also TF expression was drastically reduced at higher HU concentrations.

Table 2.

* Results are expressed as mean ± SE of the percent of basal value (in the absence of HU).
** Results are expressed as mean ± SE of the proportion of TF-positive cells in PMN cluster.

On the other hand, the effect of HU to prevent the formation of platelet-PMN aggregates was independent of platelet number. These results are in agreement with previous indications that HU may prevent thrombotic complications in MPD. This effect could be due not only to the reduction of platelet number but also to HU effects on leukocyte activation and platelet-PMN interactions.

MYELOMA AND PLASMA CELL DYSCRASIAS

C037

THALIDOMIDE, DEXAMETHASONE AND PEGYLATED LIPOSOMAL DOXORUBICIN IN REFRACTORY/RELAPSED MULTIPLE MYELOMA

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Pegylated liposomal doxorubicin combined with vincristine and dexamethasone has shown to be highly effective in multiple myeloma (MM) patients. We have explored the efficacy and toxicity of this combination in patients with refractory/relapsed MM, replacing vincristine with thalidomide in order to improve the results.

All patients received thalidomide 100 mg/day (continous), dexamethasone 40 mg days 1-4 and 9-12 of each month and pegylated liposomal doxorubicin 40 mg/sqm on day 1. ThaDD combination was administered every 4 weeks for 4-6 cycles and for eligible patients transplantation was planned. All patients received antithrombotic and antimicrobial prophylaxis with warfarin (0.25 mg/day) and ciprofloxacin, respectively. Toxicity was graded according to the WHO criteria. At February 2005, 45 patients were enrolled on this prospective, multicentric study and 37 are valuable. Median age was 68 years (range 41-82) and 16 patients (45%) were older than 70 years. Twenty-four patients (63%) had IgG MM, 6 IgA (16%), 4 light-chain (10.5%) and 4 non-secretory MM (10.5%). Thirty-three patients (87%) had stage III MM and 10 (31%) had/grade 2 performance status. Moreover, 24 patients (75%) had intermediate-high risk disease according to IPI-MM and 10 (26%) unfavourable cytogenetics. Finally, the patients had received a median of 3 previous regimens (range 1-6) and 14 (42%) had relapsed after high-dose therapy and transplantation. Ten patients (26%) achieved a CR, 3 (8%) a VGPR, 16 (42%) a PR and 3 patients (8%) a MR resulting in an overall response rate of 84%. One patient had stable disease, 3 (8%) progressive disease and 2 (5%) died early. Three patients set going to high-dose therapy. Of 14 patients who had relapsed after transplantation, 7 (50%) achieved a CR and 5 (36%) a PR (1 died early and 1 obtained a MR). After a median follow-up of 12 months (range 4-24), 9 patients showed progressive disease and 7 died. Projected 2-years PFS, EFS and OS were 50%, 41% and 74%, respectively. Overall, we administered 162 courses of ThaDD combination. No patients stopped treatment and none required treatment reduction but 10 (26%) delayed therapy because of infections in 7 patients, neutropenia in 2 and pulmonary embolism in the last one. The most common more grade 2 adverse events were fatigue (37%), constipation (26%) and tremors (10%). Grade 2 peripheral neuropathy occurred in 3 patients (8%) and 4 patients (10.5%) developed DVT. No patients experienced PPE or alopecia more than grade 2. Grade 3-4 neutropenia and thrombocytopenia occurred in 11 (29%) and 4 (10.5%) patients, respectively. Out of 162 courses of ThaDD administered, 13 (8%) were complicated by more grade 2 infections but no patients died because of them.

In conclusion, in patients with MM relapsed after standard or high-dose therapy, ThaDD combination seems to be really effective with a manageable toxicity.

C038

OSTEOPONTIN AND ANGIOPOIETIN EXPRESSION BY MYELOMA CELLS CORRELATES WITH BONE MARROW ANGIOGENESIS IN MULTIPLE MYELOMA PATIENTS

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Bone marrow angiogenesis in increased in Multiple Myeloma (MM) patients and correlates with disease progression and patient survival. It is well established that myeloma cells produced the main endothelial growth factor VEGF, recently we have demonstrated that myeloma cells may also produce factors with angiogenic properties as angiopoietin-1 (ANG-1) and osteopontin (OPN). In MM mouse models it has been also shown that the lack of CD45 expression by myeloma cells correlates with VEGF production and the angiogenic properties of MM cells, however the biological mechanisms by which myeloma cells stimulate blood vessel formation are not completed elucidated. In this study we have investigated in a cohort of 121 newly diagnosed MM patients the expression of the angiogenic molecules VEGF, ANG-1 and OPN and their correlation with bone marrow (BM) angiogenesis and CD45 expression by MM cells. VEGF, OPN and ANG-1 mRNA expression was checked by RT-PCR on fresh purified CD138+ MM cells sorted by immunomagnetic method. BM angiogenesis was evaluated on bone biopsies by CD31 expression performed with immunoistochemistry. CD45 mean fluorescence intensity was detected on CD138+ plasmacells by flow cytometry on BM samples.

We found that 90% of $CD138^+$ MM cells tested were positive for VEGF mRNA. On the other hand we found that 50% and 40% of MM patients were positive for ANG-1 and OPN mRNA respectively. Using the previously published cut off for CD45 expression (Moreau P. *et al*, Haematologica 2004) we found that 61 out of 121 MM patients were positive for CD45 and 60 out of 121 were negative for CD45 expression. Any correlation was not observed between VEGF expression and BM angiogenesis in MM patients, whereas the number of microvessels per field was higher in Ang-1 positive patients in comparison with Ang-1 negative ones (mean±SE: 6.23±0.2 vs. 2.94±0.1, median: 6.21 vs. 2.79; p=0.001,) and the micrivascular density (MVD) was significantly increased (32.98±1.7 vs. 14.55±1.3, median: 34.69 vs. 13.04; p<0.01; capillaries: 26.73±1.3 vs. 10.42±0.8, median: 24.06 vs. 9.04; p<0.01, small venules: 9.56 ±0.5 vs. 4.14±0.5, median: 10.60 vs. 3.65; p<0.01). Furthermore a significant positive correlation between Ang-1 expression and MVD was found (Pear-

son Chi-square: p=0.036, Cochran's Linear Trend: p=0.01). A significant higher MVD was also observed in the group of patients positive for OPN, (mean±SE: 29.1±0.7 vs. 17.55±0.37; p<0.01) and similarly, the number of microvessels per field was higher in OPN positive patients in comparison with OPN negative ones (mean±SE: 6.7±0.15 vs. 4.28±0.04; p=0.05). A multivariate analysis showed that ANG-1 expression by $CD138^+$ cells was more tightly correlated as compared OPN with BM angiogenesis. When we analyzed CD45 expression in relationship with ANG-1 and OPN mRNA expression by MM cells any difference was not observed between CD45 positive and CD45 negative patients (Chi-Square: p=NS). In addition we did not find any significant difference in BM angiogenesis in CD45 positive patients as compared with CD45 negative ones (p=NS)

In conclusion our data indicate that ANG-1 and OPN expression by MM cells are the critical determinants involved in the increase of BM angiogenesis that occurs in MM patients.

C039

ZOLEDRONATE VS OBSERVATION IN EARLY-STAGE, ASYMPTOMATIC MYELOMA: RESULTS FROM A MULTICENTER, RANDOMIZED TRIAL IN THE FIRST 90 PATIENTS WITH AT LEAST 2 YEARS OF FOLLOW UP

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Bisphosphonates (BP) are widely accepted as a useful adjunct to manage bone disease during the treatment of active myeloma receiving conventional or high dose therapy. The role of BP in patients with asymptomatic, otherwise untreated early-stage myeloma is less clear. We have previously reported that pamidronate, a second generation BP, is not useful to prevent or delay the need of chemo-radiotherapy in these patients, although such a treatment may reduce the number of cases developing skeletal events at the time of disease progression. Zoledronate (ZOL) is a third generation, more potent BP, which significantly decreases skeletal events in active myeloma and has been demonstrated to have possible anti-myeloma *in vitro* and *in vivo* effects. On June, 2001, we started a randomized clinical trial comparing administration of ZOL vs simple observation in patients with monoclonal gammopathy fulfilling the diagnostic criteria of asymptomatic, stage IA, IIA or smouldering myeloma, not requiring further treatments. Patients strictly diagnosed as having true MGUS were excluded. Accrual was completed on June, 2004. One-hundred-twenty-eight patients were enrolled and randomized (1:1) to receive (n. 64) or not (n. 64) ZOL (Zometa, Novartis Pharmaceuticals. Origgio, Italy) for one year, on an out-patient basis, at the dose of 4 mg as 15 i.v. single monthly infusion. The two groups were comparable for stage, time from diagnosis, levels of M-component and marrow plasmocytosis. Ninety patients have now at least two years of follow-up and represent the object of the present communication. Fever was the most frequent adverse event of ZOL and caused the interruption of the drug administration in two patients. One patient developed osteonecrosis of the jaw under ZOL therapy. Asymptomatc hypocalcemia, without need of stopping the treatment and promptly corrected by oral substitutive therapy, occurred in twelve of ZOL-treated patiens. In the observational arm, four patients were lost at follow-up after 6-20 months. No significant reduction of M-component (>25%) was observed throughout the study in both groups. After a follow-up ranging from 24 to 45 months, there have been 6 (13%) progressions in the ZOL group and 10 (22%) within the controls. Median time-to-progression was 19 and 15 months, respectively. Among the 13 patients who required chemo-radiotherapy in both arms, bone lesions and/or hypercalcemia at the time of progression were observed in 6/8 (75%) of controls, and in 2/5 (40%) of ZOL-treated patients. Our still preliminary data suggest that the use of ZOL in patients with early-stage, asymptomatic myeloma could reduce the cases developing skeletal events at progression. The possibility that ZOL may also decrease the number of and/or the time to progressions requires longer follow-up and evaluation of the total number of patients enrolled in this study.

C040

GENE EXPRESSION PROFILING OF PLASMA CELL DYSCRASIAS REVEALS MULTIPLE MYELOMA MOLECULAR HETEROGENEITY

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Multiple myeloma (MM) is the most common form of plasma cell dyscrasia, characterized by a marked heterogeneity of genetic lesions and clinical course. It may develop from a premalignant condition (monoclonal gammopathy of undetermined significance, MGUS) or progress from intra-medullary to extra-medullary forms (plasma cell leukemia, PCL). Although recent advances have contributed towards establishing a molecular classification of the disease, many issues remain to be clarified such as the molecular events leading to the transition from MGUS to MM, and the contribution of the genes deregulated by 14q32 immunoglobulin heavy-chain (IGH) locus translocations to the biological and clinical variability of MM. To provide insights into the molecular characteriza-

tion of plasma cell dyscrasias and to contribute to a better understanding of the changes in gene expression associated with the neoplastic transformation of plasma cells (PCs) in MM, we analyzed highly purified PCs of patients affected by different forms of plasma cell dyscrasias (7 MGUS, 39 MM and 6 PCL) using high-density oligonucleotide microarrays and we correlated the gene expression profiles with clinical parameters and distinct types of chromosomal lesions. Our data indicated that MMs are highly heterogeneous at transcriptional level: MM and PCL samples are not distinguishable, and some MM cases show features closely related to MGUS. Conversely, MGUS are defined as a clearly recognizable group, differentiated from PCLs and the majority of MM cases on the basis of the expression of a large number of genes mainly involved in controlling cell proliferation and DNA metabolism. Interestingly, the majority of genes distinguishing MGUS from MM were also differentially expressed in MGUS versus PCL, suggesting that their expression may be progressively modulated during the transition of PCs from a pre-neoplastic to a fully malignant phenotype. The analysis restricted to MM cases showed that their clustering was mainly driven by the different types of associated-IGH translocations, while no correlation was found either with clinical or prognostic parameters. Finally, we identified a set of cancer germ-line antigens specifically expressed in a sub-group of MM patients characterized by an aggressive clinical evolution, a finding that could have implications for patient classification and immunotherapy.

C041

RISK-ADAPTED APPROACH TO MELPHALAN CONDITIONING IN AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR PRIMARY (AL)AMYLOIDOSIS

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High-dose melphalan (MEL) with autologous peripheral blood stem cell transplantation (ASCT) has proven to be effective in selected patients with amyloid-light chain (AL) amyloidosis (approximately 60% hematologic remission rate). The procedure is however characterized by relevant toxicity and mortality (up to 40% peritransplant death rate). Candidate selection and personalized MEL dose may be crucial to minimize these negative aspects. On the other hand, lowering the MEL doses may jeopardize the superior efficacy of the procedure. A phase II bicenter study of patient-adapted MEL dose (ranging from 100 to 200 mg/m^2 according to the theoretical transplantation risk was applied to 22 consecutive AL amyloid patients. Age, performance status, number of organs involved, heart, renal, pulmonary, gastrointestinal and hepatic functions were criteria for eligibility and dose. Median age was 51 (29-65, M/F 16/6). Ten (45%) patients had single organ involvement. The main organ involved was kidney in 11 cases (50%), heart in 5 (23%). Conditioning regimen was MEL 180-200 in 67%. Treatment was frequently accompanied by manageable, but considerable toxicity and peritransplant (3 months) death rate was 14%. With a median follow-up of 3 and a half years (41 months, range 13-84) of the patients surviving at least one year post-transplantation, 11/22 (50%) of the autografted patients are still alive. Median overall survival is 5 years and 6 months. At +12 months, intention to treat hematologic response was 59% [complete (immunofixation negative), 36%; partial (>50% decrease of monoclonal component),23%]. Remission of the plasma cell dyscrasia at +3 months was associated with significantly prolonged survival (complete +partial remission, median not reached, projection more than 7 years), by contrast, lack of any response was associated with a poor outcome (median 17 months) (p=0.011, log rank test). Clinical involvement of more than 1 organ was also indicative of a worse prognosis (p=0.016, log rank test). Hematologic remission was usually accompanied by organ response (77%). The prolonged follow-up of this patient population allowed demonstrating that durable responses are possible, but late relapses may occur [3 of 13 responsive patients (23%): 2 from CR, +37 and +30 months, and 1 from PR, +38 months]. The results of this patient-adapted approach to MEL dose show that it is possible to obtain acceptable toxicity and mortality in ASCT, while preserving the overall efficacy of the procedure with significantly prolonged survival and improvement/restitution of organ function. Since unresponsive patients at 3 months post-transplantation rarely gain subsequent improvement, prompt initiation of alternative therapy should be recommended.

C042

ROLE OF 18F-FDG PET IN THE STAGING OF MULTIPLE MYELOMA

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Multiple myeloma (MM) is a malignant B-cell and plasma cell disorder which involves the skeleton in more than 80% of patients at diagnosis. For almost four decades bone lesions have traditionally been detected by Whole Body X-Ray (WBXR) survey and the number of osteolyses has been one of the staging parameters included in the Durie and Salmon system. Over the last years, newer methods of evaluation, including magnetic resonance imaging (MRI), have been increasingly used for the detection of MM bone disease, particularly of the spine. Recently, MRI has been integrated in the new Durie and Salmon PLUS staging system. MRI has proven to be more sensitive than WBXR, although its partial field of view (FOV) is a main limitation in clinical practice. 18F-FDG PET/CT is a non invasive and total body imaging method that uses the emitter fluoride deoxy glucose (FDG). This newer technique may be usefully employed to explore bones and soft tissues in MM, as well as in assessing the degree and extent of active MM bone disease by monitoring the metabolic activity of bone lesions.

Aim of this study was to compare WBXR survey, MRI of the spine and pelvis, and 18F-FDG PET/CT scanning in a series of patients with newly diagnosed MM. Twenty

eight consecutive patients (21 M, 7F; mean age 55 yo) with previously untreated, symptomatic MM entered the study and underwent WBXR, MRI and 18F-FDG PET/CT within 2 months from start of primary treatment. PET/CT scan was carried out with standard procedure and dedicated tomograph. Skull, superior limbs and femurs were included in the field of view. Focal FDG uptake was considered positive for MM bone disease if the SUV max based on body weight was > 2.5 and a lytic lesion could be recognized on CT correspondent images. Findings of 18 F-FDG PET/CT were compared to those of WBXR and MRI in terms of number and site of lesions detected.

Results of comparison 18 F-FDG PET/CT vs WBXR were as follows: in 16/28 pts (57%) 18 F-FDG PET/CT detected more lesions, all of whom were located in the skeleton; notably, 9 of these 16 patients had a completely negative WBXR survey. In 12/28 pts (43%) both methods were superimposable.

Results of comparison 18 F-FDG PET/CT vs MRI were as follows: in 7/28 pts (25%), 18 F-FDG PET/CT detected more lytic bone lesions which were all located out of the FOV of MRI (6 bone lesions, 1 soft tissue lesion); in 13/28 pts (46%) 18 F-FDG PET/CT and MRI detected the same number of lesions in the spine and pelvis; in 8/28 pts (29%) MRI detected an infiltrative pattern of the spine, without evidence of lytic lesions, whereas 18 F-FDG PET/CT was negative.

In conclusion, 18 F-FDG PET/CT appears to be more sensitive than WBXR for the detection of small lytic bone lesions, whereas it has the same sensitivity than MRI in detecting osteolytic bone disease of the spine and pelvis. At the opposite, MRI may be superior over 18 F-FDG PET/CT in several challenging situations, as distinguishing between a pathologic vs. an osteoporotic vetebral compression fracture or showing signal enhancement in areas of marrow replacement. Therefore, careful evaluation of MM bone disease at diagnosis should include both MRI of the spine and 18 F-FDG PET/CT.

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ANEMIAS

C043

CLINICAL, HAEMATOLOGICAL AND MOLECULAR CHARACTERISTICS OF 61 CASES OF PYRUVATE KINASE DEFICIENCY

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PK deficiency, transmitted as an autosomal recessive trait, is the most frequent enzyme abnormality of the glycolysis associated with chronic non-spherocytic hemolytic anemia; the prevalence of the defect, as assessed by gene frequency studies, is 51 cases per million white population. We report the clinical, haematological and molecular features of 61 PK deficient patients from 54 families (44 of Italian origin, 4 from Northern Europe, 1 Gypsy, 5 from Australia, Pakistan, Tunisia, Oman, Guinea respectively) referred to our Centre in the last 30 years. Median age at diagnosis was 16 years (range 1 day to 65 years). Haematological parameters and red cell enzyme activity were determined according to standard methods. The entire coding region, intronic flanking regions and promoter of PK-LR gene were automatically sequenced.

Clinical data of the 61 PK deficient patients are reported in the Table.

Sex	28M, 33F
Consanguinity	4/59
Anaemia	55/61
Jaundice	43/61
Neonatal jaundice	33/56
Exchange transfusion	25/56
Splenomegaly	47/58
Splenectomy	18/61
Colecystectomy	14/56
Aplastic chrisis	1/61
Transfusions	38/59
Desferal treatment	16/58

The degree of anaemia varies widely, ranging from very mild or fully compensated anaemia to life-threatening neonatal anaemia and pronounced jaundice necessitating exchange transfusions. One patient died during exchange transfusion soon after birth and in another family a history of intrauterine death was present. The early onset of symptoms was usually associated with a severe clinical course of the disease: 16/25 exchange-transfused newborns subsequently required multiple transfusions and/or splenectomy. Median haemoglobin concentration was 9.8 g/dL in not splenectomised patients and 7.3 g/dL in candidates to splenectomy. Splenectomy usually resulted in stabilisation of the haemoglobin to a slightly higher levels, with a median increase of 1.8 g/dL (range 0.4-3.4), and in a 5-fold rise of reticulocytes. Iron status parameters were increased in 33/49 patients, 15 of whom never transfused: 18 had increased serum ferritin (SF) alone, 14 increased SF and transferrin saturation (TS), 1 TS alone. Autohaemolysis and red cell osmotic fragility were normal in 75% of patients. Red cell PK activity was decreased in all patients

but four (median 35% of normal).

Molecular characterisation of PK-LR gene was performed in 53 patients, leading to the detection of 43 different mutations among 97 mutated alleles: 28 missense, 7 splice site, 1 nonsense, one 4 nucleotide duplication, 4 small deletion; two large deletions have been identified, the "Gypsy" variant which results in the loss of exon 11 and a deletion of 5006bp resulting at the cDNA level in the loss of exons from 4 to 11; 14 patients were found to be homozygous. In three patients no PK-LR gene mutations were detected and in three only one mutated allele was identified. The most frequent mutations in unrelated Italian patients were 1456T (19 alleles), 994A (7 alleles) and 1529A (6 alleles). A severe syndrome was commonly associated with some missense mutations (in particular 994A and 1529A) at the homozygous state, or with disruptive mutations such as stop codon in the first part of the protein (for example 721T), frameshift, splicing mutations or large deletions, or with missense mutations involving the last part of the protein.

C044

FERROCHELATASE GENE: MOLECULAR ANALYSIS IN ITALIAN PATIENTS WITH ERYTHROPOIETIC PROTOPORPHYRIA

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Erythropoietic protoporphyria (EPP, MIM 177000) is an autosomal dominant disease with incomplete penetrance, due to reduced activity of ferrochelatase (FECH; EC 4.99.1.1), an enzyme located in the inner mitochondrial membrane that catalyzes the chelation of ferrous iron into protoporphyrin IX, the final step in the heme biosynthetic pathway. Clinical manifestations have a childhood onset and include skin photosensitivity, mild anaemia and, in 5-10% of the cases, progressive hepatic failure. Diagnosis of EPP can be supported by the presence of fluorescent erythrocytes in peripheral blood smears and increased protoporphyrin levels in erythrocytes, plasma and faeces. The human ferrochelatase gene (FECH) maps to chromosome 18q21.3; it spans 45kb with a total of 11 exons encoding for a precusor of 423 amino acid residues, the first 54 of which are the putative mitochondrial leader sequence. A single promoter directs both housekeeping and erythroid expression, but two polyadenylation sites produce two mRNAs of different length. So far molecular analysis of FECH gene has allowed the identification of more than 90 different mutations responsible for EPP, showing a high genetic heterogeneity. Phenotypic expression of EPP required coinheritance of a null FECH allele and a wild-type low expressed allele carring the IVS3-48C polymorphism. In this study we searched for molecular defects in FECH gene, in order to identify the prevalent FECH mutations in Italian patients affected by erythropoietic protoporphyria. We investigated twenty unrelated Italian EPP patients and their relatives. The diagnosis of EPP was based on skin photosensitivity, over-normal flurocytes count and abnormal fluorescence emission in plasma and red blood cells. We applied a two-step screening strategy for promoter region and all 11 exons plus intron-exon boundaries of FECH gene. Denaturing gradient gel electrophoresis (DGGE) was followed by direct sequencing of PCR fragments showing abnormal migration patterns compared to control. Ten different molecular defects in FECH gene have been identified in twenty patients. Five were previously reported in caucasians (IVS1+5 G>A, 213insT, 400delA, 843delC, 899delTG). Two out of five (IVS1+5 G>A and 213insT) result the most common in our EPP group, identified in three and seven patients, respectively. Five mutations are new findings: -250 G>C mutation in the promoter region, responsible for loss of SP1 binding site, a 14 bp deletion in exon 5 (488-501) causing a protein truncation at amminoacid 170; a missense mutation (791 C>T) in exon 7 resulting in a 264 Ser to Leu amino acid substitution; two nonsense mutation (892 C>T) in exon 8 and (930 G>A) in exon 9 responsible for creation of a stop codon at amino acid 298 and 310, respectively. These mutations were also identified in six symptomatic relatives and twelve asymptomatic carriers. All symptomatic subjects displayed on the other allele the polymorphism IVS3-48C, responsible for allele low expression. This is the first molecular study aimed to identify FECH molecular defects in Italian EPP patients. These results allowed the identification of five novel mutations, so far limited to Italian population, and confirmed the heterogeneity of mutations responsible for EPP phenotype. Among our EPP group 213insT results the most common molecular defect as in other countries.

C045

APLASTIC ANEMIA, LARGE GRANULAR LYMPHOCYTE-LYMPHOCYTOSIS AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA SHARE A COMMON IMMUNE PATHOPHYSIOLOGY

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The damage of hematopoiesis as occurs in bone marrow failure syndromes may be due to different mechanisms, which may be intrinsic or extrinsic to the hematopoietic stem cells. Immune-mediated mechanisms have been documented in idiopathic aplastic anemia (AA), even if etiology and putative antigens are still lacking; much less is known about the pathophysiology of marrow failure in paroxysmal nocturnal hemoglobinuria (PNH) and large granular lymphocyte (LGL)-syndromes. We aimed to identify Ag-driven immune responses by demonstration of in-vivo dominant T-cell clones in patients with various marrow failure syndromes. We compared the findings obtained in large cohorts of AA (n=39), LGL (n=45) and PNH (n=24) patients. Our strategy included a fine analysis of the circulating T-lymphocyte pool at the level of the T-cell receptor (TCR)- chain, combining flow cytometry analysis of the Vβ; usage with molecular analysis of the complementarity determining region 3 (CDR3). Flow cytometry analysis of the TCR-Vβ usage demonstrated

oligoclonal expansions in AA patients, with two or three overutilized in each lymphocyte subset (CD4+ and CD8+ T-cells). RT-PCR amplification of the TCR-β-CDR3 pool was performed using a common constant (Cβ) primer and the specific Vβ primer to confirm their clonality. Amplicons were then studied by size analysis (spectratyping) followed by cloning and sequencing of several single colonies. In AA patients, the Vβ subsets found overexpressed by flow cytometry showed a monoclonal pattern by molecular analysis. In LGL patients, flow cytometry analysis documented a unique dominant Vβ with a $CD8^+$ phenotype in most cases; as expected, these populations appeared monoclonal by molecular studies; in a few patients, two clonal populations were found. PNH patients showed a more heterogeneous pattern; in general, they paralleled AA patients, with oligoclonal T-cell expansions found in most cases. However, the occurrence of extremely large expansions was surprisingly high, quantitatively and phenotypically resembling those found in LGL patients; even in these cases clonality was molecularly confirmed. All identified clonotypes resulted patient-specific, with no preferential usage of particular Vβ or Jβ, nor evident structural homology, regardless the specific disease group. This was not surprising, given the extreme HLA-class I disparity; however, two AA patients sharing 3 out of 4 class I antigens showed almost identical clonotypes (98% homology), suggesting a public HLA-restricted immune response.

In conclusion, likely Ag-driven clonal immune responses may be demonstrated in various marrow failure syndromes, regardless the primary disease. They appear moderate in AA and PNH patients, who often have an acute-onset aplasia, and larger in LGL patients, who generally have an indolent marrow failure dominated by the clonal lymphocytosis. We suggest that a common pathogenic Ag-driven immune response is responsible for the anti-hematopoiesis attack occurring in AA, LGL and PNH patients, with the specific clinical presentation depending upon the nature of the specific Ag(s), the efficiency of the target killing and additional biological features of dominant T cell clones.

C046

BONE MARROW TRANSPLANTATION FOR THALASSEMIA MAJOR

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In this study we report the long-term results of bone marrow transplantation (BMT) in 112 patients (M 56, F 56) with thalassemia major (TM) who were given 116 transplants in Pescara between May 1983 and February 2005. The median age was 10.06 years (0.11-28.11). One hundred and eight patients received their marrow from HLA identical siblings, 3 from HLA identical parent and 1 from HLA identical uncle. All patients received the same preparative therapy consisting of Busulphan (BU) (13-14 mg/Kg) and Cyclophosphamide (200 mg/Kg), preceded by an hypertransfusion regimen for 2-3 weeks. For graft-versus-host disease (GvHD) prophylaxis, 38 patients were given Cyclosporine (CSA) alone and 74 received CSA and short course Methotrexate. The median number of transplanted nucleated cells was $4.7x10^8$/Kg (2.3-10.1). Marrow engraftment was evident in 108 patients. The median time to achieve $0.5x10^9$/L neutrophils and $50x10^9$/L platelets was 19 (11-37) and 24 (10-55) days respectively. Four patients showed graft rejection and were given a second BMT from the same donor. The probability of rejection including early and late graft failure and autologous reconstitution was 6,7%. The actuarial probability of developing acute GvHD grade II-IV and cumulative chronic GvHD was 19% and 17% (7% limited, 10% extensive) respectively. BMT related mortality was 9%.Ten patients died for BMT related causes: pneumonia in 4 patients, heart failure in 3, encephalopathy in 2, aGvHD in 1. The median day of death was day 41 (12-212). Two late deaths occurred. One patient died of septic shock 54 months post-BMT. One patient died for parotitis carcinoma 138 months after BMT. As of March 2005, 100 patients are alive. Ninety six are cured after a median follow-up of 164 months (1-258). Four patients had an autologous reconstitution and are currently alive under transfusion therapy. To-date, 2 patients are receiving immunosuppression for active chronic GvHD. The 22-year actuarial probability of survival and disease-free survival (DFS) was 89% and 86% respectively. In multivariate analysis, no adverse risk factor affecting survival and DFS was identified among recipient-donor age and sex, number of pre-BMT transfusions, level of ferritin, type of chronic hepatitis, grade of liver fibrosis, serum GPT level, HBV and HCV serology, dose of BU, type of GvHD prophylaxis, marrow cell dose.

This study confirms the feasibility of curing the majority of patients with TM by BMT.

C047

CARDIAC FUNCTION RECOVERY AFTER INTENSIVE IRON-CHELATION IN THALASSEMIA MAJOR WITH ACUTE HEMOSIDEROTIC HEART FAILURE

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Introduction. Heart failure (HF) is a well known iron overload complication in transfusion-dependent thalassemic patients. Hemosiderotic HF (HHF) benefits from iron chelation with i.v. infusion of high doses desferioxamine (DFO). However, delayed efficacy makes this treatment inadequate in acute severe HHF cases. Although a sinergic iron chelation activity of deferiprone (DFP) and DFO has been demonstrated, whether combining these two treatments improves the prognosis of acute severe HHF is undetermined.

Case report. A 25 years-old male with beta-thalassemia major was admitted to the Cardiological Semintensive Unit in September 2003 for the abrupt onset of NYHA class IV HF. The patient had been regularly transfused since he was 1 year old. Iron chelation treatment with desferioxamine (DFO) 20-50 mg/Kg/day had been administered from the age of 3. By the age of 15, however, poor compliance to DFO had resulted in iron overload with steadly increased serum ferritin levels, hepatopathy, hypogo-

nadism and diabetes mellitus, although serial cardiological evaluations displayed only high takeoff at electrocardiography (ECG). At admission, the patient complained dyspnea on minimum exertion. Physical exam showed leg oedema and pulmonary congestion. ECG showed sinus tachycardia with negative T waves in most derivations. Echocardiogram demonstrated diffuse hypokynesis, 15% left ventricular (LV) ejection fraction (EF), and severe mitral regurgitation with increased LV telediastolic diameter, suggesting dilated cardiomyopathy. Cardiac magnetic resonance imaging showed severe iron overload. The patient was started on furosemide, captopril, digoxin and low-doses carvedilol, and on intensive iron chelation therapy with continuous i.v. infusion with DFO (up to 40 mg/Kg/day). Six days after i.v. DFO initiation, deferiprone (DFP) 75 mg/Kg/day p.o. was added. At discharge, 20 days after admission, clinical conditions were steadly improved and LVEF was 34%. Cardiological therapy, DFO 50 mg/Kg/day continuous s.c. infusion and DFP 75 mg/Kg/day p.o. were continued. In March 2004, further cardiological improvement allowed furosemide and digoxin suspension. In October 2004, echocardiogram showed a 67% LVEF, and mild mitral regurgitation with LV diameter at the upper limit of normal. Since serum ferritin level was 300 mcg/L, DFO was tapered and withdrawn. At present, the patient is doing well with moderate physical activity under ramipril, carvedilol and DFP.

Conclusions. Combined aggressive chelation therapy with i.v. DFO and DFP should be promptly administered, along with cardiological treatment, in thalassemic patients with acute HHF, in order to assure rapid cardiac function improvement.

C048

NEW INSIGHTS INTO THE FUNCTION OF N-TERMINAL 11 AMINO ACIDS OF BAND 3 (AE1) FROM STRUCTURAL AND FUNCTIONAL STUDY OF A NATURALLY OCCURRING BAND 3 VARIANT

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The 911 amino acid human erythroid AE1 (eAE1) Cl-/HCO3- exchanger SLC4A1 (band 3) is the major intrinsic membrane protein of red cells. The N-terminal cytoplasmic domain of eAE1 is thought of as the anchoring site for membrane-associated proteins including ankyrin, protein 4.2, protein 4.1, some glycolytic enzymes, haemoglobin, and hemichromes. We identified a son of consanguineous marriage with severe band 3 deficiency. Both parents were affected by a mild form of autosomal dominant hereditary spherocytosis due to eAE1 deficiency. SDS-PAGE and immunoblotting analysis of the proband red cell membrane proteins showed approximately 12 4% of band 3 and protein 4.2 compared to controls. Direct nucleotide sequence of SLC4A1 gene demonstrated a single base substitution (T->C) at position +2 in the donor splice site of intron 2 (Band 3 "Neapolis" variant). The mutation causes an altered splicing with the consequent formation of two different mature mRNAs, one including the intron 2 and one skipping the exon 2. While the first spliced form leads to premature translation termination, exon 2 skipping causes the usage of a new start site during eAE1 mRNA translation. The purification of band 3 at homogeneity and its characterization by MALDI mass spectrometry demonstrated the lack of the first 11 amino acids in band 3 Neapolis. The protein truncation resulted in the complete absence of membrane bound aldolase while other glycolitic enzymes (i.e. GAPDH) still remain linked to the altered protein. Experiments in intact erythrocytes demonstrated that mutated band 3 cannot be phosphorylated, implying an absolute requirement of the first 11 amino acids for the complex eAE1 phosphorylation process. In summary, the identification of a naturally band 3 mutant and its structural and functional characterization enabled us to definitely identify pivotal roles for the 11 N-terminal amino acids in eAE1 function and, in turn, in red cell membrane physiology.

Posters I

Anemias and Erythrocyte Disorders

P001

PYRUVATE KINASE DEFICIENCY: MOLECULAR AND FUNCTIONAL CHARACTERISATION OF A LETHAL VARIANT

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Pyruvate kinase (PK) deficiency is the most common hereditary enzyme defect of the glycolytic pathway. To date about 150 different mutations associated with chronic non spherocytic haemolytic anaemia have been identified in the PK-LR gene. The availability of a system to easily obtain recombinant mutants of PK helps to better define the molecular basis of the defect and to correlate genotype to clinical phenotype. We describe a case of severe haemolytic anaemia due to PK deficiency caused by a missense mutation and a large deletion in the PK-LR gene. To investigate the contribution of the missense mutation in the severity of clinical pattern we have functionally characterised this variant by mutagenesis and in vitro expression of the protein. The patient, an Australian baby, presented at birth hepatomegaly, haemoglobin 8.9 g/dL, bilirubin 275 micromol/L, and ferritin levels > 4000 ng/mL; the baby died soon after birth during a double exchange transfusion. The study of red cell metabolism performed in the parents showed intermediate values of PK activity compatible with an heterozygous state. Molecular analysis of PK-LR gene in the propositus led to the detection of a missense mutation G409A (Ala137Thr) and a large deletion of 5006bp extending from intron 3 to the last nucleotides of exon 10, which result at the cDNA level in the loss of exons 4 to 11. Expression and purification of the Ala137Thr mutant protein was performed according to the method previously reported (Valentini et al, 2002, JBC, 277). The kinetic, allosteric and thermostability parameters were evaluated and related to the clinical pattern of the patient. Ala137Thr mutant enzyme purified to homogeneity exhibited a specific activity of 320U/mg. The oligomeric state of the mutant protein was identical to that of the wild-type enzyme. Similarly, the kinetic parameters and the thermostability properties appear to be substantially unaffected by the Ala137Thr replacement. Furthermore the enzyme is less sensitive to ATP inhibition (IC50 2.3 mM vs 0.53 mM for the wild-type enzyme). In conclusion, the large deletion of 5006bp could be considered one of the most severe abnormality in PK deficiency so far reported. However, the biochemical data obtained for the recombinant mutant enzyme Ala137Thr build up by identical subunits would not explain the very severe clinical pattern found in the patient hemizygous for this mutation. We can suppose that in the PK-deficient erythrocyte the aberrant subunits generated by the deleted allele could interfere in the tetramer assembly of Ala137Thr enzyme, that would be rapidly degraded; alternatively, additional defects other than PK deficiency could exacerbate the clinical pattern.

P002

MOLECOLAR ANALYSIS OF ALPHA HEMOGLOBIN STABILIZING PROTEIN (AHSP) GENE IN CAUCASIAN BETA THALASSAEMIC SUBJECTS

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Beta thalassaemia syndromes are inherited haemoglobin disorders characterized by absence or reduction in the syntesis of beta globin chains. The pathophysiology and the severity of anaemia reflect the degree of globin chain imbalance; the excess of alpha globin chains that precipitate causes oxidative damage in red cell precursors and induces their premature destruction in the bone marrow (ineffective erythropoiesis). Although the phenotype of beta thalassaemias can be modified by inherited factors, such as mild beta mutations or different number of alpha globin genes or increase in fetal haemoglobin production, other mechanisms could be involved in the globin chain imbalance. Recently, a protein named alpha hemoglobin stabilizing protein (AHSP), that acts as a molecular chaperone specifically for free alpha globin chains, preventing their precipitation in red cell precursors, has been identified. To establish weather AHSP might have a role in the clinical variability of beta thalassaemias, we analyzed the AHSP gene in 70 Caucasian beta thalassaemic subjects: 26 patients with a genotype corresponding to Thalassaemia Major, 24 patients with Thalassaemia Intermedia and 20 patients affected by Thalassaemia Intermedia but with only one mutation in the beta globin gene and a normal alpha globin genotype. In all subjects, we have performed Denaturing High-Performance Liquid Chromatography (DHPLC) of the three exons and the direct genomic sequencing of coding and noncoding regions (~ 1.5 kb) of AHSP gene were performed. No mutation able to modify the structure or function of AHSP was found. Instead we identified eight single nucleotide polymorphisms (SNPs) spanned along the whole gene that segregate in four different haplotypes. The allele frequency of every single haplotype of beta thalassaemia subjects was compared to that observed in a group of 33 Caucasian normal controls. No statistically significant association was proved. Even though the loss of AHSP aggravates the beta thalassaemia

phenotype in mice, in Caucasian population the AHSP apparently doesn't make changes in the clinical severity of beta thalassaemia confirming the results found in Thai population.

Haplotype 1 (%)	Haplotype 2(%)	Haplotype 3(%)	Haplotype 4(%)
Beta-thal alleles	50.7	24.3	12.8 12.2
Normal alleles	40.9	30.4	12.1 16.7

P003

BIOCHEMICAL CHARACTERIZATION OF RED CELL MEMBRANES IN 40 PATIENTS AFFECTED BY HEREDITARY ELLIPTOCYTOSIS

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The presence of elliptically shaped red cells in peripheral blood is characteristic but not specific for Hereditary Elliptocytosis (HE), since it is known that elliptocytes may occurr in other chronic hemolytic disorders. HE is a clinical and heterogeneous disorder resulting from different defects in the skeletal proteins, namely spectrin and band 4.1. The diagnosis is mainly based on red cell morphology and family history, since hemolysis may be minimal or absent and red cell osmotic fragility is normal in a high proportion of cases. The minimal criteria for the diagnosis of HE are even not yet defined. The analysis of red cell membrane proteins and functional study of spectrin may be helpful particularly when the percentage of elliptocytes in peripheral blood is lower than 10-15%.We studied 40 HE subjects belonging to 26 different families. 15/40 displayed less then 10% elliptocytes at the blood smear examination, and 6/40 less then 5%.The results of the hematological and biochemical characterization, grouped according to the type of defect, are reported in the table. Among the described defects, carriers of alpha /I74 variant of spectrin were asymptomatic with no signs of hemolysis, although with a high number of elliptocytes. Band 4.1 deficient patients were the most severely affected with the highest number of elliptocytes. In six patients, no defect in red blood cell membrane was found. In conclusion, we describe the heterogeneity of this disorder and the importance of biochemical analysis of red blood cell membrane proteins to make the diagnosis of HE.

Defect (no of cases)	Hb (g/dL) (n.v.12.2-16)	Retics (x10⁹/L) (n.v.24-84)	Elliptocytes (%)	D/T (%) (n.v.<15.9)	band4.1/band3 (n.v.0.11-0.20)
Spectrin					
-alphal/74 (6)	14.1 (12.1-14.6)	25 (9-89)	23 (4-80)	50 (44-54)	0.15 (0.12-0.17)
-alpharr/I65 (3)	10.4 (10.2-12.3)*	149 (54-271)	15 (5-33)	6.3 (5.4-29.1)	0.16 (0.16-0.17)
-alpha Lely (2)	13-14	17-66	4-6	5-29	0.15-0.18
-double def (7)**	12.8 (6.4-16.1)	64 (43-349)	7 (5-28)	22 (16-34)	0.16 (0.12-0.19)
Band 4.1 (16)	10.6 (7.8-13.9)	130 (52-186)	33 (6-90)	9.9 (7.4-15.7)	0.09 (0.05-0.11)
Undetected (6)	13.7 (10-15.8)	31 (14-148)	10 (5-23)	11.8 (6.7-15.1)	0.14 (0.13-0.18)

** Median values and range are indicated;** Double defect: one patient with alpha Lely associated with alpha I/74; three patients belonging to the same family with alpha I/48 associated with alpha I/74; three patients with alpha I/50 and alpha I/46.*

P004

TWO UNUSUAL CASES OF HEREDITARY STOMATOCYTOSIS

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Hereditary Stomatocytoses (HSto) are genetic defects of the erythrocyte membrane that result in abnormal permeability to Na^+ and K^+. The diagnosis is based on the presence of hemolytic anemia, macrocytosis, stomatocytosis, abnormal cation cytoplasmic concentrations and typical abnormalities of the osmotic gradient ektacytometry curves. We describe two patients affected by HSto diasplaying unusual ektacytometry patterns on both whole blood and density-fractionated erythrocytes. Some hematological data before and after splenectomy are summarized in the Table below.

Table.

	A		B	
Parameters	**before splen**	**after splen**	**before splen**	**after splen**
Hemoglobin (g/dL)*	10.3	11.2	10.9	11.8
MCV (fL)	95	100	90	93
Reticulocytes (x10⁹/L)	550	217	162	41
Stomatocytes (%)	12	33	22	18
Target cells (%)	8	13	0	0
Spherocytes (%)	0	0	8	7
Osmotic fragility	decreased	decreased	increased	increased
Stomatin	slightly decreased	N	N	N
$[Na+]_i$	n.d.	13	18	13
$[K+]_i$	n.d.	96.4	77	77
Ektacytometry curve	n.d.	abnormal	n.d.	abnormal

**Reference values: Hb 12.2-16.0 g/dL; MCV 81-100 fL; Reticulocytes 24-84 x10⁹/L; $[Na+]_i$ 9.1-10.1 mM/L RBC; $[K+]_i$ 98.4-105.7 mM/L RBC. N, stays for normal, n.d. for not done.*

Patient A (female, 33 yrs old) was diagnosed as having HSto at the age of 23 yrs. She underwent cholecystectomy and splenectomy at the age of 24 yrs because of cholelythiasis and marked splenomegaly. The osmotic gradient ektacytometry curve (osmoscan) on the whole cell population showed the expected pattern for the Dehydrated Hereditary Stomatocytosis (DHS). Surprisingly, the densest RBC which were presumably the youngest (Chailley et al. Scan J. Haematol. 27(1981)365) revealed a striking left shift with a minimum (50% hemolysis) significantly below 130 mosM. Such an extreme cellular dehydration state has never been reported in the literature. Patient B (female, 33 yrs old) was diagnosed as HSto at the age of 27 yrs. She underwent splenectomy at the age of 28 yrs because of severe idiopatic thrombocytopenic purpura. She displayed both stomatocytes and spherocytes at the peripheral blood smear examination. SDS-PAGE analysis of red cell membrane proteins was normal. The osmoscan showed features shared by both HS and HSto: the minimum of the osmoscan was shifted to the right and the maximal DI was lower than normal, as found in HS patients. In contrast to HS RBC, the cellular hydration was increased similar to what observed in Overhydrated Hereditary Stomatocytosis.

P005

UNNOTICED ABNORMALITIES, MISINTERPRETATIONS AND PITFALLS IN AUTOMATED BLOOD CELL ANALYSES IN A CASE OF ANEMIA, INFREQUENT AND ACQUIRED IN AN UNUSUAL WAY : MICROSCOPIC EXAMINATION OF BLOOD FILM IS STILL, OFTEN, IRREPLACEABLE

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A 54 years old male, barkeeper, was admitted on January 2003 for progressive asthenia, anorexia, nausea, colicky abdominal pain and diarrhoea of three months duration. At physical examination, he was pale with a slight scleral icterus; abdomen was soft and not tender; liver edge was appreciated at 4 cm below costal margin and spleen was not enlarged. Echography documented liver steatosis and cholelitiasis. Laboratory data (Table 1) documented normochromic normocytic anemia with high reticulocyte count; slight elevations of bilirubinemia (prevalently unconjugated), of transaminases and of gamma G T; lactate dehydrogenase and haptoglobin levels were normal.

Table 1. Hematologic Laboratory data on admission (Blood cells counts were performed with Coulter Counter MAX M).

Variable	Value	Normal range
Hemoglobin g/dL	8.4	14-17
Mean corpuscular volume(fL)	86	75-95
Mean corpuscolar hemogl.conc. g/dL	34.2	30-36
White cells /mm^3		
Neutrophils	4.900	2.200-4.800
Lymphocytes	2.900	1.300-2.900
Monocytes	600	300-800
Eosinophils	100	0-200
Basophils	0	0-100
Platelets /mm^3	195.000	150.000-400.000
Reticulocytes, thousands/mm^3	284	25.000-50.000
Bilirubin: total/conjugated mg/dL	1.31/0.27	0.00-1.00/0.00-0.20
Lactate dehydrogenase U/L	157	80-285
Serum iron microg/dL	86	53-167
Total iron binding capacity microg/dL	198	200-360
Ferritin ng/mL	562	28-365
Haptoglobin mg/dL	168	30-200
Serum aspartate aminotransferase U/L	58	< 37
Serum alanine aminotransferase U/L	77	< 40
Gamma glutamyltransferase UI/L	63	10-49

Direct and indirect Coomb's tests were negative. High reticulocytes count, being not documented blood losses, pointed to an hemolytic nature of the anaemia, although haptoglobin and lactate dehydrogenase were on normal range. Microscopic examination of a May-Grunwald-Giemsa stained blood film evidenced erythrocytes of normal size and shape; of them, about 10% presented numerous, fine and coarse, dark blue, round granules, evenly distributed within the cytoplasm. Brilliant cresyl blue supravital stain evidenced blue granules in about 10% of red blood cells, with less than 0,2% of them presenting a reticulum network. Iron stain was negative. Thalassemias, other hemoglobinopathies, megaloblastic anemias and myelodysplasias were ruled out on the basis of clinical and laboratory data and of erythrocytes morphology; lead poisoning was suspected, being also supported by presence of abdominal symptoms. The patient denied any occupational exposure to lead, but recalled that, about five months before, some lead weights, used as ballast for diving, fortuitously fell into a barrel containing home-made wine, that he and other family member usually drank. High blood lead levels were documented in the patient and in two family members (Table 2), as well as in the wine(7.56 mg/L; normal value <0.3).

Table 2

	Lead Blood level microg/dL	Urinary delta aminolevulinc acid mg/L urine	Erythrocytic Free Protoporphyrin IX microg/dL
Patient	116	32.8	126
daughter	60	5.2	124
Son-in-law	57	3.7	55
Reference value	0.1-4.5	< 60	

The family members did not show anemia nor other clinical symptoms referable to lead intoxication. The patient and the two other intoxicated family members underwent treatment with Na2CaEDTA (1.5 g i.v.). Abdominal problems promptly disappeared, hemoglobin reached normal values after 4 months. Patient's lead blood levels decreased very slowly, requiring a second treatment course and reached values lower than 35 micrograms /dL after 1 year, while this value was reached after 2-3 months in the other two intoxicated family members. Lead intoxication, usually due to occupational or environmental exposure, may also be caused by ingestion of foods, water and other beverages contaminated with lead coming from pipes or containers; home-made wine, in the Mediterranean area, represents the most common source of non occupational lead poisoning.[1] In the present case, wine resulted contaminated by the metal easily released, in the acid environment of the wine, from lead weights, fortuitously fallen into it. Red blood cells punctate basophilia is due to the staining, with basic dyes, of pre-existing abnormal aggregates of ribosomes with mitochondria within the erythrocytes. It is just one in a long list of blood cells morphologic abnormalities that are missed by automated blood cell counters. Moreover, adding a pitfall to a missed data, these abnormal cells are erroneously enumerated by these machines as reticulocytes. Indeed, all the red cells that have, into their cytoplasm, any type of nucleic acid (being it the residual ribosomes of the reticulous filamentous substance in the true reticulocytes, nuclear remnants like Howell Jolly bodies and Cabot's ring, intra-erythrocytic microorganisms like Malaria and Babesia, pre-existing aggregates of ribosomes and mitochondria as in lead poisoning, ribosomal RNA bound to hemoglobin precipitates) take up the reticulocytes stains used in these machines(New Methylene Blue, in this case) and, thus, can be regarded by them as reticulocytes and so enumerated.[2] Documentation of punctate basophilia in erythrocytes represented, in this case, the fundamental diagnostic clue. It would have been missed was not followed an old rule(not to be forgotten in this era of automated blood cells analyzers!) that states: *a careful and expert microscopic examination of a well prepared and well stained blood film must be included, on principle, in the*

diagnostic work-up of each patient having any kind of hematologic problem, irrespective of results given by automated blood cell analysers. As this case demonstrates, such a cheap and fast laboratory procedure *may permit to acquire important morphologic aspects of blood cells that the presently used counting machines are not able to put into evidence. *May also unmask some of their misinterpretations, that could be diagnostic pitfalls

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P006

FAMILIAL (CONGENITAL) POLYCYTHEMIA: A PARADIGMATIC CASE (FAMILIAL SAN DIEGO HAEMOGLOBIN)

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In 2004 we studied two sisters (D.B. and R.O.) affected of polycythemia.Their father (R.B.), as well, was treated for polycythemia (considered secondary to COPD) with phlebotomies and hydroxyurea in another hospital. Their fatherly grandmother had been phlebotomized with leeches for a not well defined illness. These findings were consistent with a familial disease.

The all three patients had pathological values of red blood cells mass, normal serum erythropoietin (EPO) levels, normal EAB (specifically arterial oxygen saturation), normal abdominal echography and thorax x-rays. D.B. was the only one with a slight neutrophilic leukocytosis. Only the two sisters agreed to do more specific exams. So, D.B. and R.O. underwent to a bone marrow biopsy, which result was the following: erythroid hyperplasia; cytogenetic analysis: normal female karyotype. The count of $CD34^+$ circulating cells was normal. Congenital polycythemia vera was excluded; in the two sisters erythropoiesis was not clonal, besides there was not spontaneous growth of myeloid and erythroid colonies and only a poor growth adding exogenous EPO in the medium was observed. This result permitted us to rule out hypersensitivity to EPO or EPOR. Congenital polycythemias due to oxygen sensing defect were ruled out, too, in the evidence of the normality of both the alleli of von Hippen-Lindau [VHL gene] (used to make diagnosis of Chuvash polycythemia [CP] or due to other VHL genotypes).

Afterwards, we tested the two patients and their father for disorders of altered oxygen affinity of haemoglobin: hight affinity haemoglobin disorders and 2,3-diphosphoglycerate deficiency.

As abnormal haemoglobin fraction could not be shown by standard electrophoresis we performed HPLC. We found a variant haemoglobin called "San Diego haemoglobin type". At the moment, gene anaysis is in progress to find the definitive prove of the presence of this hight affinity haemoglobin. This, we described, would be the first case of familial San Diego haemoglobin ever quoted in literature. Until now, the sons of the two women do not have any hematological alteration. The three patients are still being phlebotomized to maintain Hct 45-47%.

P007

APPROACH TO IRON DEFICIENCY ANEMIA IN PREMENOPAUSAL WOMEN: USEFULLNESS OF A MULTIDISCIPLINARY TEAM

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Background. In premenopausal women, iron-deficiency anemia (IDA) is common and the menstrual flow is often considered responsible. The usefulness of gastrointestinal (GI) evaluation in these woman is not clear and still not advocate, whereas gynaecological assessment is often not accurate.

Aim. To prospectively investigate whether premenopausal women with IDA benefit from GI and gynaecological evaluation regardless of GI symptoms and menstrual flow.

Methods. 167 consecutive premenopausal women (median age 38 yrs), over a two yrs period, with recurrent unexplained IDA (Hb median 10.7 g/dL, ferritin median 4,2 ng/dL) and GI asymptomatic after haematological assessment were referred for gastroenterological evaluation. All patient were requested gastroscopy with biopsies to exclude celiac disease and chronic H.pylori related gastritis. 12 patients refused upper GI endoscopy and were excluded from the study. In 92 patients were requested gynaecological evaluation with an accurate assessment of the menstrual flow and transvaginal ultrasonography. *Results.* A likely cause of IDA after gastroenterological evaluation was found in 99 (64%) patients. In 92 (93%) patients the cause of IDA was related to iron malabsorbtion due to celiac disease (n=29) H. Pylori chronic pangastritis (n=50), and atrophic body gastritis (n=13). In the remaining 7 patients a GI bleeding-related cause of anemia was found. After gynecological evaluation, that was performed in 76 patients, menorragia was diagnosed in 57 (75%) patients, and only in 40% of patients due to organic cause (uterine polyps, myoma, fibroma). However the sole menorragia was considered the cause of anemia in only 21 (28%) patients.

Conclusions. In IDA premenopausal women referred from haematologist, a likely cause of IDA has been diagnosed in a large percentage (78%) of patients only after a combined accurate gastroenterological and gynaecological evaluation. Then the approach to IDA patients should be performed by multidisciplinary team including haematologist, gastroenterologist and gynecologist.

P008

PRESENCE IN ITALY OF A RARE BETA THALASSAEMIA NON-SENSE MUTATION [CODON 59 (AAG->TAG)

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We have examined four members of an Italian family: the proband, PM, a 33 years old male, his parents, PMA and BO and his sister, PP. The proband presented a beta-thalassaemic trait associated to a relevant level of Hb F. Molecular analysis showed the presence in heterozygosity of a rare non-sense point mutation at the codon 59, changing the codon (AAG) to a non-sense codon (TAG), a stop signal of the synthesis of beta globin chains. The only description of this mutation in literature was reported by Patterson *et al.,* 2003 in association with the common IVS-I-110 (G->A) but the family members were not analyzed, and so the phenotypic expression of this mutation could not be evaluated. In our family, reticulocyte count was always higher respect standard values (PM: 17‰, PMA: 13‰, BO: 15‰, PP: 13‰) and the most common alpha thal mutation resulted absent. Mother and sister showed normal haematological indices. The proband and his father, who is also a carrier of the codon 59 mutation, showed the haematological finding picture of a severe beta-thal, with evident reduced MCV and MCH (PM: 63 micrograms and 20.1 pg; PMA: 65 micrograms and 20.7 pg respectively), and red cell morphology alterated with typical mycrocytosis, hypocromia and anisopoikilocytosis. In addition, they showed an increased level of Hb A2 (5.2% and 6.2%), and a decreased osmotic fragility (65% and 70%). The only haematological difference between the two individuals was the increased level of Hb F (1.0% in the father and 3.3% in the proband). The globin chain synthesis analysis of the proband showed an imbalanced alpha/beta-globin ratio (1.50, corresponding to beta thal heterozygosity). To explain the high level of Hb F in the proband, we have also extended the molecular analysis to the promoter region of the G-gamma gene (-158 C→T) and to the (AT)x(T)y repeat motif in the promoter of beta globin gene. These two polymorphic segments have been shown to correlate with a higher Hb F expression under the mild erythropoietic stress in beta-thal heterozygous individuals. In fact there are reports that the co-inheritance of the –158 (C→T) G-gamma polymorphism and the (AT)9(T)5 repeat motif showed a higher Hb F expression (Ferrara M *et al.*, 2003). The patient and his father were homozygous for G-gamma –158 (C→T) polymorphism, the mother and the sister were heterozygous but all the subjects were homozygous for the (AT)7(T)7 wild-type repetitive motif. Finally, we have also investigated the polymorphisms present in the HS2 and HS4 hypersensitive sites of the LCR promoter, associated to a thalassaemia intermedia phenotypes. Our results suggest that the higher Hb F level (3.3%) in the proband is probably due to the regulatory properties of the polymorphic site HS2 in the LCR co-inherited with the –158 (C→T) polymorphism in homozygosity. The study stresses the importance of extending the screening also to rare mutations as codon 59 (A→T) to offer a better genetic counselling in at-risk couples and the importance of performing molecular genetic analysis also in regulatory elements of different globin loci to improve the genotype-phenotype correlation in non classical beta-thalassaemia patients.

P009

HB ABRUZZO: DESCRIPTION OF A NEW FAMILY AND REVIEW OF THE LITERATURE

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A young man (E.G.), aged 27 years, born in Campobasso (Molise, a region in the center of Italy) was referred to our observation for persistent erythrocytosis (Hb18.3 g/dL; Ht 51.4%; RBC 5.83 10^{12}/L MCV 88.2 fl; MCH 30.1 pg; MCHC 34.2 g/dL. Reticulocyte, WBC and Platelet counts were in normal range (42.4 10^9/L: 0.7%, 6.03 10^9/L, 166 10^9/L respectively) as far as differential WBC count. No signs of hemolysis were present: serum total bilirubin, LDH, aptoglobin were in the normal range and no haemoglobin in the urine was present as far as no abnormality of the common serum parameters except low folate level. Liver and spleen size was normal at physical examination and the echoghraphic evaluation confirmed this finding. The patient underwent the common screening for erythrocytosis which includes also HPLC hemolysate analysis (Variant II-BIORAD) and serum erythropoietin assay. The first test showed an abnormal haemoglobin band (51.9%) with the same retention time of Hb A2 (3.53 min); HbA0 was present. This abnormal hemoglobin migrated faster than HbS but lower than HbF at pH 9 cellulose acetate electrophoresis. Serum erythropoietin was 10.8 mU/mL. In the following weeks, we examined all the family (parents, one brother and three sisters); only the father has the same abnormal haemoglobin and the Complete blood count (CBC) was similar to that observed in the propositus. To identify the abnormal haemoglobin, the DNA sequence of β-globin gene has been performed; it revealed an heterozygosity for the CAC-->CGC mutation at codon 143, resulting in a histidine->arginine aminoacid substitution, corresponding to Hb Abruzzo [β143 (H21)]. This mutation was successively confirmed by ARMS-PCR methods. Furthermore, the oxygen affinity of our patient with Hb Abruzzo has been determined by the tonometric method at 37°C, in the absence and presence of organic phosphates (2,3 BPG) in a range of pH between 6.8 and 7.8. The results obtained showed a normal affinity compared with HbA in the absence of organic phosphates while the presence of BPG at physiological concentration (3-5 mM) increased significantly the capacity to bind the oxygen, showing at pH 7.2 a value of p50~20mmHg (HbA value = 28 mmHg). In

this contest a normal Bohr effect was found both in the absence and in the presence of organic phosphates. These results are in agreement with the aminoacidic substitution characterising Hb Abruzzo. Until now, Hb Abruzzo has been reported three times: the first family was from Schiavi degli Abruzzi (2 brothers); the second in a man born in Campobasso, the main city of Molise and the third report concerned four members of a family from Troia (Puglia). In one member of this family and in the first 2 cases, the abnormal haemoglobin was together with a β-0 thalassemia. For the presence of the latter, they showed a clinical picture inducing to suspect an hemoglobinopathy (mild hemolysis, splenomegaly, microcytosis and hypochromia). However, the clinical sign of Hb Abruzzo, when it is inherited alone, is erythrocytosis. We retain that in a screening for polycythemic conditions with normal or elevated s-EPO the presence of abnormal haemoglobin with high oxygen affinity should be investigated. The best method is to measure haemoglobin oxygen dissociation curve, but the equipment is seldom avalaible. The calculation of P50 from Blood gas analysis could give some information. On the other hand, the Hb Abruzzo gives a cleary alterated HPLC hemolysate analysis; so this test is able, in a first diagnostic step, to identify its presence. For this reason and for the fact that this haemoglobin seems to have a common origin in southern Abruzzo, in northern Puglia and in Molise, we retain that in the last region (a part of central Italy located exactly halfway between Abruzzo and northen Puglia), the HPLC hemolysate analysis must be regularly performed in polyglobulic patients, overall if other signs of myeloproliferative disorder are not present (leukocytosis and/or thrombocytosis)

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P010

MOLECULAR BASIS OF ALPHA THALASSEMIA IN CALABRIA: ANOTHER KNOWLEDGE

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Defects of alpha-globin genes result from the loss of function of one (alpha +) or both (alpha zero) alpha genes. They are more rare and less serious than beta-globin defects in Mediterranean area, and yet not much known. In our Region, foetal hydrops (alpha zero omozygosis) was never documented, while HbH anaemia is frequently found the most serious form. The alpha thalassemia diagnosis can be estimated either at birth by the presence and quantitation of Hb Bart's in cord blood or late in life for microcytic anaemia with HbH inclusion bodies or for the observation of microcytosis associated with a normal value of HbA2. More often, the haematological indices are only slightly altered and overlap with those of normal individuals. In the last two years, we have share in a scientific network for the study of molecular epidemiology of alpha-thalassemia in Southern Italy. We carried out haematological screening, collection of personal data, DNA isolation and gap-PCR protocol for molecular screening of the most common (-alpha$^{3.7}$, --MED, --alpha$^{20.5}$) thalassemia deletions, in according to a flow-chart of the Institute of Genetics and Biophysics (IIGB) in Naples. All DNA samples were analysed at IIGB using gap-PCR techniques, DGGE, ARMS, DNA-Sequencing and the restriction mapping of DNA genomic. We present the results obtained by a single Centre study on 291 subjects from Calabria, who belonged to 147 unrelated probands (or family). A pool of 276/291 samples has been characterized for alpha gene defects, while 15 samples are still waiting for a final analysis. Among 276 samples, 150 showed an alpha thalassemia trait in heterozygous (51.1%), or homozygous (2.5%) or compound heterozygous (0.7%), and 126 (45,7%) had normal genotype. The aim of the present study was to identify the frequency of the alpha-thalassemia mutations in the population of Calabria. The most commons mutations noted included: alpha$^{3.7}$ (59.1%), --MED (5.7%), alpha$^{3.7}$-AC (6.3%), alpha$^{20.5}$ (6.9%), HphI (7.5%). Rare alpha talassemia mutations were: NcoI (3.1%), PolyA (3.1%) (alpha$^{4.2}$ (2.5%), --CAL (0.6%). Interestingly, two new point mutations were found: Codon 23 (1.3%), Codon 108 (3.8%). Finally, compound heterozygotes have been found: an HbH phenotype (alpha$^{20.5}$/alpha$^{3.7}$) and a simple alpha zero form (HphI/PolyA). In conclusion, through this study the alpha-mutation spectrum has been enlarged, disclosing a remarkable alpha-thalassemia genotypic heterogeneity in Calabrian areas and the high frequency of alpha$^{3.7}$ deletion has confirmed. Furthermore, the detailed knowledge of the alpha gene defects help for an efficient genetic counselling.

P011

ANAEMIA AS MANIFESTATION OF SOLID TUMOUR'S BONE MARROW METASTASIS: EXPERIENCE OF A SINGLE CENTER

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Bone marrow is one of the most involved organs by metastases of solid tumours. Anaemia, leukoerytroblastic picture of peripheral blood, thrombocytopenia are the most frequent signs of bone marrow infiltration by solid tumour cells. For the laboratory evaluation of a metastatic lesion the specimen from the bone marrow has to be adequate, representative and properly preserved. In the past literature some authors have discussed the diagnostic role of bone marrow aspirate compared to needle biopsy in the detection of metastatic neoplasm, with discordant results. Aim of this study was to evaluate the diagnostic power of bone marrow aspirate for the detection of metastases. To assess the frequency of different primitive solid tumours metastasised in bone marrow in our Center, we retrospectively evaluated bone marrow aspirates (10500 samples)

from January 2000 to December 2004. We selected 120 patients, on the basis of clinical data and queries from our archive. The selection criteria included: normocromic normocytic anaemia (haemoglobin (Hb) median values 11g/dL), severe anaemia (Hb<7g/dL), thrombocytopenia, leukoerytroblastosis, cytopenia, disseminated intravascular coagulation (DIC), carcinocythemia. Bone marrow aspirates were harvested from the superior posterior iliac crest. Microscope analysis was carried out on May-Grümwald-Giemsa stained bone marrow smears. Among the 120 selected aspirates, 74 resulted negative, while 46 positive for metastatic lesions. Analysing the 46 positive bone marrow specimens, the primitive tumour lesion showed this distribution: breast 28%, unknown 28%, lung 19.5%, stomach 11%, prostate 6.5%, others 7% (kidney, pancreas, melanoma). Of the 46 positive aspirates, 26 biopsies were performed, 22 resulting positive for tumour metastasis. Of the 74 negative aspirates, 21 biopsies were made, none resulting positive for bone marrow tumour's involvement. Comparing data about 47 patients submitted to both bone marrow aspirate and biopsy the sensibility and the specificity for bone marrow aspirate were, respectively, 100% and 84%. Looking for the queries, 104 patients with a known cancer were submitted to bone marrow aspirate in the suspicion of a metastatic invasion; in 16 cases, instead, the aspirate, requested in the suspect of a haematological or infectious problem, revealed a bone marrow metastatic infiltration. Four out of 104 patients with cancer showed secondary diseases at bone marrow examination (3 myelodysplastic syndromes, 1 hairy cell leukaemia). Our data confirm the complementarity of bone marrow aspirate and biopsy in the detection of metastases and show the advantage of aspirate as a reliable, rapid and useful procedure to identify positive samples and, if possible, accelerate the diagnostic and therapeutic course.

P012

CA 15-3 A MARKER FOR THE MEGALOBLASTIC ANAEMIA?

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Ca 15-3 is a glycoprotein present in the cells of the mammary carcinoma and in some epithelial cells. Is a marker used for the monitoring of the breast and gastrointestinal carcinoma. The megaloblastic anaemia is an anaemia characterized by deficit of absorption of Vitamin B12 and is associated with gastritis atrophic and the target cell is the parietal gastric cell. In our institution, from June 2003 to December 2004, the level of Ca15-3 and of others tumour markers (CEA; Ca125; Ca19.9; alfa-FETO) they have been tested in the serum of 16 patients (9 male and 7 female with median age of 64,5 and range of 37-80 years) with de novo megaloblastic anaemia, 2 patients were gastrectomized. In all patients has been effected: esophagogastroduodenoscopy and control anti-parietal gastric cells antibody (APCA). Increase level in the serum of CA 15-3 with normal level of other tumour markers have been found in 14/16 patient with median value of 61 U/mL (range 35-100 U/mL) in two patients (gastrectomized) the value of CA 15-3 was normal. Besides in 12/14 patients have shown positivity for the APCA, only in two patients has been diagnosed a gastritis atrophic, in the other patients has been observed a normal gastric mucous. After a median observation of 24 months any patient has developed a mammary or gastro-intestinal carcinoma These results indicate what the increased level of CA 15-3 antigen in patient with megaloblastic anaemia is positively correlated with APCA and with the presence of a normal gastric mucous. These clinical conditions make to suppose the destruction from the APCA of the parietal gastric cells with the liberation of this glycoprotein and this is shown in two patients gastrectomized with presence of APCA and not increased level of the CA 15-3. In conclusion the CA15-3 antigen is probably an specific marker for the diagnosis of megaloblastic anaemia and is probably associated with the destruction of parietal gastric cells.

P013

Δ-CHR IMPROVES THE IDENTIFICATION OF ANEMIC SYNDROMES AND THE EVALUATION OF HEMOGLOBIN SYNTHESIS

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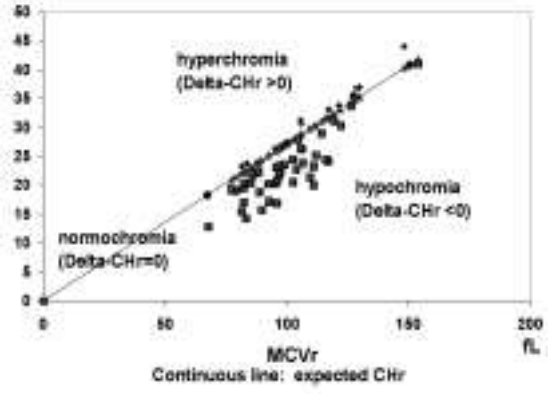
Many investigators believe that reticulocyte parameters can help to differentiate iron deficiency anemia from thalassemia, providing insight into bone marrow erythroid activity without expensive or invasive procedures. The potentialities of reticulocyte indexes derive from the short mean life of peripheral blood reticulocytes (24-48 hours), which mirrors the late phases of hemoglobinogenesis. Reticulocyte hemoglobin content (CHr) is considered an index of iron status, helpful in the differential diagnosis of microcytoses. We tried to improve the diagnostic potential of CHr in anemic syndromes and as index of erythroid activity by applying dynamic reference values (CHr-e), to possibly overcome the limitations verified in cases of severe micro- or macrocytosis. We studied 487 anemic subjects (M=187 and F=300): 40 with normocytic hypochromic anemia (hypochromic erythrocytes > 8%), 7 with normocytic normochromic anemia, 11 with normocytic hyperchromic anemia (hyperchromic erythrocytes > 1%), 221 with microcytic anemia (MCV < 80 fl > 5%), including 145 having the β-thalassemia-trait (BTT) and 75 without the β-thalassemia-trait (NBTT, i.e. presumptive iron-deficient subjects). A single patient with iron deficiency anemia was repeatedly examined during oral iron therapy. Two hundred and seven normal subjects were studied as controls. Full blood counts, including erythrocyte indexes and reticulocyte count, were performed by a H3-Bayer auto-analyzer. Microcytic cases were studied for HbA2 percentage estimation by the Variant Hemoglobin Testing System (VHTS, Biorad, Milan, Italy). Estimation of MCVr, CHCMr, MCV and CHCM were obtained by H3-Bayer with light scattering for small and big angle. CHr was automatically calculated multiplying CHCMr by MCVr, which are directly measured by the autoanalyzer. Considering that CHr and MCVr are directly proportional, we built a

line starting from mean CHr and MCVr values for the reference group and then inferred the expected CHr (CHr-e) for each MCVr in the range of 45÷150 fL. CHr-e increase was be proportional to MCVr. ΔCHr was calculated as the difference between measured CHr and the CHr-e for the corresponding MCVr (ΔCHr=CHr–CHr-e). By plotting CHr and MCVr values from the first group of 58 anemic subjects on a MCVr/CHr graph, we were able to identify three subgroups of patients: hypochromic, normochromic and hyperchromic subjects were located to the right, on the line and to the left of the line, respectively (see fig.). These differences are better highlighted when expressed as ΔCHr: hypochromic, normochromic and hyperchromic subjects have ΔCHr below, equal to or above zero, respectively (mean ± SD of normochromics: 29.72±8.68, of hyperchromics: 32.13±6.21, of hypochromics: 22.58±5.29; Student's t test: hypo vs. hyper <0.05, hypo vs. normo p= 0.2, hyper vs. normo p= 0.6). As for the 221 microcytic subjects: a) CHr and ΔCHr values of BTT and NBTT subjects were both below the reference values (p<0.001); b) ΔCHr values of BTT significantly differed from those of NBTT (p<0.001); c) CHr-e of NBTT subjects were close to reference values (p=0.5), while CHr-e of BTT subjects were significantly lower than in controls (p<0.05). As for the subject with iron deficiency anemia, although Hb reached the normal range at 30 and 45 days, a negative ΔCHr suggested persistently deficient erythropoiesis, requiring further treatment which was followed by further Hb increase. We demonstrate that the difference between measured CHr and CHr-e (ΔCHr) is helpful to differentiate the anemic syndromes and, in particular, δ-talassemia vs. presumable sideropenia. ΔCHr can also indicate when to interrupt iron supplementation. ΔCHr allows an insight into the erythropoiesis of thalassemic and sideropenic subjects, as indicator of the reduced hemoglobin production and ineffective erythroid activity in these conditions.



P014

HIGH FREQUENCY OF CONGENITAL POLYCYTHEMIA DEPENDENT ON VON HIPPEL LINDAU GENE MUTATIONS IN CAMPANIA REGION

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Chuvash form of familial polycythemia (MIM 263400) is a non-benign autosomal recessive disorder characterized by high hemoglobin level, relatively high serum erythopoietin content and premature mortality mostly due to cerebral vascular events and peripheral thrombosis. The disease is due to Von Hippel-Lindau (VHL) gene (C598T->Arg200Trp) mutation which leads to an increased HIF-1 alpha activity and, in turn, to an abnormal erythrocyte production in response to physiological O2 blood level. This polycythemia is endemic in Chuvashia with a frequency of about 0.057, while the estimated worldwide frequency is around 0.00137. The identification of an identical haplotype in all patients allowed the hypothesis that the disease originates from a single ancient event occurred from 15000 to 60000 years ago. In this study, we investigated for the first time the VHL gene 598C>T transition in the italian polycytemic patients. In particular, we analyzed the frequency of mutation in Campania, a region of the South Italy. 22 patients belonging to 13 families with a presumed congenital Chuvash-like polycythemia were included in the study. We identified five families affected by this form of congenital polycythemia. We have also investigated the haplotype of few of the Chuvash polycythemic patients and observed that it was identical to that observed in Chuvashia. This finding confirms the probable occurrence of a single founder. In conclusion, we identified a high incidence of Chuvash erythrocytosis not localized in Chuvashia. This allows the hypothesis that this form of familial polycythemia might be endemic in other areas of world and that the (C598T->Arg200Trp) VHL mutation, in heterozygosity, gives some important advantage(s).

MYELOPROLIFERATIVE SYNDROMES I

P015

IMATINIB-INDUCED APOPTOSIS IN EOSINOPHILS OF PATIENTS WITH A HYPEREOSINOPHILIC SYNDROME PREDICTS RESPONSE TO TREATMENT

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The detection of the FIP1L1-PDGFRA (F/P) fusion gene in patients with a hypereosinophilic syndrome is the most accurate predictor of response to Imatinib treatment, even if some patients who lack the fusion gene and respond to Imatinib have long been recognised. While the response criteria in all previous studies were not identical and the duration of follow-up was limited, a common feature observed in responsive patients, is a very typically rapid response to therapy. Eosinophil cytoreduction occurs in a few days and is generally achieved with a 100 mg daily dose of Imatinib. In vitro, the data have shown that Imatinib not only inhibits phosphorilation of the FIP1L1-PDGFRA fusion protein, but also potently induces the apoptosis of cells expressing this protein. Surprisingly in a clinical setting, to our knowledge, there have been no reports of induced eosinophil apoptosis by Imatinib. Could this feature be predictive of response? Utilizing the Flow Cytometric analysis after the staining of eosinophils with Annexin V fluorescein and propidium iodide in combination with light and electron microscopy, we studied eosinophil apoptosis in the peripheral blood of hypereosinophilic patients , before and during treatment with Imatinib. Our study included four patients with FIP1L1-PDGFRA fusion and seven hypereosinophilic patients without a F/P fusion. Their median age was 48 years and all patients were males. The median eosinophil count at baseline was 22,500/mm^3 (range 8,965 to 58,000/mm^3) and all patients were symptomatic with current evidence or history of tissue involvement, mostly cardiovascular or neurologic. Patients were started at a dose of 100 mg Imatinib taken orally once a day with food; if no response was observed after one week of treatment, patients were treated at 400mg/day. At 0 h all patients showed a low degree of eosinophils apoptotic cells with a mean value of 4.1+ 0.9%; at 96h after treatment, the percentages of apoptotic cells showed a peak with a mean value of 68.1+3.2% in seven patients; and 7.2+1.1% in the others (p=0.000), while the seven patients(4 F/P+, 3 F/P-) with a raised apoptosis at 96h achieved a sustained complete response with Imatinib Mesylate therapy the others did not. In non responding patients Imatinib was escalated to 400mg/day without a response. Our data suggest that a significant raised apoptosis of eosinophils in HES patients after a 96h Imatinib therapy can be predictive of response to treatment not only in patients with FIP1L1-PDGFRA fusion but also in a subset of patients without a detectable gene fusion. These remarks could be useful for clinicians who do not have access to the techniques necessary to carry out a molecular diagnosis

P016

CLINICA COURSE OF FIFTY-EIGHT PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA

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Background. Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder, characterized by bone marrow reactive fibrosis, extramedullary hemopoiesis, progressive anemia and marked splenomegaly. Overall median survival ranges from 3,5 to 5,5 years, according to the presence or absence of adverse prognostic factors, with final evolution toward disease progression (DP) or leukemic transformation (LT).

Patients and methods. we analysed 58 MMM patients (pts) referred in our hematology unit from 1999 to 2004, in order to provide information about initial features, treatment, clinical course and survival.

Results. The median age at diagnosis was 66 years (range 39-79) with 17 pts (29%) < 55 years and a M/F ratio of 37/21. 32 pts (55%) presented idiopathic MMM, 26 (45%) MMM secondary to Polycythemia Vera (7) or Essential Thrombocythemia (19). At diagnosis, spleen enlargement (median 4,5 cm, range 1-22) below costal margin was present in 46 pts (79%). The median value of WBC was 10,9x10^9/L, of Hb was 11,6 g/dL, of platelets was 367 x 10^9/L. 43 pts (74%) had circulating myeloid precursors; 18 (31%) pts had blasts. The median value of LDH was 912 U/L and the median count of CD34$^+$ cell was 57,8 x 10^6/L (evaluated on 31 pts). According to disease status, 13 pts (22%) received no treatment, 10 (17%) supportive care alone, 8 (14%) androgens or steroids, 19 (33%) anti-platelet drugs and 25 (43%) myelosuppressive agents alone or in combination with the above treatment. Eight pts (14%) underwent splenectomy after a median of 11,5 months from diagnosis. Only one patient underwent allogeneic stem cell transplantation. 47 pts (81%) are actually alive after a median follow-up of 26,5 months (range 3-72). According to Dupriez scoring system, 4 pts (7%) were assigned to high risk (HR), 21 (36%) to intermediate risk (IR) and 33 (57%) to low risk (LR) group. The median survival was 8, 20 and 28 months for HR, IR and LR group, respectively. The median survival in pts <55 years was longer (38 months) with significative worse outcome for patients with Hb <10 g/dL and/or WBC > 30x10^9/L. 11 pts (19%) died, 3 for LT, 3 for thrombosis or bleeding, 3 for DP and 2 for heart failure. LT occurred with a median of 26 months (range 2-48) after diagnosis in 5 pts (9%), 3 of which <55 years aged. At time of LT and DP, most pts worsened organomegaly and constitutional symptoms and presented higher LDH levels at diagnosis.

Conclusions. Our experience confirmes different outcome of MMM pts according to Dupriez score. The median survival in younger pts is longer with worse outcome for anemic and leucocytosic pts. DP and LT correlate with increasing splenomegaly, onset of constitutional symptoms and higher LDH levels at diagnosis.

P017

THE IL-8 INHIBITOR REPARIXIN BLOCKS THE MIGRATION OF CD34+ CELLS OF NORMAL DONORS AND MYELOFIBROSIS PATIENTS

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Background. The chronic myeloproliferative diseases (CMD) are clonal haematological tumours derived from a multipotent haematological stem cell which shows proliferation and differentiation into several lineages in a relatively normal manner. Idiopathic myelofibrosis (IM) is a form of CMD characterised by bone marrow fibrosis, ineffective hematopoiesis and myeloproliferation with extramedullary hematopoiesis, particularly in spleen and liver. Increased numbers of CD34+ cells are present in the blood of IM patients. The molecular mechanisms that are responsible for the abnormal extravasation of CD34+ cells from the bone marrow to the blood, and migration into spleen and liver, are still unclear. IL-8 has been shown to play a role in the regulation of normal CD34+ cells mobilisation and proliferation and in the control of megakaryocytic proliferation and differentation in IM. Reparixin is a small molecule inhibitor of IL-8 (CXCL8) which locks the IL-8 receptor CXCR1/2 in an inactive conformation. AIMS. We have therefore investigated the spontaneous and IL-8 induced chemotaxis of CD34+ cells from normal donors and myelofibrosis patients.

Methods. CD34+ cells from normal donors and IM patients bone marrow were purified by positive selection. Chemotaxis was performed using Boyden chambers with PVP free 5 micron filters. 50000 CD34+ cells were plated in each well and migration was measured after 6 hours at 37°C.

Results. CD34+ cells from both normal donors and IM patients showed spontaneous migration (mean 61 and 97, respectively) and a variable response to 50 ng/mL IL-8 (mean 66 and 83, respectively). In contrast, SDF-1 induced a consistent 2-3 fold increase in CD34+ cell migration, as expected. Interestingly, preincubation of CD34+ cells for 15 minutes with Reparixin at 10nM significantly inhibited the spontaneous migration of normal and IM CD34+ cells by 41% and 60%, respectively (n=5 in each case) as well as the IL-8 induced migration. Moreover, a dose response experiment showed that the effect of Reparixin was dose-dependent. The mechanism of action of the migration block is under investigation, since CD34+ cells from either normal donors or IM patients did not express detectable levels of the CXCR1 and CXCR2 IL-8 receptor. *Conclusions.* Reparixin deserves further in vitro studies for its possible application to inhibit the motility of CD34+ stem cells *in vivo*, with particular interest in the context of myelofibrosis.

P018

RELATIONSHIP BETWEEN PERIPHERAL BLOOD AND BONE MARROW CD34-POSITIVE CELL COUNTS IN PATIENTS WITH PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDER AND ITS POTENTIAL CLINICAL IMPLICATIONS

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Background. Philadelphia (Ph)-negative chronic myeloproliferative disorders (CMDs) include polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis with myeloid metaplasia (MMM). A peripheral blood (PB) CD34-positive cell count > 15 x 10^6/L easily allows MMM to be distinguished from the remaining CMDs classified according to PVSG (PV, ET) and Italian criteria (MMM) (Haematologica 2003;88:1123-28). WHO classification recognizes a prefibrotic stage of MMM characterized by bone marrow hypercellularity without fibrosis. A recent paper (Br J Haematol 2005;128:42-8) reports normal circulating CD34-positive cell counts in patients with cellular phase MMM.

Aims. To study the relationship between PB and bone marrow (BM) CD34-positive cell counts in patients with CMD. Patients and *Methods.* From April 2002 to October 2004, 77 paraffin embedded bone marrow biopsies and concomitant PB CD34 counts were available from 66 patients with CMD. For this analysis, in order to avoid any influence of cytoreductive treatments on parameters in study, we consider only 45 samples of patients who never received cytoreductive treatment. Diagnosis of CMD was according to WHO classification: 11 patients had PV, 5 ET, 6 prefibrotic MMM and 23 fibrotic MMM. PB CD34 counts were assessed by flow cytometry analysis as previously described. In order to identify BM CD34 cells, we examined 10 randomly selected fields at 400 magnification from paraffin sections immunostained for anti-CD34. BM CD34 counts were expressed as percentage of all the hematopoietic nucleated cells in the area. In the same fields, the absolute number of CD34-positive vascular structures was recorded. Non-parametric Mann-Whitney U test was applied to compare PB and BM CD34 counts between groups of patients. Results. Median PB CD34 counts were 3.1x10^6/L (range 0.8-7.2) in PV, 3.4x10^6/L (range 0.8-6.6) in ET, 5.4x10^6/L (range 3.9-10) in prefibrotic MMM and 24.5x10^6/L (range 2.3-1102) in fibrotic MMM. Mann-Whitney test showed that PB CD34 count significantly distinguished PV from prefibrotic (p=0.009) and fibrotic (p=0.0001) MMM, ET from fibrotic MMM (p=0.007), and prefibrotic from fibrotic MMM (p=0.02). Median BM CD34 counts were 0.9% (range 0.4-1.4) in PV, 0.9% (range 0.9-1.1) in ET, 0.7% (range 0.5-0.9) in prefibrotic MMM, and 0.5% (range 0.2-1) in fibrotic MMM. Mann-Whitney test showed that BM CD34 count significantly distinguished PV from fibrotic MMM (p=0.0004), ET from prefibrotic (p=0.04) and fibrotic MMM (p=0.001), and prefibrotic from fibrotic MMM (p=0.02). Finally, there was an inverse relationship between PB CD34 count and BM CD34 count (r=-0.49; p=0.001). Conclusions. This study shows that in CMD the higher the CD34 count in PB, the lower the

CD34 count in BM. The two tests are clearly useful to distinguish patients with fibrotic MMM from those with PV and ET. Our findings also indicate that patients with ET have lower PB CD34 count and a significantly higher BM CD34 count than patients with prefibrotic MMM. The combination of PB and BM CD34 cells might be useful in the diagnostic work-up of patients with CMD.

P019

LEUKEMIC TRANSFORMATION OF POLYCYTHEMIA VERA: A SINGLE CENTER STUDY OF 23 PATIENTS

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Background. Polycythemia vera (PV) belongs to the group of Philadelphia-negative chronic myeloproliferative disorders. Acute leukemia (AL) may occur as late event of PV with an incidence of 5.3x1000 person-years. Studies with long-term follow-up indicate that transformation to AL is part of the natural evolution of PV. Myelosuppressive agents as hydroxyurea or pipobroman, when used as the sole treatment, seem not to increase the natural risk of AL. Aims To define the presenting features and outcome of 23 patients who developed AL within a cohort of 414 consecutive PV patients (3208 person-years of follow-up, from 1970 to December 2004).

Patients and methods. This study includes 23 patients with AL post-PV diagnosed at the Division of Hematology, IRCCS Policlinico San Matteo, Pavia between 1981 and November 2004. Diagnosis of PV was made according to the criteria in use at the time of first observation. Treatment of PV consisted of pipobroman alone (n=14, 61%), sequential use of two or more myelosuppressive agents (n=6, 26%), phlebotomy and pipobroman (n=2, 9%), phlebotomy alone (n=1, 4%). Diagnosis of AL was according to World Health Organization (WHO) criteria, with 20% blast threshold for diagnosis. Results Median age was 68 years, and 18 patients (78%) were >60 years old. At diagnosis of AL, most patients had WBC count >10x10^9/L (n=17, 74%), Hgb <10 g/dL (n=13, 57%), and platelet count >50x10^9/L (n=17, 74%). Leukemia was of myeloid origin in 22 out of 23 patients (96%). The most frequent subtype was M0-M1. Of 14 patients in whom cytogenetic analysis was available at leukemic transformation, 12 showed high-risk abnormalities including complex karyotype (n=10), del (7)(q22) sole (n=1) and del (X)(q26) sole (n=1), while 2 had a normal karyotype. In patients whose karyotype was available at diagnosis of PV, cytogenetic evolution was documented at progression to AL. Treatment consisted of supportive care and/or low-dose chemotherapy (n=15), or induction chemotherapy (n=8) with standard-dose cytarabine plus idarubicin, high-dose cytarabine, or a fludarabine-based regimen. Of 8 patients treated with induction chemotherapy, only one (13%) obtained a complete hematological response. Allogeneic stem cell transplantation was offered to a single patient, who is alive at day +70. The outcome of patients was poor with a median survival of 2.9 months (range 0.6-20.1), with no significant differences between palliation (median 2.5 months, range 0.6-20.1) and intensive treatments (median 5.6 months; range 0.7-8.3). Conclusions This study shows a consistently poor outcome in patients with AL post-PV irrespective of the treatment employed. It may result from distinct clinical and biological features of this type of AL: namely, relatively old age, and unfavorable cytogenetics. Intensive regimens do not translate into a significant increase of survival. Allogeneic stem cell transplantation might be the treatment of choice for the rare patients achieving complete response after induction chemotherapy.

P020

CLINICAL UTILITY OF SERUM OSTEOPROTEGERIN LEVEL IN PATIENTS WITH PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. Myelofibrosis with myeloid metaplasia (MMM), polycythemia vera (PV) and essential thrombocythemia (ET) belong to the group of Philadelphia (Ph)-negative chronic myeloproliferative disorders (CMD). All these three disorders have an elevated risk of vascular complications. Distinctive biological features of MMM include, in addition to bone marrow fibrosis and myeloid metaplasia, also osteosclerosis, which is not found in PV and ET. Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor (TNFR) superfamily that acts as a decoy receptor for the receptor activator of nuclear factor-kB ligand (RANKL), inhibiting RANK/RANKL interaction on osteoclasts and osteoclastogenesis. Murine models indicate that osteosclerosis predominantly occurs via an up-regulation of OPG in thrombopoietin-induced myelofibrosis (Blood 2003;101: 2983-9). Recent clinical studies have also reported a correlation of OPG with vascular diseases.

Aims. To prospectively assess the clinical utility of serum OPG levels in 166 patients with Philadelphia (Ph)-negative CMD. Patients and Methods From February to November 2004, blood samples from 166 consecutive patients with Ph-negative CMD were collected. In detail, 35 patients (10 at clinical onset and 25 at follow-up; 18 under treatment and 17 out of therapy) had MMM, 71 patients (10 at clinical onset and 61 at follow-up; 39 under treatment and 32 out of therapy) had PV; 60 patients (16 at clinical onset and 44 at follow-up; 31 under treatment and 29 out of therapy) had ET. At the time of serum OPG measurement, thrombotic complications were observed in 11 patients with MMM, 22 with PV and 28 with ET. Osteoprotegerin was measured in a blind manner by enzyme immunoassay (Biomedica Gruppe. Wien) following the manufacture instructions. Bone marrow biopsies from 45 patients (21 at clinical onset; 24 at follow-up) were evaluated in a blind manner at the time of OPG measurement to assess bone marrow fibrosis and osteosclerosis.

Results. Median serum OPG levels were 5.23 pg/mL (range 1.91-20.61) in MMM, 5.6 pg/mL (range 0.99-21.13)

in PV, 5.44 pg/mL (range 0.4-28.23) in ET and 4.48 pg/mL (range 2.8-8.5) in age-matched healthy control. Kruskal-Wallis ANOVA did not show a significant difference in serum OPG levels between different patient groups. Non-parametric Mann-Whitney test did not reveal any significant correlation between serum OPG levels and degree of osteosclerosis and bone marrow fibrosis. A general linear model showed that serum OPG levels correlate with history of thrombosis (p=0.023) and age (p=0.008). Conclusions. Serum OPG is not a surrogate marker of osteosclerosis and does not allow MMM to be distinguished from PV or ET. The relationship with history of thrombosis rather indicates that OPG might be useful to predict vascular complications in patient with Ph-negative CMD.

P021

SDF1-3'A GENE POLYMORPHISM: RISK FACTOR FOR THROMBOTIC EVENTS IN PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. SDF1-3'A polymorphism, containing a G to A transition at position 801 in the 3' "untranslated region" (3'-UTR) of the human SDF-1 gene, is associated with an increased production of SDF-1 chemokine which is reported to play a key role in angiogenesis and atherothrombotic process. In fact SDF-1 protein is involved in the neovascularisation/angiogenesis process which occurs during the unstable atherosclerotic plaque formation and it induces an increased VEGF production, which in turn promotes the endothelial activation, with a subsequent switch to a predominant prethrombotic phenotype. Aim: The purpose of this study was to evaluate the impact of the SDF1-3'A polymorphism on susceptibility to develop thrombotic complications in Philadelphia-negative chronic myeloproliferative disorders (CMDs), which are characterized by a high incidence of thrombotic events, the mechanisms of which are still not yet defined.

Materials and methods. We studied 82 consecutive patients with CMDs, aged 71±10 years, (45 with Polycythemia Vera, 28 with Essential Thrombocythemia and 9 with Idiopathic Myelofibrosis). Thirty-one patients had previous thrombotic complications. The SDF-1 polymorphisms were determined by PCR and RFLP.

Results. The genotype distribution in subjects with a positive history of thrombotic events was GG=14; AG=9; AA=8, while in those without previous thrombosis was GG=29; AG=19; AA=3. A allele frequency was 0.39 in patients with previous thrombotic complications and 0.24 in the others. Genotype distribution of SDF-1 resulted statistically different between subjects with or without thrombotic events (chi-square=6.59; p=0.037). SDF1-3'AA genotype was strongly associated with development of thrombotic events in CMDs, in fact subject homozygous for A allele had about five fold higher risk of thrombotic complications than AG+GG (OR, 5.56; CI, 1.35-22.95; p=0.017).

Conclusions. Our results allow us to speculate that the homozygosity for SDF1-3'A polymorphism might be considered a genetic component which contributes to thrombotic events in CMDs.

P022

ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISMS (G894T AND T786C) IN ESSENTIAL THROMBOCYTHEMIA

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Introduction. Endothelial NO synthase (eNOS) continuously generates NO in the vascular endothelium so contributing to vascular tone regulation and to inhibition of platelets and leukocytes endothelium adhesion. Mutations in the eNOS gene are referred to influence NO production with consequent functional vasal abnormality. Different polymorphisms of this gene have been described and both G894T on exon 7 and T786C on promoter have been reported to be strong risk factors for vascular disease. Aim: Since subjects with Essential Thrombocythemia (ET) have an increased risk for thrombotic events, we studied eNOS polymorphisms G894T and T786C in these patients in order to evaluate a possible association between the presence of such polymorphisms and thrombotic events. *Materials and methods.* we genotyped 25 consecutive subjects with ET (mean age 65±12 years). Thirteen out of 25 had a positive history of thrombotic events. eNOS polymorphisms were determined by PCR and RFLP.

Results. Genotype distribution of G894T polymorphism in ET subjects with respect to thrombotic events was: GG=2, GT=10, TT=1 in subjects with a previous thrombotic event and GG=7, GT=5, TT=0 in those without thrombotic event. T allele frequency was 0.46 in subjects with thrombotic events, and 0.21 in the others. The occurrence of thrombotic events was significantly different between GT+TT and GG subjects (chi-square=4.99; p=0.041). Subjets homozygous or heterozygous for T allele have about eight fold higher risk for thrombotic events than GG subjects(OR 7.7; 95% CI, 1.15-51.17). No difference was observed about T786C polymorphism. *Conclusions.* The presence of the T allele for G894T polymorphism seems to be associated to a higher occurrence of thrombosis in ET subjects and might be a predictive factor for vascular damage and help in better understanding thrombotic events in these patients.

P023

PLASMA HOMOCYSTEINE AND OXYDATIVE STRESS IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA. EFFECT OF FOLIC ACID SUPPLEMENTATION

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Background. An increase in plasma homocysteine (Hcy) has been associated with augmented oxidative stress as a consequence of autoxidation of Hcy which exceeds NO bioavailability. An augmented oxidative stress may be hypothesized in subjects with Polycythemia Vera (PV) and Essential Thrombocythemia (ET) where increased plasma Hcy levels have been previously reported. It is well known that folic acid is an effective and safe agent for lowering plasma Hcy, and more recently it has been referred to have direct antioxidant capacity.

Aim. We investigated plasma Hcy and quantified oxidative stress measuring serum Reactive Oxygen Species (ROS) in subjects with PV and ET. Moreover, we evaluated the effect of folic acid supplementation on both Hcy and ROS concentrations.

Material and methods. Nineteen patients with PV (aged 65±11 years) and 15 patients with ET (aged 67±11 years) were examined at baseline for plasma Hcy, serum ROS and folate. All these subjects were daily supplemented with 5 mg folic acid for 4 weeks, while 6 out of 19 patients with PV and 5 out of 15 patients with ET carried on folic acid supplementation for 8 weeks more; plasma Hcy and serum ROS were measured after 4 and 12 weeks from the beginning of folic acid treatment. Forty-two healthy controls, matched for sex and age, were studied for plasma Hcy, serum ROS and folate. Plasma Hcy was performed by FPIA Hcy Reagent IMx System (ABBOTT), serum ROS were detected by d-ROMs test (Diacron), serum folic acid was assayed by RIA test in solid phase no boil assay (DPC).

Results. Plasma Hcy levels resulted significantly higher in PV (15.5±4.0 micromol/L, $p<0.001$) and ET subjects (14.4±3.2 micromol/L, $p=0.001$) than in healthy controls (11.0±3.1 micromol/L). Serum ROS were significantly increased in PV (345±65 U/Carr, $p=0.022$) and in ET (358±75 U/Carr, $p=0.014$) compared to controls (286±85 U/Carr). Serum folic acid resulted lower in PV (5.3±2.2 ng/mL) with respect both to ET (7.0±2.8 ng/mL, $p=0.002$) and to healthy controls (6.9±3.3 ng/mL, $p=0.033$), even if it was in normal range. There was no correlation between plasma Hcy and serum folate in all subjects studied. Serum cobalamin was in normal range in all subjects. After 4 weeks supplementation with folic acid, plasma Hcy was significantly reduced both in PV (26%; $p<0.001$) and in ET (19%; $p<0.001$), while ROS were unchanged. No reduction in plasma Hcy or modification in serum ROS were observed after folic acid supplementation for 8 weeks more.

Conclusions. Our results show that: 1. in PV and ET subjects with high plasma Hcy there is an augmented oxidative stress, 2. folic acid supplementation reduces plasma Hcy but does not influence ROS, thus suggesting that oxidative stress is unrelated to plasma Hcy, 3. further investigation are needed to define the causes and the implications of increased oxidative stress in PV and ET.

P024

THE REGISTRO ITALIANO TROMBOCITEMIA: EPIDEMIOLOGICAL, CLINICAL AND BIOLOGICAL ASPECTS. A GIMEMA PROJECT

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The Registro Italiano Trombocitemia (RIT) is a project approved and supported by the GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) Foundation. The RIT is electronically structured and besides a general section open to everyone (public area), comprehends a database of the Essential Thrombocythaemia (ET) patients diagnosed in Italy since January 2004. The ET patient registration will be done, respecting the privacy rules, by the Haematological Centres belonging to the GIMEMA Group and, subsequently, by the other Centres of Internal Medicine, Blood Transfusion, Angiology, Cardiology, Paediatrics, Gynecology, etc. The epidemiological, clinical and biological data will be validated and analysed by various Expert Subcommittees on Cellular and Molecular Biology, Genetics, Haemostasis, Histopathology, Pregnancy, Pediatric and Familial ET, Conventional and Experimental Drugs. Specific objectives of the RIT are: - to check the ET diagnostic criteria utilised in the Italian Centres, with the aim to improve the appropriateness and the homogeneity of the diagnostic approach, with particular interest in the Bone Marrow Histopathology. - to identify the therapeutic approach adopted by the Centres and their compliance to the therapeutical Guide Lines of SIE, SIES, GITMO. - to monitor the ET patient, particularly those receiving experimental molecules as Interferon alpha and Anagrelide (observational study with annual updates). - to evaluate the incidence and the diagnostic-therapeutic approach of Pregnancy, Paediatric Age and Familiarity in ET.- to identify the prognostic values of the new biological parameters.- to obtain information useful to design new clinical and biological studies.The RIT, coordinated by the Haematology Unit of Reggio Emilia, has been active since March 2005.

P025

SURVIVIN EXPRESSION, APOPTOSIS LEVELS AND PROLIFERATIVE ACTIVITY IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Survivin is an inhibitor of apoptosis of the IAP gene family, that has a role both in counteracting apoptosis and in regulating cell division. It is overexpressed in all the most common human solid tumors *in vivo*. In neoplastic patients survivin expression has been associated with reduced tumor cell apoptosis *in vivo*, enhanced proliferative activity, accelerated rate of recurrence and shortened survival. Recently, we detected survivin in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) bone marrow cells, but not in normal bone marrow. In MDS, we observed a tendential inverse correlation between survivin and apoptosis levels, whereas survivin expression was independent of the proliferative rate. Chronic myelomonocytic leukemia (CMML) is a clonal disorder of a bone marrow stem cell characterized by the association of myelodysplastic and myeloproliferative features. Abnormalities in the regulation of the myeloid pathways for cellular proliferation, maturation and survival are the most important pathophysiological mechanisms. We analyzed the expression of survivin in bone marrow cells from patients with CMML to evaluate possible differences in comparison with other myelodysplastic and myeloproliferative syndromes, and to investigate a possible correlation between survivin expression and altered apoptosis, as measured by TUNEL technique, or altered proliferation, as evaluated by MIB-1 immunostaining. We also evaluated whether abnormalities in survivin expression were associated with relevant laboratory and clinical findings. Survivin was detected by an immunoalkaline phosphatase method using a primary murine monoclonal antibody raised against human recombinant survivin (clone 8E2, NeoMarkers) on bone marrow smears from 27 patients with CMML (14 MDS-CMML and 13 MPD-CMML), 66 patients with MDS (26 RA, 14 RARS, 17 RAEB and 9 RAEB-t), 24 patients with AML, 19 patients with chronic myeloproliferative disorders (MPD) and 25 non hemopathic subjects. In CMML survivin levels higher (median 24%, IQR 14-36%) than in MDS (median 8%, IQR 5-15%) (p=0.0000) and AML (median 14.5%, IQR 3-23.5%) (p=0.01), but similar to those found in MPD (median 18%, IQR 8-34%) were observed. In CMML and MDS apoptosis was significantly higher than in normal controls and all other subtypes of leukemias (p=0.0000). Proliferation was similar in normal controls, MDS and CMML; the lowest levels were observed in AML and MPD (p=0.0004). In CMML there was no correlation between survivin expression and blast cell percentage, apoptosis or proliferation. Survivin expression and apoptosis did not differ significantly between the CMML FAB or WHO subgroups, whereas proliferation was higher in the MDS-CMML subtype as well as in the subgroup with less than 10% bone marrow blasts, and did correlate with overall survival. In conclusion, CMML, like MDS but differently from MPD, is a disorder characterized by high proliferation and apoptosis. Survivin overexpression, by disrupting the balance between cell proliferation/differentiation and apoptosis, may play an important role in the pathophysiology of this disease. Moreover, the detection of survivin deregulated expression may provide a useful tool for diagnosis and a possible target for experimental treatments.

P026

PLATELET FACTOR 4, VASCULAR ENDOTHELIAL GROWTH FACTOR AND PROTHROMBIN FRAGMENT 1+2 LEVELS IN ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE

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Essential thrombocythemia undergoes to thrombotic phenomena linked to increased platelet turnover and angiogenesis. In vitro studies demonstrated that youngest platelets are most functionally active and that vascular endothelial growth factor (VEGF) promotes thrombin-mediated platelet activation. In consequence, platelet turnover and angiogenesis constitute a reason for cytoreduction and antiplatelets. The standard treatment includes the use of alkylating agents, hydroxyurea (HU) and interferon-alpha (IFN-a) alone or in combination with antiaggregants. Anagrelide (ANA) is a new agent which has been demonstrated to decrease platelet turnover and to inhibit the platelet activation. Therefore, we studied platelet factor 4 (PF4), as marker of platelet activation, VEGF and prothrombin fragment 1+2 (F1+2), as indicator of thrombin generation, in 22 patients (12 males and 10 females, mean age 50 years) with ET diagnosed according to PVSG criteria. The mean duration of disease was 4.8 years. All patients were on cytoreductive agents either HU (6 patients) or IFN-a (4 patients) and ANA (12 patients). HU was used at doses ranging between 1 and 1.5 g/day. The dosage of IFN-a was 3 MIU, 3 days/week. ANA was initially administered in dose of 0.5 mg/day, with increases of 0.5 mg/day every 7 days until the platelets decreased below 500 x109/L and with a average maintenance dosage of 2.2 mg/day. All patients received antiplatelets either aspirin (ASA) (15 patients) or indobufen (IND) (6 patients) and dipirydamole (DYP) (1 patient). Platelets, PF4, VEGF and F1+2 were measured before cytoreduction and to complete response defined as platelets £ 500x109/L. Platelets were determined by automated analyser. PF4, VEGF and F1+2 were assayed by ELISA. Before treatment all patients had marked platelet count (1060±356x109/L) and higher PF4, VEGFPLT and F1+2 (157±83 IU/mL, 1.2±1 pg/mL and 3.6±3.7 nmol/L, respectively) than controls (1.6±0.9 IU/mL, 0.3±0.2 pg/mL and 0.6±0.3 nmol/L, respectively) ($p<0.0001$, $p=0.002$ and $p<0.0001$, respectively). After a median time of 4.5 months from cytoreduction all patients had platelet count £ 500x109/L (430±70 x109/L). PF4, VEGFPLT and F1+2 were elevated (121±72 IU/mL, 3.5±4.6 pg/mL and 3.5±3.8 nmol/L, respectively) in the HU- and IFN-a-treated patients and normal in the ANA-treated patients (7.4±3 IU/mL, 0.9±0.5 pg/mL and 1.1±0.4 nmol/L, respectively). These measurements were repeated in another sample collected after 1 o 2 months and showed concordance for PF4, VEGFPLT and F1+2 concentrations. A positive correlation we found between PF4 and VEGFPLT and F1+2 (p=0.019 and

p=0.020, respectively). The present data, if confirmed in larger studies, might suggest that ANA therapy decreases platelet turnover and VEGF-dependent platelet-clotting activation.

P027

ANAGRELIDE- ASSOCIATED CARDIOMYOPATHY, ATRIAL THROMBOSIS AND PULMONARY EMBOLISM IN A PATIENT WITH ESSENTIAL THROMBOCYTHEMIA

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Anagrelide is an oral imidazoquinoline derivative effective in lowering platelet count, in a spectrum of chronic myeloproliferative disorders(CMPD),including Essential Thrombocythemia (ET) and Polycythemia Vera(PV),with a proposed mechanism of action that interferes with the post-mitotic maturation of the bone marrow megakaryocytes. The most common adverse events are headache, nausea, diarrhea, palpitations and edema. Here we describe a case of an echocardiogram-documented cardyomyopathy with congestive heart failure that post-dated the treatment with anagrelide. A male 40 years old, suffering from TE, who failed the prior therapy with hydrohyurea and ifn, on September 2003 started a treatment with anagrelide,at a daily dose of 2mg/day,and ticlopidine hydrocloride at a daily dose of 250 mg every 12 hours.The patient had a baseline ejection fraction(EF) of 70%. On February 2004, after three months of treatment, a platelet count was 1400000/microliter,then an escalated dose of anagrelide at 3 mg/day was given. On march 2004, the patient developed progressive dyspnea, palpitations, peripheral edema; promptly hospitalised, a trans-thoracic echocardiogram (TTE) showed an EF of 40% .Diagnosis of Congestive Heart Failure was made. Anagrelide was discontinued and diuretic therapy was started, leading to a rapid improvement in symptoms. At the discharge a treatment with ticlopidine and hydroxyurea was started. One month later a TTE showed a normal EF (58%) and a thrombus in the right atrium. Thrombophilic screening (ATIII, Protein C, Protein S, activated Protein C resistance, Prothrombin variant G 20210A,Factor V Leiden, Lupus anticoagulant ,and Anticardiolipin Antibodies, Homocysteine) was normal, then a treatment with warfarin was started. He was in good health till January 2005,when was readmitted in hospital for the onset of dyspnea. Pulmonary Embolism was documented. Deep Vein Thrombosis (DVT) was not documented. TTE showed an EF of 60% and an evident reduction of the atrial thrombus, the platelet count was 600000/microliter.Treatment with sodic heparin was started with a rapid improvement in symptoms. To date the patient is in good health and continues the treatment with hydroxyurea, warfarin and aspirin. This case suggests a potentially reversible drug-induced cardiomyopathy in an anagrelide treated patient.The mechanism of action is currently unknown but may involve the drug's known cardiovascular effects including positive inotropism, vasodilatation, and tachyarrhytmia.

P028

BONE MARROW EVALUATION ACCORDING TO THE PVSG AND WHO CRITERIA IN 90 ESSENTIAL THROMBOCYTHAEMIA PATIENTS TREATED WITH PEG INTERFERON ALPHA-2B. PRELIMINARY RESULTS

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Ninety ET patients diagnosed according to the PVSG criteria were enrolled in a phase II study (sponsored by the Schering Plough Company) designed to evaluate the efficacy, safety and tolerability of a two years treatment with PEG Interferon α-2 b (PEG Intron). The patients, observed in 16 Italian Centres belonging to the GIMEMA Cooperative Group and judged at high risk, had been previously treated with cytoreductive (97%) and antiplatelet (91%) drugs. At the study start the patients, 60 F and 30 M, mean age 45 years, showed splenomegaly in 22% of cases. The Hematological Response (HR: PLT<500 x10^9/L) was observed in 64/81 (79%) and 48/55 (87%) of the patients on PEG Intron treatment at the end of the first and second year, respectively; the spleen enlargement disappeared in 75% of cases. The Bone Marrow data reported in the study CRFs document that the baseline increase of cellularity, of granulopoyesis, of megakaryocytes (MK) number, size and ploidy significantly decreased during PEG Intron treatment, while the MK displasia and fibrosis rate globally increased. In these patients a revision of the Bone Marrow biopsy slides was blindly performed by an Expert Pathologist Panel by applying the WHO criteria. The evaluation of two-thirds of cases showed the following distribution at the baseline: true ET (23%), IMF-0 (pre-MF 17%), IMF-1 (early MF 40%), IMF-2/3 (classical MF 3%), MPD-U (17%). Interestingly, the true ET patients never showed spleen enlargement that, otherwise, was present in IMF-0 (20%) and IMF-1/2/3 (42%) patients. The patients with true ET did not show any bone marrow evolution during the PEG Intron treatment, while the IMF-1/2/3 picture significantly increased. Moreover, the rate of the HR at the end of the first year was higher in true ET(93%) respect to the IMF-0 (60%) and IMF-1/2(71%) patients. These preliminary data show that the patients classified as true ET (WHO Criteria) are not splenomegalic, have no myelofibrotic evolution and reach a better response to the IFN treatment.

P029

THROMBOPHILIC RISK FACTORS IN PATIENTS AFFECTED BY MYELOPROLIFERATIVE DISORDERS

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Patients affected by myeloproliferative disorders present an increase in thrombotic events, either arterial or venous. Many thrombotic risk factors are described in association to hematologic diseases with not univocal results. In our Institution from january 2003 to February 2005, we observed 57 patients affected by myeloproliferative diseases: 25 Essential Thrombocythemia (ET), 24 Polycythemia Vera (PV), 8 Idiopathic Myelofibrosis (IMF). Out of these 57, 10 (17.5%) experienced a thrombotic event. In order to evaluate additional thrombophilic risk factors, we studied in 25/57 patients affected by ET PV and IMF, aged from 34 to 89 yrs (median 63), AT III, Protein C (PC), Protein S (PS), homocystein, Factor V Leiden, prothrombin G20210A, Lupus Anticoagulant (LAC), Anticardiolipin Antibodies (ACA)IgG and IgM. 12 patients were affected by PV, 12 by ET and 1 by IMF. Among all these patients, 1 (4%) presented a PC partial deficiency, 9 (36%) an increase in homocystein, 4 (16%)were ACA and/or LAC positive. Out of 25 patients, 9 presented a thrombotic event, before, at diagnosis or during the outcome of disease: 5 were arterial episodes, 4 were deep venous thrombosis (DVT).Three of them resulted positive for a thrombophilic factor: one was PC defective and experienced a myocardial infarction, 2 presented hyperhomocysteinemia and experienced a DVT and a myocardial infarction respectively. Furthermore, only 2/9 patients were on chemotherapy treatment at thrombothic event. In conclusion: 1)in our serie we confirm the increase in thrombotic events in myeloproliferative diseases (17.5%)2)We found an high incidence of inherited or acquired trombophilic risk factors (56%), in particular hyperhomocysteinemia 3)One-third of patients who experienced a thrombotic event presented an association to thrombophilic factor. More patients are needed to estabilish an association between thrombotic events and thrombophilic risk factors.

P030

THE STEM CELLS MOBILIZATION CAPACITY HAS NOT PROGNOSTIC RELEVANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Peripheral blood stem cells (PBSCs) are increasingly used for autologous stem cell transplantation (ASCT) in acute myeloid leukemia (AML). Recently, some reports have pointed out that the stem cell mobilizing capacity in AML directly correlates with relapse risk (RR), being a surrogate marker of insufficient *in vivo* purging. In order to evaluate these findings, we analyzed 109 consecutive non M3 AML patients mobilized with consolidation therapy after complete remission (CR) achievement from 1997 to June 2004 at our Institution. There were 63 males and 46 females, median age: 50 years (16-78). In 17 cases (16%) AML was diagnosed as secondary, while cytogenetics were classified in the 97 evaluable patients as favourable, intermediate, or unfavourable in 12 (12%), 68 (70%), and 17 (18%) cases, respectively. Furthermore, in 9 patients CR was obtained after 2 induction courses, and number of courses from induction to mobilization were 2 in 79 patients and 3 in 30 patients. A successful $CD34^+$ cells mobilization in PB ($\geq$ 20/microl) was obtained in 94 cases, median $CD34^+$ cells collection being $8.27x10^6$/kg (1.98-152.6) after a median of 2 apheresis (1-4). Finally, ASCT was actually performed in 67 patients. Results concerning overall survival (OS) and disease free survival (DFS), median 30 and 18 months, respectively, on the whole population, differed as expected when taken into account well recognized prognostic factors such as age, cytogenetics, previous MDS, and number of courses to CR. On the contrary, RR was not significantly affected by maximum $CD34^+$ cells collection per single apheresis, neither by taking into account the median value of maximum $CD34^+$ harvest in our population (<5.04 vs $\geq$ 5.04, *p*:0.80), nor the value suggested by the experience of the EORTC-GIMEMA group (<7 vs $\geq$ 7, p:0.52). Of note, patients failing to mobilize had a similar outcome in terms of CR duration (*p*:0.28). In the attempt of excluding a main prognostic factor such as cytogenetics, we separately evaluated 68 patients with intermediate cytogenetics; once again, no significant difference in RR was found by using the above cut-off values (*p*:0.20 and 0.10, respectively). In addition, different peak values of $CD34^+$/microl in PB were also considered (<20 vs >20, <50 vs >50, <100 vs>100) and no difference in CR duration was found for any of the threshold investigated (*p*:0.25, 0.73 and 0.59, respectively). Finally, we considered the percentage of $CD34^+$ cells in the harvest by adopting the median value found in the present series (1.71%, range 0,25-19) and the value previously reported as effective for relapse prediction (0.8%, Feller et al, Leukemia 2003). In both cases, no difference was found (*p*:0.33 and 0.65, respectively). In conclusion, we were not able to confirm previous reports on the correlation between mobilization capacity and risk of relapse in AML. These discordant results can be due to the not uniform cytometric evaluation in multicenter studies as well as to the lack of data concerning the possible contamination of the grafts by leukemic $CD34^+$ cells, which was ruled out in this study by evaluating minimal residual disease in most patients.

P031

PROGNOSTIC FACTORS AND THERAPEUTIC OPTIONS FOR PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA

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Chronic myelomonocytic leukemia (CMML) is a malignant blood disorder characterized by increased monocytes in the bone marrow and peripheral blood, variable degree of dysplastic abnormalities and terminal transformation in acute myeloid leukemia (AML). CMML affects predominantly elderly people and standard therapy involve hydroxyurea and supportive care, aiming at control of leukocytosis and correction of cytopenia, respectively. In younger patients, AML like chemotherapy has been reported as able to induce complete remission (CR) in nearly half of the patients. Here we describe the clinical characteristics and therapeutic options adopted in a series of 42 consecutive patients observed at our Institution between 2000 and 2004. There were 30 males and 12 females, the median age was 70 years (range 38-84). Twenty-one patients (50%) presented with a concomitant disease requiring specific treatment, including two cases affected by non-hematologic malignancies. Therapeutic selection was operated according to performance status, age by itself and white blood cell count (WBC) at diagnosis. Hydroxyurea (HU) was used when patients presented with WBC higher than 15 x 10^9/L. Overall, 17 patients received supportive therapy only, 20 were given HU and only 5, aged less than 60 years, were treated with fludarabine, cytarabine and G-CSF (FLAG), aiming at CR achievement. Among these, 4 achieved CR and 3 of them, after consolidation with an additional FLAG course, were allografted from a HLA compatible donor in CR1; one was refractory to FLAG and then managed with supportive treatment. The median WBC count at presentation was 11 x10^9/L (3.5-110), while the median Hb value was 10.2 gr/dL (6,1-14.2). In all patients dysplastic abnormalities were found at bone marrow examination. Cytogenetic data were available in 34 cases, trisomy 8 being the more frequent aberration (5 out of 34, or 15%). Overall, 8 patients had adverse cytogenetics, while 26 had a normal karyotype. The median survival of the whole patient population was 26 months. In the univariate analysis adverse prognostic factors were adverse cytogenetics (p:0.0001), IPSS >1 (p:0.01), Hb <10 gr/dL (p:0.0009) and abnormal LDH value (p:0.001), while WBC count < or > 15x10^9/L and age less or more than 70 years had no prognostic impact. In the multivariate analysis, only unfavourable cytogenetics and abnormal LDH were found as having significant adverse prognostic relevance (p:0.002 and 0.04, respectively). In particular, patients presenting with the two above characteristics had a median survival of only 6 months. Finally, unfavourable karyotype was the only factor significantly related to transformation in AML (p:0.02). We conclude that CMML patients with adverse prognostic factors at presentation, in particular those with poor cytogenetics and/or high LDH value at diagnosis should be enrolled into experimental clinical trials based on new drugs with mechanism of action alternative to conventional chemotherapy. Data concerning results of aggressive induction with FLAG need to be confirmed in larger series.

P032

RELEVANCE OF CURRENTLY AVAILABLE BIOMARKERS IN THE WORK-UP OF PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS

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Since Philadelphia negative chronic myeloproliferative disorders (MPD) lack specific genetic and molecular markers useful for the differential diagnosis among themselves and with reactive conditions, different cellular and molecular assays, including Endogenous Erythroid Colonies (EEC), Clonality Status, platelet c-MPL expression, circulating $CD34^+$ cell count and PRV-1, have been variably proposed as surrogate markers with diagnostic implication. This scenario is likely to change soon following the recent description of JAK2 mutation in almost all patients with PV and a proportion with ET or IM. The aim of this study was to analyze the diagnostic relevance of these different laboratory tests in a heterogeneous group of patients with Ph-negative disorders. Between January 2003 and January 2005, 156 unselected consecutive patients (46 males and 110 females; median age 54 years, range 16–78 years) were enrolled. The diagnosis of PV and ET was established according to the WHO criteria, and the diagnosis of IM according to Italian Consensus Conference Criteria. There were 28 PV, 102 ET and 26 IM; we also included 4 patients with secundary erythrocytosis and 5 with reactive thrombocytosis Patients were either newly diagnosed or established cases in follow-up. Clonal hemopoiesis was evaluated with HUMARA assay, circulating CD-34 positive cell count were determined using flow-cytometry, platelet MPL expression was quantified by immunoblotting, EEC from peripheral blood was determined in methylcellulose, and granulocyte PRV1 mRNA was quantified by real time RT-PCR. The different assays were conducted blinded. Clinical, laboratory and follow-up data were reviewed to confirm specific diagnosis. The results of different assays are detailed in Table 1.

Table 1. Percentage value indicates the fraction of patients showing positive assays, while the number of patients evaluated is shown within brackets

	PTS	ABNORMAL PRV1	EEC^{+VE}	CLONAL HEMATOPOIESIS	REDUCED PLATELET c-MPL	PB $CD34^+$ range	PB $CD34^+$ >15x10^6/L
PV	28	64% (n=28)	60% (n=25)	---	---	0.1-31.3	37% (n=8)
Sec. Erytroc.	4	50% (n=4)	75% (n=4)	---	---	---	---
TE	102	35% (n=98)	46% (n=76)	62% (n=86)	72% (n=68)	0.01-18.8	7% (n=13)
Sec Thromb.	5	75% (n=4)	20% (n=5)	0% (n=3)	---	0.01-4.5	0% (n=3)
IM	26	81% (n=11)	40% (n=5)	---	---	9.6-1943	88% (n=25)

In ET patients there was no correlation among different assays but a strong association between thrombotic events and clonal hemopoiesis, as already reported (Vannucchi AM et al, BJH 2004) was furtherly confirmed. Among the 53 pts with clonal hemopoiesis, 19 thrombotic events were documented (17 at diagnosis and 2 in follow-up) as compared to 5 events among the 33 pts with polyclonal myelopoiesis (4 at diagnosis and 1 during follow-up). In

patients with reactive thrombocytosis, no thrombotic events were described and all evaluable patients presented policlonal hemopoiesis. The most reliable correlation among different assays was observed in PV patients. In fact 15/25 pts (60%) showed EEC growth whereas PRV1 overexpression was detected in 18/28 (64%); of the 15 patients found positive for EEC, 12 (80%) had PRV-1 overexpressed. However, neither PRV1 nor EEC were associated with thrombotic events (recorded in 4 cases). Concerning IM patients, the median number of circulating CD34 cells was 54 x10^6/L (range 9,6-1943), with no difference between primary and secundary forms nor treated and untreated patients. However, 2/22 pts (9%) had values lower than the diagnostic cut-off of 15x10^6/L. From these data we conclude that EEC, CD34 count and clonality assay may have relevance in the work-up of patients with PV, IM, and ET, respectively, while PRV-1 does not add. We are currently evaluating in parallel the results of these tests with JAK2 mutational status.

P033

MOLECULAR CYTOGENETIC STUDIES OF MYELODYSPLASTIC/ MYLOPROLIFERATIVE DISEASES

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The WHO has recognized myelodysplastic/myeloproliferative diseases (MDS/MPD) i.e. chronic myelomonocytic leukemia, atypical chronic myeloid leukemia, juvenile myelomonocytic leukemia, and the so-called unclassifiable myelodysplasti/myeloproliferative syndromes as a separate category of myeloid disorders. Genetic diagnosis excludes the Philadelphia translocation and identifies specific changes such as the t(5;12)(q33;p13) translocation which involves the PDGFRB gene or abnormalities in the signal transduction of the RAS pathway. As little is known about cytogenetics and molecular abnormalities in MDS/MPD this study investigated the genetic profile in a series of patients.

Materials and methods. We investigated 13 MDS/MPD patients by cytogenetics, FISH and gene mutation analysis. Interphase FISH was used to study rearrangements of PDGFRA/4q12 (RP11-3H20 and RP11-120K16 or RP11-24O10), PDGFRB/5q33 (cosmids 4-1 and 9-4), FGFR1 (RP11-350N15), ABL1/9q34 (RP5-1132H12, RP5-913J14, RP5-888H11), ETV6/12p13 (cosmids 179A6 and 148B6), D14S258 locus at 14q24 (RP11-1I11), and TAF15/17q11 (RP11-689E22). 200 nuclei were scored for each probe using a fluorescence microscope (Provis, Olympus). The cut-off limits for monosomy/deletion and trisomy/splitting were the highest values obtained in normal samples. Genomic DNA from blood/bone marrow cells was used for mutational analysis of PTPN11 (ex 3, 11); NRAS (ex 1); KRAS 2 (ex 1); bRAF (ex 11, 15); RAF1 (ex 10, 14); and FLT3 internal tandem duplication (ITD) and FLT3 activating loop mutation. PCR products were analyzed by DHPLC (Wave® System, Transgenomic Inc., Omaha, Nebraska, USA). Analysis gradient and temperature were determined according to the PCR product nucleotide sequence using Wavemaker® software 4.3.1. Electrophoregrams from patients were compared with a normal control. Amplimers with abnormal denaturing profiles were purified using ABI BigDye Terminator Sequencing Kit (Applied Biosystem) and an ABI Prism 310 Genetic Analyzer (Applied Biosystem). Sequencing results were analyzed using the Sequencing Analysis version 3.6.1 and AutoAssembler version 2.1 software packages (Applied Biosystem). Results. Karyotype was normal in 8 evaluable cases. Hybridization patterns were normal for PDGFRA in 12/12 patients, for PDGFRB in 11/11, for ABL1 in 6/6, for ETV6 in 13/13, for D14S258 in 6/6, and for TAF15 in 6/6. The FGFR1 probe was normal in 8/9 patients and detected trisomy 8 in one case as confirmed with the alpha satellite probe for chromosome 8 (D8Z2). Mutational analysis detected the NRAS mutation C181A (Glut61Lys) in 1/10 and the KRAS mutation G34C (Gly12Arg) in 1/10. Conclusion. Despite this integrated approach, genetic lesions emerged only in 3/13 MDS/MPD patients with normal or failed karyotype (23%). Chromosome 8 trisomy was detected in one MDS/MPD without available karyotype. NRAS and KRAS mutations were each found in a patient with the myeloproliferative variant of CMML. Given that known myeloid genetic abnormalities were found in so few patients in this series, new candidate genes need to be tested to delineate the genetic profile of MDS/MPD. Acknowledgements This work was partially supported by CNR-MIUR, FIRB, Fondazione Cassa di Risparmio Perugia.

HEMOPOIETIC DISORDERS, CYTOKINES AND STEM CELLS

P034

AGE IS CORRELATED WITH PBSC MOBILIZATION'S ABILITY ONLY AFTER "G-CSF ALONE" BUT NOT AFTER "CHEMO + G-CSF"

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Whether patient's age is an important predictive factor for CD 34+ mobilization's efficacy, it's still an unsettled issue.In fact some authors have found that age is a predictive factor of CD34+ mobilization ability in normal donors as well as in patients, while others have not (Brown RA 1997, Anderlini P 1997, Morris CL 2003). We have analyzed correlation between age and CD34+ peak in peripheral blood during mobilization in a series of 168 subjects, 40 healthy donors and 128 lymphoma patients, mean age was of 40 years with a range from 8 to 70 years, 91 were male and 77 female. PBSC mobilization regimens were: - "G-CSF alone" at the dose of 10 micrograms/kg in 70 subjects (41,7%); G-CSF alone was used in 100% donors (40/40) and in 23,4% of lymphoma patients (30/128) - "Chemotherapy + G-CSF " in 98 subjects (58,3%) The correlation between age and P.B. peak of CD 34+ was studied: - in all cases - in the group of lymphoma patients mobilized with "G-CSF alone" (Figure1) - in the group of healthy donors mobilized with "G-CSF alone" (Figure 2) - in the group of lymphoma patients mobilized with "chemotherapy + G-CSF".

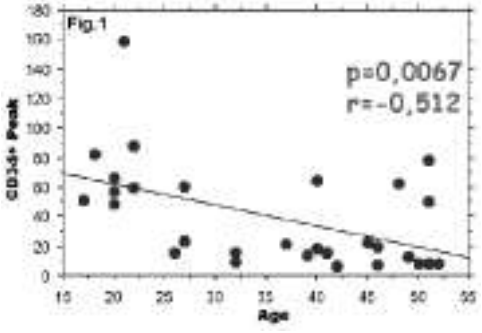

Figure 1.

Table 1.

	CD 34+ Peak in P.B.	
Donors below 45 y	91,515/mmc	p=0,003
Donors over 45 y	62,821/mmc	

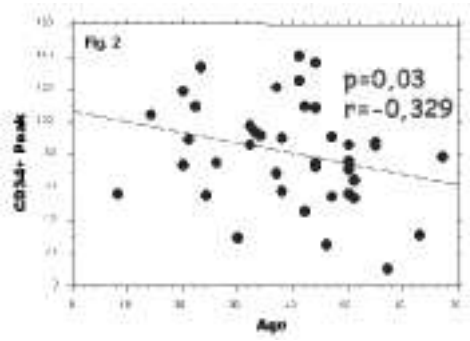

Figure 2.

In the population as a whole we found a weak inverse correlation between age and CD34+ peak (p=0,050 ; r=-0,15). The correlation between age and CD34+ peak was statistically significant in the lymphoma's group mobilized with "G-CSF alone"(p=0,006 ; r=-0,512) (Figure 1). However this correlation was not found when lymphoma's group mobilized with "Chemotherapy + G-CSF" was analysed (p=0,16 ; r=-0,14). Moreover an inverse correlation between age and CD34+ peak was again found in the group of normal donors after G-CSF alone mobilization (p=0,03 ; r=-0,329) (Figure 2). We also investigated results of PBSC mobilization with G-CSF alone in two "age subgroups": over 45 y and below 45 y and in the strata of normal donors we found that the group older than 45 years had a lower mobilization efficacy in respect to younger (p=0,003) (Table 1) We conclude that in normal subjects and in lymphoma patients correlation between CD34+ peak in P.B. and age is evident only when " G-CSF alone" mobilization schedule is used. Normal donors older than 45 years old of age have a reduced mobilization ability.

P035

QUANTIFICATION OF ORGANIZATIONAL NEEDS IN AN HSC HARVEST CENTER FUNCTIONING ACCORDING TO JACIE CRITERIA

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JACIE dictate a highly demanding standards in the organization of an HSC Harvest Center and it is conceivable that this will determine a increase amount of resources needed. Quantitative estimation of resources required in a HSC Center is thus an open issue and it is likely that it will vary in different transplantation program, depending on clinical and physical integration that are possible in each different situation. We have settled in accordance to JACIE standard an HSC Harvest Center integrated with our transplantation Unit and Hematological ward, main activity are the harvests of PBSC (HPC-A) and harvests of Bone Marrow (HPC-M), a mean of 100 HSC products were harvested per year and numbers of HSC transplants done using those products were 65 per year. We have described Flow-Charts for each process, prepared 61 documents and worksheets and assigned responsibility to each professional figures involved. Quality management plan focused on Adverse Event report and analysis, documentation of

acquired experience, data recording, quality index from statistical analysis of aggregated data. We have thereafter calculated during a 3 months period workload of each activity done by each professional (Table 1) As far as patients receiving autologous transplant are concerned the activities more time-consuming were monitoring of mobilization after administration of chemotherapy plus Growth Factors (35 hours), clinical evaluation of eligibility of patients (4 hours), restaging of underlying hematological disease (3 hours). In a HSC Harvest Center the role of hematologist is prominent in terms of workload, the integration of a Center for HSC Harvest with Hematological Ward is therefore a mean to allows an easy exchange of data and information thus improving efficiency.

Table 1.

	Hours of Works required/HPC-A	Hours of Works required/HPC-M
Clinical hematologist 50 h.	25 h.	
Medical manager	7 h.	10 h.
Apheresis	10 h.	1 h.
Quality responsible	8 h.	8 h.

P036

FUNCTIONAL ALTERATIONS OF GLYCOSYLPHOSHATIDYL-INOSITOL -DEFECTIVE GRANULOCYTES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA PATIENTS

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PNH is an acquired haematopoietic stem cell disorder in which the affected cells are the clonal progeny of a mutated progenitor, unable to synthesise the GPI anchor. The majority of peripheral monocytes and granulocytes, playing a critical role for effector functions in innate immunity, are usually GPI-defective in PNH patients. Here we analyse bacterial dependent intracellular ingestion and the consequent activation of oxidative burst in GPI-defective granulocytes from PNH patients. In comparison the functional behaviour of normal granulocytes was studied in a population of age- sex matched healthy controls. Our results indicates that GPI-defective granulocytes from PNH patients show a significant increase in their ability to ingest opsonised bacteria and an impaired respiratory burst effectiveness in response to two independent bacterial stimuli represented by the N formyl MetLeuPhe (fMLP) synthetic bacterial peptide and E. Coli. The latter alteration was maintained after triggering with phorbol 12 myristate 13 acetate (PMA), a pharmacological stimulus able to recruit and to extensively trigger intracellular Protein Kinase C (PKC). In PNH the lack of GPI-linked molecules CD55 and CD59 on the cellular membrane produces an altered control of activated complement fractions. The consequent excess of C3 activated molecules could act as a continuous stimulus, probably depleting the intracellular PKC stores. This condition could be involved in the pathogenesis of both the increased phagocytic effectiveness and the impaired oxidative burst generation observed in GPI-defective granulocytes. We hypothesise that such functional alteration of PNH granulocytes could account, together with cytopenia, for the increased susceptibility to infections observed in PNH patients.

P037

SPONTANEOUS MOBILIZATION OF BONE MARROW-DERIVED HEMATOPOIETIC AND ENDOTHELIAL PROGENITOR/STEM CELLS AFTER ORTHOTOPIC LIVER TRANSPLANTATION

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This study characterized the peripheral blood (PB) stem/progenitor cells compartment of 24 adult patients before (day –1) and after (days 1, 3, 7 and 14) orthotopic liver transplantation (OLT) for liver failure (10) and/or hepatocellularcarcinoma (14). Twelve healthy donors served as normal controls. The time-course phenotypic evaluation of circulating cells after transplant demonstrated the significant mobilization of $CD34^+$ cells (day +7; p 0.017), together with a significant increase of primitive $CD34^+$/CD90+ cells (day +7; p 0.043). We also observed the significant increase of circulating mature committed progenitors (CFU-C) on day +14 after transplantation (p 0.028). The number of circulating endothelial CD133+ and $CD34^+$/KDR+ stem/progenitor cells, which contribute to neoangiogenesis after tissue ischemia and organ regeneration in animal models, significantly augmented following OLT (day +3; $p< 0.05$). When we analyzed the serum level of cytokines involved in stem cell mobilization and/or liver repair after OLT, we observed the significant increase of Stem Cell Factor (SCF), Granulocyte Colony-Stimulating Factor (G-CSF), Interleukin-6 (IL-6) and Vascular Endothelial Growth Factor (VEGF). The serum level of stromal cell-derived factor-1 (SDF-1) significantly decreased after transplant at days +1 and +7 (p less than 0.05). Interestingly, the baseline levels of IL-6 and Hepatocyte Growth Factor (HGF) were significantly higher in patients with liver disease than in healthy controls (p 0.002). Cytogenetic and molecular analyses showed the host origin of mobilized bone marrow-derived cells and the co-expression of liver-specific epithelial markers (CK-19 and alpha-fetoprotein) in a representative case of sex-mismatch transplant. This is the first study reporting the mobilization of both hematopoietic and endothelial stem/progenitor cells after OLT. Our data suggest that stem cell recruitment to non-hematopoietic organs may be a physiological process in case of tissue injury and underline the potential role of bone marrow-derived cells for cell therapy of liver diseases.

P038

INCREASED ERYTHROPOIETIN PLASMA LEVELS IN PATIENTS WITH ISCHEMIC HEART DISEASE AND METABOLIC SYNDROME

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Background. Several studies suggest that erythropoietin (EPO) is not only the main factor involved in erythropoiesis, but also an emergent growth factor that offers protection against apoptosis in a wide variety of tissues and affects endothelial progenitor cells proliferation and differentiation. However, the levels and significance of plasma EPO in patients with ischemic heart disease with and without the metabolic syndrome (MetSy) have been poorly investigated. Objective. To determine EPO plasma levels in patients with ischemic heart disease, with reference to the presence of the MetSy. *Methods.* Thirty-two consecutive patients with ischemic heart disease (62+7 yrs, 27 males) were investigated: 11 admitted with acute myocardial infarction (AMI), 12 with unstable angina (UA) and 9 with chronic stable angina (CSA). We also enrolled 16 control subjects (61±10 yrs, 10 males). The MetSy was defined according to the National Cholesterol Education Program III criteria. Reasons for exclusion were: presence of non-ischemic heart disease (valvular, myocardial, pericardial), blood transfusions, haemoglobin<11 g/dL, treatment with EPO, haemodialysis. Informed consent was obtained from all partecipants and venous blood samples were taken 4.6+2.9 days after admission. EPO concentrations were measured by ELISA (RDS, mUI/mL, mean+SEM). Results. Patients with and without the MetSy and control subjects did not differ significantly with respect to age, gender, haemoglobin concentrations and creatinine clearance. EPO levels did not differ significantly among patients with admission diagnosis of infarction, unstable angina or stable angina. EPO levels were significantly higher in all patients with ischemic heart disease compared with controls (27.3+4.3 vs 11.9+1.2mUI/mL, p=0.04) and in the 18 patients with MetSy compared to the 14 patients without the syndrome (33.7+9.4 vs 12.5±2.5 mUI/mL, p=0.001)); however, they did not differ among patients with AMI, AI or CSA. EPO concentrations were significantly related to the presence of hypertension (r=0.37, p=0.03). Conclusions. These results suggest that ischemic heart disease and the alterations that characterize the MetSy may be involved in determining EPO plasma levels. Alternatively, or additionally, EPO may have a physiopathological role in ischemic heart disease course and, particularly, in the MetSy.

P039

SOLUBLE UROKINASE ACTIVATOR RECEPTOR IN STEM CELL MOBILIZATION

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Mobilized peripheral blood hematopoietic stem cells (PB-HSC) are currently the major source of stem cells for autologous and allogeneic stem cell transplantion (SCT). G-CSF is the most common HSC mobilizer in patients and normal donors. HSC release from bone marrow (BM) involves also proteolytic enzymes, which are able to cleave SDF1 and its receptor CXCR4. Many reports have shown the involvement of the urokinase-type plasminogen activator (uPAR) receptor in cell adhesion and migration of normal and malignant cells. Despite the increasing use of mobilized HSC, the mechanisms governing HSC trafficking from and to BM are not yet well defined. We investigated the involvement of the uPAR in G-CSF-induced mobilization of CD34$^+$ HSC from 16 healthy donors. Flow cytometric analysis showed that G-CSF induced a significant increase of uPAR expression on CD33+ myeloid and CD14$^+$ monocytic precursors released from BM into circulation during HSC mobilization, whereas CD34$^+$ cells, T and B lymphocytes remained uPAR negative. In steady state conditions, uPAR expression on granulocytes was high and remained unchanged during G-CSF administration. Western blot analysis with an anti-uPAR polyclonal antibody confirmed a progressive increase in uPAR expression in all donors during G-CSF treatment and showed that PBMNC expressed only the intact form of uPAR. Up-regulation of cell-surface uPAR expression in CD33$^+$ and CD14$^+$ monocytic cells coincided with the increase of the soluble form of the receptor (suPAR) in almost all donor sera; suPAR levels increased to the maximum extent at days 3-5 of G-CSF stimulation when CD34$^+$ HSC also peaked into the circulation. suPAR detected in the serum of G-CSF-treated donors was also analyzed by an immunoprecipitation assay to determine whether the shed receptor was in the intact or in the cleaved form. The analysis showed not only the presence of the intact form of suPAR that increased during G-CSF treatment, but also the appearance or the strong increase of cleaved forms of suPAR (c-suPAR) in all analyzed sera. c-suPAR was able to chemoattract CD34$^+$ HSC, as documented by their in vitro migratory response to a chemotactic suPAR-derived peptide (uPAR84-95). uPAR84-95 induced CD34$^+$ HSC migration by activating the high-affinity fMLP receptor (FPR). In addition, uPAR84-95 inhibited CD34$^+$ HSC in vitro migration toward the stromal derived factor 1 (SDF1), thus suggesting the heterologous desensitization of its receptor, CXCR4. In bone marrow long term cultures (LTC), we found that the chemotactic peptide uPAR84-95 induced increased release of erythroid and myeloid clonogenic progenitors from the stroma layer. Preliminary data seem to confirm that the chemotactic peptide uPAR84-95 was able to induce stem cell mobilization also in BALB/c mice. All together, our data document that, during G-CSF-induced HSC mobilization, uPAR expression is up-regulated on CD33$^+$ and CD14$^+$ cells, thus leading to increased uPAR shedding in the serum. suPAR could be rapidly cleaved by increased serum proteases, thus generating the chemotactically active form of suPAR. Serum c-suPAR could chemoattract BM CD34$^+$ HSC, thus inducing their

migration into the circulation. In addition, c-suPAR could be generated also in BM, by the proteolytic action of neutrophil cathepsin G and elastase, where it could inactivate CXCR4 by cross-desensitization and promote HSC release from BM. These data suggest a new, unexpected role for c-suPAR in HSC mobilization.

P040

THE 67 KDA LAMININ RECEPTOR IS INVOLVED IN HEMATOPOIETIC STEM CELL MOBILIZATION AND HOMING

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Autologous and allogeneic stem cell transplantations (SCT) are increasingly performed with granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs) because of technical advantages and shorter time to engraftment. Several adhesion molecules, such as LFA-1, VLA-4, VLA-5 and CD44, have been suggested to participate both in the homing to and in the release from bone marrow (BM) of hematopoietic stem cells (HSCs). The 67 kDa laminin receptor (67LR), a non-integrin cell surface receptor, mediates a crucial step in the metastasis cascade: the adhesion of cancer cells to basal membranes of epithelia and endothelia. Expression of 67LR is increased in cancer cells and directly correlates with enhanced invasive and metastatic potential. We investigated the expression and function of the 67LR in receptor in G-CSF-induced mobilization of CD34+ HSCs from 20 healthy donors. Using a western blot analysis with a polyclonal anti-67LR and two color flow cytometry, we documented that G-CSF-mobilized CD34+ HSC, including CD34+CD38- cells, showed increased expression of 67LR (% of CD34+ cells expressing 67LR after G-CSF: 34.1±4.6). Expression of 67LR on G-CSF-mobilized CD34+ HSC peaked at day 4-5 of cytokine treatment, when also CD34+ cells reached the higher level of mobilization into PB. By contrast, CD34+ BM-resident HSC expressed low levels of 67LR, which could be up-regulated by in vitro G-CSF treatment. G-CSF mobilized CD34+ PBSC expressed a functionally active 67LR able to mediate their chemotaxis to laminin, whereas adhesion of BM CD34+ cells to laminin was 67LR-independent. Laminin was able to chemoattract G-CSF mobilized CD34+ cells by 67LR engagement, and 67LR activation also regulated stem cell migration toward the chemoattractant stromal cell derived factor 1 (SDF1). Long-term colture initiating cell (LTC-IC) assays were also performed on mobilized PBMNC at various time-points during G-CSF treatment in mobilized donors. A dramatic inhibition of LTC-IC growth was observed when the 67LR-mediated cell binding to laminin was inhibited by its competing soluble polypeptide 37LRP (number of LTC-IC/10E3 CD34+ at day 0, +3 and +5 after G-CSF mobilization: 3.5±1 vs 1±1, 15.5±4.5 vs 4.7±3.5 and 26.6±8 vs 11.7±7.6 in absence and in presence of 37LRP, respectively; all $p<0.001$) suggesting that 67LR also regulates PBSC adhesion to laminin of the bone marrow microenvironment. Our findings demonstrate that G-CSF-induced HSC mobilization is associated with up-regulation of 67LR expression on circulating CD34+ HSC, which could contribute to their chemotaxis and recruitment into BM following transplantation.

P041

MEDIUM FLUORESCENCE RETICULOCYTE PERCENTAGE AND MYELOPEROXIDASE INDEX OBTAINED BY ADVIA 120 MIGHT EVALUATE MYELOPOIETIC FUNCTION BEFORE CHEMOTHERAPY AND INDIVIDUATE A PATIENT SUBSET WITH LOW RISK OF NEUTROPENIA

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Chemotherapy frequently involves, in neoplastic patients, severe myeloid suppression. Myeloid suppression is sometimes the main cause of therapy recycling delay, deep, severe and prolonged neutropenia, anaemia and thrombocytopaenia. Neutropenia is associated with an increased sanitary expense, number of febrile episodes and days of hospitalization. The aim of our study is to verify if a correlation between reticulocytary fraction, reticulocytary indices, myeloperoxidase index (MPXI) and postchemotherapy myelopoietic function and severe postchemotherapy neutropenia was present. Our study is a monocentric, prospectic, nonrandomized, open label study. 70 paients (M/F:40/30, median age 58 years), 36 with lymphoma or myeloma and 34 with solid neoplasms with bone marrow micrometastases were treated with chemotherapy (CT). After CT 36 patients had neutropenia (ANC<500/mcl) for a median of 7 days (range 3-21). Before CT, myelopoietic function was assessed by above mentioned parameters using hematologic automated analyser ADVIA 120 (BAYER, Diagnostic Division, Tarrytown, NY). Patients with B12 and/or folate and/or iron deficiency or receiving growth factors and/or transfusions were excluded from our study. Among tested parameters, MPXI negative value was related to neutropenia with an OR=3.714 (CI95%:1.36-10.08, Chi square 5.67 p0.017). Subsequently we assigned to patients with MPXI value positive and MFR>10.7% a score=1, a score=0 was assigned to remaining patients. Patients with score=1 showed a lower number of neutropenic events (only 4 on 19 patients) than those with score=0 (32 on 51 patients), with a Chi square test of 8.03 with $p=0.005$, a sensitivity of 0.89 (CI95%: 0.79-0.95), a specificity of 0.44 (CI95%: 0.34-0.51), a positive predictive value of 0.63 (CI95%:0.56-0.67), a negative predictive value of 0.79 (CI95%:0.60-0.91) and an OR of 6.3 (CI95%:1.89-20.8). MPXI and MFR might be used in myelopoiesis assessment before CT administration, independently from type of tumor, CT regimen and number of CT cycle, with the aim to identify a patient subset with a lower risk of neutropenia post CT.

P042

COMPARISON OF COMMERCIAL COMPLETE MEDIA WITH OR WITHOUT SERUM, WITH AND WITHOUT ERYTHROPOIETIN, FOR ANALYSIS OF ENDOGENOUS ERYTHROID COLONY GROWTH IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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The assay of endogenous erythroid colony formation (EEC), a characteristic of polycythemia vera and essential thrombocythemia, is not standardized. In this study, we compared the colony growth obtained with semisolid, serum-free methylcellulose based media commercialized for the EEC assay (MethoCultTM StemCell, Canada SH4436 with erythropoietin (epo) and SH 4536 without epo) with the standard media normally used in our laboratory for the progenitor assays (MethoCultTM StemCell, Canada GH4434 with epo and GH4534 without epo). *Materials and methods.* Peripheral mononuclear cells (PMMC) from 7 subjects (2 patients with polycythemia vera, 3 with essential thrombocythemia, 2 normal volunteer donors) were grown using the above reported media. In particular colony assays were performed in parallel in serum free conditions and with fetal calf serum, with and without epo.

Table 1.

	SH4436		SH4536		GH4434		GH4534	
	SF BFU-E	SF CFU-GM	SF WE BFU-E	SF WE CFU-GM	BFU-E	CFU-GM	WE BFU-E	WE CFU-GM
PV	53	5	18	4	47	9	12	7
PV	97	6	4	5	64	10	1	13
PV	107	4	6	15	68	7	5	13
ET	91	2	0	9	73	6	0	17
ET	104	17	12	11	71	25	3	15
nor	42	3	0	15	37	14	0	6
nor	82	7	0	20	52	11	0	19

*SF*serum free, WE= without epo, PV= polycythemia vera, ET=essential thrombocythemia, nor= health volunteer donor.*

Results. (Table 1) In SF and standard media without epo EEC formation was specific, as it was not observed in cultures of normal subjects. None statistical differences (p=0.09) were observed between the serum free and standard media in the setting "without epo". The SF media show instead remarkable increments of BFU-E (p=0.004) when compared with the growth observed in standard conditions. On the contrary CFU-GM were lower in SF than in standard media in both conditions, with and without epo (p<0.005). In conclusion these data may help to utilize the commercial media, also considering the marked difference of price existing between the tested media, e.g. using front line the standard medium (GH4434 and GH4534) and reserving the serum free media more erythroid "oriented" (SH4436, SH4536) when the standards give uncertain results.

P043

IN VITRO HAEMOPOIETIC PROGENITOR CELL GROWTH AND CD34+ PERIPHERAL CELLS BEHAVIOUR IN HIGH GRADE LYMPHOMA RECEIVING DOSE-DENSE R-CHOP SUPPORTED WITH PEGFILGRASTIM

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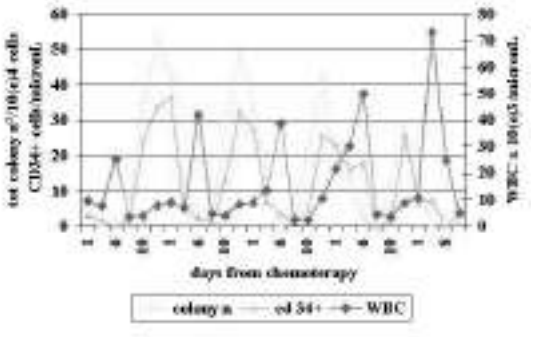
The aim of this study was to evaluate the in vitro growth of peripheral hemopoietic progenitors (CFU-GM, CFU-GEMM, BFU-E) and CD34+ peripheral cells behaviour after dose-dense chemotherapy (R-CHOP-14) supported with pegfilgrastim in high grade lymphoma. In 8 diffuse large B cell lymphoma patients (5 male, 3 female, age 18-51) planned to receive as first line treatment 6 courses of dose-dense chemotherapy with Rituximab and CHOP14 supported by pegfilgrastim, we monitored white blood cells (WBC), GFU-GM, CFU-GEMM, BFU-E and CD34+ cell counts. The CHOP regimen was delivered every 14 days, preceded on day 1 by rituximab (375 mg/m^2) and followed on day 3 by pegfilgrastim (6mg per cycle). In each course of immuno-chemotherapy the analysis were performed before treatment (day 1) and on days +3, +6,+10 and +13. Hemopoietic progenitors were assayed in a complete StemCell medium (MethoCultTM GF H4434 StemCell, Canada) and counted with an inverted microscope after 14 day incubation at 37°C- 5%CO2. CD34+ cells were evaluated with Beckman Coulter cytofluorimeter.



Until now, data are available in all patients for the first course, in five for the second, and in three for the third and fourth course of immuno-chemotherapy. The data collected show a distinct behaviour of WBC and progenitor cells (colonies and CD34+ cell number)(see graph). In fact, after each cycle of therapy, when WBCs reach the nadir (between day +8 and +10), colonies and CD34+ cells start to increase, reaching the maximum value on day +13. The WBC zenith, instead, is reached between day +3 and +6 corresponding to the progenitor nadir. The highest number of colonies and CD34+ cells was reached on day +13 after the first course of R-CHOP + pegfilgrastim (CD34+/µL: 33±3, CFU-GM/10^5 cells: 136±36, CFU-GEMM/10^5 cells: 7±4, BFU-E/10^5 cells: 382±74). Peaks were progressively lower in subsequent courses. However, the number of CD34+ cells after the third course (day +13) was still over 20/µL in two of three cases. At the same time WBC count was 10.3±5x10^3/µL. These results can be helpful in view of

peripheral blood stem cell collection in patients treated with dose-dense protocols supported with pegfilgrastim.

P044

PROTEIN ENRICHED DIABETES CONTROLS B-CHRONIC LYMPHOCYTIC LEUKEMIA CELL DEATH

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Chronic lymphocytic leukaemia (CLL) is the most frequent form of leukemia in Western countries. It is characterized by the clonal expansion and accumulation in the blood, bone marrow, lymph nodes and spleen of long-lived B lymphocytes blocked in Go/G1 of the cell cycle. The accumulation results from a failure of cells to undergo apoptosis rather than from by excessive cell proliferation. The mechanisms underlying apoptosis resistance of B-CLL cells are presently unknown. Therefore, present research on B-CLL pathogenesis and therapy points to the identification of molecules involved in the regulation of the cell death pathway. In this work we have explored the molecular mechanisms of TRAIL-cell death resistance in B-CLL. TRAIL (TNF related apoptosis-inducing ligand) has the unique characteristic of inducing apoptotic cell death only in transformed cells and not in normal cells, and thus may represent a novel therapeutic strategy for many forms of cancer. The mechanism underlying TRAIL-resistance may be related to the function of inhibitory molecules, such as PED. PED is a DED-containing protein that interferes with signalling at the level of TRAIL receptors. We explored the possibility that PED expression levels correlate with TRAIL-resistance. Our analysis was conducted on peripheral blood lymphocytes from 35 B-CLL-affected patients. To down-modulate PED expression levels we treated the cells with phosphorothioate antisense oligodeoxynucleotides (ODNPEDα) or with the protein synthesis inhibitor, cycloheximide (CHM). PED expression, analyzed by western blot and immunofluorescence, was markedly reduced by both these treatments. The percentage of basal apoptosis, measured as hypodiploidy in flow cytometry, was 15% in the cells incubated only in medium o with control scrambled oligonucleotide (ODNs) and 35% in cultures kept with ODNPEDalfa. Thus, PED down-modulation determined a significant increase of basal apoptosis (p=0.05). In basal conditions, B-CLL cells were resistant to TRAIL-induced apoptosis; interestingly, the addition of ODNPEDalfa determined increased apoptosis, up to 100%, upon TRAIL treatment. No appreciable variations were detected with the control scrambled ODNs. Finally, we observed that cycloheximide sensitized B-CLL cells to TRAIL-induced apoptosis and this effect correlated with down-regulation of PED expression. In conclusion, PED down-modulation determined a significant increase of both basal and TRAIL-induced apoptosis. Thus, modulation of PED expression could represent an important tool for improving TRAIL sensitivity in B-CLL.

P045

BEHAVIOUR OF CIRCULATING CD34+ CELLS IN PATIENTS WITH BONE MARROW METASTASES FROM SOLID TUMORS

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Circulating CD34 (cCD34$^+$) cell count is at the present a routine test carried out in patients undergoing autologous stem cell harvest, to monitor stem cell mobilization after G-CSF administration. High levels of cCD34$^+$ cells are also detected after chemotherapy, after the administration of various types of growth factors and in patients with idiopathic myelofibrosis. We investigated cCD34$^+$ cell levels in patients with myelophthisis, which is a condition characterized by a damaged bone marrow microenvironment due to cancer micrometastases. We studied eleven patients with diffuse metastatic cancer. Myelophthisis was suspected in cancer patients presenting uni-, bi-, or trilinear cytopenia not related to chemotherapy, and was documented by the finding of non hematopoietic neoplastic cells at bone marrow aspirate and/or biopsy. Marrow fibrosis was detected in all the four patients with myelophthisis, who had undergone bone biopsy ; the fibrosis was focal, surrounding the metastatic lesions. In six patients suffering from metastatic cancer (breast, lung, stomach) with pancytopenia and micrometastases detected by bone aspirate or biopsy, cCD 34$^+$ cells were 8.1, 9.7, 60, 20, 48 and 149/ microL. In a control group of five patients with diffuse metastatic cancer without bone marrow involvement, cCD34$^+$ cells were 0, 4, 2, 0 and 0/microL. The mean number of cCD34$^+$ cells was 49.1 and 1.2 in the two groups, respectively. Stem cell mobilization by growth factors and/or chemotherapy is mainly due to modifications of membrane-bound molecules causing detachment of CD34$^+$ cells from the stroma. So far, the only disease in which a high number of cCD34$^+$ cells has been described in the absence of any treatment is idiopathic myelofibrosis. The reason for this findings is poorly understood; both altered bone marrow microenvironment and stem cell surface abnormalities may be involved. A slight increase in cCD34$^+$ cells has been described in patients with head or neck cancer and attributed to GM-CSF production by the neoplastic tissue. Raised levels of cCD34$^+$ cells in patients with myelophthisis is a new intriguing finding, which requires additional investigation. By converse, a high number of cCD 34$^+$ cells in cancer patients may indicate the presence of bone marrow involvement.

P046

HDAC INHIBITORS IN COMBINATION WITH HYPOMETHYLATING AGENTS INDUCE SPECIFIC CHROMATIN MODIFICATION AND TRANSCRIPTIONAL ACTIVATION IN CBF/AML CELLS

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Acute myeloid leukaemia (AML) characterized by the presence of AML1/ETO fusion protein, or by other

rearrangements involving core binding factors, share a common leukemogenic mechanism, determining transcriptional repression of target genes involved in myeloid maturation via permanent recruitment complexes containing HDACs, DNMTs and co-repressor molecules. We demonstrated that butyrates are able as single agents to restore histone acetylation and to reinduce gene expression allowing granulocytic maturation in AML1/ETO pos Kasumi-1 cell line, as well as in primary CBF-AML blasts. Core histone proteins can be acetylated by histone acetyl transferases (HAT) or methylated by histone methyltransferases (HMT) at N-terminal lysines, at different residues which are specifically associated to the transcriptional state of the nearby chromatin. Transcriptionally active chromatin is characterized by histones with acetylated lysine tails, and methylated lysine 4 of histone H3, whereas dimethylated lysine 9 of H3 and deacetylated lysines in H4 and H3 seem associated with transcriptional silence . We investigated the acetylation and methylation pattern of lysine residues of histone H4 and H3 in our AML1/ETO pos cell model, prior and after exposure to butyrate as HDAC inhibitor alone or combined with azacitidine, as DNMTi. Cells were lysed after 6-12-72 and 96 hrs of culture and whole cell lysate was analysed by SDS-PAGE electrophoresis and Western blot, with antibodies specific to different lysine residues of the two histones. K5 and K16 residues of histone H4 were not acetylated in the absence of butyrate and their acetylation strongly increased after 6 h of butyrate administration. K8 and K12 residues showed a basal acetylation which was slightly enhanced by the co-treatment with HDACi and DNMTi . Lysine methylation of histone H3 was also evaluated. Butyrates determined a significant reduction of di-methylated K9 H3. Di-methylated K4 H3 was, on the other hand, unmodified after butyrate administration. Azacitidine was not influencing the lysine methylation. These histone epigenetic modifications were paralleled by an increase of p21/cip1 protein expression and a decrease of p27/kip1 protein expression. Appearance of a conspicuous amount of p57/kip2, an as yet uncharacterised oncosuppressor, after 72-96 h treatment with butyrates was also observed. Moreover, CEBP family expression and phosphorilation were induced. In parallel, AML1/ETO mRNA and protein expression was markedly reduced after D1 exposure.

P047

DONOR CD3+ LYMPHOCYTES RECONSTITUTION OF RECIPIENT PERIPHERAL BLOOD WITHOUT GVHD IN A CASE OF APLASTIC ANEMIA FOLLOWING LIVER TRANSPLANTATION

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Tolerance is an area of active study in the setting of solid organ transplantation. The fact that donor T-cell microchimerism in the peripheral blood of a recipient is associated with lower graft rejection rates is particularly interesting, as the HLA of a transplanted liver is usually different from that of the recipient. On the other hand, high rates of graft-versus-host disease (GVHD) have been reported in the case of donors who are homozygous for a shared HLA haplotype. GVHD typically presents with fever, skin rash, diarrhea, or pancytopenia within 2 to 6 weeks after transplant, and is usually characterised by substantial donor lymphoid chimerism. Differential diagnosis may be difficult and includes cytomegalovirus disease, drug reactions and aplastic anemia, all of which require different and specific treatments that involve increasing or decreasing immunosuppressive therapy. We here report the case of a 61-year-old male who underwent orthotopic liver transplantation for alcohol-induced liver cirrhosis in July 2004, and developed nearly complete donor lymphoid chimerism without GVHD. The recipient was IgG anti CMV positive(IgM negative), HCV negative, and anti-HBc antibodies positive. The donor was a female of the same age (62 years old), with identical ABO blood group and different HLA. The patient was discharged 13 days after the transplantation with normal hepatic function, and the prescription of standard tacrolimus and methylprednisolone immunosuppressive therapy. Moderate anemia (Hb 86 g/L) was diagnosed during the fourth post-transplantation week and, two weeks later, the patient became severely anemic and neutropenic (Hb 50 g/L; WBC 0.4 x 10^9/L). He was admitted to our hematology ward and also developed severe thrombocytopenia (PLT 10x10^9/L) after 10 days. A diagnosis of CMV pneumonitis was made on the basis of the results of a high-resolution lung CT scan, the presence of CMV pp65 antigenemia and CMV DNA positivity. The findings of a bone marrow trephine biopsy were compatible with bone marrow aplasia. Microchimerism analysis revealed that 80% of the CD3+ lymphocytes in peripheral blood and 15% in bone marrow were of donor origin. There were no signs of GVHD (particularly diarrhea and skin rash) and none subsequently developed. The treatment consisted of the administration of G-CSF, immunosuppression reduction and supportive care with transfusions of packed red blood cells (for Hb<80 g/L) and platelets (for PLT<20x10^9/L); the CMV infection was treated with gancyclovir. The patient's WBC, PLT and Hb levels slowly improved over the following month, when microchimerism analysis showed that the level of donor CD3+ lymphocytes in peripheral blood had decreased from 80% to 20%. Eight months after discharge, the patient's peripheral blood counts were normal. We interpreted the clinical picture of the patient as an aplastic anaemia in the course of CMV infection. It is therefore possible that the high CD3+ chimerism observed emerged following the deep immunosuppression of the patient, caused by the CMV-correlated pancytopenia, and the immunosuppressive therapy. This hypothesis is supported by the fact that the progressive reduction of the immunosuppressive therapy paralleled the reduction of the donor chimerism. The other rather surprising aspect of this case is that the high degree of CD3+ lymphocytes chimerism was not associated with signs of GVHD. In conclusion, in the context of an immunocompromised patient, it is possible to obtain a two way tolerance (recipient-host and host-recipient), without GVHD. This case confirms a number of observations suggesting that the engraftment of all

kinds of organs and bone marrow cells involve host-versus-graft (HVG) and graft-versus-host (GVH) reactions with the reciprocal induction of variable degrees of specific non-reactivity (tolerance).

P048

MAJOR CLONAL T-CELL EXPANSIONS WITH LARGE GRANULAR LYMPHOCYTE FEATURES IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Paroxysmal nocturnal hemoglobinuria (PNH) is due to acquired somatic mutations within the phosphatidylinositol glycan class A (PIG-A) gene, which impair the glycosylphosphatidylinositol (GPI) anchor biosynthesis, resulting in the lack of all GPI-linked proteins from the cell surface. While it is known how the absence of these molecules explains defective complement inactivation on red cells with subsequent intravascular hemolysis, the pathogenic mechanisms of marrow failure in PNH are still debated. We investigated the T-cell repertoire in 24 PNH patients, 14 with concomitant marrow failure and 10 with purely hemolytic disease. The initial aim was to identify clonal T-cell expansions eventually documenting pathogenic Ag-driven immune responses paralleling those described in AA patients (Risitano *et al.*, Lancet 2004). Flow cytometry analysis of TCR-Vbeta usage was utilized in combination with molecular characterization of the complementarity determining region 3 (CDR3); this method allows a fine definition of T-cell clonality. Vbeta utilization as determined by flow cytometry was abnormal in all 24 PNH patients, with over-representation of particular Vbeta subsets compared to the normal range established in 10 normal donors; on average, two or three Vbeta-subfamilies were expanded in each patient. Both CD4+ and CD8+ T cell pools showed expansion, and no common pattern of specific Vbeta-subfamilies was identified; no difference emerged in Vbeta subfamily expansions between patients with hemolytic PNH and those with aplastic PNH. Four patients showed a distinctive pattern, with an extreme expansion of a single Vbeta family, accounting for more than 25% of the total lymphocyte pool. These four expansions were all CD8+, and harbored an effector phenotype (CD56+, CD16+, CD28-); in all cases, the blood smear revealed typical lymphocytes with abundant cytoplasm full of azurophilic granules. One patient was diagnosed as having a concomitant large granular lymphocyte (LGL)-leukemia, all the others harboring a subclinical chronic LGL-lymphocytosis. The molecular analysis of the CDR3 by RT-PCR followed by size analysis, cloning and sequencing documented the monoclonal nature of these expanded lymphocyte populations. This study documents a surprisingly high frequency of clonal LGL-like expansions in PNH patients; in addition to the association between clinical/subclinical LGL-disease and PNH, this observation raises intriguing pathophysiological interpretations. In PNH, the presence of an Ag-driven immune response suggests that, besides the PIG-A mutation occurring in the stem cell, an immunological pressure shapes the hematopoiesis. In this context, the PIG-A mutation might represent a rescue of hematopoiesis, leading, together with the nature of the immune response, to the individual clinical balance among marrow failure, clonal T-cell expansion and overgrowth of the PNH clone.

P049

CTLA-4 GENE EXPRESSION IN HAEMATOLOGICAL MALIGNANCIES

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Cytotoxic T Lymphocyte Antigen 4 (CTLA-4) is a critical downregulatory molecule expressed on T cells that plays a major role in inhibiting T cell activation and peripheral tolerance. A soluble form of CTLA-4 (sCTLA-4) has been shown to possess a similar function as a membrane bound CTLA-4 molecule and is constitutively expressed in non-stimulated human T cells. The CTLA-4 genetic locus has been implicated in multiple autoimmune diseases as well as in blood disorders, including Non Hodgkin's Lymphomas, and genetic variation in the expression levels of sCTLA-4 have been associated with susceptibility to autoimmune disease. Since available data on CTLA-4 expression are limited, the aim of the present study was to analyze the distribution pattern of CTLA-4 expression in bone marrow cells from patients with haematological malignancies. Umbilical cord blood (UCB) units were also examined. Expression of CTLA-4 specific transcripts was investigated by nested RT-PCR with a set of primers that reveals both CTLA-4 full length coding sequence (flCTLA-4) and the transmembrane deleted transcript which corresponds to the sCTLA-4. Two main mRNA transcripts of 354 bp and 246 bp referred to the flCTLA-4 and the sCTLA-4, respectively, were detected on 4% agarose gel. Preliminary data showed that both flCTLA-4 and sCTLA-4 are present in 11 out of 15 bone marrow samples from patients with haematological malignancies, while 1 of the bone marrows expressed the sCTLA-4 alone and 3 expressed the flCTLA-4 alone. CTLA-4 expression was also investigated in bone marrow cells at clinical remission. Three out of 8 presented both CTLA-4 transcripts, while 1 had the flCTLA-4 and 4 the sCTLA-4. When both transcripts were detected, they were expressed at comparable levels. When UCB were examined, both CTLA-4 variants were present in 5 out of 8, with overexpression of the sCTLA-4 transcript. The flCTLA-4 alone or the sCTLA-4 alone were present in 1 and 2 UCB, respectively. An additional transcript of roughly 290 bp was detected together with the flCTLA-4 and sCTLA-4 in 1 Non-Hodgkin's lymphoma and 1 UCB. We found that sCTLA-4 is the most abundant transcript on UCB and bone marrow cells from patients at remission, while in malignant bone marrow both CTLA-4 variants are equally expressed indicating that the expression pattern of CTLA-4 splice variants is variable in distinct cellular status. The relationship between the presence or level of specific CTLA-4 splice variants and disease status needs further investigation. The knowledge of the bio-

logical significance of CTLA-4 isoforms may contribute to understand the potential involvement of CTLA-4 in the malignant process.

P050

DEFECTIVE CHARACTERISTICS IN BONE MARROW STEM CELLS ARE ASSOCIATED WITH IMPAIRED VASCULOGENESIS IN SYSTEMIC SCLEROSIS

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Systemic Sclerosis (SSc) is a connective tissue disease characterized by early generalized microangiopathy and culminating in systemic fibrosis. The pivotal steps of the disease are endothelium injury, immune activation, and collagen deposition by activated fibroblasts. Clinical and biological evidences strongly support the hypothesis of a unique vascular injury as an important and primary process in scleroderma. In addition, recent studies have provided evidence that in patients with SSc the formation of new blood vessels, despite tissue ischemia, seems to be insufficient to replace the damaged vessels. We investigated whether impaired vasculogenesis in SSc is due to defective characteristics in BM microenvironment. Circulating endothelial progenitors (CEPs) were evaluated in the peripheral blood (PB) of 63 patients suffering from SSc by flow cytometry and characterized as $CD34^+/CD133+$. In addition, bone marrow (BM) samples were collected from 12 SSc patients and evaluated by means of various assays in order to study hematopoiesis, and the results compared with normal bone marrow controls. No patient had received cytotoxic drugs. CD133+ bone marrow cells were isolated by immunomagnetic sorting and grown in order to induce endothelial differentiation. Long-term bone marrow cultures (LTBMC) were assessed: the cells in the harvested medium were weekly counted and the stroma formation was evaluated as the percentage of the flask surface covered by stromal cells. The number of stromal clonogenic precursors was evaluated by means of a CFU-F (colony-forming unit fibroblast) assay. Mesenchymal stem cells (MSC) were separated by means of immunomagnetic selection for nerve growth factor receptor (NGF-R)+ cells: the cells were grown in order to assess the clonogenic potential and the proliferative capacity, whereas their multipotential differentiation ability was determined after culture in adipocytic, osteoblastic and endothelial conditioned media. Phenotypic analysis of bone marrow mononuclear cells and NGF-R+ MSC was performed by flow cytometry. Phenotypic analysis of BM mononuclear cells showed a greater expression of the surface markers P1H12 and CD105 TGF-beta receptor (1.2%±0.6 vs 0.5%±0.1, p=0.01 and 9.9%±5 vs 4.7%±3, p=0.02 respectively), but a lower percentage of CD133+ cells (0.36%±0.4 vs 1.2%±0.8 in normal controls, p=0.05), as confirmed by the low frequency of the immunomagnetically sorted CD133+ cell fractions. On the contrary, the absolute number of CEPs in PB was higher in patients with SSc than in healthy controls (mean values 2.1 cells/microL vs 0.26 cells/ microL, p=0.04). When BM CD133+ cells were grown in the presence of VEGF, only 3/12 cases gave endothelial differentiation, moreover with a reduced proliferative ability. All of the patients showed a defective stromal cell compartment and a reduced number of bone marrow NGF-R+ stromal cell precursors (0.73±0.5 vs 1.61±0.6 in normal controls, p=0.02), as detected by the CFU-F assay (4%±3.2 vs 43%±19.8 in normal controls/$1x10^6$ LDMNCs seeded) and by the lower frequency of the immunomagnetically separated stem cells. In the LTBMCs, the stroma formation was always deficient and never reached confluence, with a lower total number of recovered hemopoietic cells. NGF-R+ MSC overexpressed KDR and CD117 (26.4%±7.4 vs 4.6%±1.7, p=0.01 and 87.7%±5.1 vs 57.6%±11, p=0.03 respectively). Only in two cases NGF-R+ cells grew and formed a confluent layer with fibroblastic morphology, but with a reduced proliferative capacity. Interestingly, in the presence of VEGF these two NGF-R+ fractions gave rise to endothelial colonies, while on the contrary they never gave rise to adipogenic or osteoblastic differentiation. The results of this study provide evidence that patients with SSc 1) have an increased mature endothelial subset in the BM, as previously described in SSc PB, and 2) have defective both hematopoietic and stromal cell compartments. The higher expression of KDR and CD117 on NGF-R+ cells suggests a role for VEGF in inducing endothelial differentiation of MSCs, responsible for the depletion of the stromal progenitors pool in BM. These findings are consistent with recent studies in which CEPs detected in the PB of SSc patients were functionally impaired and not able to respond adequately to angiogenic factors. At this regard, the detection of high CEP numbers in the PB of the same patients further suggests an extreme attempt at revascularization and healing of ischemic tissues. Furthermore, the continuous recruitment of endothelial progenitors to sites of vascular damage might lead to the irreversible BM alterations we observed. In conclusion, this study provides new insights into the pathogenetic role of defective vasculogenesis in contributing to vasculopathy in SSc and the possible role of circulating endothelial precursors as a novel target for therapeutic strategies.

HEMOSTASIS AND THROMBOSIS I

P051

ATYPICAL HAEMOLYTIC-URAEMIC SYNDROME AT ONSET OF ACUTE PROMYELOCYTIC LEUKAEMIA

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A 68-year-old woman was admitted with fever and pancytopenia (WBC count 0.9x10^9/L, haemoglobin 8 g/dL, platelet count 9x10^9/L). Bone marrow examination revealed acute promyelocytic leukaemia (APL); cytogenetic and molecular analysis showed t(15;17) and a PML-RARalpha fusion protein (BCR3 transcript). Laboratory findings at diagnosis: blood urea nitrogen 30 mg/dL, creatinine 1.8 mg/dL, uric acid 6.5 mg/dL, lactate dehydrogenase 2259 U/L, total bilirubin 2.4 mg/dL, unconjugated bilirubin 1.6 mg/dL, haptoglobin less than 8 mg/dL. Direct and indirect Coombs' tests were negative, fibrinogen, prothrombin time and activated partial thromboplastin time were normal with a D-dimer of 8456 microg/mL. The peripheral blood smear showed some schistocytes. Urinalysis revealed microscopic haematuria and proteinuria. The patient was started on induction chemotherapy with all-trans retinoic acid (ATRA) 60 mg/daily followed by Idarubicin 10 mg/m^2 (four doses) according to AIDA-GIMEMA protocol. Despite chemotherapy and appropriate supportive therapy, including hydration and erythrocytes transfusions, the acute renal failure worsened with increase of creatinine (7.2 mg/dL) and progressive oliguria for which hemodialysis was started (three times a week). Kidney ultrasound examination was normal. A renal biopsy showed thrombotic microangiopathy with predominant glomerular involvement (arteriolar congestion with thrombi and endothelial damage) without cortical atrophy or necrosis. Direct immunofluorescent microscopy showed a diffuse IgM deposition in the mesangium and glomerular capillary subendothelium and focal deposition, along capillary walls, of IgA and C4 with a marked subendothelial widening with double contours. The clinical and histological findings were in accordance with an Atypical (without dirrhoeal prodrome) Haemolytic-Uraemic Syndrome (HUS). Despite the treatment with dialysis, dexamethasone, plasma infusion and plasma exchange, acute renal failure did not improved and the patient died, five weeks after the diagnosis of acute leukaemia, in a condition of haematologic remission. Atypical HUS, altough rare, can be a fatal complication at onset or during induction chemotherapy of acute myeloid leukaemia.

P052

SUCCESSFUL TREATMENT WITH DERMATAN SULPHATE OF HEPARIN-INDUCED THROMBOCYTOPENIA AND BILATERAL PULMONARY EMBOLISM AFTER HIP ARTHROPLASTY

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A 70-year-old woman underwent major orthopedic surgery for elective hip arthroplasty. She received nadroparin thromboprophylaxis, 4000 U subcutaneosly every 24 hours with first dose 12 hours prior to surgery. The medical history of this patient reported previous exposure to low molecular weight heparin for knee arthroplasty without adverse reactions. On the sixth postoperative day, her platelet count fells to 34 x 10^9 cells/L. The preoperative blood work showed a normal platelet count with 270 x 10^9 cells/L. Meanwhile the clinical course was complicated by several and sudden dyspnea without hypotension. The D-dimer level was 800 microg/L. The chest radiography was normal. Spiral CT scan revealed bilateral perfusion defects of pulmonary artery main branch on the right and lower lobe tributary branch on the left. The echocardiography showed right atrial and ventricular dilatation with severe pulmonary hypertension (PAP: 60 mmHg). Previous echocardiography, carried out the week before, was normal. We suspected HIT and stopped the low molecular weight heparin. An alternative anticoagulation therapy with dermatan sulphate (Mistral®, Mediolanum Farmaceutici) was started immediately (0.5 mg/kg/h continuous infusion without IV bolus) before laboratory confirmation. The result of an enzyme-linked immunoassay ELISA (Diagnostica Asserachrom Roche) for heparin-induced PF4 antibodies was very strongly positive. Dermatan sulphate is an indirect thrombin inhibitor enhancing cofactor heparinic II activity. Dosage of dermatan sulphate was regularly monitored by aPTT evaluation. Oral anticoagulant therapy with warfarin was started only when aPTT ratio reached a protective value of 1.5 times the control normal value. Dermatan sulphate was stopped when INR settled around 2 for two consecutive days. This off label therapeutical approach was well tolerated and no hemorrhagic nor other complications were observed. After ten days from the initiation of dermatan sulphate therapy, the platelet count rose to normal value and the patient was discharged from the hospital while receiving warfarin therapy.

P053

MANAGMENT OF PATIENTS AFFECTED BY INHIBITOR OF FACTOR VIII: REPORT OF FOUR CASES

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Acquired hemophilia (AH) due to acquired inhibitor of factor VIII (iFVII) is a rare event, sometimes presenting as life-threatening because of severe bleeding. Its incidence

of one per million is probably underestimate, due to fatal events first to diagnosis. It is more frequently in the elderly, could be associated to malignant conditions (lymphomas, solid tumors), related to autoimmune diseases or post-partum. Best therapy is still debated probably because the different pathogenesis of the disease. Complete remission after therapy is possible, especially if diagnosis and treatment are not delayed. Here we report our experience of the last two years in the diagnosis and therapy of this disease. From January 2003 to January 2005 4 patients received diagnosis of acquired inhibitor of FVIII in our institution. The onset of coagulopathy was represented by soft tissue emorrage, particular of muscles of the limbs and anemia. Main characteristics of patients are summarized in Table 1.

Table 1.

Pat. N.	Sex	Age	PTT (n.v.:20-35 sec.)	iFVII (Bethesda Units)	Sites of Hemorrhage	Hb (gr/dL)	Concomitant conditions
1	Male	67	67	1,92	Muscle: legs and arms	5,7	CLL, IRC, diabetes
2	Female	34	79	3,4	Muscle: legs and arms	11,3	Post-partum (fifth month)
3	Male	91	63	2,1	Abdomen wall, scrotus, muscles of the legs	7,5	None
4	Male	82	77	3,2	Abdomen wall, scrotus, peritoneum, and arms muscles of legs	6,5	None (ischemic cardiopathy)

Main characteristic of patients at diagnosis

Treatment was started with 1,5 mg/kg of metilprednisone and desmopressin eV. Response to treatment was valued as regression of the ecchymosis, the absence of new hemorrhagic manifestations, the normalization of PTT and the disappearance of detectable inhibitor of FVIII. Results were as follows: Case n. 1: 10 days after the start of methilprednosone (1 mg/kg) because of leucocytosis due to the underline disease (CLL) cyclophosphamide 150 mg per os was started. Clinical and laborathory response were obtained in 1 month. Case n. 2: Patient had only partial response to corticosteroids and desmopressin after 1 month (complete clinical response, slight reduction of iFVIII and PTT) so cyclophosphamide 200 mg per os was started 2 months later the onset of coagulopathy. A complete laboratoristic response was achieved in 3 months, and is still present. Case n. 3: Complete response was achieved in 1 month and is still maintained. Case n. 4: After 3 days patient presented large hemorrhage of the abdomen wall, peritoneum and scrotum with anemization (hb: 5.5 gr/dL), atrial fibrillation and cardiac failure. Surgical intervention was needed, but was fatal. It is to underline that diagnosis of iFVIII in this patient was delayed for two weeks after the onset of ecchymosis. In our experience, no toxicity related to the treatment was observed. In › patients complete remission was obtained and no relapse has been observed. iFVIII is undetectable and titer of FVIII ise actually normal in all three patients. It is important to make diagnosis in the shortest time in order to start treatment as soon as possible. Only with the beginning of the treatment massive bleeding could be avoid. Treatment with corticosteroids with or without other immunosuppressive agents and desmopressin could result in good response and mild or none toxicity, even in elderly patients, but with the availably of recombinant factors the best treatment is still to be codified.

P054

SUPERIOR SAGITTAL SINUS THROMBOSIS AFTER LUMBAR PUNCTURE IN A PATIENT WITH T-CELL LYMPHOBLASTIC LYMPHOMA: ROLE OF THE PROTHROMBIN MUTATION G20210A AND GENOTYPE 4G/4G

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Introduction. The superior sagittal sinus (SSS) thrombosis is a rare complicance described in haematological diseases and other disorders, with variable neurological symptoms, signs and clinical outcome. There have been few reports of SSS thrombosis occurring after lumbar puncture in patients with aggressive lymphoma, and the physiophatological explanation remains still unclear. The crucial point could be investigate if lumbar puncture and dural sinus thrombosis is a causal or casual association. Case report A 28-year-old man was diagnosed with T-cell lymphoblastic lymphoma and mediastinal bulky mass. The patient started anticoagulation treatment with low-molecular-weight heparin (5,700 IU nadroparine per day) and was admitted to our hospital. A lumbar puncture showed immunotyping positivity for T-lymphoblasts. The patient received induction chemotherapy with daunorubicine, vincristine and prednisone. Methotrexate (MTX) 15 mg were administered intrathecally. A second positive immunotyping diagnostic lumbar puncture was performed on day +7. After 2 hours from procedure, the patient noticed tension headache. Later he became disorientated with psychomotor disturbance and presented moderate aphasia. A cerebral MR scan revealed SSS thrombosis. Coagulation profile studies showed normality of PT, PTT, fibrinogen, concentrations of D-Dimer and antithrombin III activity. A thrombophilia screening was also performed. It consisted of the following tests: antithrombin, protein C, protein S activities, plasmatic homocysteine, Kaolin Clotting Time and dRVVT (dilute Russell Viper Venom Test) for Lupus anticoagulant screening, factor V Leiden and prothrombin mutation (G20210A). The patient showed positive results for the G20210A prothrombin and for the 4G/4G genotype. Dosage of nadroparine was increased to 11400 IU per day with a regression in few days of neurological symptoms and signs. Intrathecal treatment was discontinued and the patient was treated with systemic chemotherapy. After 15 days a cerebral MR scan revealed a partial resolution of thrombosis. Induction cycle was completed and a new diagnostic lumbar puncture showed immunotyping negativity for T-lymphoblasts. Since the resolution of mediastinal bulky mass was achieved, the patient underwent a consolidation cycle. Discussion Possible explanation for the cerebral vein thrombosis in our patient requires to take into account several factors: the lymphoblastic CNS involvement, the endothelial damage induced by previous MTX intrathecal injection, the decrease of venous blood flow secondary to lumbar puncture, an hypercoagulable

state indicated by the contemporaneous presence of G20210A prothrombin and an hypofibrinolytic state. In fact, previous findings showed an increased risk (13.4 Odds Ratio) for a thrombotic episode in subjects with the concomitant presence of the prothrombin mutation and genotype 4G/4G. This is responsible for the higher plasma plasminogen activator inhibitor–1 (PAI-1) level, conditioning an hypofibrinolytic state and leading to an increased resistence of the fibrin clot to fibrinolysys. Intersting, the concomitant heparin prophylaxis started in the patient was not sufficient to prevent SSS thrombosis. We suggest that in patients with aggressive lymphoma and involvement of CNS that requires intrathecal treatment, a therapeutical dosage of heparin could be administered; moreover a prothrombin-gene mutation and 4G/4G genotype study could be useful in all patients developing thrombosis.

P055

PROGNOSTIC IMPACT OF METYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISM ON VENOUS THROMBOSIS IN PEDIATRIC PATIENTS

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Background. The venous thromboembolism is a serious and complex pathological condition especially for its potential complications. It is related to both congenital and acquired factors. Among the congenital factors, different mutations have been identified leading to a reduced activity of enzymes (even higher than 50%) involved in the homocysteine-methyonine methabolic pathway, such as the methylenetetrahydrofolate reductase enzyme (MTHFR). We describe 6 clinical cases of children presenting a serious and complex thrombosis. The only risk factor found was the MTHFR mutation. Aims The aim of this study is to define the real role of MTHFR mutations and increased risk of trombotic events. Methods and Results Between december 2002 and december 2004 six children (median age 7, range 3-11) were admitted into our Department. They were affected by deep venous thrombosis. The MTHFR polymorphism C677CT was found in all the patients and also in their parents. The polymorphism was identified by polymerase chain reaction (PCR) followed by restriction enzyme digestion and separation on a 3% agarose gel. Four of the children were homozygous for the genetic mutation, the other two were heterozygous. Two heterozygous and two homozygous patients presented increased plasmatic and urinary levels of homocysteine. The thrombotic events were diagnosed by CT angiography, ecocolordoppler and digital cavography. One patient showed venae cavae thrombosis involving the renal veins bifurcation. Two patients showed a mesentheric thrombosis followed by an intestinal infarction requiring a bowel resection. One patient presented a thrombosis of left lower limb, one of sovrahepatic vein, one of the spinal cord vascular funnel. Initially, five patients were treated with low molecular weight heparin. Afterwords, they continued with oral anticoagulant therapy lasting from 6 to 12 months; after stopping this therapy, three of them started a treatment with folic acid and B group vitamins. Two children are event free since more than two years, one showed two months ago a new thrombotic event. Conclusions The association between the homozygous MTHFR mutation and thrombosis is still not clear. It seems that not always this polymorphism leads to high plasmatic level of homocysteine. On the other hand, hyperhomocysteinemia spikes could occur in particular circumstances that might not be detectable at all times but clinically significant. Recent data demonstrated that MTHFR C677T homozygosity with hyperhomocysteinemia is not necessarily associated with VTE (Frederiksen J. *et al.,*-2004). Nevertheless, our cases do not seem to confirm this considering that two patients presented both homozygosity and high level of plasmatic homocysteine with significant thrombotic events. Further investigations are required in order to understand the real link between MHTFR mutation and the increased risk of thrombosis.

P056

SUCCESSFUL TREATMENT OF RECOMBINANT FACTOR VIIA FOR PARANEOPLASTIC FACTOR XI INHIBITOR

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A 79-old-year Caucasian woman was admitted in our hospital undergoing surgery for left renal carcinoma. During preoperative assessment, we found a prolongation of aPTT. Her clinical history was not significant for hereditary coagulopathy; she previously underwent surgical procedures and delivery without haemorragic complications. Bleeding time, platelet count, INR and prothrombine time were normal. At diagnosis she didn't show bleeding signs nor anaemia. Laboratory investigations showed a PTTratio of 1.67; factor XIc of 14%; mixing text did not normalize after incubation for 2 hours at 37°C; FXI inhibitor titer was 2,4 U.B. The kaolin clotting time (KCT), RVVT test and inosithin neutralization test were done to detect lupus anticoagulant. The anticardiolipin and anti beta2 glicoprotein antibodies were negative. Factor XII, factor IX, factor VIII were all in normal range. We concluded for acquired paraneoplastic factor XI inhibitor. To allow radical nephrectomy, we administered a standard initial bolus of intravenous rVIIa (90 microg/kg) 2 hours before surgical procedure followed by low dose continuous infusion of 16 microg/kg/h for five days during the perioperative period. In the meanwhile we started oral immunosuppressive therapy with prednisone 1 mg/kg/d and cyclophosphamide 150 mg/d. The above-mentioned open surgery procedure was not accompanied by any bleeding complication nor thromboembolic event. After two months the inhibitor disappeared definitively, the factor XI rose to 63% and aPTT ratio returned to normal value of 1,07. To our knowledge, this is the first case reported in literature of successful treatment of acquired factor XI inhibitor with recombinant factor VIIa.

P057

MITRAL VALVE INVOLVEMENT IN PRIMARY ANTIPHOSPHOLIPID SYNDROME

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The anti-phospholipids autoantibodies (APL) are a heterogeneous group of allo and autoimmunoglobulins that recognise complexes of protein-phospholipid in vitro laboratory assay. The antiphospholipid syndrome (APS) may be defined as the occurrence of thrombosis, recurrent miscarriages, or both in association with laboratory evidence of persistent APL, either Lupus Anticoagulant (LA) tests, including Kaolin Clotting Time (KCT) and Dilute Russel's Venom (Drvvt) or anticardiolipin and anti-beta 2GPI antibodies. In APS cardiac involvement includes coronary occlusion, cardiomyopathy, intracardiac thrombosis, valvular heart disease and Libman-Sacks nonbacterial endocarditis. In particular mitral valve prolapse represents the common form of the valvular heart disease in APS. To assess the prevalence of cardiac disease in the presence of APL clinical findings we studied through cardiological noninvasive methods patients with primary APS. Between January 1998 and December 1999 30 consecutive patients with APS have been diagnosed and managed at the Institute of Haematology of the University "La Sapienza" of Rome. All the patients had elevated levels of antiphospolipid antibodies tested by enzyme linked immunosorbent assay and confirmed three times consecutively every 8 weeks. 27/30 pts, 11 males and 16 females, median age 37 years (range 19-72) gave the informed consent to perform doppler echocardiography study. Recurrent fetal abortions, arterial thrombosis and venous thrombosis were documented in 3/27, 9/27 and 11/27, respectively. The control group consists of 88 age-and-sex–matched subjects (43 males and 45 females- median age 47, range 15-72) affected by myeloid acute leukaemia undergone to echocardiography in the same period to perform chemotherapy. A valvular abnormality was detected on physical examination in 5/27 (18%) APS pts; all patients had a normal electrocardiogram. Mitral valve abnormal echocardiographic findings were documented in 17/ 27 cases (62%): mitral valve prolapse was present in 5/27 pts, the enlargement and redundancy of the valve leaflets in 12/27 pts; all valve mitral disease was regurgitant with mild (4/5 pts) to moderate (1/5 pts) haemodynamic repercussion; stenotic lesions were not evidenced . In the control group 10/88 pts (11,3%) had valve disease with mitral prolapse with mild insufficiency; arterial or venous thrombosis events were not recorded. Our data evidence that mitral valve involvement is commonly represented in APS and the echocardiographic study should be performed in initial assessment followed by regular controls in the follow-up.

P058

VENOUS THROMBO EMBOLISM: A COMMON AND SOMETIMES THREATENING COMPLICATION OF ACUTE LEUKEMIA AND CHEMOTHERAPY. A CLINICAL EVALUATION OF SCHEDULED DOSES OF ENOXAPARIN

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This investigation was meant to testing a safe and effective therapy for thrombotic occourrencies in Acute Leukemias (ALs), also when thrombocitopenia and hemorrages are present. Deep Venous Thrombosis (DVT) and Pulmonary Embolisms (PE) are expected complications of ALs, mostly during chemoterapy, and are often related to the use of venous central catheter (VCC). The current antithrombotic therapies suffer from the concern about the risk due to the significant co-occurrence of trombocytopenia and hemorrages with the VTE, and about the necessity to discriminate this from disseminated intravascular coagulation (DIC), before that the data of fine analyses are available. 42 patients were evaluated. Between November 2001 and November 2004, they received diagnosis of AL and chemoterapy, and did not take any other antithrobotic drugs contemporarily. "Frale" and older than 70 year old patients were excluded, because they were under a different observational protocol. We also excluded Acute Promyelocytic Leukemia and full-blown DIC. D dimer, regional ultrasonography, echocardiogram, routinary blood tests for AL, first blood count chiefly, PT, aPTT, Fibrinogen assays, in addition to assiduous clinical examinations were performed twice a week when symptomatic VTE occurred. The therapy was thus scheduled: DVT. patients received 200 iu of enoxaparin/Kg/day until resolution of acute symptoms (generally fifth-seventh day), an halved dose for 30 days and further halved was prescribed for the following 60 days. For PE patients starting doses were 400 iu/kg/day, halved doses after clinical resolution (10-15 days) for 30 days; a further halved dose was proposed for at least 3 months. Patients with previous obstructed and restored VCC, received 4000 iu dayly until the removal of catheter, when infusional need ceased. We used Enoxaparin for all the patients. 8 VTE, 2 PE, 4 blocked VCC (on wich, once removed, some thrombous were found on most of the VCC surface) were registered. 2 VTE were already observed at admission, 4 over chemotherapy, 2 during the aplasia and simultaneous with severe bacterial infections. 1 PE became evident on the second day of chemoterapy, in a patient with previous acute myocardial infarction. The second EP befell three months after chemotherapy - as a consequence of a trauma to the left arm - in CR of disease, in a patient with chronic HCV related hepatitis. Among the 42 patients who received chemotherapy, 30 had VCC. Only in six cases, in spite of the accuracy of maintenance, the removal was needed. The 70% of surface of the catheter, once pulled out, was coat by thrombous. DVT affected 2 patients with less than $40x10^9$/L Platelets (PLTs)and significant hemorrages (haematuria and gums bleeding, rectoraggies); 2 patients with PLTs ranged between $40x10^9$/L and $80x10^9$/L (one had hypermenorrhoea); 4 DVT cases showed more than $80x10^9$/L PLTs. The conditions of patients treated with doses of Enoxa-

parin scheduled for DVT rapidly improved (in 5-7 days) and in about 60 about attained almost total functional recovery. The patients affected by PE were shifted on intensive care for breath and circulatory monitoring and for supports, nevertheless the scheduled administration of Enoxaparin was kept. Clinical conditions of a patient ameliorated critically and after 15 days he was discharged. He was followed on home care over 3 months, assuming Enoxaparin at scheduled doses. Another patient ameliotared too (Echocardiogram and D dimer) during 15 days in intensive care room. So he was moved to ordinary care, where he received scheduled doses of Enoxaparin; he completed chemotherapy (Ara C x 10 days), but unfortunately he died 5 days after, because of atrial fibrillation and acute heart failure. The profilaxis with Enoxaparin for the patients with a restored VCC allowed it to be hold until the completion of the infusional therapy, without further complication. In no cases did we register a thrombocitopenia unequivocally related to Enoxaparin administration, notably during chemoterapy. The hemorragic occurrences that happened together with thrombotic events did not appear significantly worsened by Enoxaparin administration, consistently with the aPTT trends that were longer only in the patients who had received higher doses, but who did not get any bleedings. VTE appears to be a common complication of ALs, mostly during chemotherapy, and sometimes is really threatening. Anticoagulant or antithrombotic therapies are problematic because of the high incidence of contemporay thrombocitopenia or bleedings. In our study Enoxaparin seems to be effective and safe, not inducing thrombocitopenia, not influencing the trend of minor bleedings. Moreover, we conclude that it is mandatory to assess every and each factor of thromboembolic risk at the same time of the diagnosis of leukemia in order to start with a prompt and adequate profilaxis.

P059

FIRST LINE THERAPY WITH FEIBA FOR THE INTRACRANIAL HEMORRHAGES IN HAEMOPHILIA PATIENTS WITH INHIBITORS

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To date, the intracranial hemorrhage (IH), such as subaracnoideal bleeds or intracerebral hematomas, in haemophiliacs with inhibitor to FVIII remain of great concern. The treatment is very difficult if we consider that the hemostatic agents should across haemato-encephalic barrier and achieve a local effective haemostasis to stop bleeding. We report our observation regarding IH episodes (n=18) in haemophiliacs (n=5) with inhibitors (2 pts with long standing inhibitors, 2 pts high responders and 1 pt low responder) all of them treated with FEIBA during 1980-2004. Five IH episodes were late diagnosed owing to small symptoms; 13 IH were promptly suspected on the basis of the increasing clinical findings. Computered tomography (CT) scan documented 12 intracranial hematomas and 6 vaste subaracnoideal hemorrhages. FEIBA was administered in bolus modality at mean dosage of 50 IU/kg three times a day for three day. Thereafter the drug was administered twice a day for two weeks. On the fifth day CT scan pictures showed a remarkable reduction of the intracranial hematomas or subaracnoideal hemorrhages. No subjective symptoms were also seen. Generally, no later than one month, according to CT scan findings, FEIBA was infused until the complete recovery of the IH, at the same dosage once a day (from fifteenth day). During this period in all patients the FVIII inhibitor levels unchanged. A slight activation of the coagulation was documented by standard plasma measurements of FPA, Prothrombin F1+2, D-dimer. No adverse event occurred by FEIBA therapy. In our opinion, FEIBA may be considered as first line successful and safe therapy for intracranial hemorrhages in haemophilia A patients with inhibitor. In addition, by using the rediscovered thromboelastography technology, we safely can monitor the '*in vivo*' effect of the treatment in order to further optimizing and individualizing the therapy with FEIBA (Varady K. *et al.,* Haemophilia 10:17-21, 2004, suppl. 2).

P060

SUBCUTANEOUS CONCENTRATED DESMOPRESSIN IS VERY EFFECTIVE FOR TREATMENT OF MENOMETRORRHAGIAS IN WOMEN WITHOUT INHERITED OR ACQUIRED BLEEDING DISORDERS

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Even if inherited and acquired disorders of coagulation and haemostasis are considered in the differential diagnosis of menorrhagia and abnormal uterine bleeding(ACOG Committee Opinion 263:1185,2001),often the medical treatment options have a profound impact on the health and quality of life of larger number of women(Philipp CS. J Thromb Haemost 1:477,2003)without any documented coagulopathy.In this regard,prostaglandins synthetase inhibitors(nonsteroidal antiinflammatory drugs),hormones(oral contraceptives), danazol as well as antifibrinolytics(tranexamic acid) are commonly employed.But they often fail or limit clearly long-term use. So,transfusion therapy is inevitably adopted to correct an acute menometrorrhagia in these subjects. Recently,wide experience has demonstrated that the subcutaneous infusion of the synthetic analogue desmopressin is an effective therapy for menstrual bleedings in women with von Willebrand disease (VWD) as well as in many patients with platelet functional dysfunction (PFD) (Mannucci PM. Blood 90:2515,1997). We here report that subcutaneous desmopressin (EMOSINT, Sclavo, Italy) is an effective treatment for menometrorrhagies among adolescent girls of reproductive age and in peri-menopausal women without congenital (or acquired) coagulation abnormalities. In 62 healthy women (22 teenagers and adults 40,aged 42-54 yrs), previously treated with different therapeutical compounds to control their menstrual bleedings,even transfused for consequent severe anaemia, EMOSINT Sclavo (0.3 µg/kg)was employed in occasion of hypermenorrhagic episodes (n=108). Basal plasma prothrombin time,activated thromboplastin time, fibrinogen and thrombin time showed normal values.Bleeding time(Ivy method),platelet count,ristocetin-induced platelet aggregation and platelet

responses to several inducers were in normal average. Lupus anticoagulant was negative. Moderate hyperfibrinolytic status was also present. In our preliminary experiences(n=19)carried out during in-hospital, factor VIII-complex related activities, as expected, were increased (2-3 fold)after subcutaneous desmopressin infusion. Mild face flushing,with or without headache, was referred from 60% of subjects.No other side-effects were reported.Afterwards,all women were required to self-evaluate for menorrhagia recording the number of pads used to that of previous untreated hypermenorrhea and days of the menstruation during home treatments.According to all women,in 85% of cases the clinical response to desmopressin was scored as effective(20%)or very effective(65%).The reduction in pad consumption was remarkable in 95 treated menstrual bleeding.The patient's compliance was good.From these observation,we suggest that EMOSINT may represent a novel therapeutical mean to treat the menometrorrhagias of healthy women.

P061

SICKLE CELL DISEASE, REDUCED ENDOTHELIAL NITRIC OXIDE SYNTHETASE AND TOTAL NITRIC OXIDASE SERUM LEVELS, ABNORMAL HUMAN ENDOTHELIN-1 RELEASE, VASCULAR ENDOTHELIUM SUFFERING AND THROMBOEMBOLIC RISK

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Cumulated evidences have showed that a thrombophilic state is present in sickle cell disease (SCD) with microvascular occlusive events leading to painful crisis. Although the precise pathophysiology of vaso-occlusive complications is still understood only incompletely in SCD, several investigations have addressed the possibility that abnormal activation of sickled erytrocytes, platelets, white cells and endothelial cells (EC) with the complex perturbations of plasma protein components and soluble receptors might cause micro- (and macro-) vascular occlusions and multiorgan damage (Lubin BH, NEJM 1997; 27:1623). Keeping in mind that expression of the endothelial nitric oxide synthetase (eNOS) occurs in response to factors as shear stress, chronic ipoxia, inflammation and atherosclerosis, we investigated its circulating amount in SCD, during the steady state and sickling (ELISA method, R&D Systems). Parallely, nitric oxide (NO), produced by the eNOS in EC, which functions as a vasodilator thereby regulating blood flow and pressure was also measured (ELISA method, R&D Systems). In this scenario, plasma endothelin-1 (ET-1), a vasoconstrictive peptide produced by EC which is involved in long-term changes of the endothelium in response to repeated sickling stimuli was assayed (R&D Systems). 16 SCD patients (9 females and 7 males, age ranging 20-63 yrs, δ+ thalassemia/S trait, 7 previously splenectomized, without renal nor liver dysfunctions) both in steady state and during painful episodes (n=26) were studied. 13 healthy subjects, sex and age comparable, served as controls. Our data confirm that in SCD patients a chronic endothelium suffering is present also in steady state. In this regard, in SCD patients the VWF was highest as well as an increased ET-1 release was observed, thus indicating EC chronic damage. Therefore, the observed impaired NO production or signaling could enhance platelet adhesion/aggregation via a reduced eNOS activation in SCD. In our opinion, we suggest that a spectrum of abnormal EC responses together with chronic endothelium suffering/damage might contribute to the impaired microcirculatory reperfusion with increased *in vivo* thrombin generation leading to district 'no-reflow' phenomena and thromboembolic complications often observed during the episodic punctuation of SCD.

P062

PROPOSAL OF IMMUNOTOLERANCE INDUCTION IN HAEMOPHILIACS A WITH INHIBITORS UNDERGOING TO PRESSING ELECTIVE SURGERY BY USING FVIII/VWF-ENRICHED PURE PLASMADERIVATES

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To date, an urgent elective surgery in haemophiliacs A (HA) with inhibitor even in those low responders or with long-standing inhibitor (LSI), not undergone in the past to immunotolerance treatment (ITT), remains a big concern. In fact, even if widely advised from physicians is often refused. In our opinion, ITT may be really performed in expectation of a pressing elective surgery. On the basis of our previous positive ITT result in an adolescent HA with LSI-high responder type treated with pure FVIII/VWF-enriched plasma concentrate (Fanhdi, Grifols, Italy), we here report a further success of ITT in an adult severe haemophiliac (43 yrs old), with low responder inhibitor (4.7 UB/mL, lasting 15 yrs)(HCV+ and HIV-) and recurrent hemartroses, undergoing to urgent elective surgery. In August 2003 the patient experienced frequent painful colics for congenital retention of the right testicle in the abdomen. The computered tomography confirmed his pathology. The video-laparoscopic laser-surgery was successfully performed by bolus infusion of Fanhdi (8.000 UI, Grifols, Italy). During the post-operative period the concentrate was infused (2.000 UL, twice/day) for one week. No bleeding was observed and he was discharged after 5 days. The patient enthusiastically continued ITT (Fanhdi, 8.000 UI/week) at his home for 6 months. The inhibitor progressively sloped and the *in vivo* recovery (IVR) was normal (>6 h) 15 days after the operation. At present, the inhibitor remains negative; the IVR is normal. No hemartroses have been seen in these last 16 months; he actually gained his articular motions and remains on therapy with the same concentrate (4.000 UI/week) to have a normal quality of his life. On the basis of recent reports regarding the favourable achievement of ITT by using plasma FVIII/VWF-enriched pure derivates, we suggest that ITT induction with these products can be considered in haemophiliacs with inhibitor undergoing to an urgent elective surgery.

P063

EVIDENCES FOR VASCULAR ENDOTHELIUM DAMAGE/PROLIFERATION AND ABNORMAL NEOANGIOGENESIS BY ENDOTHELIAL GROWTH FACTOR PLATELET DERIVED GROWTH FACTOR, INTERLEUKIN-6 INCREASED LEVELS IN SICKLE CELL DISEASE

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Abnormal circulating endothelial cells (EC)(Samuel O Sowemino-Coker *et al.,* American J Hematology 31; 1989), as well as vascular endothelium chronic damage with abnormal cytokines release (Musso R. *et al.,* 6th Intern. Conference on Thalassemia and Hemoglobinopathies, Malta, 1997) have previously been reported in sickle cell disease (SCD). We here report that vascular endothelial growth factor (VEGF), the most important growth and survival factor for endothelium, is increased in SCD, thus suggesting an important role in the regulation of the vasculogenesis. We further carry evidence that human platelet derived growth factor AB (PDGF), the major mitogenic factor, is also raised in the serum of SCD patients. 18 SCD patients (10 females and 9 males, age ranging 21-60 yrs, β+ thalassemia/S trait, 7 previously splenectomized, without renal nor liver dysfunctions), both in steady state and during painful episodes (n=19) were studied. 12 healthy subjects, sex and age comparable, served as controls. Human VEGF by ELISA method (R&D Systems), VWF (Dade Behring) as index of EC function, human PDGF-FAB (ELISA, R&D Systems), as indicator of platelet release and of chemotaxis for neutrophils and fibroblast in conjunction with Interleukin-6 (IL-6), well known cytokine-mediator of coagulation process, were assayed. Our results showed that raised VEGF and IL-6 circulating levels were present in SCD even in the steady state. Human PDGF-FAB, VWF and D-dimer were parallely elevated (p<0.001, vs controls). A further significant increase of these parameters was seen during the painful crisis in all patients (p<0.001 vs baseline values). Among the several effects induced by VEGF, it is reported that this cytokine increases micro-vascular permeability, vasodilatation, EC proliferation and cell migration, abnormal platelet release with elevated platelet count and influences the neo-angiogenesis and vasculogenesis. VEGF also promotes extravasation of plasma fibrinogen, leading to fibrin deposition and promotes the migration of macrophages and fibroblasts and EC proliferation. By considering that abnormal plasma levels of VWF and PDGF with elevated D-dimer, in conjunction with raised amounts of IL-6, were observed in SCD, we suggest that a proneness to increased thrombogenic risk is constantly present. In addition, a multifactorial antithrombotic therapy is warranted in these patients.

P064

INHIBITION OF INCREASED SENSITIVITY OF PLATELETS TO PLATELET-ACTIVATING FACTOR BY ISOSORBIDE DINITRATE IN SICKLE CELL DISEASE

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Previously we reported that *in vitro* platelet-activating factor (PAF) at subcritical concentration induces a marked increase of platelet aggregation (PA) in sickle cell disease (SCD) (Musso R. *et al.,* Thromb Haemost 1991, 65 (6):1108). In this disease, the continous injury of vascular endothelium and/or chronic red blood cells lysis determine abnormal PAF release which in turn may contribute to the platelet hyperaggregation constantly observed *in vivo*. Isosorbide dinitrate (ISDN), one of the most commonly used nitrate vasodilators, has been shown to possess direct antiplatelet properties restricted to ADP and ionophore A 23187-induced platelet PA. The pourpose of our study was to verify if ISDN was active at low concentrations and if these effects were restricted to PAF-acether induced PA. Citrated platelet rich plasma (PRP) was obtained from 11 SCD patients (δ+ thalassemia/S trait), 6 females and 5 males, age ranging 17-57 yrs, both during painful episodes (n=21) and at least 6 weeks since last sickling crisis. Twelve healthy volunteers of comparable sex and age were the controls. PA was performed by using a computerized four channels aggregometer (Aggrecoder PA 3220, Menarini) in presence of threshold concentration of PAF-acether (C 18:0, Biochemia). Our results showed the PAF-induced PA was significantly inhibited (p<0.001, n=11) in a dose dependent manner from 78% inhibition at 100 μM ISDN to 26% inhibition at 0.1 μM ISDN when normal PRP was assayed. When SCD platelets in steady state were tested the dose-response curve ranged from 50% inhibition at 100 μM ISDN to 11% inhibition at 0.1 μM ISDN, while PAF-induced PA was inhibited in a dose manner from 36% inhibition at 100 μM ISDN to 4% inhibition at 0.1 μM ISDN (p<0.001) during painful episodes (n=21). From our results it emerges that SCD platelts more sensitive to PAF are less inhibited by ISDN than the normal ones. Anyhow ISDN may also account for '*in vivo*' antiplatelet activity. In our opinion, ISDN may contribute to its beneficial effects on sickling manifestations. Therefore, the potential therapeutical role of ISDN in SCD is hopefully warranted.

P065

ANTI-PHOSPHOLIPID ANTIBODY SYNDROME MANIFESTED BY SEVERE IDIOPATHIC THROMBOCYTOPENIC PURPURA AND RECURRENT ACUTE MYOCARDIAL INFARCTION

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Background. APS is a clinical condition characterized by thrombosis and/ or pregnancy loss and persistence of lupus anticoagulant (LA) and/ or anti-cardiolipin antibodies. Mild and asymptomatic thrombocytopenia with ITP findings, rarely requiring medical treatment, is a frequent aspect of the disease. However, caution is suggested in its treatment in consideration of the thrombotic risk that some treatments used for ITP, such as splenectomy but also i.v. high dose immunoglobulins (HDIg), could produce in APS. Here we describe the case of a young woman who developed three acute MI (AMI) during remission of severe thrombocytopenia attributed to ITP and whose course revealed the existence of APS. CASE REPORT: A 37 y.o. woman

was admitted on October 7th, 2001 to Niguarda Ca' Granda Hospital, Milan, because of petechiae, spontaneous ecchymoses and severe thrombocytopenia (platelet count 5 x 10^9/ L). Her family history was positive for MI; she was a heavy smoker. She referred a fetal loss at the 16th week of gestation (1983), cutaneous discoid lupus (1992), oral contraception (from September 2000), and transitory pains in the joints, oral aphthous ulcers and Raynaud's phenomenon (August 2001). A diagnosis of ITP was made and she was treated with steroids (methylprednisolone 5 mg/Kg/ day i.v. for 4 days, followed by prednisone 0.4 mg/ Kg/ day orally tapering) and i.v. HDIg (400 mg/Kg/ day for 5 days) with complete remission (platelet count 145x10^9/ L). In November 2001 she experienced angina-like chest pains. ECG and enzymes were consistent with inferior AMI. A percutaneous transluminal coronary angioplasty (PTCA) with stenting of the right coronary artery was carried out. Laboratory tests showed heterozygous G20210A FII mutation and mild hyperhomocysteinemia (12.7 umol/ L); platelet count 333x10^9/ L. She was treated and discharged with atenolol, enalapril, clopidogrel for a month, and acetylsalicylic acid (ASA) 100 mg. In January 2002 the laboratory data were consistent with LA diagnosis; no IgG and IgM anti-cardiolipin antibodies. ASA was substituted with oral anticoagulant therapy (OAT) by warfarin, maintained in spite of the absence of LA in two successive controls and platelet counts between 60 and 70x10^9/ L. On February 15th 2003 the patient was admitted to our department because of spontaneous ecchymoses and menorrhagia. Laboratory data: severe thrombocytopenia (platelets 4 x 10^9/ L), normocytic anaemia (Hb 10.6 g/ dL with MCV 84 ft), iron and folate deficiency and PT and APTT ratios 1.9 and 1.28 respectively. Warfarin was discontinued. ITP treatment: steroids (methylprednisolone 1 mg/ Kg/ day i.v. for 4 days, then prednisone 0.8 mg/ Kg/ day orally) and i.v. HDIg (400 mg/ kg/ day for 5 days) with normalisation of the platelet count. Laboratory tests for infective and autoimmune diseases were negative. Prednisone was progressively tapered and discontinued on May 15th. On July 23rd 2003, with ITP still in remission (platelet count 215x10^9/ L), the patient experienced a new constrictive thoracic pain. ECG evidenced a 1-2 mm ST segmental depression in D2, D3 and aVF derivation with minimal alterations of cardiac enzymes (troponin T 1.91 ng/ mL). Coronarography documented an in-stent neointimal proliferation with a critical stenosis in a right ventricular branch of the right coronary artery. Therapy: oral atenolol (50 mg), clopidogrel (75 mg), ASA (100 mg), folate (5mg), and transdermic glyceryl trinitrate. Three months later, the presence of LA was confirmed in two subsequent determinations at six weeks apart. Clopidogrel was stopped and substituted with warfarin, associated with ASA. In the summer of 2004, while ITP persisted in remission, the thoracic pains reappeared, and on October 10th, 2004 she experienced an intense thoracic pain with increased values of myocardial necrosis enzymes. After voluntary discharge from another hospital casualty department, she underwent, on December 2004, a new coronarography with transluminal angioplasty and three medicated stenting of the right coronary artery and the descending anterior branch of the left coronary artery. So, after temporary suspension of OAT, she was discharged with a treatment including oral atenolol (50 mg), double anti-platelet therapy by ticlopidine (250 mg twice a day) for 6 months and ASA (100 mg) indefinitely, plus OAT by warfarin. At the time of writing (March 11th 2004), the patient is without symptoms. DISCUSSION: The case here reported is that of a young woman with severe and recurrent thrombocytopenia, who, after remission of ITP, experienced two acute MI before a definitive diagnosis of APS. A third AMI occurred while ITP resulted in remission for more than a year and she was receiving anti-platelet therapy with ASA associated with OAT. Considering that thrombosis is a multifactorial event, the unusual recurrence of AMI in this APS patient can be attributed to the concomitant presence of other risk factors for thrombosis, like smoking, G20210A mutation in the prothrombin (FII) gene, contraception and mild hyperhomocysteinemia. However, important roles on the development and recurrence of AMI were also played by: (i) bad compliance of the patient, (ii) ITP overtreatment, (iii) underestimated importance of anti-platelet treatment required by coronary stenting, and (iv) uncertainties in APS diagnosis and treatment.

P066

PREVALENCE AND CLINICAL SIGNIFICANCE OF IGG AND IGM ANTI-PROTEIN S AND ANTI-ANNEXIN V: RETROSPECTIVE AND PROSPECTIVE ANALYSIS OF THE WAPS STUDY

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Anti-protein S (aPS) and anti-annexin V (aAV) antibodies belong to the family of antiphospholipid antibodies (aPL). Since their prevalence and clinical significance in the antiphospholipid syndrome (APS) has not yet been defined, we analysed the association of aPS and aAV antibodies with thrombosis in a group of patients whose serum samples were available at enrolment in the WAPS (Warfarin in the Anti-Phospholipid Syndrome) Study. They were 112 patients, 24 males and 88 females, aged 23-81 (median, 42) years. Ninety-one were lupus anticoagulants (LA)-positive. Thirty-two suffered from autoimmune disease, and 87 from APS. Eighty-one had a history of arterial and/or venous thrombosis, and 17 women had suffered from one or more abortions. During a median follow-up time of 4 years, 15 patients experienced arterial or venous thrombosis. IgG and IgM antibodies were measured by prototype ELISA's kindly provided by Diagnostica Stago. Values were expressed in mOD and grouped by tertiles. Odds Ratio with 95% Confidence Intervals and p values were calculated by contingency tables. Range and (median) values were: aAV-G, 63-387 (105) mOD; aAV-M, 47-1526 (106) mOD; aPS-G, 43-214 (93) mOD; aPS-M, 53-1462 (97) mOD. The following associations with clinical events occurring prior to registration were found: high vs low tertile of aAV-G and abortions (p=0.02); high vs low tertile of aPS-M and abortions (p=0.05). High vs low tertile of aPS-M were also marginally associated with thrombosis occurring during follow-up (p=0.0577). In conclusion, these data suggest that the measurement of aAV and aPS antibodies (both G and M iso-

types) may be useful in aPL-positive women with recurrent abortions. Whether their presence represent a risk factor for thrombosis remains to be established.

P067

THROMBOTIC THROMBOCYTOPENIC PURPURA (MOSCHOWITZ DISEASE) IN OVARIAN CANCER, A CASE REPORT

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Thrombotic Thrombocytopenic purpura (TTP), first described by Moschowitz in 1924, is defined by the classic pentad of fever, microangiopathic hemolytic anemia (MAHA), thrombocytopenia and renal and neurological impairment. While most cases are held to be idiopathic, its association with malignancy is long well recognized, but infrequent. A about 5% of neoplastic patients can develop TTP, and it being a sign of disseminated disease Neoplastic patients have been noted to have a deficiency of von Willebrand factor (VWF) cleaving protease, ADAMTS-13. The very low levels of this protease are said to be similar to those found in patients with idipatic thrombotic thrombocytopenic purpura (ITTP). Recent observations showed that patients with disseminated tumors had lower mean plasma levels of ADAMTS-13 than those with localized tumors. However, in no patient was the level of ADAMTS-13 below 18% of normal. This is a risk factor to develop a thrombotic microangiopathy. We report a patient with the association of TTP displayed as the first manifestation of a ovarian cancer. Our patient, a 63-year old female with no previous significant pathologic event or contact with drugs, presented (November 2003) with a 1-week history of abdomen-radiated lumbar pain, anorexia and sweats. The diagnosis of TTP was made based on thrombocytopenia and microhemangiopathic hemolytic anemia characterized by an elevated LDH and the presence of schistocytes on the peripheral blood smear. A CT scan of abdomen showed an ovarian neoplasm. The patient were treated with plasma exchange (PE), using fresh frozen plasma and cryoprecipitate poor plasma as replacement fluid, without result. In consideration of the progressive clinical worsening, the patient was transferred in surgical unit.. Platelets count restoring after the ovariectomy, and the value was normal during the six cycles of carboplatin chemotherapy. A surgical "second look" (July 2004) showed an absence of metastatic (nodal or extranodal) disease. In February 2005, the patient showed a relapse of hemorrhagic purpura, without evidence of metastatic lesions in whole body CT scan. Also this time, plasma exchange and fresh frozen plasma therapy was ineffective. The patients died for renal and hepatic failure. The post-mortem evaluation showed diffuse nodal metastasis in lomboaortic lymphonodes In conclusion, the finding of TTP compels a search for an occult neoplastic disease (or relapse), especially when associated with ineffective standard therapy

Infections I

P068

EFFICACY OF CASPOFUNGIN AND FILGRASTIM AS FIRST LINE THERAPY OF PULMONARY INVASIVE FUNGAL INFECTIONS IN NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Invasive Fungal Infections (IFI) remain a severe and major complications among patients with hematologic diseases, but recently new agents (echinocandins and new azoles) have become available, improving the chance of cure. Caspofungin (Cancidas®-Merck) is a large lipopeptide molecule able to inhibit the enzyme complex 1,3-D-glucan synthetase; this action specifically damages the fungal cell wall. Caspofungin (CAS) is active, in vitro and *in vivo*, against most Candida species and Aspergillus species. Herein we report our experience with this drug as a first-line therapy for pulmonary proven or probable IFI in neutropenic patients with hematologic malignances. Thirty-four consecutive patients (pts) have been treated with CAS (28 acute leukemias, 3 lymphomas, 2 chronic leukemias and 1 myeloma): 21 males and 13 females with a median age of 51 yrs (range 22-72). 17/34 (50%) pts had a relapsed or resistant hematologic disease, while 12 pts were in complete remission and 5 pts were at onset of disease; 9/34 (26%) developed IFI after a Transplant (BMT) procedure. Out of 34 pts, 9 (26%) had proven pulmonary IFI (2 Candidiasis and 7 Aspergillosis) and 25 (74%) had a probable IFI (defined according to international consensus), all 34 cases with pulmonary localization. 31/34 (91%) pts had less than 1000 granulocytes/µL at onset of infection. CAS was given at the dose of 70 mg on day 1, followed by 50 mg/daily. Median duration of CAS therapy was 20 days (range 8-72); 31/31 neutropenic pts (100%) received also G-CSF (Filgrastim). The overall response rate was 59% (20/34) with 13/20 complete responses, 7/20 partial responses; 2/34 pts had a stable disease. Twelve out of 34 pts (35%) did not respond and six of them (50%) died for mycotic infection. Univariate analysis showed that granulocytes recovery (>500/µL vs <500/µL) and status of hematologic disease (remission/onset vs refractory/relapsed) were significantly associated to favourable outcomeof IFI. No adverse clinical effects were reported and only a grade I°-II° transient increase of alkaline phosphatase and/or transaminases occurred in 4/34 (12%) pts. After CAS therapy six non-responders and 6 pts with a partial or stable response were rescued with voriconazole. Two out of 6 pts (33%) in the former group and 6/6 (100%) in the latter obtained a complete resolution of IFI. Our experience suggests a good efficacy of CAS in combination with G-CSF, as first-line treatment of proven or probable IFI with lung localization. The drug was well tolerated and there were no significant hepatic adverse events even in pts receiving CAS with cyclo-

sporin after a BMT. A significant proportion of non-responders or partial responders to CAS plus G-CSF can be rescued with a subsequent voriconazole-based therapy.

P069

INCIDENCE AND OUTCOME OF EARLY AND LATE INFECTION COMPLICATIONS AFTER SPLENECTOMY IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Inytoduction. In this retrospective study we revised the data regarding 50 patients (pts) with Idiopathic Thrombocytopenic Purpura (ITP) unresponsive to steroids or other conventional therapies who underwent laparoscopic (LS) or open (OS) splenectomy. This analysis has been made in order to evaluate the incidence of early and late infection complications after splenectomy for ITP.

Patients and Methods. From June 1990 to June 2001, 289 cases of ITP were diagnosed at our Department and in 17% of them (50/289) a splenectomy has been performed; 29 out of 50 pts were female and 21 males with 27 years as median age at diagnosis; the median age at splenectomy was 34 years (range 14-85). All pts were relapsed or refractory to first linee therapy that was standard or HD of steroids in the majority of cases (steroids alone 30/50, steroids/immunosopressive therapy 2/50, steroids plus immunoglobulins 15/50, other in 3/50). The median platelet count before splenectomy was 30.000/mmc (range 6-416.000). 31/50 (62%) pts had received anti pneumococcus and anti meningococcus vaccination. Definition of Response after Splenectomy. Complete Remission (CR) was defined as platelet count >150.000/mmc lasting for 3 months or longer without treatment, Partial Response (PR) as platelet count between 50 to 150.000/mmc for the same period of time.

Results. 39/50 (78%) of pts received a LS while 11/50 (22%) an OS. LS was completed in 37/39 (95%) pts with a conversion rate (OS) of 5%. Operative and postoperative complications occurred in four (8%) pts; no deaths were attributed to the procedure. A response (CR plus PR) was obtained in 48/50 (96%) of pts, only 2/50 (4%) were not responders (PLT count after splenectomy lower than 50.000/mmc). Of responsive pts 30/48 (62%) were in continuous CR after a mean follow up of 64 mths and 6/48 (13%) were in PR after a median follow-up of 79 mths; 12/48 pts (25%) relapsed after a mean disease free of 13 mths. The OS at 73 mths was 97% and the probability of Relapse free survival at 48 mths was 71%. The probability of CR at 96 mths was 52%. Early infections complications (within the first 60 days from surgery) occured in 4/50 (8%) pts (1 HZ, 2 pneumonia, 1 abdominal abscess) and a FUO occured in 3/50 (6%) of cases; 5/7 of these pts are older than 50 yrs, 6/7 had received a prolonged immunosuppressive therapy and 5/7 pts had been vaccined before surgery. The mortality infection related was 0%. No significant late infection complications have been recorded.

Conclusions. 1)Splenectomy is a safe and effective option for ITP unresponsive to steroids or other therapies. 2)The early infection complications can be possible in a small number of cases (less than 15%) with a favourable outcome. 3)We have not seen significant late infection complications and we think that largest studies are necessary to revised the role of vaccination before splenectomy for ITP and in order to evaluate if the vaccination could be reserved for a subset of cases (older pts or those with longer immunosuppressive therapy or concomitant diseases).

P070

PLATELIA ASPERGILLUS TEST IN THE DIAGNOSIS OF ASPERGILLUS SPECIES INFECTION : A 4-YEAR SINGLE CENTER EXPERIENCE

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Background. early diagnosis of Invasive Aspergillosis (IA) has been shown to improve the antifungal treatment outcome in immunocompromised hosts. We report here the single-institution experience of sequential serum Galactomannan (GM) monitoring associated with weekly high-resolution TC Scan (HRTC) in IA diagnosis and treatment outcome.

Materials and methods. from January 2000 through December 2003, sequential, twice weekly GM monitoring by Platelia Aspergillus tes (Bio-Rad®) associated with chest HRTC, performed once weekly upon fever onset, or increase of GM optical density (OD) index, were prospectively and consecutively performed in the following categories of immunocompromised hosts: 1) acute leukemia (AL) induction/consolidation treatment, 2) autologous stem cell transplantation (autoSCT), 3) reduced intensity conditioning allogeneic stem cell transplantation (RIC alloSCT), 4) Fludarabine or Cladribrine based regimens for chronic lymphocytic leukemia (CLL) and Hairy Cell Leukemia (HCL), 5) salvage treatment for Hodgkin's lymphoma (HD) or non Hodgkin's lymphoma (NHL), 6) immunosuppressive treatment for aplastic anemia/ myelodisplastic syndrome (AA/MDS). We performed 2480 Platelia Aspergillus tests for 161 patients, accounting for 260 consecutive treatment episodes. Treatment episode was defined as hospital admission for AL treatments (95), autoSCT (80), RIC alloSCT (35), miscellaneous treatments (Misc) in AA/MDS, CLL, HD, NHL and HCL (50). The mean number of tests/episode was 15.18 (range 5-32). Two consecutive positive tests were required for a positive value. The cut-off value of GM OD index for a positive test decreased over the years from 1 to 0.5. False-positive Platelia Aspergillus test results related to semi-synthetic penicillin were ruled out. Episodes were classified according to the EORTC/MSG criteria in 11 proven IA (4.2%), 30 probable IA (11.5%), 27 possible IA (10.4%), 192 not IA (73.8%). Results : the categories based on clinical risk were as follows : 11 proven IA in 2 RIC alloSCT, 6 AL, 3 Misc(1 NHL patient, 1 MM patient and 1 MDS patient); 30 probable IA in 12 RIC alloSCT, 13 AL, 2 autoSCT, 3 Misc (3

relapsed/refractory HD patients); 27 possible IA in 2 RIC alloSCT, 15 AL, 2 autoSCT, 8 Misc (1 HCL patient, 1 HD patient, 3 CLL patients, 2 MDS patients, 1 refractory MM patient). Antifungal treatment was given in proven and probable IA episodes. In 22 out of 27 possible IA episodes antifungal treatment was administered mostly because of clinical failure of broad spectrum empiric antibiotic treatment. In 27 patients with an episode of possible IA, only 2 proven IA were subsequently recorded. Lethality of IA among RIC alloSCT recipients was 76%; no evidence of IA was found among patients undergoing autoSCT for a chemosensitive disease or during first line phase of treatment. Sensitivity, specificity,efficacy, positive and negative predictive value of Platelia Aspergillus test for proven plus probable IA cases were determined using 1, 0.7, and 0.5 cut-off value, respectively. Sensitivity, specificity, efficacy, positive predictive value and negative predictive value for GM OD index 1 were 0.46 [0,31; 0,62],98] , 0.99 [0,96; 1,00], 0.90 [0,85; 0,93], 0.90 [0,68; 0,98] , 0.90 [0,85; 0,93] , respectively. Sensitivity, specificity, efficacy, positive predictive value and negative predictive value for GM OD index 0.7 were 0.68 [0,52; 0,81], 0.96 [0,92; 0,98] , 0.91 [0,86; 0,94], 0.78 [0,60; 0,89] , 0.93[0,89; 0,96] , respectively. Sensitivity, specificity, efficacy, negative predictive value and positive predictive value for GM OD index 0.5 were 0.71 [0,54; 0,83], 0.87 [0,81; 0,91],0.84 [0,78; 0,88], 0.54 [0,40; 0,67] , 0.93 [0,88; 0,96], respectively. Results are depicted in Table 1.

Table 1.

GM OD index cut-off value of 0.7 was better than the other values in terms of specificity, efficiency, positive and negative predictive value, respectively; as proven by the ROC curve (Figure 1).

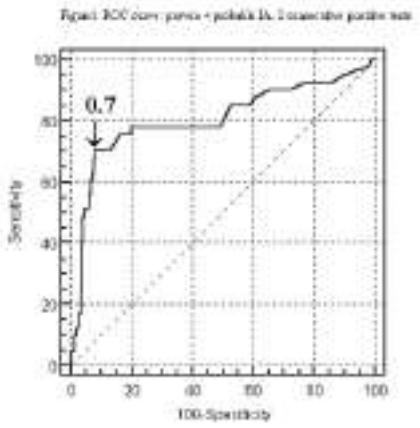

Figure 1.

Conclusions. 1) the risk stratification of the patients based on diagnosis and risk treatment is feasible; 2) AL patients and RIC alloSCT recipients are at high risk of IA; 3) patients a with a possible IA generally do not develop proven IA during further episodes and therefore should not be given antifungal treatment; 4) chemoresponsive MM and LNH patients treated with high dose chemotherapy at diagnosis are not at risk of IA; 5) relapsed/refractory HD patients and advanced CLL patients older than 60 years treated with fludarabine-based regimen seem to be at low-intermediate risk of IA (generally as a terminal event); 6) GM OD index cut-off value of 0.7 is the best cut-off value.

P071

NOSOCOMIAL INFECTIONS IN ACUTE LEUKEMIA:COMPARISON BETWEEN YOUNG AND ELDERLY PATIENTS

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Background and objective. The progressive decline in immune functions render elderly individuals more susceptible to infections than the young. To evaluate potential differences between young (<60yr) and elderly (>60 yr) acute leukemia patients (pts), febrile episodes during antineoplastic regimen were reviewed.

Methods. Febrile episodes were classified according to established criteria (EORTC). In case of isolation of skin saprophytes, 2 positive blood cultures were required. Fungal infections were defined according to IFICG-EORTC-MSG criteria. Results. From 2001 to 2003, 128 febrile episodes occurred in 196 pts (65%): 63 in 95 young pts (66%), mean 45 yr and 65 in 101 elderly pts (64%), mean 75yr. The number of febrile episodes was 161: 90 in < 60 yr, 71 in > 60 yr , occurring respectively in 45% and in 50% during induction treatment, in 32% and in 13% during consolidation, in 23% and in 37% during relapse (p 0.001). Febrile episodes during severe neutropenia (ANC < 100/l) occurred in 47% of < 60 yr pts and in 18% of > 60 yr pts (p 0.017). CVC was present in 68% and in 42% respectively (p 0.001). Microbiologically documented

infections were recorded in 53% and in 44% of febrile episodes, clinically documented infections (mainly pneumonia) in 12% and in 21%, FUO in 35% and in 35% respectively (p ns). Gram-positive cocci were isolated in 78% and in 68% of bloodstream infections, particularly staphilococci coagulase negative (66% and 68%), frequently responsible for CVC related infections due to strain slime producer; gram-negative in 17% and in 29%, fungi in 5% and 3% (p ns). Death directly attributable to infection occurred in 17% and in 23% respectively (p ns); no significant difference was documented in death-responsible organisms (mainly gram-negative bacilli and fungi). Conclusion.Elderly patients do not seem to be more susceptible to infections than young patients; type of febrile episodes, pattern of nosocomial infections and mortality are similar, although the lower frequency of risk factors such as severe neutropenia and CVC must be considered.

P072

PROVEN AND PROBABLE ASPERGILLOSIS IN PATIENTS WITH ACUTE LEUKEMIA AND INFLUENCE OF HOSPITAL RENOVATION IN A HAEMETOLOGIC UNIT

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Background and objective. Invasive infections from Aspergillus fumigatus and other Aspergillus species are a major threat to leukemia patients with cytotoxic therapy-induced neutropenia. Moreover, hospital outbreaks of invasive aspergillosis have been associated with the renovation and construction of buildings. To evaluate the effect of Aspergillus airborne contamination during hospital renovation adjacent to our hematologic unit, we conducted a strict program of fungal environmental surveillance. *Methods.* From November 2003 to January 2005 , two haematology wards were monitored in order to make both a qualitative and quantitative evaluation of fungal burden in the air. Air samples were taken from the hospital rooms of patients, in the corridors of their wards and outside the building (260 total air samples).All severe neutropenic patients with acute leukemia were allocated in multiple-bed rooms with restricted access to visitors and without HEPA filtration.The diagnosis of proven or probable fungal infections was based on clinical, radiographic and microbiological data, according to established IFICG-EORTC-MSG criteria.

Results. Total fungal concentration resulted higher outside, lower in the corridors and even lower in the rooms. The Aspergillus genus was isolated in about 40% of air samples, especially from the corridor between the two haematology wards. Aspergillus fumigatus was isolated in about 16% of air samples and its concentration was very low in neutropenic patients' rooms (from 0 to 0,16 CFU/m3), higher in the corridors and outside of the hospital building (from 0 to 3,13 CFU/m^3).During this period, 2 proven cases of aspergillosis (Aspergillus fumigatus) and 4 probable cases of fungal pneumonia in 97 examined patients (6%) were documented . In a previous clinical surveillance, performed in 2000-2002 period in the same patient population (100 patients) and in absence of building construction work, we documented only two possible fungal pneumonias (2%). Conclusions. Although A.fumigatus has not been isolated frequently,construction work did increase the rates of Aspergillus colonization in the hospital and adjacent wards and may contribute to development of invasive infection. A standardized protocol of aerobiological survey is useful to facilitate prevention of nosocomial aspergillosis.

P073

INCIDENCE OF CYTOMEGALOVIRUS INFECTION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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This study was performed to evaluate incidence, risk factors and outcome of CMV infection in stem cell autologous transplantation (ASCT) with the aim to perform preemptive therapy in patients with antigenemia. Starting from 2001, 171 consecutive ASCTs were performed in 136 patients; 102 of them were seropositive at the onset of haematological disease. In all these patients a CMV pp65 antigenemia assay was determined weekly, starting from the day when absolute neutrophil count raised over 500/µL, and until day 60 after ASCT; subsequently, antigenemia was determined only when a CMV infection was suspected. Forty among the 136 transplanted patients (29.4%) presented a positive antigenemia; all of them were seropositive for CMV before ASCT; no cases of primary infection were seen. The incidence of CMV infection in seropositive population was 40/102 (39.3%) 6 patients (5 with multiple myeloma and one with non-Hodgkin's lymphoma) who received two ASCT, developed CMV infections after both transplantations, so that positive antigenemia developed after 46/171 (26.9%) transplantations. First positive antigenemia presented a median of 32 days (range 7-57) after stem cell reinfusion. The median antigenemia level at the first appearance was 2/200.000 (range 1 –1000) Clinical picture: Enteritis was present in 5 patients; 2 of them had also fever, one of them had also thrombocytopenia. In 5 patients fever without any other clinical signs or symptoms was present; 30 patients were asymptomatic. Prognostic factors: when the two groups (patients with or without CMV infection) are compared, no significant difference in other parameters was present: particularly no difference was present in age, sex, number of infused stem cells. Therapy Therapy was immediately begun when at least one of the following was present: a) antigenemia > 30 cells/200.000; b) known or suspected disease; c) increase of antigenemia levels; d) fever, pancytopenia, liver or kidney function tests alterations, if not due to different cause. In the other cases antigenemia was determined after 3 days and then weekly until disappearance or therapy. Overall 14 patients were treated with anti CMV drugs, while in 26 asymptomatic patients antigenemia became negative without therapy. Six patients were treated only with ganciclovir (GCV) at doses of 5 mg/kg every 12 hours for two weeks; in the third and fourth week, GCV was administered at a

dose of 5 mg/kg once a day for five days a week in 4 patients while two patients received oral valganciclovir (900 mg x 2/day); 5 patients received oral valganciclovir from the beginning and for four weeks. 3 patients were treated with cidofovir (CDV); they received a first dose of 5 mg/kg, followed by four doses of 3 mg/kg at weeks 2,4,6,8. All patients received probenecid with the following schedule: 4 cps four hours before the infusion, 2 cps after 2 hours and 2 cps after 8 hours from the end of infusion. Concomitant fluid load with 2 liters was given. CMV reactivation was successfully treated in all patient and no patient died for CMV disease.

P077

INCIDENCE OF INVASIVE ASPERGILLOSIS IN ALLOGENEIC STEM CELL TRANSPLANTATION PATIENTS : AN ITALIAN PROSPECTIC MULTICENTER STUDY

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The incidence of post-engraftment invasive aspergillosis (IA) in allogeneic stem cell transplant (ASCT) recipients increased during the 1990s. Recently, GVHD and lymphopenia have been reported as risk factors of IA after engraftment. Between January 2003 and May 2004, every consecutive patient admitted for HSCT in four institutions was monitored for circulating galactomannan (GM) with Platelia Aspergillus assay (Bio-Rad, Marnes-La-Coquette, France) twice weekly from admittance until day 100, and then once weekly until day 365 if the patient was on treatment with cyclosporin A only. In case of occurrence of GVHD requiring treatment after day 100, serial screening with Platelia Aspergillus assay was still performed twice weekly. The cut-off value of GM optical density (OD) index used to define positivity was 0.7. Following fever occurence or GM optical density index increase, screening with weekly high resolution chest computed tomography (CT) scan was started. Diagnosis of IA was made according to the EORTC/MSG criteria. Eighty six patients accounting for 91 ASCT were analyzed, and 126 entered by now in the present study. Clinical characteristics of the patients are reported in Table 1.

We observed 10 cases of probable IA and 3 cases of possible IA. IA occurred before day 40 in 3 patients (median, day 13 after ASCT), between day 40 and day 180 in 8 cases (median, day 109 after ASCT), and after day 180 in 2 patients. Among the probable IA group of patients, 7/10 had GVHD, 6/10 were on treatment with steroids, and 3/10 had fever. Two consecutive positive Platelia Aspergillus test results were observed in the sera of 6/10 patients, GM was detected only in BAL in 3/10 cases and 1 patient had GM detected only in a central nervous system (SNC) fluid sample.

Table 1.

Clinical characteristics of patients	Total
Patients	86
Disease	
Multiple Myeloma	27 (31,4%)
Acute Myeloid Leukemia	13 (15,1%)
Non-Hodgkin's Lymphoma	9 (10,5%)
Myelodisplastic syndrome	6 (6,9%)
Hodgkin's Disease	7 (8,1%)
Chronic Myeloid Leukemia	6 (7%)
Chronic Lymphatic Leukemia	6 (7%)
Acute Lymphoblastic Leukemia	5 (5,8%)
Chronic Myelo-Monocitic Leukemia	3 (3,5%)
Aplastic anemia	1 (1,2%)
Renal cell carcinoma	1 (1,2%)
Mean age years (range)	49 (19-70)
ASCT	91*
HLA-identical RIC	62 (68,1%)
MUD RIC	6 (6,6%)
HLA-identical conventional	16 (18,6%)
Syngeneic conventional	2 (2,2%)
MUD conventional	5 (5,5%)
MUD conventional (UCB)	1 (1,1%)
Stem cell source	
PBSC	81 (89%)
Bone marrow	9 (10%)
UCB	1 (1%)
Follow up days	
Median	200
Min	25
Max	668
GVHD	
Acute	63/91 (69%)
Chronic	39/89 (37%)
Chronic extensive	17/39 (44%)
CMV Reactivation	44/91 (48%)
Mortality	25/86 (29%)

Chest CT scan was diagnostic in 8/10 cases (halo sign or air crescent sign), in 2 patient CT scan revealed aspecific signs and in another patient CNS RMN scan showed lesions consistent with IA. 100% of patients were lymphopenic at the time of IA diagnosis with an absolute count <450/mm^3. Only 1 patient was neutropenic. Three patients out of 10 had CMV reactivation during the episode of IA and 4/10 experienced CMV reactivation in the 100 days preceding IA diagnosis. Overall incidence of IA at day 200 was 14,3%, and the probability of developing IA reached 22% at 1year after aSCT. The cumulative incidence of IA in the RIC group was 11,7%. Eight patients out of 13 died of IA, with a median survival of 19 days (range 1-83). The 1-year survival in the IA group was 16% and 69%in the non-IA group (Figure 1).

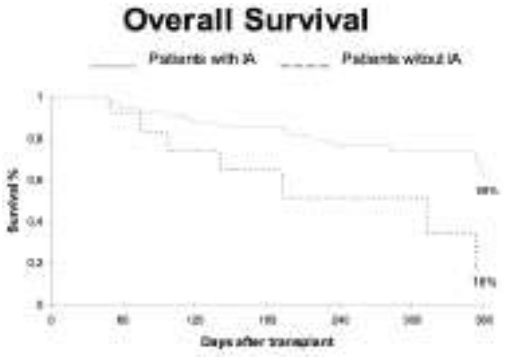

Figure 1.
The bimodal incidence distribution of IA after ASCT was confirmed, 23% of IA cases were diagnosed early (< day 40) after ASCT, 62% were diagnosed between day 40 and day 180, and an additional 15% developed later after

day 180. This report appears to confirm the role of GVHD and lymphopenia as important risk factors during the late post-transplantation periods. The relatively high incidence of IA could be a consequence of increase of IA in the recent years and of diagnostic improvement (CT+GM). We suggest that the use of serial screening for circulating GM with Platelia Aspergillus assay should be continued after day 100 especially in patients lymphopenic or with GVHD. There is a clear need for new diagnostic and therapeutic stragies for HSCT patients as IA accounts still for an unacceptable high portion of non-relapse-related deaths.

P075

SYSTEMIC NECROTIZING LYMPHADENITIS, EVANS SYNDROME AND NODAL T-CELL NON-HODGKIN LYMPHOMA IN EBV AND CMV CO-INFECTION

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Background and introduction. The role of EBV in B- or T-cell lymphoma-genesis is known and can be observed also in immune defects out of post-transplant setting. Evans syndrome or other autoimmune disorders like ITP or AIHA associated with EBV and/ or CMV infections have been described. Here we describe the case of an elderly patient with EBV and CMV co-infection who developed diffuse necrotizing lymphadenitis, Evans syndrome and nodal T-cell non-Hodgkin lymphoma subsequently.

Case Description. A 78-year-old woman came to our attention on August 26th, 2002, because of severe Coombs positive anaemia (Hb 6.1 g/dL) and thrombocytopenia (platelet count from 47 to $20x10^9$/L). Her clinical history revealed: arthroplasty of the right knee because of osteoarthritis, some years before, and hospitalisation, a month before, in another hospital because of fever and systemic lymphadenitis, attributed to EBV and CMV co-infection. Laboratory tests, carried out during this hospitalisation, revealed that IgM anti-EBV and -CMV were positive, and CMV antigen and necrotizing lymphadenitis were respectively present in circulating leucocytes and at a cervical lymph node biopsy. After spontaneous remission of symptoms and discharge from this hospital, she referred, on August 24th, 2002, intense asthenia and dispnea. After packet red cell transfusions, carried out in another hospital because of severe and isolated anaemia (Hb 5.3 g/dL), she was admitted, two days later to our hospital, where a diagnosis of Evans syndrome was made. So, she received therapy including packet red cell transfusions, i.v. steroid and HDIg. During hospitalisation, IgM anti-EBV and anti-CMV were still present, while IgG and IgM anti-B19 parvovirus presented respectively a titre of 1/ 64 and 1/18. Confirmed CMV infection through presence of Pp65 antigen (in 14 cells) and PCR in bone marrow cells and Pp65 (in 50 cells) and P72 (in 40 cells) antigens in peripheral leucocytes, steroid was tapered and the patient was treated with i.v. infusion of ganciclovir. On October 25th, 2002, remission of Evans syndrome and disappearance of Pp65 antigen in peripheral leucocytes were obtained and the patient was discharged. Meantime, a bone marrow biopsy revealed only high eosinophil number and increased reticulin formation, and an attempt at axillary lymph node biopsy, carried out because of transitory lymph node enlargement, was unsuccessful (fat tissue). Three months later (January 8, 2003), while Evans syndrome persisted in remission, the patient was readmitted to our hospital because of appearance of inguinal lymphadenopaty. CT scan confirmed the systemic nature of lymphadenopaty, while an inguinal lymph node biopsy revealed the existence of diffuse large-medium size T-cell (CD 20-, CD79a-, CD3+ , CD4-, CD8+, CD68-, CD138-, MUM1-, CD56-, Bcl6-, ALK-, MIB 30%) non-Hodgkin lymphoma associated with EBV presence (EBER) in some B-cells. Bone marrow biopsy showed a nodular infiltration by T-lymphoma (CD3+, CD8+ medium size cells). Meantime, IgM anti-CMV were negative and Pp65 CMV antigen was absent in circulating leucocytes, while IgM anti-EBV persisted positive, and IgG and IgM anti-B19 parvovirus presented respectively a titre of 1/ 512 and 1/ 32. The patient was discharged again with a therapeutic program including monthly treatment of oral prednisone and cyclophosphamide. However, after two cycles of this therapy, she was readmitted in our hospital because of septic shock caused by Staphilococcus aureus right knee prosthesis infection associated with reactivation of CMV infection caused by severe post-chemotherapy leucopenia. In spite of adequate treatment, cachexia syndrome developed and she died on June 27th, 2003.

Considerations. We speculate on the etiological role produced by EBV on the development of necrotizing lymphadenitis, Evans syndrome and T-cell lymphoma. Patient ageing, CMV coexistence, and Evans syndrome steroid treatment, in our opinion, contributed subsequently. CMV and EBV co-infection impairing, through diffuse necrotizing lymphadenitis, the already compromised immune system of the elderly, can have favoured the appearance of Evans syndrome. The consequent steroid treatment of autoimmune disease, worsening the immune defect, contributed to the lymphoma-genesis that can characterise EBV infection. The same B19 parvovirus infection, observed during the development of T-cell lymphoma, can be considered as an epiphenomenon of the severe immune defect produced by EBV and CMV co-infection, and Evans syndrome steroid treatment in this elderly patient.

P076

FATAL REACTIVATED HEPATITIS B INFECTION IN A PATIENT DURING RITUXIMAB MAINTENANCE FOR NON HODGKIN'S LYMPHOMA

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In HbsAg positive patients reactivation of hepatitis B virus is a well documented complication of cytotoxic therapy, but in literature it is described in few case reports after rituximab therapy as single agent. Actually there isn't a significant increased incidence of opportunistic infections during rituximab treatment. Here we report a case of a 47 year old man who on October 2001 was diagnosed as follicular lymphoma grade 2 according to WHO classification. At diagnosis his disease was stage II. Since 1981 he had been

HbsAg positivity, without clinical and biological liver abnormalities. Before treatment serum analyses showed: HbsAg positive, HbsAb negative, HbcAb positive, HbeAg negative, HbeAb positive, HBV-DNA quantitative negative, liver and coagulation tests normal. He received chemotherapy according to CEOP protocol for 6 cycles every 21 days, obtaining a partial response. On September 2002 for disease progression he started FCR (fludarabine, cyclophosphamide, rituximab) regimen for 4 courses every 21 days. He had a complete remission and a good compliance to therapy, his liver and coagulation tests were always normal. At the end of chemotherapy HBV-DNA quantitative was negative. The patient was eligible for maintenance therapy with monthly Rituximab at 375 mg/m^2 as single agent. After 7 doses his hepatic tests were : AST 80 UI/l, ALT 167 UI/l, ALP 196 UI/L, Bilirubine 0.42 mg/dL, HBV-DNA quantitative 1.200.000 copies/mL and HCV- RNA negative. Rituximab therapy was stopped and lamivudine administration was begun. Nevertheless antiviral treatment he developed progressive severe hepatic failure and after 4 months he died.

Conclusions. We have shown that after 11 months from the end of chemotherapy and after 8 months from the start of a monthly maintenance with rituximab as single agent an Hepatitis B reactivation could happen later. We suppose that the reactivation depended on a cumulative toxicity that occurred only after rituximab multiple doses without any previous pathological evidences. We suggest that there is direct relationship between the drug cumulative dose and the immunological derangement. It is likely that the immunological damage was so severe that the antiviral therapy with lamivudine couldn't control the infection. Therefore rituximab should be used cautiously in HbsAg positive patients and the prophylaxis with lamivudine should be offered not only before the start of chemotherapy, but also before the beginning of any exclusive immunotherapy.

P077

ACUTE FULMINANT HEPATITIS B VIRUS REACTIVATION IN HBSAB POSITIVE PATIENT RECEIVING RITUXIMAB

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Acute fulminant hepatitis B in HBsAg positive patient during or after cytotoxic therapy is not uncommon, but it is really uncommon in HBbAb positive receiving rituximab as single anti-cancer drug. On February 2000 a 51 year old man was diagnosed as B Cell Chronic Lymphocytic Leukemia (CLL) stage II according to Rai classification with multiple lymphadenopaties and abdominal bulk. At presentation he was: HBsAg negative, HBsAb positive, HBcAb positive, HBeAg negative, HBeAb positive, anti HCV negative, liver and coagulation tests normal. He received different regimen of chemotherapy (CEOP, Fludarabine, ProMace/CytaBom) until February 2001 because of refractory disease. On April 2003 for a new disease progression he received Rituximab at 375 mg/m^2 for two doses every week and later started FCR regimen for 4 courses. He obtained a good control of disease with poor related toxicity then the patient was eligible for another consolidation therapy with Rituximab. After 4 course of immunotherapy was detected a moderate deterioration of liver tests (AST: 36 UI/L, ALT 57 UI/l, ALP:156 UI/L, Bilirubine 0.50 mg/dL) and reduced serum HBsAb titer. Unfortunately he developed a quickly progressive liver failure for B virus reactivation and died because of fulminant hepatitis. It was demonstrated HBsAg positive, HBsAb negative, HBcAb negative, HBeAg positive, HBeAb negative.

Conclusions. Here we have reported an unusual case of HBV reactivation under rituximab in a HBsAg negative, HBsAb positive and HBcAb positive patient. Even if theorycally we could consider this patient as cured for HBV infection, he developed a fulminant HBV hepatitis under rituximab. In literature few similar cases of HBV reactivation are described early from the start of rituximab, while in this report it happened about ten months later from the end of chemotherapy and after total ten doses of rituximab. We suppose that rituximab reduces the antibody titer for HBV thus inducing an immunological impairment which leads to HBV reactivation. Therefore we suggest that rituximab should be used cautiously also in HbsAb positive patients and for this reason the prophylaxis with lamivudine should probably be offered them also during and after treatment.

P078

LINEZOLID AS POTENTIATION OF ANTI-GRAM+ BACTERIAL INFECTION THERAPY

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In the last decade important changes in the etiology of the nosocomial infections have been recorded with the increase of resistant bacteria (methycillin-resistant Staphylococcus, Enterococci etc.) and a corrispondent decrease of the sensitive pathogens (E.Coli, Proteus Mirabilis, Klebsiella Pneumonia etc.). Moreover, Gram+ bacteria have become the main cause of the nosocomial infections. Gram+ bacteria often behave a multiresistant phenotype that confer them a reduced sensitivioty or a total resistance to glycopeptides. In details, Staphilococcus aureus often displays a reduced sensitivity to vancomycin and Enterococcus faecium is resistant to vancomycin and, sometimes, to teicoplanin. Linezolid, derived from oxalidones antibiotic family, is a new antibacterial agent not correlated to any known antibacterial agent. Linezolid does not show cross-resistance with other antibiotics with a reduced occurrence of *in vivo* resistance. Linezolid has a wide anti-bacterial activity agains the main gram + bacteria (Stafilococci, Streptococci, Pneumococci, methycillin-resistant Staphylococcus, penicillin-resistant Pneumococci and glycopeptide-resistant Enterococci) with a 100% per os bioavailability comparable to the parenteral administration. We have administred Linezolid to eight patients with low/intermediate risk acute myeloid leukemia when they developped

febrile neutropenia lasting 10 days. In all the patients standard prophylactic treatment with cyprofloxacin (1 g) and fluconazole (100 mg) was performed before the beginning of the induction chemotherapy accordino to the GIMEMA protochol. At the beginning of the neutropenia ceftriaxone and amikacyn were administred according to the EORTC 97 protochol. However, the fever was not solved at day 5 and therefore, in absence of cultures positive for Gram+, teicoplanin was added and fluconazole was increased at the dose 400 mg x 2/day iv. At day 10 of febrile neutropenia the patients did not show any respiratory syndrome and chest Rx was negative for fungal infections, but blood culture was positive for staphylococcus aureus. Linezolid administration was begun at the dose of 600 mg x 2/day at the day 10. The treatment was well tolerated and the fever disappeared after 4-5 days from the beginning of linezolid administration in all the patients. Linezolid was continued per os for three days as out-patient maintainance therapy. Therefore, on the basis of its clinical activity we suggest the use of Linezolid as an important therapeutic option in the treatment of nosocomial infections by Gram+ Staphyloccoccus, Streptococcus or Pneumococcus.

P079

INFECTIOUS MORBIDITY OF PATIENTS WITH MULTIPLE MYELOMA RECEIVING THALIDOMIDE COMBINED WITH CHEMOTHERAPY

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No studies have specifically focused on the infectious complications occurring in patients with multiple myeloma (MM) treated with thalidomide and chemotherapy. We have explored the incidence, the type and the outcome of infections in 78 MM patients (45 male and 33 female) treated in the outpatients setting with the combination thalidomide 100 mg/day (continous), dexamethasone 40 mg days 1-4 and 9-12 and pegylated liposomal doxorubicin 40 mg/sqm on day 1 (ThaDD) within the prospective, multicentric study. This combination was given every 4 weeks for 4-6 cycles to 37 newly diagnosed MM patients older than 65 years and to 41 relapsed/refractory patients. Median age was 71 years (range 41-82) and 51% of patients were older than 70 years. All patients received antimicrobial prophylaxis with ciprofloxacin and no patients had central venous catheter. A febrile event occurred in 30/306 (10%) courses of ThaDD regimen and it resulted higher than grade 2 WHO in 14 episodes (4.5%). Overall, the median duration of fever was 5 days (range 1-18). Pneumonia accounted for 50% of infections and the radiologic patterns for pulmonary infiltrates detected on chest-x ray were consolidative (60%) or interstitial (40%). Twelve episodes (40%) were classified as FUO and only one patient developed a septic shock due to E. Coli. The last two cases experienced ocular abscess and tracheobronchitis, respectively. Only in 2 episodes (6%) neutropenia was present at onset of infection while hypotension/shock were documented in 4 cases (13%) and respiratory failure in 8 (27%). Most infections (80%) occurred during the first 3 courses of treatment; however, only one patient discontinued therapy because of infections and no deaths were related to them. In 13 episodes (4%) hospitalization was required and the most commonly used empiric antibiotics were cephalosporins (53%) and quinolones (46%). One patient who developed retinitis and interstitial pneumonia with respiratory failure received foscarnet in the suspicion of viral infection. Out of all parameters analised as potential risk factors, related to patient and to disease, only male gender was statistically associated with the development of infection (p=0.027). In conclusion, ThaDD regimen can be managed as an ambulatory basis in most patients. However, despite the normal neutrophyls count in nearly all patients, they can develop life-threatening infection, mainly pneumonia. It is likely that in MM patients treated with chemotherapy associated with steroids and thalidomide, apart from neutropenia and other clinical parameters, biological factors related to the activity of these combinations on the immune system could predispose to infections. Further studies need to improve knowledge about immunomodulatory effect of these combinations.

P080

SUCCESSFUL SURGICAL TREATMENT OF FUNGAL SINUSITIS IN ONCO-HEMATOLOGICAL PATIENTS

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Invasive fungal rhinosinusitis (IFR) is one of the major causes of morbidity and mortality in immunocompromised patients, the most frequent causative agents of which are Aspergillus, Mucoraceae, and Fusarium. In particular, zygomycosis has been increasingly reported in onco-hematological patients, in whom rhino-orbital-cerebral involvement leading to rapid cerebral necrosis is frequently lethal even if appropriately treated. Five onco-hematological patients affected by IFR documented by computed tomography and microbiological tests (Aspergillus, Fusarium and Mucoraceae) were treated by means of surgical debridement of the paranasal sinuses (and orbital cavity when required) plus systemically and locally administered antimycotic drugs. At the time of the onset of IFR, two were suffering from severe aplastic anemia, two from acute myeloid leukemia (one undergoing induction chemotherapy, the other after an allogenic bone marrow transplantation), and one from acute lymphoblastic leukemia in complete remission treated with imatinib. A combination of amphotericin B (deoxycholate or liposomal) and voriconazole was given in three cases. Local therapy with amphotericin B deoxycholate and saline solution was administered by means of external drainages until the complete resolution of IFR. Although all of the patients

were deeply immunosuppressed and three were in aplastic phase, no adverse events were observed during the follow-up. The IFR completely reresolved in all cases and the patients completed their scheduled chemotherapy or immunosuppressive treatment; three underwent a second chemotherapy course. Three of the five patients are still alive; neither of the deaths was attributable to the recurrence of IFR. On the basis of these data, we conclude that the combination of surgical debridement of the paranasal sinuses (and orbital cavity when required) plus systemically and locally administered amphotericin B is feasible for the treatment of IFR in immunocompromised patients, including those in aplastic phase. It also allows the delivery of immunosuppressive or cytotoxic therapy without IFR relapse.

P081

RETROSPECTIVE ANALYSIS OF CYTOMEGALOVIRUS INFECTION FOLLOWING NONMYLOABLATIVE COMPARATED WITH MYLOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION

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Human cytomegalovirus (HCMV) infection is the most frequent infectious complication after allogeneic hematopoietic stem cell transplantation. Preliminary data suggest a faster immune recovery following non-myeloablative stem cell transplantation (NST), but the real impact on post-transplant infectious complications remains unknown. We retrospectively analysed the incidence of HCMV infection in 40 patients following reduced intensity allogeneic stem cell transplantation. Results were compared with 186 patients receiving myeloablative transplants. We monitored weekly both groups of patients for HCMV antigenemia; the median follow up was 13 months (range 2-29) in non myloablative setting and 18 months (range 2-127) in myeloablative setting; we excluded patients who were seronegative before transplant and had a seronegative donor, in which we did not ever find HCMV infection or disease. The incidence of HCMV infection following reduce intensity transplant was 58% slightly higher than in myeloablative transplant (48%), but this difference was not statistically significant. Median age in patients receiving NST was higher than in patients receiving myloablative transplant (56 versus 41 years) (p=0.02); the median number of $CD34^+$ cells and $CD3^+$ cells infused in non myeloablative setting were $5.5x10^6$/kg and $18.2x10^7$/kg respectively, both higher then in myloablative setting ($2.7x10^6$/kg and $13.0x10^7$/kg, respectively); anyway this differences were not statistically significant. Recurrent CMV infections were observed in both cohort of patients, with a similar incidence in myeloablative transplant and NST (30% and 26% respectively). The median time between NST and infection was longer than in the myloablative setting: 90 days (range 30-365) vs 66 days (range 25-630) (p=n.s.). The association between graft-versus-host disease (GVHD) and HCMV infection was stronger in patients undergoing myloablative transplant than NST (80% vs 60%; p=0.02). In non myloablative group none of the patients died for infection nor developed CMV disease; the incidence of HCMV disease was 13% in the myloablative setting. In conclusion our data showed a trend towards an increased incidence of HCMV infection following NST, with most cases occurring after day 100; nonetheless, the incidence of HCMV disease is lower in NST patients. The risk of CMV reactivation seems to be more influenced by the immunosuppressive agents administered and preparative regimen than GVHD.

P082

PSEUDOMONAS AERUGINOSA OUTBREAK CONTROL WITH THE USE OF STERILE FILTER IN A HAEMATOLOGICAL CARE UNIT

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Pseudomonas aeruginosa (Pa) infections are relevant cause of morbidity and mortality in severe neutropenic onco-haematologic patients. Any hygienic and sanitary measure to avoid such infections is essential to warrant as much as possible the safety of intensive chemotherapy programs. Approximately 30% of febrile/infectious that occur in neutropenic onco-haematological patients is sustained by or associated with a bacteremia, mostly of nosocomial origin. In our ward we observed a significant increase of Pa bacteremias during the first half of 2002 (40 positive specimens, as compared to the 20 ones observed during the whole previous year. To investigate this issue we performed an accurate microbiological sampling (85 samples) from different potential sources: cleaning solutions (4 samples), aria (11), handle of bath doors (6), WC siphon water (6), WC board (6), flush buttons (6), water taps (17), shower head (6) and siphons (23). Twenty-nine out of fourty-six specimens obtained from water taps, shower head and siphons turned out to be positive for Pa (17 multisensitive and 12 resistant to Ciprofloxacin and Gentamicine). These results prompted us to take the following measures: 1) weekly surveillance cultures, including pharyngeal and rectal swabs in high risk patients 2) Use of tap water after running the tap for five minutes at least 3) Use of weekly replaced sterile filters (PALL, Acquasafe) in all taps and showers. These procedures resulted in a significant decrease of Pa bacteremias (19 cases in the second half of 2002; 4 cases in 2003 and 9 in 2004). Moreover we observed a significant reduction in Pa- positive surveillance cultures: 9/169 pharyngeal swabs and 8/166 rectal swabs were positive for Pa along the four months preceding the onset of anti-Pa measures, as against 2/570 pharyngeal swabs and 5/563 rectal swabs positiva for Pa along the following 9 months (p=0.0001 and 0.0008, respectively). Weekly replacement of steryle filters resulted in an increase of annual costs of 34.500 Euro. On the other hand, this additional cost was counterbalanced by the reduced morbidity for Pa, which resulted in a lower cost for antibiotics and shorter time of hospitalization.

P083

FUNGAL INFECTIONS IN HEMATOLOGICAL MALIGNANCIES: SEIFEM-2004 STUDY (SORVEGLIANZA EPIDEMIOLOGICA INFEZIONI FUNGINE NELLE EMOPATIE MALIGNE

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Background/Aims. To evaluate the incidence and the outcome of fungal infections in patients affected by hematological malignancies (HM) and admitted in Italian centres. *Methods.* A retrospective study, conducted over 1999-2003, in HM patients, admitted in 18 hematology divisions in tertiary cares or university hospitals, who developed fungal infections.

Results. Our population included 11,802 patients: 3,012 with AML (25.5%), 1,173 with ALL (9.9%), 596 with CML (5%), 1,104 with CLL (9.4%), 1,616 with MM (13.7%), 3,457 with NHL (29.3%), 844 with HL (7.2%). Patients who underwent autologous or allogenic HSCT were included in a specific different analysis. A proven or probable fungal infection occurred in 538 patients, with an incidence of 4.6%; in particular we registered 346 episodes sustained by moulds (2.9%) and 193 by yeasts (1.6%). The incidence rate depends upon underlying malignancy (12.3% in AML, 6.5% in LLA, 2.7% in CML, 0.6% in CLL, 0.5% in MM, 1.6% in NHL, 0.9% in HL). Among moulds, the detected etiological agents were Aspergillus spp (310 episodes, 2.6%), Mucorales spp (14 episodes, 0.1%), Fusarium spp (15 episodes, 0.1%), and other rare fungi (7 episodes, 0.1%). Among yeasts we registered only septicemia sustained by Candida spp (175 patients, incidence 1.4%). Other yeast infections were caused by Cryptococcus spp (8 pts, incidence 0.1%), Tricosporon spp (7 pts, 0.1%) and other rare agents (2 pts). As for aspergillosis, the identification of the specific subtype of agent was possible only in the 108 cases (35%); A. fumigatus was identified in cases (15%), A. flavus in (12%), A. terreus in (5%), A. niger in (2%). It is worth noting that the number of infections caused by A. flavus increased from 1999 (5 pts, 8.8% of the total cases of aspergillosis registered during the year) to 2003 (14 pts, 18.4%); relative risk was about 2.10 (IC95% 0.8-5.49; *p*-value 0.117). Conversely all other subtypes showed a stable incidence. The letality rate registered in the population was about 39%, with differences between aspergillosis (42%) and candidemia (33%). In particular the letality due to aspergillosis ranged from 40% in 1999 to 45% in 2003 without significant variation (RR 1.11; IC95% 0.74-1.66; *p*-value 0.613), as well as the letality in patients affected by candidemia not significantly increased from 30% in 1999 to 37.5% in 2003 (RR 1.25; IC95% 0.67-2.32; p-value 0.478).

Summary/conclusions. Our study confirms the general trend already described for hematological patients: infections due to moulds continue to be more frequent than those caused by yeast. Among all fungi, Aspergillus spp remains the main etiologic agent. AML represents the most frequently involved cathegory. The mortality rate is actually about 40%, with a remarkable decrease when compared to past years.

P083a

EPIDEMIOLOGY OF FUNGAL INFECTIONS IN HEMATOLOGICAL STEM CELLS TRANSPLANTED PATIENTS: SEIFEM 2004 STUDY

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Background/Aims. To evaluate the epidemiology and the outcome of fungal complications in patients who underwent autologous or allogeneic hemopoietic stem cells transplantation (HSCT) and admitted in multiple Italian centres.

Methods. A retrospective study, conducted over 1999-2003, in bone marrow transplant recipients, admitted in 13 hematology divisions in tertiary cares or university hospitals, who developed fungal infections.

Results. we evaluated 4,139 patients who underwent HSCT: 1,505 (36.4%) allogeneic and 2,634 (63.6%) autologous transplant recipients (TR). A fungal infection occurred in 78 patients, with an incidence of 1.9%; in particular we registered 59 episodes sustained by moulds (incidence 1.4%) and 19 by yeasts (incidence 0.5%). The incidence rate depends upon the type of transplant (3.8% in allogenic HSCT, 0.8% in autologous HSCT). Among moulds, the detected specific etiological agents were Aspergillus spp in 58 episodes. Among yeasts, we registered 19 episodes caused by Candida spp. As for aspergillosis, the identification of the specific subtype was possible only in the 31% of cases; A.fumigatus was identified in the 6 cases (10.4%), A. flavus in 2 (3.4%), A. terreus in 7 (12%), A.niger in 3 (5.2%). The mortality registered in our population was about 64%, with differences between allogenic TR (56%) and autologous TR (7.7%). The etiologic agent also influenced the patients outcome: the mean mortality rate due to Aspergillus spp was about 69% (78% in allogenic TR and 12.5% in autologous TR), while that one due to Candida spp was about 47.4% (66% in allogenic TR and 38.5% in autologous TR).

Summary/Conclusions. Among HSCT recipients, fungal infections represent a common complication, in particular in patients undergoing allogenic transplantation. Aspergillus spp is the most frequent agent detected in our

population. The mortality rate due to fungal infection was higher in allogenic TR than in the autologous one. Patients affected by aspergillosis have a poor outcome more frequently than those affected by candidemia.

P084

EPIDEMIOLOGY OF PATHOGENS ISOLATED FROM HAEMATOLOGICAL PATIENTS: INFLUENCE OF ANTIBIOTIC PROPHYLAXIS. A SINGLE INSTITUTION'S EXPERIENCE

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Background. An increasing number of antibiotic-resistant germ strains seem to be the result of prophylaxis with fluoroquinolones (Fq) in neutropenic haematological patients.

Aim, Patients and Methods. To clarify the possible role of prophylaxis in inducing resistance, we investigated the epidemiology and the susceptibility to antibiotics of different bacterial pathogens in 325 patients admitted to our Institution during a period of seven months (June-December 2004). Prophylaxis with levofloxacin was administered to patients with >7 days expected neutropenia; a breakthrough infection was defined as emerging during a broad-spectrum antibiotic therapy. Results. An infective cause was detected in 79 of 132 episodes of fever (59.8%). It was microbiologically documented in 59 cases (44.7%); the incidence of Gram+ and Gram- was similar (31/59, 52.5% and 29/59, 49.1% respectively). In 8/59 cases a mycosis as infective cause was detected. Twenty-nine/59 cases (49.2%) were on prophylaxis with levofloxacin. There were 9/59 (15.3%) breakthrough infections. Patients on prophylaxis had a non significant frequency reduction of G+ and G- infections (12/29 vs 12/21 and 13/29 vs 13/21 respectively). Ten mixed infections were observed: 5 among patients out of prophylaxis (26.8%), 4 as breakthrough infections (44.4%), only 1 in the group on prophylaxis (3.4%). The more frequently isolated pathogens were E. coli (16/59, 27.1%), S. aureus (11/59, 18.6%) and coagulase-negative staphylococci (10/59, 16.9%); less frequently Enterobacteriaceae other than E. coli (7/59, 11.9%), Enterococcus spp (5/59, 8.5%), Pseudomonas spp (4/59, 6.8%). E. coli was similarly detected in the two groups with or without prophylaxis (10/29 [34.5%] vs 6/21 [28.6%]), whereas S. aureus was less frequent among patients on prophylaxis (1/29 [3.4%] vs 5/21 [23.8%]). In 5/9 (55.6%) cases a breakthrough S. aureus infection was observed during adequate antibiotic therapy for febrile neutropenia. Coagulase-negative staphylococci were not significantly different in the two groups (5/29 [17.2%] vs 2/21 [9.5%]). Enterobacteriaceae other than E. coli (3 Klebsiella spp, 2 E. cloacae, 2 S. marcescens) were reduced among patients on prophylaxis (1/29 [3.4%] vs 5/21 [23.8%]), whereas Enterococci were increased (4/29 [13.8%] vs 1/21 [4.8%]). Bacteraemia was present in 23/29 (79.3%) cases on prophylaxis and in 11/21 (52.3%) without prophylaxis (p=0.067). All E. coli bacteraemias were in the prophylaxis group. Resistance of E. coli to Fq was observed in 14/19 cases (73.7%): 2/6 out of prophylaxis (community-acquired infection) and 12/13 on prophylaxis (p=0.017). All E. coli strains revealed susceptibility to ceftriaxone and amikacin, the first-line antibiotic therapy adopted in our Institute. No resistance to Fq was observed among Enterobacteriaceae other than E. coli. Conclusions. Prophylaxis with levofloxacin does not appear to modify frequency and distribution of G+ and G- infections. It shows a trend in reducing S. aureus and Enterobacteriaceae infections, but may predispose to enterococcal infections. It seems to be associated with an increased incidence of bacteraemia with respect to other forms of infection, particularly E. coli bacteraemia. The widespread use of Fq selects a subset of patients colonized with resistant E. coli strains.

Acute Lymphoblastic Leukemia

P085

FLOW CYTOMETRY TO IDENTIFY RESIDUAL LEUKEMIC CELLS IN ADULT B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA

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Flow cytometry is a powerful tool to assess the persistence of minimal residual disease (MRD) in childhood B-lineage acute lymphoblastic leukemia (ALL). On the other hand, the value of this method in adult ALL is still debated. We have used a multi-parametric flow cytometry approach to assess the frequency of some leukemia-associated immunophenotypes (LAIP) and the sensitivity of flow cytometry in detecting residual leukemic cells. Then, we have evaluated the stability of LAIP in leukemic cells after co-culture with a BM mesenchymal stem cell (MSC) monolayer. Finally, we have estimated the prognostic value of immunophenotypic MRD detection in 10 adult patients. We have analyzed 20 normal or regenerating bone marrow (BM) samples to quantify the expression by normal CD19+ B cell precursors of some antigens (CD34, CD10, CD38, CD45, CD58, CD22, CD21, CD13, CD33, CD15, CD65, CD66c, CD56, NG2, TdT). We have identified the normal distribution of B cell precursors inside the BM mononuclear cells (MNC) and we have drawn some "normality templates"; by them, we have compared the expression of the same markers by leukemic cells and thus identified the presence of LAIP. The sensitivity of immunophenotypic MRD detection has been tested by scalar dilutions of leukemic blasts with normal BM-MNC. BM blasts obtained from 5 cases of adult B-lineage ALL with suitable LAIP, have been diluted with MNC from healthy BM donors (ratio 1:10) and co-cultured with a MSC monolayer. After 14 and 21 days of culture, immunophenotypic analysis has been carried out to verify the stability of LAIP. Finally, from January 2003 to January 2005, we performed the immunophenotypic MRD monitoring in 10 adult B-lineage ALL patients and we correlated the flow-cytometric analysis with the clinical outcome. Of 64 patients analyzed, 61 (95.3%) had at least one LAIP. Of them, 26 (40.6%) had only one marker suitable for MRD monitoring; the remaining 35 cases (54.7%) had at least two LAIP. We could identify at least 1/1,000 leukemic cells (10-3 sensitivity) in all the cases and 1/10,000 leukemic cells (10-4 sensitivity) in 72% of cases. However, just considering the LAIP with over- or down-expression of normal markers (such as CD38 or CD45), the frequency of false negative at 10-4 dilution was only 11%, as compared to 44% with LAIP based on aberrant antigens (such as CD13 or CD33). The LAIP were stable, as they were still detectable at the end of the co-culture of blasts with MSC. Clinical follow-up: 4/10 patients are in complete remission (CR) without MRD. Four patients have relapsed following positive MRD controls. Two patients are in CR with a positive MRD detection (median follow-up: 39 weeks, range 23- 91). Immunophenotypic MRD detection can be considered a potential tool to detect residual leukemic cells in adult B-lineage ALL, with the same efficiency observed in childhood B-lineage ALL. However, a bigger number of patients is necessary to better assess the prognostic value of immunophenotypic MRD detection.

P086

CD1D EXPRESSION ON B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA SUBSETS WITH POOR PROGNOSIS

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Acute Lymphoblastic Leukemia (ALL) is the most frequent malignancy of childhood. Although therapeutical advances have been achieved, some ALL subgroups still fare poorly. CD1d is a monomorphic molecule that provides a suitable target for immuno-therapy in view of the characterization of a glycolipid, alpha-galactosylceramide (alpha -GalCer), capable of being presented to CD1d-restricted T cells with cytotoxic potential. We investigated CD1d expression in 80 pediatric B-cell precursor (BCP) ALL cases defined according to immunophenotype, cytogenetic features and age at onset. CD1d was detected on ALL cells in 15% of the patients. CD1d+ ALLs were significantly associated with infant leukemia, pro-B phenotype and mixed-lineage leukemia (MLL) gene rearrangement. Accordingly, the overall survival of patients with CD1d+ ALL was significantly shorter. CD1d+ leukemic blasts were able to present alpha-GalCer via CD1d to cytotoxic CD1d-restricted T cells which induced apoptosis of ALL cells that was inhibited by mAb to CD1d. CD1d+ blasts loaded with alpha-GalCer elicited cytokine secretion by CD1d-restricted T cells. Analysis of bone marrow (BM) cells derived from normal donors revealed that CD19+/CD1d+ cells were mostly mature B lymphocytes. However, a minority of BCPs expressed CD1d. Thus, expression of CD1d in ALL cases heralds an adverse prognosis but may provide a therapeutic tool.

P087

ACUTE LYMPHOBLASTIC LEUKAEMIA CELLS CARRYING THE T(12;21) TRANSLOCATION ARE HIGHLY SENSITIVE TO ALEMTUZUMAB (CAMPATH-1H) MEDIATED CELL LYSIS

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Background. Alemtuzumab is an anti-CD52 humanised IgG1 antibody used for the treatment of B-CLL and for non-myeloablative conditioning in allogeneic bone marrow transplantation. CD52 is expressed on mature T and B lymphocytes, monocytes, monocyte derived dendritic cells and on most leukaemia and lymphoma cells derived from these haematopoietic cell populations, including B-CLL. Aims. 1) To determine the levels of expression of CD52 in acute lymphoblastic leukaemia (ALL) cell lines and freshly isolated samples of different subtypes. 2) To investigate the capacity of alemtuzumab to lyse ALL cells through complement activation.

Methods. CD52 expression was analsed by standard imnmunophenotyping and FACS analysis. Complement dependent cytotoxicity (CDC) was measured in vitro using human serum as source of complement. Cell death was determined with the alamar blue vital dye as well as by FACS analysis.

Results. CD52 was expressed in 4 out of 9 ALL cell lines studied, with 62-100% of the cells staining positively in these. However only two of these lines, the pre B-ALL REH (t(12;21)) and the B-ALL Silti (t(8;22)) expressed CD52 at relatively high density (MFI 351 and 274, respectively). Analysis of alemtuzumab induced CDC of the four cell lines showed that only the REH cell line was lysed efficiently, with 50% lysis in presence of 10 microgr/mL alemtuzumab. The other CD52+ cell lines all showed either weak or no lysis (0-8%). Lysis was complement dependent. We then analysed CD52 expression in a panel of 56 freshly isolated adult or pediatric ALL samples. CD52 was expressed at varying levels on 84% of cases. CD52 expression did not correlate with leukamia subtype, except that all t(4;11) pro-B ALL cases were CD52 negative (4/4 cases). Furthermore all t(12;21) ALL cases were CD52 positive and expression levels in this subgroup was on average slightly higher than in all other subgroups (MFI 684 compared to 400). CDC was then analysed: interestingly 8/8 cases of t(12;21) cases showed very high CDC (mean 96% lysis with 10 microgr/mL alemtuzumab, range 85-100%) compared to a mean of 14% in the other 20 CD52+ cases bearing other or no translocations (0-50%). In t(12;21) cases, efficient CDC was obtained with as low as 1 microgr/mL alemtuzumab. Measurement of deposition of the complement components C3 and C9 indicated that more efficient initiation of the complement cascade took place in t(12;21) cells compared to non t(12;21) cases. Furthermore the CD55 and CD59 complement inhibitors were expressed at similar levels in all leukemia subtypes amd were not responsible for poor lysis in resistant ALL cells. Although t(12;21) cells express on average relatively high levels of the CD52 antigen, this factor alone does not fully explain the particularly high susceptibility of these cells to alemtuzumab mediated CDC compared with other leukaemia subtypes.

Conclusions. pre B-ALL cell lines and freshly isolated samples bearing the t(12;21) translocation express CD52 and are particularly sensitive to alemtuzumab and complement mediated lysis in vitro. We thank Schering SpA (Segrate, Italy), AIRC (Milan, Italy), the AIL-Paolo Belli section (Bergamo, Italy), and MIUR (Firb project to JG, Rome Italy) for their financial support.

P088

HUMAN T-LYMPHOBLASTIC LEUKAEMIA CELLS PRODUCE IL-8 IN RESPONSE TO CXCL12 SECRETED BY NORMAL BONE MARROW STROMA

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T acute lymphoblastic leukaemia (T-ALL) cells arise inside the thymus and in most cases, early during the clinical course, migrate to bone marrow (BM). In this study, we explored whether the chemokine CXCL12, produced by BM stromal cells, could affect cytokine production of T-ALL cells. Treatment of cells derived from adult T-ALL patients with CXCL12 induced a significant increased production of IL-8 but was ineffective on IL-1, TNF, or IL-10 production. Real Time PCR and colorimetric quantification assay confirmed the IL-8 increased expression also at mRNA level. We then investigated the role of CXCL12 produced by BM stromal cells in inducing IL-8 in primary T-ALL cells. To address this issue, we performed lymphostromal co-cultures between normal human BM stroma and primary T-ALL cells. After co-culture, T-ALL cells were isolated from BM cells by Fluorescence Activated Cell Sorting and analyzed for the presence of IL-8 mRNA. The interaction with BM stroma induced increased expression of IL-8 mRNA in T-ALL patients. The presence in the co-culture of antibody blocking the CXCL12 receptor, CXCR4, completely inhibited the IL-8 induction mediated by BM stroma. Our results implicate a novel function for CXCL12 in regulating IL-8 production in T-ALL within the BM microenvironment, and point out the role of bystander cells in influencing cytokine expression profile of T-ALL.

P089

DE NOVO ACUTE TRILINEAGE LEUKEMIA: A CASE REPORT

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Acute leukemias are classified as myeloid or lymphoid on the basis of morphological, cytochemical and immunological criteria. In rare cases, the coexpression on the same cells of myeloid and lymphoid antigens determinates acute biphenotipic or hybrid leukemias. Hybrid leukemias can occur de novo or can emerge after a therapy for AML or ALL. According to the type of myeloid and lymphoid markers expressed by the blasts, four groups can be distinguished: the most common coexpresses myeloid and B-lymphoid, less often T-lymphoid antigens; trilineage differentiation, with expression of B, T and myeloid markers and coexistence of B and T markers are uncommon. Our group has followed several de novo acute hybrid leukemias; we report a recently observed rare case, pointing the rational of our therapeutic choices and the peculiar biological behaviour of the illness. A young 25-year-old woman, with recent episode of mononucleosis complicated with thrombocytopenia, showed diffuse joints pain, awkward gait, weight loss, icterus, chronic DIC, metrorrhagia, bulky ovarian tumefaction. Blood samples showed anaemia (Hb 8.4 g/dL), thrombocytopenia (Plt 28,000/cmm), leukocytosis (WBC 24,000/cmm) with blastic elements. Peripheral blood and bone marrow immunophenotyping enabled the diagnosis of acute hybrid, trilineage, leukemia; pathological cells coexpressed the following antigens: $CD34^+$/DR+/TdT+/CD13+/CD33+/CD11b+/CD10+/CD19+/CD24+/CD5+/CD7±. The subsequent cytogenetic and biomolecular samples showed the presence of Ph chromosome – t(9;22) – with hybrid bcr-abl rearrangement encoding for P-190 protein. The initial good response to steroid treatment, with reduction of pelvic mass, suggested the opportunity of an antiblastic treatment with an ALL-like regimen; thus vincristine, daunorubicine and prednisone were administered. After a 3-course treatment molecular remission was obtained with absence of bcr-abl. In consideration of initial unfavourable prognostic factors (trilineage phenotype, Ph+), consolidation therapy with AML-ICE regimen was performed; then patient underwent autologous stem cell transplantation, due to lack of familial compatible donor. A research for MUD was started. Three months after transplant, disease relapsed as ALL-B2 FAB (CD10+/CD19+/$CD34^+$ phenotype), in presence of bcr-abl. The persistence of P-190 suggested the opportunity of treatment with Imatinib, at a dosage of 600 mg/d, obtaining prompt blasts clearance and chariotype normalization. Twelve months after diagnosis and three months after the beginning of Imatinib-based therapy, patients underwent MUD allogeneic transplant. Post-stem cell infusion phase was complicated by a severe respiratory GVHD which ended with patient's death. Hybrid leukemias are aggressive diseases and no standard therapeutic strategy is yet disposable for them. Our group experience pointed out that such leukemias can have a therapeutic-induced evolution. On the basis of that, an improvement in survival rates could be obtained in the future administering combined treatment including intensive regimens, immunotherapy (allogeneic transplant, monoclonal antibodies) and, possibly, specific molecular therapy.

P090

DE NOVO ACUTE BIPHENOTYPIC LEUKEMIA: REPORT OF A SUCCESSFULLY TREATED CASE

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Around 5% of acute leukemias remain difficult to classify; this is due to myeloid and lymphoid antigens coexpression on pathological cells. Such cases are defined as hybrid or biphenotypic acute leukemias. According to the expression of myeloid and lymphoid markers on the blasts, four groups can be distinguished. The most common coexpresses myeloid and B-lymphoid or less often T-lymphoid antigens; trilineage differentiation, with expression of B, T and myeloid markers and coexistence of B and T markers are uncommon. Hybrid leukemias can occur de novo or can emerge after a therapy for AML or ALL. The overall probability of survival at 2 years for the patients affected by such hemopathies is less than 40%. Our group has a survey of acute hybrid leukemias; we report a rare case of de novo acute hybrid leukemia observed in the year 1999, pointing the peculiar homing mechanisms of pathological cells and the biological illness behaviour according to therapeutical choices. A 22-year-old woman came to our observation with the diagnosis, performed on lymphnode sample, of T-cell lymphoblastic NHL. Bone marrow immunophenotyping showed the presence of pathological cells only partially overlapping to those found in the lymphnode: over 80% of marrow blast coexpressed myeloid and T-lymphoid antigens (CD2+/CD7+/MPO+/CD33+/$CD34^+$/TdT+). Thus a diagnosis of acute hybrid, biphenotypic, leukemia was performed. Biomolecular analysis excluded the presence of bcr-abl rearrangement. In consideration of the wide nodal involvement and the highly expressed T-lymphoid antigens, patient underwent ALL GIMEMA 7/96 regimen. After the provided three courses, despite a complete regression of lymphoadenopaty, no aplastic phase was obtained, with the persistence of biphenotypic clone; thus an AML-ICE regimen was administered. After three months from diagnosis, in complete remission phase, patient underwent compatible familial donor allogeneic bone marrow transplant. Six years after diagnosis, complete remission of leukemia still persists. Our experience shows that hybrid leukemias are aggressive diseases and no standard therapeutic strategy is yet disposable for them. Furthermore, they show cellular homing features related to neoplastic subclones. Thus we purpose an intensive treatment based on the illness biological features.

P091

ACUTE LYMPHOBLASTIC LEUKEMIA OF THE ELDERLY AGE

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Acute Lymphoblastic Leukemia (ALL) is uncommon over than 60 years of age. We review the our experience of 43 consecutive cases of ALL of elderly age collected over a fifteen years period. Median age was 66 years (range 61-83). L2/L1 FAB classification: 37/6; Median WBC was $15x10^9$/L (range 1-180); Male/Female ratio was: 25/18. Thirty-nine cases (90,7%) belonged to B cell lineage (pre-pre-B 11, common 23, pre-B 5) and 4 (9,3%) to T cell lineage (pre-T stage); CD34 expression was observed in 26/33 cases (78,8%); CD33, CD13 and CD15 surface expression was positive in 17/34 (50%), 13/33 (39,4%) and 5/23 cases (21,7%), respectively; on the whole, CD13 and CD33 were co-expressed on 9/33 cases (27,3%). Philadelphia chromosome was present in 13 patients (30,2%). Of the 43 revisited patients, 31 younger patients (median age 65 years (range 61-77), good performance status and without other diseases) received an intensive treatment such as 0183 or 0288 GIMEMA Protocol. In the remaining 12 older patients (median age 77 years (range 61-83) and serious co-existing cardiac, pulmonary, renal and hepatic failures) were utilised prednisone and vincristine (and maintenance with 6MP and MTX). Six patients (19,3%) of the group treated with curative intent dead during the induction phase; 19 patients (61,3%) achieved a CR, and at present, 3 patients are live at +6, +42 and +101 months. Out of 12 patients receiving less intensive and supportive treatment, only 4 (33%) achieved a short CR. Our data demonstrate that the patterns of immunophenotypes and caryotype of these patients differs from those usually seen in children and adults with ALL confirming the presence of a stem cell disorder and an extremely poor prognosis. In addition, in our experience emerged that to the biologically younger patients who can well tolerate an aggressive therapy this approach should not be denied because of is possible to achieve long survivals.

P092

BONE MARROW-DERIVED STROMAL CELLS INHIBIT SPONTANEOUS APOPTOSIS AND INDUCE PROLIFERATION IN BLASTS OF T ACUTE LYMPHOBLASTIC LEUKAEMIA: THE ROLE OF INTERLEUKIN 7

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T acute lymphoblastic leukaemia (T-ALL) cells arise inside the thymus and in most cases, early during the clinical course, migrate to bone marrow (BM). Interleukin-7 (IL-7), secreted by BM stromal cells, promotes survival and induces cell cycle progression of T-ALL cells. In this study we explored the effect of BM stroma on T-ALL cell survival and analyzed the role of IL-7 within the microenvironment generated by stroma/T-ALL interaction. To this aim, we co-cultured cells obtained from 5 adult T-ALL patients with normal human BM stromal cells. The spontaneous apoptosis observed in cultured blasts was sensibly decreased when they were co-cultured with BM stromal cells. In addition, lympho-stromal interaction induced a considerable proliferative response (53-fold the unstimulated cells) in rescued T-ALL cells. The functional blockage of IL-7 or IL-7 receptor (IL-7R) by monoclonal antibodies reduced the stroma-mediated protection, thus demonstrating that IL-7/IL-7R system is involved in the T-ALL survival induced by BM stromal cells. Our results point to the role of BM stroma in the regulation of T blast survival and proliferation. In addition, they implicate that IL-7/IL7-R interplaying has an active role in the modulation of T-ALL survival within the microenvironment generated by BM stroma/T-ALL interaction.

P093

ADHESION MOLECULE EXPRESSION IN CHILDREN'S ACUTE LYMPHOID LEUKEMIA . PROGNOSTIC RELEVANCE OF CD11A

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Adhesion molecules on mononuclear cells play a significant role for the regulation of cell-to-cell and cell-to-extracellular matrix interactions. They are involved in several physiological and pathological mechanisms such as extravasation and dissemination of neoplastic cells. Although many data have been published on the role of the *adhesive phenotype* in several haematological diseases like Chronic Lymphocytic Leukemia, Acute Myeloid Leukemia and Multiple Myeloma, studies in ALL are still unclear. Between 1994 and 2002 at the Haematology Division of Children Hospital Bambino Gesù of Rome, 121 children aged between <1 and 16 (median age 4) have been studied at the onset of ALL. Well known prognostic clinical parameters such as WBC count, hepatosplenomegaly, extrahematopoietic tumor mass, CNS involvement, prednisone response were evaluated. All cases have also been studied for other biological charateristics such as immunological phenotype, cytogenetic alterations and molecular genetic rearrangements. All patients were treated according to protocols Interfant 99, ALL-AIEOP 91, 95, 2000. A panel of adhesion molecules has been analyzed in this group of patients, namely CD54, CD58, CD18, CD11a,b,c, CD29, CD49d, CD61, CD44, Leu8, CD62, CD80, CD86. Univariate analyses showed that WBC count (over $100x10^9$/L), cytogenetic abnormalities, splenomegaly and the expression of CD11a on lymphoblasts were significantly related to a higher incidence of relapse (p=0.009, p=0.003, p=0.037 and p=0.003, respectively). With the exception of splenomegaly, the same parameters were also related to a higher rate of mortality (p=0.06, p=0.06 and p=0.002, respectively). According to multivariate analyses, only CD11a positivity emerged as a significantly independent variable for events such as relapse and death. The EFS at

100 months is 70% in CD11a negative cases vs 56% of positive patients. Thus, in childhood ALL CD11a bears an adverse prognostic impact in the disease outcome.

P094

PROGNOSTIC IMPACT OF HYPERGLYCEMIA IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA. A STUDY ON 81 CASES

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Hyperglycemia is not an uncommon complication occurring during induction chemotherapy in patients with acute lymphoblastic leukemia (ALL). It has been reported to be an independent predictor of adverse outcome in ALL (Cancer 2004; 100: 1179) and a recent study indicates that high glucose levels can activate molecular pathways that promote survival and chemoresistance of ALL blasts (ASH 2004, #2049). The purpose of this study was to determine the prevalence of hyperglycemia and its prognostic impact on complete remission (CR) achievement and duration as well as on overall survival in a series of 81 adult patients affected by ALL treated with the GIMEMA LAL96 and LAL 2000 protocols if aged up to 60 years (n=70) and LAL0288 (n=11) if over 60 years. The median age of the whole patient population was 39 years (13-80); 25 patients (31%) had Ph+ ALL, 2 had t(1;19) and 5 had t(4;11). Hyperglycemia was defined as the occurrence of a blood glucose level higher than 200 mg/dL and was found at diagnosis in 3 patients only (4%), while it occurred during induction in 24 additional patients for a total of 27 cases (33%). Overall, CR was achieved in 68 patients (84%); of note, while the occurrence of hyperglycemia per se did not affect the CR rate (74% vs 89%, *p*:0.11), the reduction of steroids due to uncontrolled hyperglycemia significantly did (63% vs 90%, *p*:0.01). Median CR duration for the entire group was 22 months but CR lasted 10 months for patients with steroid reduction as compared to 24 months for the remaining patients (p:0.11). Median survival of the whole patient population was 21 months and 35% are alive at 5 years. Patients with steroid reduction had a shorter median survival (17 months) as compared to the remaining group (28 months), *p*:0.06, while the difference was not significant by considering hyperglycemic vs non hyperglycemic patients (*p*:0.11). We conclude that, in our series, hyperglycemia per se did not significantly affect the clinical outcome in adult patients with ALL. However, steroid dose reduction during induction chemotherapy resulted in a lower CR rate and shorter survival. A trend toward shorter CR duration was also observed in this study, but this finding needs to be confirmed in a wide series. A more sustained treatment of hyperglycemia rather than a reduction of steroid dosage seems to be advisable during induction treatment of ALL patients.

P095

GMALL REGIMEN FOR BURKITT AND B-ALL. EXPERIENCE OF A SINGLE INSTITUTION

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Results in mature B-ALL and Burkitt NHL substantially improved by short intensive chemotherapy cycles based on fractionated cyclophosphamide and high-dose methotrexate (alternating CODOX-M/IVAC). Many of B-ALL/Burkitt-NHL patiens are CD20+ (>20% of cells). A new protocol (GMALL, as proposed by Hoelzer *et al.*; Blood 1996 and 2003), which includes antiCD20 is actually used in our institution. The regimen is based on six 5-day chemotherapy courses (cycle A: methotrexate, vincristine, ifosfamide dexamethasone, cytarabine, teniposide; cycle B: cyclophosphamide, methotrexate, vincristine, doxorubicin, dexamethasone; cycle C: dexamethasone, methotrexate, etoposide, vindesine, cytarabine); 8 doses of antiCD20 (before each chemotherapy cycle and 2 after cycle 6). Patients younger than 55 years receive 6 cycles (ABCABC). Intrathecal prophylaxis is provided on cycles AB. Radiation after 6 cycles is provided in patients with residual tumor or CNS involvement. Between june 03 and february 05, 5 patients (median age 18 years, range 17-49) entered the protocol: 1 mature B-ALL, 3 Burkitt NHL, 1 relapsed ALL after 9 years of continuous complete remission (CCR) with therapy-related cardiomiopathy. Among NHL patients 3/3 had stage IV disease (abdominal mass and marrow infiltration), B-symptoms and elevated LDH, 2/3 had bulky disease, no CNS involvement was recorded. Moreover one patient affected by Burkitt NHL has been treated in ICU during 1st cycle (comatose with acute renal failure and massive gastrointestinal bleeding at the onset of disease). 3 patients have completed the six cycles, one 1 cycle, one (the oldest) in NHL group died during first cycle cause of sepsis. AntiCD20 was administered in all but one patient (CD20 negative ALL) without excess toxicity. The major toxicity was haematological: grade III/IV leukopenia and thrombocytopenia were recorded in all the patients; 3/5 developed mucositis and 2/5 mild increase of liver enzymes. Overall there have been only slightest delay on recycling. We chose not to perform CT until 2nd or 3rd cycle; so CR was documented in mature B-ALL and after 1st cycle and in 2/3 Burkitt NHL pts after 2nd and 3rd cycles respectively; patient with relapsed ALL has completed 1st cycle and is now waiting for response evaluation. 4 patients are alive after a median follow-up of 130 (25-640) days. In conclusion, despite few patients treated and the limited follow up, this short intensive chemotherapy regimen including HDMTX and HDAC is feasible without excess toxicity, could be employed in extra-hematological setting (ICU) and seems to be a reasonable option for patients relapsed after a standard regimen for ALL and with comorbidity.

P096

SUSTAINED MOLECULAR RESPONSE IN Ph+ ALL WITH CONVENTIONAL INDUCTION FOLLOWED BY ALTERNANCE OF IMATINIB-MESYLATE AND CHEMOTHERAPY: REPORT OF TWO CASES

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Adult patients with acute lymphoblastic leukemia (ALL) bearing the Philadelphia chromosome (Ph) or Bcr-Abl rearrangement are at a very high risk of treatment failure. The introduction of tyrosine-kinase inhibitors has disclosed new perspectives in the therapeutical approach of Ph positive leukemias. We have performed a sequential molecular monitoring in two ALL patients receiving a standard dose induction regimen followed by an innovative maintenance therapy with alternated administration of imatinib and conventional chemotherapy. Two patients were diagnosed as having Philadelphia positive and bcr-abl positive ALL on July and Semptember 2003. In the induction phase patients were treated with vincristine (VCR) (1,4 mg/m^2, day 1, 8 and 15), daunorubicin (DNM) (30 mg/ m^2, day 1 and 3), cyclophosphamide (CTX) (1 g/m^2, on day 15 and 17), prednisone (60 mg/m^2 days 1-30). Maintenance therapy started on day 31 from the beginning of induction. It was based on the alternate administration of imatinib-mesylate (600 mg/day for 45 days) and chemotherapy (purinethol 60 mg/m^2 / day, methotrexate 12 mg/m^2 / weekly, i.m, both for 45 days). VCR (1,4 mg/m^2, on days 1, 8 and 15), DNM (30 mg/ m^2, day 1), CTX (1 g/m^2, on day 8), prednisone (60 mg/m^2 days 1-15) were administered as reinduction therapy, every 3 months during the first year. We planned to give reinduction courses every 6 months during the second year. Maintenance therapy was scheduled for 2 years. Molecular study of patients has been performed at diagnosis, at the end of induction phase and every three months, during maintenance therapy. In each marrow sample three different markers of clonality have been studied; bcr-abl (both qualitative and quantitative analysis of p210 and p190 expression have been performed, by RT-PCR and Real-time PCR, respectively), monoclonal JH rearrangement, and WT1 expression. The results of this study are reported in table 1. Our data shows that, in Ph$^+$ ALL patients, a standard dose induction regimen can induce complete haematological and cytogenetic remission but does not achieve molecular response. As indicated by sequential real-time PCR analysis the following post induction phase of therapy was able in both patients to achieve bcr-abl negativity, that is a molecular feature associated with long lasting CRs. The detection of clonal JH rearrangement in both patients indicates that some residual leukemic cells persist. In our two patients the persistence of JH rearrangement did not predict impending relapse and was associated with a prolonged DFS and a persistent normalization of WT1 expression. The significance of WT1 expression is still under investigation but could reflect a functional state of the pre/leukemic clone, and gives information on the biological activity of residual neoplastic cells. The administration of conventional antiblastic drugs during the maintenance phase might control the growth of leukemic cells lacking or not expressing bcr-abl rearrangement and therefore insensible to imatinib. On the other hand, imatinib is able to inhibit the expansion of non-detectable bcr-abl positive clones and, furthermore, an adequate dosage and the pulsed schedule might reduce the risk of imatinib-resistance. In conclusion our molecular analysis has produced very stimulating data, which need to be confirmed and better explained in larger cohorts of patients.

Table 1: Molecular monitoring of Ph+ ALL patients

	Karyotype		p190		p210		JH		WT1	
	Pat 1	Pat 2	RT/QRT Pat 1	RT/QRT Pat 2	RT/QRT Pat 1	RT/QRT Pat 2	Pat 1	Pat 2	Pat 1	Pat 2
Diagnosis	46XX, t(9;22)	46XX, t(9;22)	pos/n.d.	pos/n.d.	pos/n.d.	pos/n.d.	pos	pos	1520,42	400,33
Post-induction	46XX	46xx	pos/108	pos/73	pos/126	pos/58	pos	pos		
3 months	46XX	46XX	neg/n.d	neg/n.d.	neg/n.d.	neg/n.d.	pos	pos		
6 months	46XX	46XX	neg/0	neg/5	neg/0	neg/3,26	pos	pos	0,57	26,55
12 months	46XX	46XX	neg/0	neg/0	neg/0	neg/0	pos	pos	1,67	44,81
last follow up	46XX	46XX	neg/0	neg/0	neg/0	neg/0	pos	pos	1,56	32

P097

BCR-ABL E1A3 TRANSCRIPT IN ACUTE LYMPHOBLASTIC LEUKEMIA SECONDARY TO CHRONIC LYMPHOCYTIC LEUKEMIA: A CASE REPORT

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Second neoplasms are frequent complications in patients with chronic lymphocytic leukaemia (CLL), as possible mechanisms responsible of the increased risk of second malignancies in CLL patients authors propose the immunodeficiency associated with disease and an enhancement by therapy of common carcinogens but the nature of the association between CLL and a second cancer remains unclearns. In this study we report a rare case of acute lymphoblastic leukemia (ALL), BCR-ABL positive, following B-CLL in complete remission after allogeneic bone marrow transplantation. In order to identify the clonal origin of the two disorders, we performed a molecular study by sequencing the VH-D-JH rearrangement. A 54 year-old woman was diagnosed with B-CLL in May 2001. Rearranged VH genes were analyzed by multiplex Polymerase Chain Reaction (PCR) and direct sequencing. The neoplastic clone showed germline VH configuration with VH4-39 D3-3 JH4 rearrangment. The disease was refractory to a variety of chemotherapic regimes and one year after diagnosis the patient underwent allogeneic transplantation. Graft failure with autologous reconstitution was documented by full-recipient chimerism, in the absence of the CLL clone. Two years after ALL blasts appeared in peripheral blood. Cytogenetic and FISH analysis performed on ALL blasts documented t(9;22) translocation; molecular analysis by RT-PCR detected the BCR/ABL 190 fusion transcript, but a lower weight amplification product was detected. cDNA sequencing of the fusion region showed an in-frame rearrangement with a break-

point in BCR exon e1 and fusion with ABL exon a3 following skipping of the entire ABL exon a2. So we identified a chimeric transcript involving the rare fusion of BCR exon 1 and ABL exon 3 (BCR/ABL junction e1a3). Immunoglobulin rearrangement, analyzed by PCR using BIOMED-2 protocols, documented the clonality. The rearranged Ig VH D JH genes of ALL clone was VH3-74 D2-8 JH4 and it was somatically point-mutated (based on comparison of its sequence with those of reported germline genes). These results clarified that the two malignancies of the patient had different VH and D genes rearranged with the same JH gene, thus suggesting that the second lymphoblastic transformation emerged from non pre-existing B-lymphocytic clone. Our data indicate that ALL and CLL occurring in the same patient had an independent clonal origin. The finding of a rare BCR/ABL chimeric transcript lacking ABL exon 2 suggests that ABL exon 2 sequences are not necessary for the pathogenesis of ALL. The concomitant presence of a rare breakpoint in the ABL gene, and its possible role in an ALL clone secondary to CLL adds further complexity to this singular case.

P098

SINGLE AMINO ACID CHANGE A91V IN THE PERFORIN GENE: A NOVEL, FREQUENT PREDISPOSING FACTOR TO ADULT ACUTE LEUKEMIA?

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Perforin plays a pivotal role in the cytotoxicity of natural killer (NK) and cytotoxic T lymphocytes (CTL). Perforin gene (PRF1) mutations have been associated with familial hemophagocytic lymphohistiocytosis (FHL, HLH). The pathogenic role of the DNA variant C272T, resulting in single amino acid change A91V, has been recently questioned by two groups, which reported in 4 and 17% prevalence among controls, suggesting this is only a neutral polymorphism. To address this issue, we studied the prevalence of A91V in our control population. Furthermore, the issue of a possible link between PRF1 mutations and lymphomagenesis was reported. We recently demonstrated that the impaired cytotoxic function of perforin machinery, resulting from the PRF1 single amino acid change A91V, correlates with an increased risk of childhood lymphoblastic leukemia. In order to assess, if this correlation is confirmed in adult patients also we screened a series of 50 consecutive patients (>18 years) with acute myeloid (n=33) or lymphoblastic (n=17) leukemia for the 272T DNA variant. We also analyzed constitutional DNA from 127 Caucasian, unrelated controls (61 healthy subjects and 66 consecutive newborns). To search for the C272T DNA variant (resulting in A91V) by "minisequencing", we amplified PFR1 exon 2. PCR products were used to perform a primer single base extension reaction (SNaPshot Kit Applied Biosystems, USA). The reaction is based on a specific minisequencing primer, which is exactly one base short of the 272 position and fluorescent ddNTP. The product obtained is separated by electrophoresis and the color of the peaks obtained makes it possible to identify the genotype (GeneScan Analysis Software, Applied Biosystems). Heterozygous C272T was found in 2 out of the 61 healthy control subjects (3.2%), and in 3 out of the 66 (4.5%) unselected newborns. Overall, heterozygous C272T was observed in 5 of the 127 control subjects, accounting for a prevalence of 3.9%. Of the 50 adult patients with acute leukemia, 7 were heterozygous for A91V (14%). This incidence was significantly superior to that of the control subjects (Odds Ratio 4.05). Seven 272T alleles were observed out of 100 chromosomes versus five 272T alleles out of the 254 chromosomes in the control group (chi-square 5.67). The pathogenic role of A91V was questioned by two recent reports: ZurStadt found that 15 of 86 (17.5%) control DNAs from healthy Caucasians were heterozygous for A91V, while Molleran Lee found it in 7 of 202 (3%). Both suggested that A91V has to be considered only a neutral polymorphism. Our finding of A91V in 3.9% of controls is in keeping with Molleran Lee. At least two recent studies demostrated the pathogenic role of A91V aminoacid change. Voskoboinik reported that in rat basophil leukemia cells A91V perforin was expressed at reduced levels compared to wild-type, resulting in partial loss of lytic capacity. Trambas *et al.,* demonstrated that A91V perforin from a patient with HLH undergoes conformational changes and impaired cleavage, likely to explain the reduced cytotoxicity in CTL and NK cells and likely to contribute to the pathogenesis of HLH. We found heterozygous A91V in 14% of a cohort of adult patients with acute leukemia. This prevalence, as previously reported in childhood setting, exceeded that of 3.9% observed in ,our fully comparable control population from the same geographic area. Since the patients were not selected for any additional criteria, including HLH-like clinical features, this finding suggests that the single amino acid change A91V in the perforin gene is significantly associated with the risk of developing acute leukemia at any age.

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P099

ADULT AND CHILDHOOD T- LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA: A TWENTY YEARS PERIOD EXPERIENCE

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T lineage acute lymphoblastic leukemia (T-ALL) accounts for 15-20% of newly diagnosed cases of ALL. It is characterized by a male predominance, high WBC count, mediastinal tumors, central nervous system involvement. Historically, T-ALL patients have a worse prognosis than

other ALL patients. In this paper we review our experience about 66 consecutive patients with T-ALL (17 children and 49 adults) diagnosed and treated in our center between 1985 and 2004. Median age of adult and childhood patients was 22 (range: 16-75) and 9 (range: 4-15) years, respectively. Male/Female ratio was 47/19 (adults 33/16; children 14/3) Based on immunophenotype, all cases were classified in three ontogenic stage-related subtypes: Subtype I or early T-ALL (immunophenotype: CyCD3+/CD7±/CD1-/CD3-): 39 patients (59,1%) belonged to this group. Adult/Childhood ratio was 33/6; Median WBC was 19x10E9/L (range 1-260); in 14 patient (35,9%) was present a mediastinal mass; CD34 expression was observed in 26/34 cases (76,5%); myeloid antigens (MyAg) (CD13 and/or CD33 and/or CD15 and/or CD65) were co-expressed in 18/35 cases (51,4%). Subtype II or cortical T-ALL (immunophenotype: CD7+/CD1+/ CD3-): 20 patients (30,3%) were included. Adult/Childhood ratio was 12/8; Median WBC was $39x10^9$/L (range 7-1000); mediastinal tumor in 13 patients (65%); CD34 was positive in 4/17 cases (23,5%) and MyAg were co-expressed in 1/16 cases (6,2%). Subtype III or mature T-ALL (immunophenotype CD7+/CD1-/CD3+): the remaining 7 patients (10,6%) were included. Adult/Childhood ratio was 4/3; Median WBC was $18x10^9$/L (range 4-480); mediastinal tumor was present in 4 patients (57,1%); no case expressed CD34 and MyAg co-expression was only present in one case (14,3%). Therapeutic approaches applied during the twenty years period of the study were those of GIMEMA (for adults) and AIEOP (for children) cooperative groups. On the whole, 51 patients (77,3%) achieved Complete Remission: (35 (71,4%) and 16 (94,1%) adult and childhood patients, respectively). Regarding to immunological groups, 27 (69,2%) early T-ALL, 18 (90%) cortical T-ALL and 6 (85,7%) mature T-ALL patients achieved CR (p 0.035); of these, at present (median follow-up 136 months - range: 5-236), 24 patients are alive in CCR (subtype I: 10 patients (37%); subtype II: 12 patients (66,7%); subtype III: 2 patients (33,3%); *p* 0.012-). Our data confirm that T-ALL may be quite heterogeneous in terms of clinical and biological features: a lower incidence of lymphomatous features was observed in the less mature subtypes of T-ALL, in which, on the contrary, an higher co-expression of CD34 and MyAg was found. In our experience come up that the immunologic classification is the most significant prognostic factor in T-ALL: in fact, in adult as well as in childhood T-ALL, the cortical subtype showed a superior outcome compared to early and mature subtype.

P100

T-CELL RECEPTOR BETA REPRESENTS A USEFUL TARGET FOR IDENTIFICATION OF CLONAL REARRANGEMENTS IN ADULT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Current minimal residual disease (MRD) studies in T-cell acute lymphoblastic leukemia (T-ALL) mainly use T-cell receptor gamma and delta gene rearrangements to generate valuable molecular probes for monitoring MRD by PCR analysis. The molecular identification of SIL-TAL1 deletion may also contributes to increase the number of patients analyzed. However, by this approach no more than 70% of T-ALL patients can be analyzed for MRD detection with one or two informative probes. Hence, we applied a recently described strategy based on the study of TCR beta gene rearrangements. TCR beta analysis was performed on Bone Marrow (BM) or Peripheral Blood (PB) samples obtained at diagnosis from 51 T-ALL adult patients, consecutively enrolled in a Phase II clinical study (ALL 09-2000) of the North Italian Leukemia Group (NILG). A DNA-based multiplex PCR analysis of TCR Beta gene rearrangements was applied using 23 consensus Vbeta, and 13 Jbeta primers for the identification of almost all complete Vbeta-Dbeta-Jbeta rearrangements; moreover 2 Dbeta and 13 Jbeta primers were used to detect the incomplete Dbeta-Jbeta rearrangements. The PCR products, run on heteroduplex polyacrylamide gel, allowed the identification of clonal bands which were subsequently sequenced to design patient specific primers for quantitative PCR analysis and MRD detection. Clonal rearrangements of TCR beta were found in 30 out of 51 (59%) T-ALL patients. Complete Vbeta-Dbeta-Jbeta recombinations were found in 22 patients (44%) while 17 cases (34%) showed an incomplete Dbeta-Jbeta rearrangement. In 12 cases (24%) at least two TCR beta rearrangements were identified, whereas in 18 (36%) only one rearrangement was observed (9 complete and 9 incomplete). The TCR beta probes used for quantitative PCR analysis of MRD were usually chracterised by a high reproducible sensitivity (10-4) and maximal sensitivity (10-5). The combined analysis of TCR beta, gamma and delta gene rearrangements in adult T-ALL allows the identification of at least one sensitive molecular probe in more than 80% of cases and in 53% of cases 2 informative probes have been available. Our approach allows the prospective molecular evaluation of minimal residual disease in the great majority of adult T-ALL patients enrolled into prospective clinical studies.

P101

CLINICAL IMPACT OF Q-RT-PCR MONITORING OF MINIMAL RESIDUAL DISEASE IN NEWLY DIAGNOSED ADULT PATIENTS WITH BCR-ABL+ ACUTE LYMPHOBLASTIC LEUKEMIA RECEIVING IMATINIB MESYLATE ALONE AS A POST-CONSOLIDATION TREATMENT: AN UPDATE OF THE ITALIAN GIMEMA LAL 0201/A PROTOCOL

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Thirty adult patients aged between 22 and 60 years (medi-

an age: 46 years) with BCR-ABL+ acute lymphoblastic leukemia (ALL) were prospectively monitored by Q-RT-PCR between August 2001 to July 2005. All patients were treated according to the GIMEMA LAL 0201/A protocol, in which Imatinib alone, at the dosage of 400 mg x 2 daily for at least six months, was administered as post-consolidation therapy in responding patients after high-dose induction and consolidation treatment. twenty patients (66.6%) harboured the p190 transcript and 10 (33.3%) the p210 with/without the p190 isoform. The main clinico-biologic features did not significantly differ between the two patient groups, including the mean number of BCR-ABL copies at diagnosis (ie: BCR-ABL/ABL x 104) that was 13,052 (range: 1,466–35,449) and 22,487 (range: 7,315–78,000), respectively. At the beginning of the treatment with Imatinib, all patients were in 1st complete hematological remission (29 patients after the first induction and consolidation course; 1 patient after a salvage treatment). Before Imatinib, 9 of the 20 p190+ve patients (45%) showed a BCR-ABL copy number reduction of ≥ 3 log compared to the levels at diagnosis, (mean BCR-ABL copies 3.6; range: 0-10), and they were defined as good responders to chemotherapy (CHT). The remaining 11 patients (55%), defined as poor responders, showed a reduction of < 3 log (mean copies 2,825.8; range: 12 – 25,245). To monitor minimal residual disease in these two groups we tested 70 samples (mean of 9 test per patient; range 4-19) and 80 samples (mean 7 per patient; range: 2-16), respectively. In the 11 poor responders, BCR-ABL copies constantly increased over time and this was predictive of an hematological relapse in 8/11 patients. By contrast, 7 of the 9 good responder patients during Imatinib treatment persistently showed level of BCR-ABL below 10. These 7 patients were in CCR maintained by Imatinib alone at 6, 9, 13, 13, 17, 21 and 24 months, respectively. In 1 patient, at 6 months from the start of Imatinib, a CNS relapse preceded a marked increase in the BCR-ABL copy numbers in BM cells. Therefore, after a median follow-up of 6 months (range 3 – 23), for poor responders, and of 13 months (range 6 – 30), for good responders, these two patient groups significantly differed for the actuarial probability of relapse that resulted of 100% and 12.5%, at 24 months (p=0.027), respectively. In the p210+ve patient group we tested 41 samples (mean 5.75 per patient; range 1-18). This smaller group of patients achieved a molecular response rate slightly different respect to the p190+ cases. In fact, before starting Imatinib, only 1 of the 10 cases (10%) analyzed showed a BCR/ABL copies reduction ≥ 3 log. However, differently from what observed in p190+ cases, in the remaining 9 patients, 2 relapsed at 6 and 8 months respectively, but the other 7 showed a decrease of the BCR-ABL copies that, in 2 cases fell below the 3 log reduction after 8 and 12 months, respectively, and are in CCR at > 2 years. Altogether, after a median follow-up of 6 months (range: 2-28), 8/10p210+ patients were in CCR maintained by Imatinib alone without transplant at 1, 2, 4, 5, 6, 19, 24 and 28 months, respectively. In conclusion, Imatinib is an highly effective post-consolidation treatment for adult Ph+ ALL patients being able to maintain and/or induce the minimum level of BCR-ABL expression, without the high morbidity and mortality related to transplants. In addition, in p190+ cases an unsatisfactory molecular response rate after CHT was a powerful predictor of a subsequent clinical resistance to Imatinib.

P102

A SINGLE CENTER EXPERIENCE IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Standard treatment in acute lymphoblastic leukaemia (ALL) with conventional protocols for adults and children generally do not include bone marrow transplantation as up-front therapy, except for those patients who have t(9;22) or t(4;11) or are at high risk for other factors. The cure is almost obtainable in about 25% of adults and 50% of children.

Aims. To demonstrate if the early therapeutic approach with bone marrow transplantation (BMT) is curative in a higher number of patients.

Patients and Methods. In our Centre we treated since 1993 a total of 43 patients with ALL with an age of 60 years or less, 11 patients were children. The GIMEMA for adults or AIEOP protocols for children were applied to all patients but those with high risk disease for t(9;22) or t(4;11) were addressed to BMT following consolidation therapy as up-front therapy and those without bad kariotypic characters were addressed to BMT at relapse following salvage therapy. The patients with high risk characters without available donor underwent ABMT in remission. Twenty-one patients received only conventional therapy, 9 patients at high risk for kariotypic characters or relapsed without donor received conventional therapy and ABMT, 13 patients at high risk or relapsed received BMT from family or unrelated donor.

Results. Twenty-one patients received only conventional therapy, 8 of those are in CR, 12 to 120 months from the therapy; 5 of them are children. One patient, relapsed from conventional therapy, is waiting for MUD transplantation and 11 patients died for disease progression and one for concurrent Gram- sepsis during induction. Among the 9 patients at high risk who received high dose therapy following remission and ABMT only one is alive and in CR 16 months from the procedure and 8 patients died for disease progression. Among the 13 patients who received BMT 8 patients are in CR 6 to 68 months from transplantation; 3 of them are children. The type of BMT was from matched family member in 5 patients with 4 of them in CR, matched unrelated in 6 patients and 4 of them in CR and two Aploidentical from family member, both died for infection. Comment and conclusions. Our experience clearly indicated that fully matched BMT is curative in a higher number of patients as compared to conventional and high dose therapy followed by ABMT. Due to low incidence of complications BMT should be recommended in young adult patients when a matched donor is available as up-front therapy. The BMT in children should be addressed following relapse to conventional therapy.

P103

PHILADELPHIA NEGATIVE ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA WITH TRISOMY 8 IS AN EXTREMELY POOR-RISK DISEASE: A REPORT FROM NILG (NORTHERN ITALY LEUKAEMIA GROUP)

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I*ntroduction.* Besides t(9;22) i.e. the Ph chromosome and t(4;11), few other chromosomal abnormalities are consistently associated with an adverse outcome in adult ALL (-7, +8, del6q, hypodiploidy). In a CALGB report (Blood 1999;93:3983), outcome of 10 patients with $Ph^{-/+}8$ ALL (estimated incidence 4%) was as poor as in Ph+ ALL with or without +8. We studied 6 more $Ph^{-/+}8$ ALL cases, to expand the limited knowledge on this rare ALL cytogenetic variant.

Methods. Patients with $Ph^{-/+}8$ ALL were identified within the multicentric, minimal residual disease (MRD)-oriented NILG-ALL 01/00 trial (activated may 2000). A known cytogenetic study result must be available, i.e. adequate metaphases to disclose clonal chromosome aberrations or normal karyotype on standard Q/G banding. For better prognostic evaluation, outcome (DFS, overall survival) of patients with $Ph^{-/+}8$ ALL was compared with Ph+ (reference poor-risk group) and normal karyotype (reference standard-risk group) ALL, respectively.

Results. Two-hundred and thirty-five patients were evaluable, of whom 175 (74.4%) were succesfully karyotyped. Of these, 6 had $Ph^{-/+}8$ ALL (3.4%), often in combination with other anomalies. Salient clinical features and therapeutic response including MRD course during the first 6 mos. of treatment are shown in the Table (survival times in mos.). With regard to outcome of $Ph^{-/+}8$ ALL, results were exactly superimposable to Ph+ ALL (n=44, DFS: median 12 mos. and 28% at 4 years, p=NS; survival: 15 mos. and 24%, p=NS), but significantly worse than in ALL with normal cytogenetics (n=70, DFS: median not reached and 51%; survival: 38 mos. and 49%, p=0.017). *Conclusions.* $Ph^{-/+}8$ ALL is a rare karyotypic variant of adult ALL, carrying an extremely poor prognosis. Although a morphological remission can be achieved, the disease usually persists at the molecular level, to herald further clinical progression and short survival. $Ph^{-/+}8$ ALL is a very high-risk condition for which early stem-cell transplantation is indicated, with MRD monitoring for subsequent DLI or other experimental therapy.

P104

THE ROLE OF SERIAL MRD MONITORING IN LONG-TERM MAINTENANCE WITH IMATINIB OF PHILADELPHIA CHROMOSOME/BCR-ABL POSITIVE ALL PATIENTS IN FIRST COMPLETE REMISSION

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The treatment of Philadelphia chromosome/Bcr-Abl positive acute lymphoblastic leukemia (Ph+ ALL) has a dismal prognosis either with standard chemotherapy or with the allogeneic bone marrow transplantation (alloBMT). In the last few years, imatinib mesilate has shown to induce remission in more than two third of relapsed/refractory Ph+ ALL patients, although for short time. In more recent studies, in Ph+ ALL patients treated with imatinib, serial monitoring of the minimal residual disease (MRD) has shown utility in predicting the relapse of the leukemia and in bridging the time to BMT. We report a long term complete molecular and clinical follow-up of 7 consecutive patients with Ph+ ALL in hematologic complete remission (hCR) after induction therapy, who were maintained with imatinib alone. We have evaluated whether imatinib may represent a valid option for the maintenance therapy of Ph+ ALL patients in first hCR, and if a strict MRD follow-up could individuate patients that may derive continue benefit from imatinib alone. MRD monitoring was performed by quantitative TaqMan reverse-transcriptase polymerase chain reaction (RT-PCR). The algorithms for MRD evaluation were as follow: - the "minimum BCR-ABL" level was defined as the lowest measured relative BCR-ABL level prior to an increase or identical value, - the "increased BCR-ABL" level was the highest value after the minimum (it was considered only if the sample was obatined at least 15 days prior to hematologic relapse), - the "magnitude" of increase was determined by calculating the log ratio between the "increased" BCR-ABL and the minimum, as defined above. - "persisting" mCR was defined as mCR sustained for at least three months - "short" mCR was defined as mCR detected in a single observation or sustained less than three months. The 2-years progression-free survival was 75%. The hazard rate was 0.00021617. Six out of 7 patients (86%) were still in hCR at the time of last follow-up. Only 1(14%) patient relapsed 456 days and dead 729 days after the start of imatinib. On the basis of serial PCR monitoring of BCR-ABL, the patients with log lasting response presented: a) "persisting" mCR, or b) BCR-ABL levels < 10-4 after best MRD value. Furthermore MRD analysis has shown that: 1) molecular relapse did not invariably mean hematologic relapse; 2) the wide and rapid increment of the transcipt values was the only factor predictive of leukemia relapse. In summary, single agent imatinib results useful for long-term maintenance of Ph+ALL patients, and serial BCR-ABL quantification results efficacious for a better assessment of treatment. However, several issues need to be addressed, including the optimal duration of treatment, whether imatinib may replace or support CHT and whether such a strict MRD monitoring may help to individuate which patients should delay or avoid BMT, especially in the case unavailability of a sibling donor.

P105

ACUTE LEUKEMIA FOLLOWING KIDNEY TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

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Introduction. The development of neoplasms as a consequence of immunosuppressive therapy is a significant complication of organ transplantation. The most common post transplantation malignancies are lymphoproliferative disorders (non-Hodgkin lymphoma of B-cell origin) and solid tumors. On the other hand, in literature it has been reported few cases of post transplant acute leukemias (PT-ALs).

Patients and Methods. In our series of 496 acute leukemia patients there were 7 cases affected by acute lymphoblastic leukemia (3 with T-ALL, 2 with B-ALL) and acute myeloid leukemia (1 with AML-M1, 1 with AML-M3) respectively, following kidney transplantation. Results. The PT-ALs patiens median age at the onset of leukemia was 39 yrs (range 20-50 yrs).The median latency period of PT-ALs appearance was 100 months (range 46-135 mo). Five patients (3 T-ALL, 1 B-ALL, 1 AML-M3) and two patients (1 AML M1 and 1 B-ALL) received azathioprine and cyclosporin A as immunosuppressive therapy, respectively. The median overall survival of the 7 PT-ALs cases was 3 months (range 1-39 mo.). Three (43%) patients (1 AML-M3 and 2 T-ALL) achieved complete remission but in all cases the relapse occurred after few months.The remaining 4 (57%) patients died during induction therapy. Infectious events occurred in all PT-ALs patients. Conclusion. Our study showed that PT-ALs had particular clinical features. In fact, poorly response due to chemotherapy toxicity and aggressive disease may make hard the management of PT-ALs. Further studies may clarify whether this subset of patients needed of a tailored induction treatment.

P106

REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION OF IGH AND TCR REARRANGEMENTS FOR THE MESUREMENT OF MINIMAL RESIDUAL DISEASE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Molecular investigations of minimal residual disease (MRD) levels and the dynamics of MRD in childhood acute lymphoblastic leukaemia (ALL) has proven superior to other standard criteria (age, sex, and WBC) for identification of patients at high, intermediate and low risk of relapse. To date clinical implications of MRD in adult ALL patients has been less widely investigated. In this study we explored the applicability of IGH and TCR gene rearrangements as targets for MRD detection by real time quantitative PCR analysis (RQ-PCR).

Patients. We included 19 ALL patients (B-lineage, n=15 and T-lineage, n=4, median age 33, range 18-54 years), recruited at ours institutions in whom an unique IGH or TCR gene rearrangement was identified at the diagnosis. Bone marrow aspirates for MRD study was performed at the diagnosis, at the end of induction therapy, at the end of consolidation therapy and during the follow-up.

Methods. RQ-PCR assays was performed on a ABI 7900 platform (Applied Biosystems). Patient specific primers and probes were designed spanning the V-D-J junction regions (Pimer Express software packages , Applied Biosystems), choosing the probe positioned in the tumour-specific sequence at the junction region (ASO probe approach) according to minor groove-binding (MGB) technology, to increase specificity. Sensitivity and accuracy of patient's specific MRD assay was determined by serial dilutions of known amounts of diagnostic DNA in normal control DNA, documenting a sensitivity 10-4, often 10-5 in all patients. Reproducibility of the method was confirmed by comparing results obtained from triplicate samples. We used a Relative Quantification method by comparing target and control genes cycle threshold (delta delta Ct). A single copy Telomerase gene was selected as control gene.

Results. Eleven patients were evaluable for the outcome analysis (median follow up 18mo, range 7-54) while eight were excluded because they either did not achieve complete remission (CR) or had a follow up shorter than 3 months. Five pts who showed a reduction of MRD level >3 log after induction or consolidation-therapy, all are in CR at a mean follow-up of 12 months. One patient achieved a 3 log reduction only after consolidation therapy but increasing level of MRD at 14 months from the diagnosis and relapsed four months later. Five pts had a lower rate decrease therefore they were considered bad responders: one patient received an allogeneic bone marrow transplantation six months after diagnosis and achieved a >3 log reduction after transplant, the others four pts showed a persistent and increasing level of MRD during the follow-up and subsequently 3 out of 4 relapsed. Conclusion: Our preliminary findings point toward a possible use of RQ-PCR for prospective risk-assignment also for adult ALL, however the ASO-probe strategy is very expensive and time consuming. Thus we are now planning a cheaper and more feasible approach by using ASO-primer and SYBR green detection for RQ-PCR.

P107

IMPACT OF UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANT (CB AND BM) ON PATIENTS WITH HIGH-RISK A LYMPHOBLASTIC LEUKEMIA: A PROSPECTIVE STUDY BASED ON THE INTENTION TO TREATMENT ANALYSIS

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Patients with Acute Lymphoblastic Leukemia (ALL) with high-risk features at diagnosis or in second remission after

an early relapse are usually candidates to be treated with an allogeneic stem cell transplant (ASCT). However, the need for a suitable HLA identical sibling restricts the ASCT to a limited number of patients. Both umbilical cord blood and bone marrow from volunteer donors represent a stem-cell source for those patients who lack a family HLA compatible donor. Herein, we report long-term results of a prospective single center study on outcome of 112 patients with high-risk ALL, aged 1-20 years and lacking an HLA identical sibling, for whom the search for an unrelated donor was simultaneously addressed towards the International Bone Marrow Donor Registries and the Cord Blood Banks worldwide, between April 1995 and February 2004. The close out date for the analysis was November 30, 2004 . At the start of the search , 29 patients with high risk ALL were in first complete remission (CR), 56 in II and 4 in >III CR , the other 23 patients were in more advanced phase of disease. A cord blood unit was found for 87 patients (77%) at a median time of 26 days (range 1-284) from the beginning of the search . A suitable identical bone marrow donor was identified for 44 patients at a median of 90 days (range, 30-365). After a median time of 5 months (range 2-58) from start of search, 50 out of 112 patients (44%) underwent a transplant: 37 from cord blood, 12 from a volunteer bone marrow donor and 1 patient from an haploidentical relative donor. At a median follow-up of 12 months (range 1-108), 33 out of 112 patients are alive (29%) and 79 died. The major cause of death was disease progression occurring prior to transplant in 46 patients at a median of 4 months (range 1-30) from the start of search. For the whole population,the 9 -year probability of survival, relapse and leukemia-free-survival (LFS) was 24%, 68% and 21%, respectively. By censoring the grafted patients at time of transplant, the 9-year probability of survival , relapse and LFS were 18% , 78% and 13% , respectively. The differences between the actuarial curves with patients censored and not censored at transplant were not statistically significant.

Conclusions. the policy of search for an unrelated donor has a positive impact on outcome of young patients with high risk ALL, even though a higher number of patients is required in order to find a significant difference between transplanted and not transplanted patients. Since the risk of death related to the disease progression for this patients' population is particularly high, ASCT must be performed as soon as possible with either cord blood or bone marrow stem cells according with the current criteria for donor selection.

P108

MINIMAL RESIDUAL DISEASE DETECTION BY FLOW CYTOMETRIC ANALYSIS CAN PREDICT OVERT HEMATOLOGICAL RELAPSE IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Multiparameter flow cytometric analysis (FCA) of acute leukemia has been recognized as a powerful tool to define antigen expression pattern of blast cells at diagnosis as well as for monitoring of minimal residual disease (MRD). When appropriate combinations of immunomarkers are used, mainly based on the presence of aberrant leukemia-associated phenotypes, and a great number of events (more than 100.000) is acquired, flow cytometric technique may in fact result much more sensitive and effective than classical morphological examination in detecting very few numbers of blast cells; however, the role of such findings in predicting relapse is still unknown. Moreover, in acute lymphoblastic leukemia (ALL), flow cytometric detection of MRD is more difficult, as aberrant phenotypes are less frequent and discrimination of blasts from normal B-cell progenitors (hematogones) is often hard. With these premises, we investigated the usefulness of this analysis method in detecting MRD and its capability to give early information about relapse in ALL patients. In our institution 56 patients (pts) with acute lymphoblastic leukemia (B-ALL: 48, T-ALL: 6, biphenotypic-ALL: 2) were enrolled from 1996 to 2004. However we considered for this study only 46 patients, for whom flow cytometric data were obtained throughout follow up. There were 39 B-ALL, 5 T-ALL and 2 BAL. 29 pts were males, 17 females. Median age was 25.5 yrs (range:1.5-79). Karyotypic analysis showed chromosomal abnormalities in 18/32 valuable cases: t(9;22) in 8 pts, t(4;11) in 1 pt, t(8;14) in 2 pt and t(8;22) in 1 pt with evidence by FISH of c-MYC disruption, t(1;19) in 1 pt. 5 pts had complex karyotypes. Molecular analysis of BCR/ABL resulted positive in 11/34 valuable pts: therefore, 3 pts were found positive despite karyotypic absence of Ph1+ (probably because of insufficient metaphases). Three-four color flow cytometric analysis was performed based on SSC/CD45 gating strategy and MRD was investigated mainly by searching the so-called MRD-phenotypes: iperexpression of CD10, lineage-infidelity markers: CD19/CD13, CD19/CD33, CD19/CD56, asynchronous expression: CD20/CD34 and ectopic expression as cyCD3/nTdT; also CD45 intensity was useful as many B-ALL blasts resulted almost CD45 negative; finally, MRD analysis took into account the antigenic expression pattern exhibited by blasts and compared to normal B-precursors. The patients received induction treatment according to different protocols: overall, 41/46 pts (89%) obtained complete remission, 5 pts being resistant to treatment (3 of them carrying Ph1+). However, only 15 of 41 pts (36.5%) remained in continuous complete remission (CCR) with a median follow up of 26 months (range 6-66), while 26 pts (63.4%) finally relapsed, with a median time to relapse of 11months (range:5-60). Importantly, BCR/ABL status was different in the two groups: 1/15 (6.6%) in CCR pts, 7/26 (27%) in relapsed pts. In CCR pts, FCA analysis during follow up resulted constantly negative in 13/15 pts: amounts of 0.06% and 0.2% of blasts have been recently found (1-2 months ago) in 2 pts in the absence of other signs of disease; too short follow up prevents us from any hypothesis about incoming relapse. More interestingly, in relapsed patients, flow cytometry showed little amounts of blasts (ranging from 0.02% to 3%) in 11/26 pts (44%) about 1 month (range 1-5) before overt hematological relapse; in those pts, also karyotype became positive at the same time, while morphological examination of bone marrow smears showed no evidence of disease. We therefore suggest that

even very low numbers of blasts revealed by flow cytometric analysis with at least 100000 acquired events, could predict overt relapse of disease in a good proportion of cases and that immunophenotypic results agree with karyotypic data to this regard. Early recognition of incipient relapse may in turn affect clinical decision about different therapeutic options.

P109

INTERPHASE FISH ANALYSIS REVEALS HIGH FREQUENCE OF PARTIAL 6Q DELETIONS IN ADULT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIAWITH NORMAL OR FAILED KARYOTYPE

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On behalf of the GIMEMA Cooperative Study Group

Karyotypic analysis in adult ALL has provided relevant insights in the prognostic significance of the chromosome abnormalities, which in turn become of potential importance for the design of risk-adapted therapeutic strategies for newly diagnosed cases. However, the detection of chromosome abnormalities may be unsuccessful in 20-25% of ALL patients due to the technical difficulties associated with conventional chromosome analysis. Among structural karyotypic changes, partial deletions of the long arm of chromosome 6 (6q) are reported to be between 4 and 6% of cases of adult ALL by conventional cytogenetics and depict a subset of patients with strict correlation with T-lineage ALL and a not favorable prognosis. Recent studies at the molecular level in childhood ALL showed 6q deletions in up to 30% of investigated cases. With the aim to more precisely define the exact incidence of 6q deletions in adult ALL, we started to investigate by interphase FISH patients with T-ALL, showing normal or failed karyotype, included in the GIMEMA LAL 2000 protocol. This trial contemplated a central handling of bone marrow samples of the enrolled patients at presentation. We identified the following bacteria-derived artificial chromosome with a know map location within the most common deleted regions of 6q, which were obtained from Children's Hospital Oakland Research Institute (CHORI): 90G9 (6q13), 12B13 (6q15), 465M14 (6q21), 81H16 (6q26), 91016 (6q27). Fifteen consecutive patients were analyzed so far by interphase FISH, of these 10 had a normal karyotype, 5 a failed cytogenetics. As expected, all cases were negative for the B-lineage ALL associated fusion genes, 1 case showed a SIL-TAL rearrangement. Among the cases with normal karyotype, we detected 1 case with a deletion in 6q21 region, whereas in the group with failed cytogenetics 2 cases with deletions spanning respectively 6q15-21 and 6q13-6q21 regions were identified. Overall, partial 6q deletions were observed in 20% of analyzed cases. The present study shows that the probe set we have developed is a useful tool for providing accurate cytogenetic diagnosis of partial 6q deletions and suggests that this abnormality could be a frequent karyotypic change in adult T-ALL. Interphase FISH analysis for 6q deletions should be included in the routine genetic screening of the T-ALL patients with failed or normal cytogenetic analysis.

Chronic Lymphoblastic Leukemia and Lymphoproliferative Syndromes I

P110

COMBINATION OF DHAP AND CAMPATH-1H FOR HIGH-RISK, FLUDARABINE-PRE-TREATED B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: HIGH RESPONSE-RATE BUT LOW FEASIBILITY OF CONSOLIDATION AUTROGRAFT AND LACK OF MOLECULAR REMISSION

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Purpose. To investigate a novel intensive chemo-immunotherapy approach to high-risk, fludarabine pre-treated B-cell Chronic Lymphocytic Leukemia (B-CLL) patients.

Patients and Methods. Ten patients with resistant/relapsed, advanced stage B-CLL (7 with fludarabine-refractoriness and 3 with advanced-stage disease after multiple relapses) were included in this pilot study. Age range was 37-60 years (median 53). All but one had an unmutated IgVH status. The treatment schedule included debulking with 2 DHAP courses followed by Campath-1H (30 mg, 8 doses); subsequent peripheral blood progenitor cell (PBPC) mobilization with intermediate/high dose cyclophosphamide and final autografting after high-dose Mitoxantrone+L-Pam were planned.

Results. The DHAP-Campath-1H combination was highly effective, with an overall response in 9 patients, 5 of them achieving Complete Remission. Following Campath-1H, PB double positive clonal CD5+/CD19+ lymphocytes dropped, with median purification rate 99.95%. Due to poor PBPC mobilization, only 5 patients underwent autografting, 3 of these experienced post-graft recurrence. Molecular evaluation also revealed persistence of minimal residual disease, assessed by PCR, both in all PBPC collections tested and in post-treatment follow-up sample.

Conclisions. The use of DHAP/Campath-1H appears useful to re-induce disease remission in relapsed/refractory, high-risk B-CLL patients. However, the addition of autograft was not usually feasible and of questionable clinical use. Other strategies should thus be considered for remission maintenance.

P111

CORRELATIONS BETWEEN PROGNOSTIC MARKERS EXPRESSION AND FUNCTIONAL FEATURES IN B-CLL CELLS

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Previous studies on small number of patients revealed a correlation between CD 38 expression by B-CLL cells and in vitro apoptotic capacities, suggesting that the cell from the CD 38-positive B-CLL subset were in a more active status already *in vivo*. The present study explored the correlations existing between CD38, ZAP-70 expression and Ig VH gene status, on one hand, and the apoptotic capacities of the neoplastic cells, on the other, in a cohort of 82 B-CLL patients. These correlations also were investigated in cell fractions separated from single leukemic clones, based upon the density of their surface CD 38. The CD 38, ZAP-70 and Ig VH gene status were in the expected concordance in 70-90% of cases depending upon the combinations considered. In contrast, a looser correlation was detected with the cell apoptotic capacities; the magnitude of spontaneous apoptosis in vitro correlated with the levels of ZAP-70 and the absence of Ig VH gene mutations., while that induced by surface IgM cross-linking correlated significantly with CD38 expression. The cell incapacity to be induced into apoptosis was only in part dependent by low expression of surface IgM or faulty signal transducing capacities by surface IgM. Strikingly, in the cell fractions from single clones, a close correlation was observed between the amount of CD38 and ZAP-70 expressed by each fraction as well as between the expression of these two markers and the apoptotic capacities, both spontaneous or induced, or the viability of the IgM-dependent signal transducing pathway. This suggests that the two markers not only are indicators of the cell activation status, but may participate in cell stimulation. The difficulties in detecting the same correlations in unfractionated B-CLL cells from a large cohort appear related to a clonal heterogeneity, which is possibly greater than previously expected. This heterogeneity is discussed for its relevance in the disease pathophysiology and for the difficulties it may pose for the precise prognostic marker assessment.

P112

ZAP-70 EXPRESSION IN B-CLL PATIENTS - STAGE 0 RAI - WITH STABLE DISEASE FROM AT LEAST 10 YEARS

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Introduction. ZAP-70 is a 70 KDa signal trasductor protein with cytoplasmatic localisation usually expressed in T and NK cells. Signal transduction is promoted by TCR activation by antigen linkage. Usually B lymphocytes did not present ZAP-70, but sometime Chronic Lymphocitic Leukemia (CLL) cells may express this protein. Particularly, the CLL subtype with unmutated IgVH genes frequently correlates with ZAP70 overexpression. Considering that unmutated IgVH LLC has more aggressive features respect to mutated IgVH-LLC, many Authors retain that ZAP-70 seems to be a good surrogate for gene sequencing and its expression is associable to worse prognosis; otherwise ZAP-70 absence would correlate with a good prognosis. In order to confirm this last aspect we evaluated ZAP-70 expression in patients with long term stable CLL.

Patients and methods. We retrospectively analysed 25 B-CLL patients with stage 0 disease (according to Rai classification) stable from at least ten years (median time of follow-up: 14 years; range: 10-26 years). ZAP-70 expression in B-cells cytoplasm has been evaluated with immunophenotyping methods. An antibodies panel including CD19 and ZAP-70 has been tested on lymphocytes gate. Analysis of mononucleated cells has been obtained from peripheral whole blood. Double expressions CD19/ZAP-70 were measured in these patients; ZAP-70 has been tested after membrane permeabilization with "Fix and Perm" kit (Caltag laboratory). B-CLL with more than 20% of CD19 cells co-expressing ZAP-70 has been considered ZAP-70 positive CLL.

Results. 23/25 patients with stable B-CLL (92%) resulted ZAP-70 negative, 22 of them showed a minimal ZAP-70 expression (ranging from 0% to 5%), only 1 showed a borderline value of 17%. Only 2/25 patients with stable B-CLL were ZAP-70 positive (8%): one is still stable after 12 years of follow up, and one is actually in disease progression after nearly 10 years in stage 0. Conclusion: ZAP-70 negativity really seems to characterize patients with good prognosis CLL. Our observation confirms in fact that patients with stable disease and ZAP-70 negativity have a good outcome. Prospective and comparative studies between patients with different stage of B-CLL and different clinical outcome needs to confirm the real prognostic impact of this marker.

P113

DIAGNOSTIC POTENTIAL OF CD38 AND ZAP-70 EXPRESSION IN PREDICTING MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Impressive progress has been recently made in defining biological determinants of risk in chronic lymphocytic leukemia (CLL) patients. The best-studied parameters are somatic hypermutation of the immunoglobulin heavy-chain variable region (VH), expression of the cellular proteins CD38 and ζ-associated protein 70 (ZAP-70). Originally, a correlation was observed between the VH mutation status and CD38 expression of the CLL cells. More recently, the expression of ZAP-70, as detected by flow-cytometric analysis, was demonstrated to be strongly associated (sensitivity 91%, specificity 100%) to CLL cells with unmutated VH genes. In this study, we analyzed the mutational status of the VH genes (samples in which < 2% of base pairs differed from those of the consensus sequence were considered unmutated) in CLL cells from a series of 135 cases and correlated the results with CD38 expression detected by flow-cytometry and ZAP-70 using Western blotting. To determine the approximate percentage of ZAP-70 positive cells in a sample, we mixed different concentrations of purified T cells with mononuclear cells from the same healthy blood donors depleted of T cells and NK cells. The areas of the band corresponding to ZAP-70 in the western blot was measured and compared to that of B-CLL cells. The CLL samples were divided into three groups of ZAP70 expression: strong (100%-40% positive cells), weak (<40%-20%) and negative (<20%). For the purpose of this study, samples showing a negative and weak ZAP-70 patterns were collectively analysed. As a first step, we determined, by ROC curve analysis, 7% as the best cut-off value of CD38 which discriminates between mutated and unmutated CLL cases (area under the curve 0.786, p<0.0001). Moreover, the usefulness of CD38 and ZAP expression in identifying VH mutational status was analysed according to the following standard diagnostic tests: sensitivity and specificity, positive and negative predicted values and accuracy, as well as by Kappa statistic. On the basis of standard diagnostic tests, CD38 expression, categorized by 7% cut-off value, had high sensitivity (85%) and high negative predictive (80%) value, while specificity (60%) and positive predictive value (68%) were relatively low in anticipating VH mutational status. Moreover, Kappa statistic revealed that the agreement between CD38 expression and VH mutational status was low although significant (K=0.45, p<0.001). On the other hand, ZAP-70 showed the best combination of diagnostic tests for VH mutational status (sensitivity, 78%; specificity, 91%; positive predictive value, 90%; negative predictive value, 81%) and a significantly high K statistic (0.69, p<0.001). Finally, we combined the value of both tests to evaluate whether CD38 improved diagnostic informational to that obtained from ZAP-70 test in estimating VH mutational status. In this regard, we obtained the following results: sensitivity, 69%; specificity, 97%; positive predictive value, 96%; negative predictive value, 76%; k statistic 0.66, p<0.001). In conclusion, our data demonstrated that ZAP showed the best combination of diagnostic tests in anticipating VH mutational status. The addition of CD38 test failed to improve the predicting model.

P114

SAFETY AND EFFICACY OF LOW-DOSE SUBCUTANEOUS CAMPATH-1H THERAPY IN PATIENTS WITH REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA

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B-CLL patients have a poor prognosis after failing alkylators and purine analogues therapy. We utilized the monoclonal antibody alemtuzumab (Campath 1-H) as salvage therapy for 16 symptomatic patients with heavily pretreated and refractory CLL. As alemtuzumab may cause substantial "first-dose" reactions when given i.v., the drug was delivered subcutaneously and, in order to further minimise adverse therapy-related effects and make the treatment more manageable, a low dose of 10 mg was administered three times a week for a prolonged period of 18 weeks. Fourteen of the patients treated in this phase II study were refractory to both alkylators and fludarabine (of whom eight were also refractory to rituximab-containing regimens), and two were refractory to chlorambucil and had presented autoimmune haemolytic anemia (AIHA) that precluded fludarabine therapy. An overall response rate (OR) of 50% (95%CI, 24.6-75.3%) was achieved, including 25% complete responses (CR). CLL cells were completely cleared from blood in 87.5% of patients in a median time of 7.5 weeks. A 1-log depletion of peripheral blood lymphocytes, as assessed by a routine haematological analyser, occurred in 15 patients after a median of six weeks of therapy (range 2-17); this reduction in PBL was achieved by non-responding patients after a median of eight weeks of therapy and by responding patients after a median of three weeks (respectively 2.5 and 6 weeks in CR and PR patients). A 62.5% OR, including 31.3% CR and 25% nodular partial response was achieved in the bone marrow in a median time of 15 weeks. An objective response in lymphnodes and spleen was obtained in respectively 50% and 42.9% of the patients. The responses were substantial even in patients with unfavourable cytogenetics, rituximab refractoriness, Rai stage IV, previous infections, and age of over 65 years. Eleven patients, including seven out of 8 responders, are alive after a median follow-up of 15 months, giving an overall survival of 68.8%; the median time-to-treatment failure was 10 months (range 2-21). It is worth noting that there were no systemic side effects, except transient fever in 25% of the patients, while short-lived grade I-II injection site skin reactions were seen in 56%. Grade III-IV neutropenia developed in one-third of the patients, but severe infections were uncommon during Campath treatment (12.5%). During the follow-up 11 patients developed major infections (5 pneumonia, 2 FUO, 1 otitis, 1 perianal abscess, 2 septicemia), four of which were lethal. Asymp-

tomatic on-therapy CMV reactivation was documented in only 2/16 patients. No hepatic flares were seen in two HBsAg-positive patients treated with alemtuzumab in combination with reverse transcriptase inhibitors. Reactivation of immune haemolysis was observed in one patient with previously documented AHIA, and de novo AHIA was documented in another patient, respectively five and fourteen months after therapy start. Subcutaneous low-dose alemtuzumab is effective in patients with poor prognosis B-CLL, has a particularly favourable toxicity profile, and its cost-effectiveness is appealing. This therapeutic approach appears to be more convenient for patients and physicians, enables the home administration of the antibody, and reduces health care costs in comparison with intravenous infusion or full-dose subcutaneous administration.

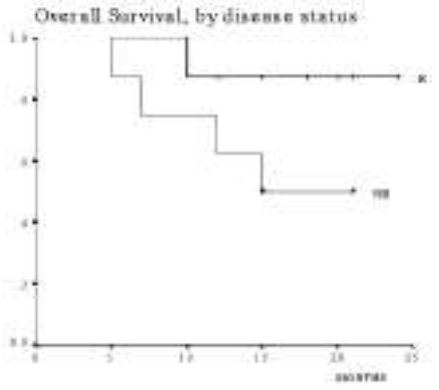

Table 1. Toxicity (%) during low-dose subcutaneous treatment with alemtuzumab in 16 patients with refractory B-CLL.

Type	WHO grading	
	I-II	III-IV
Local injection site reaction	50.1	0
Fever	25	0
Anemia	50	6.3
Neutropenia	31.2	31.2
Thrombocytopenia	6.3	12.5
Infection	12.5	12.5
CMV reactivation	12.5	

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RITUXIMAB AS SINGLE-AGENT IN INDUCTION AND MAINTENANCE THERAPY IN ELDERLY, PRETREATED AND POOR PERFORMANCE STATUS PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA: CASES REPORT

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Waldenstrom's macroglobulinemia (WM) is a low-grade lymphoproliferative disorder, in which CD20 is usually expressed on the cell surface and is characterized by a serum monoclonal immunoglobulin M and monoclonal lymphoplasmacytic expansion in the bone marrow. WM is a indolent lymphoma treatable but incurable and long-term survival is possible. The disease affects older patients and symptoms may be caused by anemia, lymphadenopathy, splenomegaly, trombocytopenia, hyperviscosity, cryoglobulinemia. The presence of symptomatic disease indicates the need for treatment. The level of serum monoclonal protein is not parameter to initiate treatment. Patients with asymptomatic WM should not be treated, because disease may remain stable for years. Systemic standard treatments with single agents are: chlorambucil, melphalan, cyclophosphamide, fludarabine, cladribine; also polichemotherapeutic regimens are used. Several studies have suggested that the anti-CD20 monoclonal antibody rituximab has activity in this disease. Starting from these considerations initiation of treatment needs to carefully balance the risks and benefits. We report three cases of WM treated with rituximab with these characteristics: symptomatic disease (anemia, lymphadenomegaly, splenomegaly, cryoglobulinemia), chemotherapeutic pretratment, progressive disease, 2 patients older than 70 years and with cardiac disease, 1 patient with history of acute hepatitis after HCV reactivation following chemotherapy CHOP and in treatment with interferon and ribavirine. Although the patients needed of chemotherapeutic regimens, these characteristics don't permitted aggressive treatments. Therefore all patients were treated with rituximab at a dose of 375 mg/mq administered intravenously at weekly intervals for 4 consecutive weeks. Patients were restaged for response and those with objective response or stable disease received maintenance rituximab courses, identical dose and schedule, at 1-month intervals for a maximum of 12 courses or until progression. Rituximab was administered by slow infusion following standard guidelines.

Results. after induction treatment all patients achieved objective response, 2 complete and 1 partial responses. Two patients with complete responses had a disappearance of the monoclonal protein by immunofixation, resolution of lymphadenopathy and splenomegaly, less than 20% lymphocytes in the bone marrow. One patient had partial response because don't achieved a complete disappearance of the monoclonal protein by immunofixation but only a reduction greater than 50% and resolution of lymphadenopaty. After 12 maintenance rituximab courses all patients remained free of progression. At present two patients remain alive and objective response is stable after 24 and 16 months respectively; one patient died because of

other disease after a complete response of 14 months. Rituximab therapy was well tolerated and was not associated with myelosuppression; no adverse events were encountered during the first treatment course. This agent is active, has low-grade toxicity and might be associated with a long period without other treatment in elderly, pretreated and poor performance status patients with WM.

P116

FLUDARABINE PHOSPHATE IN THE TREATMENT OF PATIENTS WITH REFRACTORY/RECURRENT B-CELL CHRONIC LYMPHOCITIC LEUKEMIA (B-CLL): RETROSPECTIVE ANALYSIS OF INDUCED TOXICITY AND ITS ROLE IN HEPATITIS B VIRUS REACTIVATION

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Fludarabine phosphate is currently used as monotherapy in the first-line treatment of B-cell Chronic Lymphocitic Leukemia (B-CLL) but it is even a drug consolidated for second-line therapy and however utilized in all pretreated cases with disease progression. PURPOSE This retrospective study analyzed two groups of patients (in all sixteen) previously treated with different drugs and chemotherapeutic protocols (Chlorambucil; Chlorambucil+Prednisone; Cyclophosphamide+Vincristine+Prednisone) which received fludarabine phosphate as monotherapy in disease progression (between the years 2000 and 2004). Some patients (I group) have been treated with intravenous fludarabine phosphate, others (II group) with the oral formulation. The aim of this study is to obtain significant information about the drug effects in the two different formulations and about induced toxicity.

Patients Aand Methods. Half patients (eight) received intravenous fludarabine phosphate in dosage regiment of 25/mg/m^2/daily for 3-5* consecutive days repeated every four weeks for a total of 2 to 6 cycles**; the other half of patients treated with oral fludarabine phosphate to a dose of 40 mg/m^2/daily for five consecutive days, every four weeks, for a total of 3 to 5 cycles. * Four patients made cycles for three days. ** Patients who completed only two cycles observed the stop-therapy because serious adverse events and hematological toxicity arose. The age of patients is included between 55 and 85 years (median age at diagnosis was 70) with intermediate/high Rai Staging System disease (from II to IV).

Results. The response rate to therapy was assessed using *International Workshop on Chronic Lymphocytic Leukemia* (IWCLL) and *National Cancer Institute* (NCI) criteria. The monitoring information in terms of Overall Response (OR), Complete Remission (CR), Partial Remission (PR) and No Remission (NR) was for the two groups of patients and for the two institute respectively: Tabel 1 Best results of OR achieved with the use of oral formulation of fludarabine compared to the intravenous formulation may be due partly by a less hematological toxicity, as myelosuppression induced by the drug on erytropoietic compartment. This remark comes out by a detailed observation of hematological toxicity (assessed according to WHO-Grading) in the two groups of patients. In the study of patients treated with intravenous fludarabine phosphate, hematological toxicity (according to WHO) reported in 50% of cases is of intermediate/high grade (from II to IV) including anemia, granulocytopenia, thrombocytopenia that required a support therapy with the use of red blood cell transfusions and grouth factors (G-CSF and Erythropoietin) in 37,5% of patients. In the study of patients treated with oral fludarabine phosphate, hematological toxicity (according to WHO) reported in a high rate of cases (75% of patients) is included from I to IV grade: granulocytopenia, WHO grade IV, resulted in the same rate as that secondary to the therapy with intravenous fludarabine phosphate (37,5% of patients also in this case all treated soon after with G-CSF); anemia of WHO grade II which required red blood cell transfusion occured only in 12,5% of cases (one patient). The toxicity induced on a bone marrow granulocytic component is expressed by grade IV neutropenia with the same proportion in the two groups of patients. So a more toxicity on the eritropoiesis resulted in the group of patients treated with intravenous fludarabine phosphate (anemia of WHO grade III in three out of eight patients with hemoglobinic level which required transfusions of concentrated erythrocytes) than those treated with oral formulation. As regards the lower rate of Overall Response achieved in the group of patients treated with intravenous fludarabine phosphate, it is necessary to consider also an incidence (25%) of appeared diseases such as heart disorder in a case and respiratory disorder in the other so it was impossible to continue the therapy (Atrial Fibrillation and Acute Bronchopulmonary Process due to an opportunistic infection). A significant information, obtained by the analysis of patients treated with oral fludarabine phosphate, is represented by the toxicity as possible immunosuppressive effect induced by the drug. These adverse events secondary to the immunosuppression are presented in two cases with previous infection by Hepatitis B Virus (HBsAg-negative, HBsAb-positive and HBcAb IgG-positive patients before the treatment with fludarabine) in which occurred a change in a replicative stage of HBV. In both of these cases the event was tested by the research of HBV-DNA positiveness that resulted "qualitative" and "quantitative" using the "Polymerase Chain Reaction" (P.C.R.) technique. A patient had an early stage of chronic infection by HBV with pannel of typical markers (HBsAg-positive, HBeAg-positive and viraemia in PCR of 1.800.000 copies/mL) and a normal transaminasemia, which denoted a preclinic diseased. In this situation a therapy with Lamivudine (100mg/day) is started. The other patient had an evident form of acute hepatitis B (with grade III hyperbilirubinemia and grade IV hypertransaminasemia according to WHO-Grading) and a subsequent spontaneous recovery. These events could be ascribed to a iatrogenic pathogenesis and must be considered important in terms of incidence (25%) and seriousness in relation to a small number of patients analyzed in this study.

Conclusions. Fludarabine showed a clinical efficacy in oral formulation producing a high Overall Response rate (OR = IWCLL: 87,5% NCI: 75%) and generally a good tolerability, so it always represents an important choice in the treatment of refractory/recurrent B-CLL. However it is recommended precautions in the use of fludarabine evaluating with care the patients affected by B-CLL with HBV previ-

ous infection (a condition represented not only by HBsAg-positive cases, but also by HBsAg-negative with HBsAb-positive and HBcAb-positive cases). In fact the immune system suppression induced by the drug could predispose to a reactivation with Hepatitis B Virus replication. So it is necessary to monitor HBV-DNA and to assess a prophilaxis with Lamivudine, since other researchs (about indolent non Hodgkin's lymphoma) remarked in some cases the emergence of HBV genomic mutations which cause resistance to this drug in immunocompromised patients.

Table 1.

		Intravenous FP		Oral FP	
		IWCLL %	NCI %	IWCLL %	NCI %
Overall Response	**(OR)**	62,5 (5 p)	50 (4 p)	87,5 (7 p)	75 (6 p)
Complete Remission	**(CR)**	12,5 (1 p)	12,5 (1 p)	25 (2 p)	25 (2 p)
Partial Remission	**(PR)**	50 (4 p)	37,5 (3 p)	62,5 (5 p)	50 (4 p)
No Remission	**(NR)**	37,5 (3 p)	50 (4 p)	12,5 (1 p)	25 (2 p)

p = patients

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FLUDARABINE AND CYCLOPHOSPHAMYDETHERAPEUTIC COMBINATION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA : EARLY AND LATE COMPLICATION

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Purpose. Since F+C schedule is recognized as an extremely active regimen, this association is now indicated by some authors as a gold standard for the management of first-line therapy in B-CLL. However its toxicity profile refrains from its unanimous use in untreated cases. With the aim to add informations on the toxicity profile of F+C schedule, we report our experience on B-CLL patients treated in different disease phases.

Patients and Methods. Thirthy-three patients, 20 males and 13 females, with a median age of 60 years received this treatment. Six cases (18%) were untreated, while the remaining 27 (82%) were relapsing/resistant. Among patients belonging to relapsing/resistant group, 14 cases received the F+C combination after the first and 13 after the second relapse, respectively. Moreover, 23 were treated with chlorambucil (CLB) as first line, 2 with F and 2 with CNOP, while 9 of these cases received F, 2 CNOP, 1 CLB and 1 C as second line treatment. Eighteen CLB responding cases received a maintenance therapy with the same drug given bi-weekly at a dose of 5-15 mg/day. In the F+C combination, F at a dose of 25 mg/m^2 and C of 250 mg/m^2 X 3 days were administered every 4 weeks. The median number of F+C cycles was 4, range 2-6.

Results. Two out of 6 untreated cases did not complete the therapeutic program. The first case withdrew treatment because of a FUO grade 4 experienced at second cycle. The second one as a consequence of prolonged cytopenia (lasting over six months), occurring after third cycle: bone marrow biopsy showed severe hypoplasia with histological signs of minimal residual disease. Out of 9 relapsing/resistant patients who underwent therapy without CLB maintenance, only two cases experienced haematological and/or extrahaematological toxicity grade 3-4. Finally, out of 18 relapsing patients who received F+C after CLB induction and manteinance, a haematological toxicity grade 3-4 and infectious complications grade 3 have been recorded in 61% and 44%, respectively. Notably, 3 s-MDS, 1 s-AML and 1 severe hypoplasia with a bone marrow picture of complete response have been observed in this latter group. All these 5 cases, but one, underwent to F+C program at the first relapse and the maintenance therapy with CLB ranged from 18 to 50 months and all, but one who did 5, received 6 cycles of F+C. Also, a case of Richter transformation has been registered in the group of relapsing patients undergoing CLB maintenance. Conclusion: Based on the current data, an important and not negligible early toxicity has been observed also when the F+C schedule has been administered as front-line therapy. But the maximum alert must be kept on late toxicity given by this association, because the combination of potent immunosoppressive agents and inhibitors of DNA repair as F with DNA damaging drugs as CLB and C, can produce a higher occurrence of s-MDS and s-AML.

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A PECULIAR B-CLL SUBSET, FREQUENTLY IGVH HYPERMUTATED, IS IDENTIFIED BY A SIMPLIFIED IMMUNOPHENOTYPE-FISH DIAGNOSTIC ALGORITHM

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We have evaluated the clinico-diagnostic role of a new algorithm in a group of CD5+ B-cell chronic lymphoid leukemias. The algorithm was based on fluorescence in situ hybridisation (FISH) detection of t(11:14) and on our recently presented immunophenotype classification (IC). IC can recognise classic B-cell CLL (cluster 1, C1), mantle cell lymphoma (MCL; C3) in leukemic phase and situations (SIgbright/CD5+/CD23+ or +/-) with peculiar prognosis and/or clinical behaviour; these last ones, previously defined as CLL-variant (C2), are here more completely analysed. CD5, CD23, secondary markers reactivity, and SIg intensity expression were evaluated in 69 C2-C3 cases. FISH for the presence of t(11:14) (q13;q32) was performed in all the patients; 20 pts was also analysed for immunoglobulin (Ig) heavy (H) chain variable (V) mutations. Forty-two out of 69 C2-C3 cases were re-classified as true variant CLL (C2-vCLL) and 27 as true MCL (C3-MCL). Reactivity for CD20, CD79b, CD1c, CD49d and FMC7, age, frequency of prevalent splenic involvement, degree of lymphocytosis, atypical cytomorphology and karyotype, serum LDH and beta-2 microglobulin levels,

in vCLL were more similar to C3-MCL than to a comparison group of 150 C1 cases. A mutated pattern, in the absence of a heavily biased use of VH genes, was detected in all of the 20 genetically evaluated C2-vCLL cases. C2-vCLL patients had a significantly shorter time to treatment ($p< 0.001$) and a borderline significant worse overall survival than the CLL comparison group, regardless of their IgVH mutational status. In conclusion the proposed combined phenotype-FISH diagnostic approach can identify a subset of CD5+ B-CLL with a distinct phenotype, particular clinico-ematological presentation, more aggressive clinical course regardless of their hypermutated IgVH status and the absence of an unfavourable karyotype.

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CD5 NEGATIVE CHRONIC B CELL LEUKEMIAS: A POLYCENTRIC RETROSPECTIVE STUDY

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CD5 negative chronic B-cell lymphoid leukemias (B-CLL) are disorders relatively uncommon, biologically, cytomorphologically and clinically heterogeneous; moreover histo-pathological definition is often missing for absence of lymph adenopathy. We here describe a retrospectively reviewed large group of these situations. We have evaluated 156 patients affected by CD5-CD10 negative B-CLL (median age 66; 39-86) for clinico-hematological features at diagnosis, follow-up, overall survival and variables related to prognosis were identified. Hairy cell leukemias and large cell lymphomas in leukemic phase were excluded from the study. Median follow-up was 51 months (range 12-216) and overall survival at 3 and 5 yrs was 87% and 76%; 50 patients needed therapy at diagnosis and 40 during follow-up (3-117 months). Bulky disease, B symptoms, anemia (Hb <11 g/dL), albumin and LDH levels and lymphocytosis degree significantly related with treatment starting at diagnosis. A borderline significant better survival was observed for patients without organ involvement. At multivariate analysis, LDH levels (p=0.001) and age >60 yrs (p=0.007) were significantly related to overall survival, and LDH (p<0.001), haemoglobin (p=0.007) and systemic clinical presentation (p=0.002) to time to failure (defined as need of therapy for the indolent cases and relapse/progression for treated cases). In conclusions we retrospectively describe the clinical features and the variables related to evolution in a large group of patients with CD5 negative chronic B-cell lymphoid leukemias

P119

COMPARISON OF ZAP-70/SYK MRNA LEVELS WITH IMMUNOGLOBULIN HEAVY-CHAIN GENE MUTATION STATUS AND DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) patients with progressive disease usually lack somatic mutations in their immunoglobulin heavy-chain variable region (VH) genes and express ZAP-70, a T-cell-receptor associated protein tyrosine kinase. ZAP-70 is highly homologous to Syk, which is expressed in all B-cells and is key mediator of proximal B-cell receptor (BCR) signaling.

Aims. To investigate the relative amounts of ZAP-70 and Syk mRNA in purified B-cells from 91 CLL patients.

Methods. Blood samples were collected from 91 patients with B-cell CLL, and from 5 age-matched healthy donors. Sixty two patients (68%) were male. Median age was 63 years (range 33 to 84). At diagnosis 71 patients (78%) were in Binet stage A, 15 patients (16%) in stage B and 5 patients (6%) in stage C. Median duration of follow-up from diagnosis was 48 months. Fifty six (62%) patients were never treated and 35 (38%) had received treatment at some stage during the course of the disease. Treatment was initiated according to National Cancer Institute Working Group Criteria. The patients that were treated had not received chemotherapy or steroids for a period of at least 3 months prior to the sampling. Measurement of ZAP-70 and Syk mRNA: Measurement of ZAP-70 and Syk mRNA was based upon total cellular RNA was isolated from the CD19-selected B-cells using the Trizol reagent (Invitrogen), according to the manufacturer's instructions. ZAP-70 and Syk plasmid DNA standards were prepared by RT/PCR of mRNA from the Jurkat T-cell lymphoma and BJAB B-cell lymphoma cell lines, respectively. The PCR fragments were cloned in the pDrive cloning vector using the PCR cloningplus kit (Qiagen, Valencia, CA). Plasmids containing the ZAP-70 and Syk fragments were linearized by restriction enzyme digestion and mixed in equimolar amounts.Ig VH gene sequence analysis: The PCR amplification, cloning, and sequencing of VH region genes has been widely described in detail elsewhere. Sequences with less than 2% differences from germline VH sequences were considered unmutated. Immunoblot analysis:The protein samples obtained after lysis and determination by the RC DC Protein Assay (Bio-Rad Laboratories, Hercules, CA) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred on Immobilon-P polyvinilidene difluoride membranes (Millipore, Bedford, MA). Membranes were blotted at 4oC with antibodies against ZAP-70, Syk (both from Cell Signaling Technology) and beta-Actin (Sigma, Saint Louis, MO). Immunodetection was done with anti-rabbit IgG HRP-linked or anti-mouse IgG HRP-linked antibodies (Cell Signaling Technology) and the ECL Plus enhanced-chemiluminiscence detection system (Amersham Biosciences, Buckinghamshire, UK) with BioMax MR films (Eastman Kodak, Rochester, NY). Protein bands were quantified by

laser densitometry and analyzed by ImageQuant software. Cell culture:Freshly isolated CLL B-cells were resuspended at a density of x10^6 cells/200 µL in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate (Invitrogen, Carlsbad, CA) and cultured at 37oC in a humified atmosphere containing 5% CO2.

Results. ZAP-70 was expressed in the majority of cases with unmutated VH genes (88%), but also at lower levels in a substantial fraction of cases with mutated VH genes (41%). High levels of ZAP-70, defined as ZAP-70/Syk ratios above a 0.25 threshold, correlated strongly with unmutated VH gene status. Median treatment free survival (TFS) in the group of patients with high ZAP-70 levels was 18 months, whereas it was not reached in the groups with low or undetectable ZAP-70 (p<0.001).

Conclusions. Measurement of the ZAP-70/Syk mRNA ratio can accurately predict the VH gene mutation status and risk for disease progression in CLL and represents a valuable alternative to flow-cytometry analysis of ZAP-70 expression.

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THE USE OF LOW-DOSE ALEMTUZUMAB IN PATIENTS AFFECTED BY RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Several studies reported the efficacy of alemtuzumab in previously treated CLL, at a dose of 30 mg three times weekly, but there is only one report that has evaluated the use of low-dose alemtuzumab in these patients.

Aims. To assess efficacy, hematological and extrahematological side effects, infectious disease rate, CMV reactivaction and immunological recovery after low-dose alemtuzumab in relapsed or refractory CLL.

Methods. twelve patients were included (8 males, 4 females; median age of 61.5 years), 7 were in relapse and 5 were refractory to previous treatment. All patients received alemtuzumab at 10 mg as target dose, 3 times weekly, for a maximum of 10 weeks. At study entry, one patient was in stage A/progressive, five in stage B/II and six in stage C/IV. Seven patients had unmutated IgVH genes, 4 had mutated IgVH and 1 patient was not evaluable.

Results. Two patients obtained a CR (16%) and 3 patients (25%) achieved a PR. When Binet/Rai stage was taken into account, the OR was 83% in stage A-progressive/B-II compared to 0% in stage C/IV (p=0.01). With respect to VH gene mutational status, OR was 50% in patients with mutated VH genes and 29% in patients with unmutated VH genes (p=ns). None of the refractory patients responded to alemtuzumab, whereas five of the seven patients treated in relapse responded. At present 2 patients are still in CR, without detectable MRD at 15 and 10 months post-treatment. Among patients that achieved PR, one is in continuous PR at 11 months, one died 7 months after the end of treatment while still in PR because of idiopathic pneumonia, and one is alive at 24 months with disease progression. The median response duration was 10 months. Two patients showed grade IV neutropenia and required G-CSF during the treatment. Grade III anemia occurred in three and grade III thrombocytopenia in two patients. Mild adverse infusion related events (grade I/II) occurred during the first week of treatment. Alemtuzumab was generally well tolerated with no episodes of febrile neutropenia or bacterial infection during treatment. The incidence of CMV reactivation (Ag and/or CMV DNA) was 66%, but no evidence of CMV disease was noticed. All patients were succesfully treated with oral ganciclovir 1000 mg tid for a median of 14 days. Immunological recovery was markedly delayed during and after alemtuzumab therapy. Before treatment all lymphoid subsets, except for total lymphocytes and NK cells, showed median values into the normal range. Total lymphocytes, T-helper and T-suppressor cells rapidly decreased, achieved a minimum level after 2 months, and thereafter showed a mild trend toward recovery. However, their median values were still below the normal range at the end of the study.

Conclusions. Low-dose alemtuzumab induced significant responses in these heavily pretreated CLL patients, with mild hematological and extrahematological side effects and low risk of infections, even in the presence of long-lasting severe immunosuppression. Among these patients we detected a particular sub-group (relapsed CLL, A-progressive/B-II stage, mutated-IgVH status) in which low-dose alemtuzumab achieved a higher response rate.

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ORAL FLUDARABINE PLUS CYCLOPHOSPHAMIDE AS FRONT-LINE TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Fludarabine monotherapy is an established treatment for chronic lymphocytic leukemia (CLL), achieving superior remission rates compared to other treatment regimens without purine analogous. Recently encouraging results have been reported in advanced CLL with the combination of fludarabine plus cyclophosphamide.

Aims. We tested efficacy and safety of the oral formulation of fludarabine combined with cyclophosphamide in the treatment of high-risk B-CLL as first line therapy. *Methods.* Starting from December 2002, 23 patients with untreated B-CLL (15 male, 8 female; with a median age of 67 years (range 52-75) received a regimen combining oral fludarabine (30 mg/sm) and oral cyclophosphamide (250 mg/sm) for 3 consecutive days every 4 weeks, for a maximum of 6 cycles. At study entry, 20 patients were in stage B/II and 3 in stage C/IV. Ten had unmutated IgVH genes and 7 had mutated IgVH while in the other six patients IgVH were not evaluable or not done. The median number of lymphocytes in peripheral blood (PB) was 44510/µL

(range 8700-136240 µl), and in the bone marrow (BM) 82% (range 41-93%). All patients received prophilaxis with oral acyclovir 800 mg tid and trimethoprim-sulfametoxazole 900 mg bid for two consecutive days a week.

Results. Among the sixteen evaluable patients, 8 obtained CR (50%) and 5 achieved PR (31%); 2 patients showed stable disease (SD) and one patient had disease progression (PD). Of the 13 patients that responded to chemotherapy, 3 relapsed after a median of 13 months (11-20 months). The median overall survival was 8 months (2-20 months). Among patients with unmutated IgVH, one obtained CR, 5 PR and 2 showed SD instead of patients with mutated IgVH; 2 achieved CR and one PD. At this time, all sixteen patients are still alive without further chemotherapy. Regarding haematological toxicity, 5 patients showed grade IV neutropenia and required G-CSF during the treatment. Anemia and thrombocytopenia (Grade III and IV) did not develop in any patient. Mild extrahematological toxicity, consisting of nausea and vomiting, occurred in 3 patients during treatment. No episodes of unknown febrile neutropenia or documented infection were noticed during the treatment.

Conclusions. Oral fludarabine plus cyclophosphamide as front-line therapy in CLL achieved 50% CR and 31% PR in our series of patients. Haematological side effects were mild and extrahaematological toxicity was acceptable. Moreover, the scheme was easy to administer on an outpatient basis and its tolerability was good. Therefore, oral fludarabine plus cyclophosphamide is feasible, safe and effective front-line treatment for CLL. However, a longer follow-up will be needed to define the duration of response and the effect on survival.

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RITUXIMAB TREATMENT FOR AUTOIMMUNE HEMOLYTIC ANEMIA ASSOCIATED WITH B-CELL LYMPHOPROLIFERATIVE DISORDERS

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Background. There is a well-described association between lymphoproliferative disorders (LDs) and autoimmune hemolytic anemia (AHA). While steroids represent the main treatment option, a variety of other immunosuppressive agents are employed for steroid-refractory cases. Recent studies have suggested that the anti-CD20 monoclonal antibody Rituximab may represent a very active agent for the treatment of LDs-associated AHA and autoimmune thrombocytopenia. Aims. We report the results of Rituximab treatment in 15 patients (see Table) with LDs-associated AHA, seen at our Institutions between March 2001 and December 2004.

Patients and Methods. The mean age of patients at AHA diagnosis was 68 years (range 48-87 years) and all were affected by B-cell LDs: 12 chronic lymphocytic leukemia (CLL), 1 small lymphocytic non-Hodgkin's lymphoma (SLL/NHL), 1 Waldenstrom macroglobulinemia (WM), 1 leukemic phase of a CD5-CD23- chronic lymphoproliferative disorder (CLD). They developed direct antiglobulin test (DAT)-positive AHA at a mean time of 44,5 months (range 0 – 166 months) from diagnosis of LDs. In 5 cases AHA was concurrently diagnosed with CLL (pts. no. 1, 7, 9, 13 and 15). All patients received steroids as first-line treatment (prednisone or methyl-prednisolone), while in only 1 patient (no. 10) methyl-prednisolone was concurrently administered with Rituximab. Prior of Rituximab therapy, some patients (no. 6, 7 and 8) also received cyclophosphamide (pts. No. 6, 7 and 8) while patient no. 8 was given intravenous vincristine (2 mg). All patients but one were considered refractory to steroids and/or subsequent immunosuppressive therapy and were then given weekly Rituximab (375 mg/m^2) for 4 consecutive weeks at a median time of 58 days (range 1-155 days).

Results. Table shows transfusion-independent Hb (g/dL) levels before and after Rituximab treatment. All but three patients (pts. no. 6 and 8, who died of cardiac failure and sepsis soon after the 3rd cycle, and pt. no 12, with HCV-positive chronic hepatitis, who showed increase of AST and ALT serum levels) received the scheduled 4 courses. The first infusion side effects of Rituximab were irrelevant (fever in 2 cases and chills in 1 case). All but two patients (pts no. 8 and 10) showed an increase in Hb levels in response to Rituximab with a mean increment value of 2,8 gr/dL (range 0,7–10 g/dL). Eleven patients required packed red cell transfusions before starting Rituximab and six of them became transfusions-free after the 3rd cycle. At the end of Rituximab treatment only two patients remained transfusion-dependent, even though one of them showed a 50% reduction in transfusion requirement as compared to pre-Rituximab needs. Only three patients underwent to a maintenance program with Rituximab administration with different schedules. At a mean follow-up of 17 months, 8 patients are still alive, 6 of them being transfusion-free. Conclusions. Our results clearly demonstrate that anti-CD20 monoclonal antibody is an effective and well tolerated alternative treatment option for steroid-refractory LDs-associated AHA.

Table.

Pts.	Age/Gender	Disease	Hb pre performed	Hb post	No. cycles	Follow-up (since AHA dgn)
1	57/M	CLL	11,7	13,6	4	46
2	66/F	CLL	6,6	9,6	4	27+
3	72/F	CLL	6,3	10,2	4	14
4	79/F	CLL	7,8	10,8	4	24
5	69/M	CLL	7,4	10,4	4	22+
6	82/F	CLL	7,4	8,1	3	3+
7	87/M	CLL	4,2	5,6	4	4+
8	60/M	CLL	6,4	4,8	3	3+
9	48/M	CLL	5,0	15,0	4	12
10	79/M	CLL	5,2	5,2	4	4
11	71/F	CLL	5,7	9,3	4	24+
12	56/M	CLL	5,7	9,8	3	21
13	52/M	CLD	4,0	9,0	4	38
14	56/F	WM	6,0	7,0	4	12
15	86/M	NHL	7,9	9,9	4	3+

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EVALUATION OF THE OXIDATIVE METABOLISM IN CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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Accumulation of B-cells in chronic lymphoproliferative disorders (CLD) is caused not by their higher proliferation rate but by their prolonged life-span due to dysregulation of apoptosis. Activation of intracellular caspases is carefully controlled through a delicate balance of anti- and pro-death stimuli which are integrated at the mitochondrial level for the ultimate life/death decision. Even though there are reports characterising some mechanisms of B-CLD cell apoptosis, relatively less is known about the complex regulation of this process. More recently a growing body of experimental evidence has focused attention of clinicians and basic investigators on the possible involvement of intracellular reactive oxygen (as well as nitrogen) species as potential modulators of cell growth given their recognized role of second messengers. Thus we reasoned, in this study, to characterize the features of the two main cellular ROS producers, mitochondria and plasma-membrane NADPH oxidase. The results of our analysis carried out on samples from a cohort of 9 patients with chronic lymphocytic leukaemia (CLL) and 2 with splenic villous lymphoma (SVL), can be summarised as follows: (a) The efficiency of mitochondrial oxidative phoshorylation, assessed by membrane potential sensitive probe resulted to decrease with increasing the severity of the disease; this was confirmed by the spectrophotometric analysis of the mitochondrial respiratory chain cytochrome content. Since it has been recently reported that mitochondriogenesis is enhanced in B-CLL cells under NO stimulation, our results might rationalize its occurrence as a compensatory process. (b) RT-PCR analysis on cell transcripts showed that differently from normal B-lymphocytes, which express two of the NADPH oxidase isoforms (NOX2 and NOX4), B-CLL (6/10) and B-SVL (2/2) cells did not express NOX4 which was fully recovered in patients undertaking chemotherapy. Noteworthy NOX4 gene is located on chromosome 11q14.2-q21 known to be deleted in some of the structural chromosome 11 abnormalities which have been described to be associated with CLL. The results presented will be discussed in the framework of the control exertable by the intracellular redox tone, in defining cell fate (survival vs apoptosis) and rationalised for clinical applications.

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CHRONIC LYMPHOCYTIC LEUKEMIA: EVALUATION OF INCIDENCE IN A HEALTHY SUBJECTS COHORT

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Background. Chronic Lymphocytic Leukemia is the most frequent leukemia in the Western countries (Rozman, 1995). Its incidence is generally reported around 2 - 6 cases/100000 persons-year (Linch, 1992, Brinker, 1982) but this figure based on cancer registries may be an underestimation. In fact authors reported a 37% higher incidence (Zent, 2000) in a selected population of Veterans. A reliable estimation is made difficult because of several reasons: CLL is often asymptomatic and passes undiagnosed; cases may not be recorded because of a benign course of the disease, without hospital admissions or treatment; elderly people, in whom the disease is more frequent, don't undergo complete diagnostic procedures. Aim of the study. The purpose of the study was the evaluation of CLL incidence using an approach based on a healthy people cohort which avoids any selection bias.

Materials and methods. The charts of all CLL patients recorded in the Vicenza Hospital Hematology Department data-base were traced. The Hematology Department is the only hematology facility of the area and its data-base encompasses data of all the visited subjects, even if not subsequently admitted to the hospital as in- or out-patients. We retained only CLL cases occurred in subjects belonging to a cohort of healthy people enrolled from 1993 to 1996 in a clinical survey on thrombophilia carried out in the Vicenza metropolitan area. A total of 15109 subjects aged 18 to 65 years, casually extracted from the census lists, had been evaluated: 8017 of them were females and 7092 males. Median age of the cohort at the enrolment was 43 years. Diagnosis of CLL was validated if based on morphological and immunophenotypic data according to currently accepted criteria (Cheson, 1988; IWCC, 1989; Rozman, 1995). Results. Six out of 15.109 people were diagnosed with CLL (4 males). The median follow-up for the entire cohort was 8.2 years and the total time of exposure was 121000 pts-year. Incidence of CLL is estimated to be 4.9 cases/100000 pts-year (CI: 1.8-10.7) for the entire cohort, 25/100000 pts-year in people 60 to 69 years old. Diagnosis of CLL was done from 36 to 97 months after the enrolment. Mean age at diagnosis was 66 years (61–70). At the time of enrolment in the study, all of the patients had a normal WBC. All of the patients were asymptomatic at diagnosis. One patient died 7 months after the diagnosis for unrelated causes. The median follow-up for the remaining ones was 58 months (35–64). Only one of them became symptomatic and needed a treatment, 40 months after the diagnosis. Conclusions. According to our data, the incidence of CLL appears to be higher than assessed so far. In our opinion it's noteworthy that our estimation is based on data of subjects evaluated in a clinical setting and not from tumour or disease registries. In fact it could still be underestimated comparing the young median age of our cohort with that usually reported at diagnosis (Rozman, 1995). Moreover, the comparison of our data with those available could suggest that only a part of the cases might usually be diagnosed.

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IGVH GENE REARRANGEMENT AND MUTATIONAL STATUS BEFORE DIAGNOSIS OF CLL

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Background. Chronic Lymphocytic Leukemia is the most frequent leukemia in the Western countries (Rozman, 1995) but many clinical and biologic aspects of this disease are not completely defined. The diagnosis is often made by chance and we can assume that the disease could inapparently be present even for a long time.

Materials and methods.To test this hypothesis we searched for IgVH gene rearrangement present before CLL diagnosis in a series of patients whose genetic samples were available having them been included in a clinical survey on familial thrombosis performed on 15.000 healthy people from 1993 to 1996. Moreover we planned to examine the IgVH mutational status in the hope to confirm its prognostic value (Hamblin, 1999; Damle, 1999) in case of identification of a gene rearrangement. We identified 5 subjects (4 M) with a diagnosis of CLL, according to currently accepted criteria (Cheson, 1988; IWCC, 1989; Rozman, 1995), who had participated in the study. All of them had a normal CBC count at the enrolment. Diagnosis was done 36 to 83 months after the participation. Mean age at diagnosis was 66 years (61–69) and all of the subjects were asymptomatic (Rai stage 0). One patient died 7 months after the diagnosis for unrelated causes and the median follow-up for the remaining ones was 58 months (35–64).

Results. We could see a monoclonal IgVH gene rearrangement in 4 subjects but in one case a faint fluorescent peak, not suitable for sequencing analysis, was detected. A polyclonal pattern was demonstrated in the fifth subject. The VH family gene involved in the rearrangements was identified using the fluorescent FR1 primers. The peaks were the same at enrolment and at diagnosis, done 39, 52 and 54 months afterwards. Sequencing analysis was performed in subjects with gene rearrangement: a somatic hypermutation was detected in 2 pts. In 1 pt an unmutated status was found. The sequences were the same at enrolment and at diagnosis. ZAP-70 expression by flow cytometry (Monserrat, 2001) was negative in mutated patients and positive in unmutated one. The patient with the unmutated IgVH gene population became symptomatic and needed a treatment 40 months after the diagnosis and 92 from the first identification of gene rearrangement. The patients with the mutated pattern remain asymptomatic after a follow up of 54 and 64 months.

Conclusions. Typical CLL gene rearrangements have been reported in healthy people (Ghia, 2004, Rawstron, 2002), but this is the first time they have been found in subjects subsequently developing CLL suggesting that these populations may eventually progress to overt malignancy and suggesting a causative role of gene rearrangement. A rearrangement may be present for a long time and we observed it 83 months before a clinical diagnosis. It is noteworthy that , even if the small numbers of our subjects prevent any reliable conclusion, the prognostic significance of the mutational status (and ZAP 70) seems to be confirmed. Its clinical value, however, might need further defined since we identified an unmutated clone 92 months before any treatment was required.

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ORAL FLUDARABINE IN ELDERLY PATIENTS: PROFILE OF TOLERABILITY

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Chronic Lymphocytic Leukemia (CLL) is the most common hematological malignancy in the Western world, with an incidence of more than 20 new cases per 100000 for year after the age of 60. In the subset of elderly patients, frequently the choise of treatment is conditioned by the presence of concomitant diseases and therapies. For these patients the oral formulation of fludarabine in immediate-release tablets of 10 mg is a reasonable treatment due to easiness of administration, efficacy and tolerability. We report the experience of seven hematological Italian centres that used this kind of therapy in patients over 65. End points of the study were the schedule ward and the assessment of the tolerability profile of the drug. We collected 83 patients treated by oral fludarabine as single therapeutic agent; 52 patients had diagnosis of classic CLL, 28 of low grade LNH, 3 of Waldestrom Macroglobulinemia. Schedule and line of treatment were variable: in 59% oral fludarabine was the first line of treatment, in 30% the second line. In 80% of patients the dose per body surface was respected. The days of treatment per course were 5 in 54 patients (65%) and 3 in 21 patients (25%). The number of courses was variable: in 12 cases (14%) only one course of chemotherapy was sufficient for normalization of the lymphocyte count and disappearance of lymphoadenomegaly; in 24 patients (29%) the complete schedule of 6 courses was carried out. Nausea/vomiting and diarrhea occurred in 8 patients (9%), but no patient stopped or reduced the treatment for these complications; all these events were WHO grade 1 and 2. Thrombocytopenia occurred in 14 patients (17%), anemia in 20 (24%) and neutropenia in 15 (18%); in no case transfusion with platelet or erythrocyte concentrates was needed. Supportive therapy by growth factors was utilized using G-CSF or epoetin in 12 and 11 patients, respectively. We did not observe episodes of autoimmune haemolytic anemia. Infections occurred in 20% of the patients, even if no case required hospitalisation. Three patients showed cutaneous rash with pruritus. The short follow-up does not consent an evaluation of efficacy of this treatment, even if provisional data show a longer time to retreatment for these patients, compared to historical controls treated by chlorambucyl. These retrospective data confirm that oral fludarabine is a well tolerated treatment; the easy administration produces improvement of quality of life and reduction of the medical costs. A longer follow up is needed to assess the efficacy of the treatment in this group of elderly patients, in whom the main aim is to control disease progression rather than eradicate the disease. To reach this goal maybe lower doses of this drug are required.

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EXPRESSION OF LYMPHOCYTE SURFACE MOLECULES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA IS CONSISTENT WITH AN ACTIVATED MEMORY PHENOTYPE

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B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the homogeneous coexpression of CD19, CD23 and CD5 on clonal B cells, along with dim expression of membrane immunoglobulins. Conversely, surface expression of other relevant molecules may vary depending on the particular physiopathologic state the cell is in. The aim of this study was to compare clonal B cell expression of membrane molecules involved in cell activation, differentiation, T-B cell cooperation and apoptosis between B-CLL patients and healthy subjects. The study population consisted of 29 patients (16 M/13 F; mean age: 66.5 years) with B-CLL in various Rai stages. The control group consisted of 16 sex- and age-matched healthy subjects. Phenotypic analyses were carried out by flow cytometry. The expression of the lymphocyte activation markers CD69, CD25 and CD11c was significantly higher on CLL B cells than control B lymphocytes ($p<0.01$ for all). The expression of the death receptor CD95 was significantly lower on CLL B cells when compared to normal B lymphocytes ($p<0.001$). Expression of CD27, a marker of memory B cells, was observed on the large majority of CLL B cells as opposed to normal B cells ($p<0.001$). Finally, CD80 and CD86, molecules involved in T-B cell cooperation, were inversely correlated in B-CLL patients and healthy subjects. Specifically, a significantly higher CD80 expression was found on normal B cells as compared to CLL B cells ($p<0.001$), whereas clonal B cells were shown to display higher CD86 membrane levels than control B cells ($p=0.001$). Linear regression analysis showed a positive correlation between CD80 and CD95 ($r=0.444$, $p<0.001$) and an inverse correlation between CD69 and CD11c on CLL B cells ($r= -0.506$, $p=0.04$). These results are consistent with the Hypothesis that CLL B cells are activated lymphocytes with a memory phenotype. The significance of concordant CD80 and CD95 expression and reciprocal CD69 and CD11c expression, each of which may be regulated by a common pathway, requires further investigations.

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PRESENTING FEATURES AND PROGNOSTIC FACTORS IN SPLENIC MARGINAL ZONE. A STUDY ON 145 PATIENTS

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Purpose. Splenic Marginal Zone Lymphoma is a well defined pathological entity considered to behave as an indolent neoplasm. However, data on prognostic factors are scanty and only a few large series have been published so far. Patients and Methods Clinical-pathologic features, and outcome of 145 consecutive patients diagnosed with SMZL/SLVL in four Italian centres were reviewed. Results The disease occurred mostly in elderly males (median 66 years + 10; M/F ratio 1.6). Anemia was recorded in 55% of patients and 24% showed a moderate thrombocytopenia. Leukocytosis > 30.000/L and neutropenia 1000/L were found respectively in 6% and 10% of patients and typical villous lymphocytes were found in 48% of cases. Fifteen percent of patients were HCV+ and a small monoclonal component was detected in 20% of cases. The bone marrow was infiltrated with an intrasinusoidal component in all patients. Sixty-two patients were placed on watch and see policy until the disease progressed and they are still alive 1 to 5 years after diagnosis. The remaining patients received one or more lines of treatment. Overall, 51 patients underwent splenectomy and in all cases the diagnosis of SMZL was histologically confirmed in the surgical specimens. With a median follow-up of 48 months the median survival for all the series is not reached, and 82% of patients are expected to be alive at 7 years. In univariate analysis LDH above normal value and anaemia were significantly associated to a shorter survival at seven years, while anaemia, a splenomegaly > 4 cm and a lymphocyte count above 5000 mmc3 correlated with a shorter Event Free Survival. In multivariate analysis only Hb< than 11 gr/dL proved to be associated to shorter survival. Conclusions Up to 30% of SMZL/SLVL patients have an indolent disease and can be monitored with a watch and wait policy. The bone marrow intrasinusoidal infiltration pattern may be a valuable diagnostic hallmark, thus obviating diagnostic splenectomy. Patients without anemia with a splenomegaly less than 4 cm and LDH with normal value seem to have a true smouldering clinical course. The issues regarding prognostic stratification and the best therapeutic strategy need to be addressed in properly designed prospective trials.

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DEOXYCOFORMYCIN (PENTOSTATIN) IN THE TREATMENT OF SPLENIC MARGINAL ZONE LYMPHOMA WITH OR WITHOUT VILLOUS LYMPHOCYTES

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Background. Splenic Marginal Zone Lymphoma (SMZL) is an infrequent B-cell neoplasm that pursues an indolent course. Signs and symptoms, mostly related to hypersplenism, are successfully managed by splenectomy. However, the therapy of patients who are not fit for a surgical procedure or who relapse after splenectomy, is still an unsettled issue. Patients and methods We report a phase II study on 16 patients with SMZL, three therapy naïve and 13 pre-treated,all showing systemic symptoms or progressive worsening of peripheral cytopenia, who were treated with pentostatin at a dose of 4 mg/m^2 every other week for six to ten weeks. In relapsed patients, the median interval between diagnosis and treatment was 26 months(range, 8 to 49). Results Overall, 68% of the patients showed a clinical response. Two out 3 patients, who received pentostatin as first line therapy, attained a Complete Response (CR). One CR and 7 minor or good haematological responses were recorded in relapsed patients. Treatment toxicity, mostly haematological, proved manageable. With a median follow-up of 35 months the median Overall Survival (OS) is 40 months and the median Progression Free Survival (PFS) is 18 months. Conclusion Our data show that Pentostatin administered every other week has a good degree of activity in the treatment of SMZL and suggest that this schedule could be considered a possible therapeutic option for patients who are not fit for splenectomy or have relapsed.

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ZAP-70 EXPRESSION AND HEMATOLOGICAL AUTOIMMUNE COMPLICATIONS IN B-CLL PATIENTS

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The course of B-chronic lymphocytic leucemia (B-CLL) is frequently complicated by hematological autoimmune diseases (HAD), particularly autoimmune hemolytic anemia (AIHA), autoimmune thrombocytopenia (AITP), Evans Syndrome (ES) and, more rarely, pure red cell aplasia (PRCA) and autoimmune neutropenia. The risk of HAD is higher in advanced and multi treated B-CLL, but its prognostic impact on survival is still questioned. The clinical behaviour of B-CLL is heterogeneous; a number of new biological prognostic markers, including ZAP-70 expression by leukemic cells, have been developed over the last years of possibile value for outcome prediction in B-CLL patients at presentation. In order to assess a possibile correlation between ZAP-70 expression and the risk of HAD, we retrospectively evaluated the prevalence of AIHA, AITP and PRCA in 154 B-CLL patients in whom ZAP-70 expression of leukemic cells was investigated by immunochemistry on bone marrow biopsies performed within 6 months from diagnosis. They were 95 males and 59 females, aged 33 to 83 years (median 62). At presentation 107 (69%) were Binet stage A, 39 (25%) stage B and 8 (6%) stage C. Overall, HAD was observed in 22 patients (14.3%); 14 were AIHA, 4 AITP, 2 ES and 2 PRCA. In 5 cases (23%) the complication was present at diagnosis (2 AIHA, 1 AITP, 1 ES, 1 PRCA), in the remaining it was registered after treatment (alkylating agents in 13, Fludarabine in 4). HAD was significantly more frequent in the 74 patients expressing ZAP-70 on leukemic cells than in ZAP-70 negative cases (19/74 (25.7%) vs 3/80 (3.7%)), respectively. The actuarial probability of HAD in ZAP-70 positive patients was 33% and 43% at 5 and 10 years, respectively vs 0% and 5% in ZAP-70 negative (p<0.0001). When we considered only the subgroup of Binet stage A patients at diagnosis, the probability of HAD at 10 years in ZAP-70+ cases was 49% vs 4% in ZAP-70 negative. The correlation between ZAP-70 expression and the risk of HAD was confirmed also at multivariate analysis (p=0.003). In conclusion, our data suggest that ZAP-70 expression on leukemic cells, that has been demonstrated to be a strong and reliable prognostic marker in B-CLL, can also influence the risk of autoimmune complications, possibly related to the degree of immunological deregulation produced by the lymphoproliferative disease.

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ZAP-70 EXPRESSION, AS DETECTED BY IMMUNOSTOCHEMISTRY ON BONE MARROW BIOPSIES FROM EARLY PHASE CLL PATIENTS, IS A STRONG ADVERSE PROGNOSTIC FACTOR

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In order to predict the heterogeneous clinical course of B chronic lymphocytic leukaemia (B-CLL) at presentation and to plan a risk-adapted treatment strategy, new biological prognostic factors have been developed over the last years. Among them ZAP-70 expression on leukemic cells, as evaluated by molecular analysis or flow cytometry, initially proposed as surrogate of IgVH mutational status, recently was suggested to be a promising prognostic marker in B-CLL. We evaluated the cytoplasmic expression of ZAP-70 protein in leukemic cells by immunohistochemical methods on bone marrow trephine biopsies taken within 6 months from diagnosis and before treatment in 154 patients with B-CLL. The results were correlated with age, sex, Binet stage, survival and clinical outcome. They were 95 males (62%) and 59 females (38%), aged 33 to 83 years (median 62). At presentation 107 (69%) were Binet stage A, 39 (25%) stage B and 8 (6%) stage C. Among the 125 survivors, the median follow-up period from diagnosis was 69 months (range 7 – 278 months). Seventy-four cases (48%) of B-CLL patients had cytoplasmic expression of ZAP-70. This group of patients presented higher percentage of advanced Binet stage (B-C) (p= 0.001). The ZAP-70 positivity was significantly related to lower OS (38% vs 86%

at 10 years) (p=0.0004) and PFS (6% vs 46% at 10 years) (p<0.0001). ZAP-70 expression was correlated to poorer outcome also when we evaluated only the 107 stage A patients. In conclusion, ZAP-70 expression by leukemic cells, simply detected by immunohistochemical methods on bone marrow samples obtained at diagnosis, is a reliable prognostic marker in B-CLL at presentation. This marker, in particular, can be useful in identifying among early stage patients without clinical unfavourable prognostic features cases at high risk of progression who may benefit from early and/or more intensive treatment.

Acute Myeloid Leukemia I

P132

ACUTE PROMYELOCYTIC LEUKEMIA IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA PREVIOUSLY TREATED BY CHLORAMBUCIL

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The development of acute promyelocytic leukemia (APL) in patients with chronic lymphocytic leukaemia (CLL) is extremely rare. Most cases of therapy-related APL regard patients affected by solid tumours treated by chemotherapy, frequently including topoisomerase inhibitors. We describe a case of APL developing in a 68-year-old woman, with a diagnosis of B-CLL in stage IIB (Rai/Binet) lasting from 1996 and treated with monthly chlorambucil and prednisone until March 2004. She was admitted to the hospital in June 2004 because of anemia (hemoglobin level 9.3 g/dL) with macrocytosis (mean corpuscular volume 103 fl), neutropenia with absolute lymphocytosis (white cell count $5.1x10^9$/L, neutrophil count $0.6x10^9$/L, lymphocyte count $4.4x10^9$/L) and mild thrombocytopenia (platelet count $84x10^9$/L). The coagulation profile was normal except for elevation of D-dimer. Bone marrow trephine biopsy showed a maturative arrest of myelopoiesis with about 20% of atypical ipergranular promyelocytes and a B-lymphoid infiltrate consisting of small mature cells accounting for 10% of cellularity. Immunophenotypic characterization by flow cytometry confirmed the suspicion of APL showing a 30% of promyelocytes with a negative staining for HLA-DR and CD34 and positivity for CD33 and MPO. Lymphocytes coexpressed CD19, CD20, CD23 and CD5. Cytogenetic study on bone marrow aspirate demonstrated a t(15;17) in 13% of the metaphases. Additional chromosomal abnormalities were absents. PML-RAR-alfa (bcr 3) transcript was revealed by RT-PCR. The patient was treated by ATRA alone from July to October 2004 achieving a complete morphologic remission of APL (with persistence of a CD5+/CD19+/CD23+ monoclonal lymphoid population) but no disappearance of PML-RAR-alfa rearrangement. A consolidation with idarubicin and ATRA was then started in January 2005. This report shows a unique case of APL diagnosed in a patient affected by CLL and previously treated only by alkylating agents. With respect to response to therapy and clinical outcome, the prognosis seems to be similar to that of de novo APL, despite the persistence of CLL

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PHOTOSENSITIZATION EFFECT ON DISCHROMIC CUTANEOS AREAS DURING CONTEMPORARY VORICONAZOLE AND CYTARABINE TREATMENT. REPORT OF TWO CASES

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Introduction. Rash/erythema is an azole class effect; photosensitivity reactions were also reported in subjects treated with voriconazole. Cytarabine also is described as a cause of cutaneous erythema; it is usually of mild-moderate entity, but sometime it may be severe and a generalized erythrodermia was also reported. We describe two cases of photosensitization on dischromic cutaneous areas during contemporary treatment with Voriconazole and Hi-dose of Cytarabine in patients who tolerated both single drugs.

Case Reports. Two patients affected by acute myelogenous leukemia (AML), respectively were in treatment with Voriconazole per os from 30 and 40 days respectively. The first AML patient was a female aged 50 affected by vitiligo, with many dischromic areas in different zones of the body. After a consolidation IC course (Idarubicin 10 mg/m^2 for 2 days and Cytarabine 100 mg/mm^2 x 2/die for 5 days) a pulmonary aspergillosis was bioptically demonstrated. Specific oral treatment with Voriconazole began, it was well tolerated without any relevant side effects and rapid improvement was demonstrated with CT scan. The scheduled high dose cytarabine course (2000 mg/m^2 x 2/die for five days, Idarubicin 8 mg/m^2 for the first day) was begun three weeks after. In third day of cytarabine administration a progressive increasing erythematous lesion appeared on dischromic areas, particularly on face, arms, hands and legs. These lesions were diagnosed as photosensitization in dischromic areas induced by contemporary treatment of Cytarabine and Voriconazole. Therapeutic approach included suspension of Voriconazole and steroid topic application, and led to progressive improvement of the lesions after a disepitelization phase. Complete regression of the lesions was obtained after ten days; after fifteen days Voriconazole treatment restarted without any complication. Also the second AML patient was a female aged 58 who presented some dischromic cutaneous areas. After the first consolidation course with hi-dose cytarabine (2000 mg/m^{2}x2/die for five days, Idarubicin 8 mg/m^2 for the first day), she suffered a multifocal pneumonia suggestive for filamentous fungi infection. Initially intravenous and then oral Voriconazole (200 mg x 2/die) led to a rapid improvement. When the patient began the second high dose cytarabine course, with contemporary administration of oral Voriconazole, erythematous lesion on dischromic areas on the limbs appeared also in this patients 48 hours after the start of treatment. Clinical features were similar to the previous case, and treatment was the same, with topic steroids and suspension of Voriconazole. Also in this case a progressive improvement was noted with complete resolution of the lesions.

Discussion. These two cases illustrate similar clinical situations of two patients who presented particularly sensitive cutaneous areas, as dischromic ones, and were both submitted to contemporary treatment of hi-dose cytarabine and oral voriconazole. In both patients single therapy with Voriconazole and cytarabine were well tolerated, but when they experienced the contemporary association of both the drugs some cutaneous lesions appeared on the most sensitive areas. We supposed a clinical diagnosis of photosensitization on dischromic cutaneous areas due to Voriconazole exacerbated by contemporary treatment with cytarabine. Three evidences supporte our hypotesis: first Voriconazole can cause cutaneous photosensitization and cytarabine has a cutaneous toxicity; second both our patients well tolerated cytarabine and voriconazole therapy alone in the past but never experimented the contemporary treatment; third the lesions appeared only on dischromic areas of the body regions most exposed to the light. Even if voriconazole alone can cause photosensitization, our clinical observation stress the possible synergistic effect of combination voriconazole-citarabine in causing this complication. Simultaneous administration of the two drugs must therefore cautiously evaluated in patients presenting particular cutaneous sensibility.

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ALL-TRANS RETINOIC ACID AND GENTUZUMAB OZOGAMICIN IN ELDERLY UNTREATED ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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All-trans retinoic acid (ATRA) combined to anthracycline-based chemotherapy is currently the reference treatment of patients with Acute Promyelocytic Leukemias (APL). Furthermore ATRA induces remissions in up to 90% with newly diagnosed APL and produces long-term remission in 60-80% of patients when given together with consolidation chemotherapy. Here we report a treatment with Gentuzumab Ozogamicin (GO) following induction with ATRA alone in a patient 68 years old not eligible for the use of anthracycline. A 68 years old man was referred to our institution because of severe leucopoenia (WBC: 0,65/mmc, Hb: 8,8 gr/dL, Plt: 189000/mmc) during the last month. He was chronically anticoagulated with warfarin because of mechanical cardiac valve and his Left Ventricular Efflux Fraction (L-VEF) was 35%. Bone marrow examination revealed promyelocyte blasts infiltration. Cytogenetics showed the presence of translocation 15;17 confirmed by molecular examination for PML/RARalfa fusion gene transcript of the bcr ? type. Protocol with retinoic acid alone at the dose of 45 mg/m^2 for 80 days was given as induction therapy. Complete hematological, cytogenetic and molecular remission was achieved and confirmed by bone marrow aspiration 60 days later. At day + 170 the patient was treated with consolidation treatment based on two monthly doses of 6 mg/m^2 of Gentuzumab Ozogamicin (GO). Consolidation therapy was very well tolerated and the patient, at one year from the diagnosis, remains in complete molecular remission. Actually he receives a maintenance program with ATRA 45 mg/m^2 for 15 days every three months. This treatment is planned for two years. With this therapeutic strategy we conclude that it may be possible in older patients to minimize or eliminate chemotherapy in untreated APL at low or intermediate risk by combining ATRA+GO.

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FLUDARABINE, CYTARABINE, IDARUBICIN AND ETOPOSIDE COMPARED TO FLUDARABINE, CYTARABINE, G-CSF AND IDARUBICIN AS INDUCTION TREATMENT FOR DE NOVO AML PATIENTS (<60 YEARS)

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Background. The addition of etoposide to a regimen including synergistic drugs, such as intermediate dosage Ara-C, idarubicin and fludarabine might reduce treatment failure. The relatively short duration of chemotherapy might, on the other hand, reduce toxicity and allow prompt and safe administration of high dose therapy (HDT). Patients and methods. The induction regimen (FLAIE) included fludarabine (30 mg/sqm), followed four hours later by a 2-hour infusion of Ara-C (2 g/sqm) and etoposide on days 1-5, and by a 30 minute infusion of idarubicin (10 mg /sqm) on days 1,3,5. High dose therapy with stem cell rescue was planned for all patients in first CR after high dose Ara-C and idarubicin consolidation course and in good clinical conditions. Patients' features and results were compared with an historical group of patients who received in the same center fludarabine, Ara-C, G-CSF and idarubicin (FLAG-Ida) followed by consolidation chemotherapy and HDT. Results. Patients treated with FLAIE and FLAG-Ida have been 23 and 43, respectively. Median ages were 53 and 50. Almost all patients had de novo AML. Karyotype belonged to the unfavorable prognosis group in 2 (9%) and 2 (5%) patients, respectively. An intermediate prognosis karyotype was detected in 21 (91%) and 39 (91%) patients receiving FLAIE and FLAG-Ida regimens, respectively. Days to PMN > $0.5x10^9$/l have been 17 (10-33) and 17 (10-28); days to Plt > $50x10^9$/l have been 18 (13-43) and 17 (12 - 38), respectively. Median number of erythrocyte transfusions were 5 (2-10) and 7 (2-18). Median numbers of platelet transfusions have been 5 (2-18) and 5 (1-17).No episodes of cardiovascular toxicity have been recorded in both groups. Sepsis have been reported in 6 (26%) and in 7 (16%) patients treated with FLAIE and FLAG-Ida, respectively, and pneumonia in 4 (9%) patients receiving FLAG-Ida. Deaths in induction have been 4 (17%) and 1 (2%), complete responses (CR) have been achieved in 16 (70%) and 35 (82%) patients, respectively. Allogeneic BMT has been performed in 5 (22%) and in 6 (14%) patients. Autologous PBSC transplants have been done in 1 (4%) and 17 (39%) patients receiving FLAIE and FLAG-Ida induction regimens, respectively. Relapses have been recorded in 3 (13%) and 18 (42%) patients. Median duration of survival is 9 (1-24) months and 20 (1-83) months. Median duration of DFS is 10 (2-23) and 17 (3-66) months, respectively. Conclusions. The low number of enrolled patients and the short follow up in the FLAIE group, and the historical nature of the comparison do not allow to draw any definitive conclusion on the efficacy and tolerability of the two induction regimens. Anyway, compared to our previous experience with FLAG-Ida, the addition of etoposide does not seem to increase the antileukemic efficacy in patients with intermediate risk AML and might reduce tolerability (as indicated by deaths in induction and incidence of infections).

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RELEVANCE OF AUTOIMMUNE THYROID DISORDERS IN HAEMATOLOGICAL MALIGNANCY

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The relationship between thyroid disorder and haematological malignancies is debated. To clarify this controversial issue, a prospective study on thyroid function in haematological diseases was performed. The occurrence of thyroid disease was evaluated in 40 consecutive haematological patients with different disease (23 acute leukemias, 6 bone marrow aplasias, 7 multiple myelomas, 2 chronic lymphoid leukemias, 2 non Hodgkin's lymphomas), before starting therapy and in 40 age-matched healty controls living in the same borderline iodine-sufficient geographic area. All subjects were submitted to clinical ultrasono-thyroid evaluation and serum free T4, free T3, TSH, thyroperoxidase and thyroglobulin antibody determination. Fine needle aspiration was performed in all thyroid nodules.The overall incidence of thyroid disorders was 23 in 40 (42%) haematological patients and 7 in 40 (14%) in controls ($p<0.0001$). The prevalence of nontoxic goiter was 27.4% in haematological patients and 11% in controls ($p=0.003$). Autoimmune thyroiditis was found in 16% of haematological patients and only 2% of the controls ($p<0.005$). Other thyroid disorders found in the haematological group included 1case of thyroid carcinoma and 1 of subacute thyroiditis, whereas in the control group only 1 case of Graves'disease and none of the other disorders were found. Free T4, free T3 and TSH concentrations showed no differences between haematological patients and controls. The incidence of thyroperoxidase antibodies was higher in haematological patients than in controls (23.5% vs 8%; $p<0.005$),whereas the difference of thyroglobulin antibodies was not significant. In haematological patients the presence of thyroid antibodies was more frequently associated with autoimmune thyroiditis and was more common in the younger group. In conclusion, the present study provides evidence that the overall incidence of thyroid disorders is increased in patients with acute leukemia, and indicades the opportunity of screening for thyroid disease in patients with haematological malignancies.

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SERUM TRYPTASE LEVELS IN 93 DE NOVO AML PATIENTS AT DIAGNOSIS: CORRELATION TO CBF-LEUKEMIAS

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Alpha -and beta-tryptase genes encode lineage-associated

serine proteases that are abundantly expressed in mast-cells and, in trace amounts, in basophils. Under physiologic conditions no other myeloid cells express tryptases, but in several myeloid leukemia cell lines and in myeloblasts of AML patients, tryptase expression is elevated. AML blasts are capable to produce and release tryptase in cell culture and levels of serum tryptase are also elevated in certain FAB subtypes of AML. In an attempt at correlating the levels of tryptase with FAB classification and cytogenetics, we have analyzed serum samples collected at diagnosis, from 93 AML patients (mean age 43 year, range 17-76; M/F ratio 50/43). Fifty healthy people (mean age 35 y, range 20-50) and 30 ALL patients (mean age 38 y, range 17-59) were conidered as controls. The total serum concentration was measured by UniCAP 100 and UniCAP Tryptase Fluorenzyme Immunoassay Kit (Pharmacia-Upjohn, Uppsala,Sweden). The median value of tryptase level in the control group amounted to less than 5 nanograms/milliliter. Elevated tryptase levels (more than 15 nanograms/milliliter) were found in 43 out of 93 AML-patients (46.2%). According to FAB classification, we detected high levels in 1 of 5 (MO), 8 of 22 (M1), 10 of 21 (M2), 3 of 9 (M3), 8 of 18 (M4), 9 of 10 (M4eo) and 3 of 8 (M5). According to cytogenetics we recorded elevated serum tryptase levels in 7 of 8 patients with t(8;21) and in 9 of 10 inv(16), with a median level of 89.3 nanograms/milliliter; furthermore, 3 patients with CBFL showed the highest levels of tryptase (more than 200 nanograms/milliliter). Our data suggest that elevated serum tryptase levels may play a role as a specific myeloid marker for AML subtypes.

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LONG-TERM MOLECULAR COMPLETE REMISSION WITH PULSED ATRA AS SINGLE AGENT IN PML-RARALPHA-POSITIVE ACUTE PROMYELOCYTIC LEUKEMIA

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All-trans retinoic acid (ATRA), alone or combined with chemotherapy (CHT) is widely used in the treatment of acute promyelocytic leukemia (APL). When used alone, ATRA results in a considerable proportion of complete remissions (CR). However, the continuous administration of ATRA as single therapy almost invariably leads to relapse in a short period of time (months). Thus, conventional chemotherapy often followed by autologous or allogeneic stem cell transplant (SCT) are used to maintain long term remission. Basing on pharmacokinetic evidence that that acquired resistance to ATRA is frequently suppressed by the intermittent use of the drug, we treated with "pulsed" ATRA seven APL patients who were either molecularly refractory after combined ATRA/CHT treatment, or relapsed, or at diagnosis, but not eligible for the combination treatment. They were treated with ATRA (45 mg/m^2/day) for 15 days. After 2 weeks, the treatment was then prolonged continuously for 1 week every 2 weeks. Molecular analysis was performed by qualitative and quantitative reverse transcription-polymerase chain reaction (RT-PCR). All but one patients (87%) obtained molecular CR, as assessed by qualitative RT-PCR. Quantitative RT-PCR confirmed these results, showing a progressive reduction to a 'negligible quantity' of PML-RARalpha fusion transcript (ratio PML-RARalpha/ABL x 104 ABL < 10(-1)) in all but one patient treated with pulsed ATRA therapy. One patient achieved a molecular CR by receiving a combination of arsenic trioxide and pulsed ATRA. The median progression free survival was 24 months (13-60 months). After a median follow-up of 30 months, 5/7 patients (71%) are in continuous molecular CR, in one case after allogeneic SCT. We report on very long molecular CRs obtained with intermittent ATRA alone (without chemotherapy), confirming our previous experience. This approach, if validated in larger studies, could therefore be effective in relapsed/refractory or high risk frail patients unsuitable for high-dose therapy and SCT. Furthermore, it may be proposed as induction therapy for selected older APL patients if considered not to be eligible for combined ATRA/CHT due to inadequate performance status or concurrent disease.

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IMATINIB MESYLATE IN THE TREATMENT OF NEWLY DIAGNOSED OR REFRACTORY/RESISTANT C-KIT POSITIVE ACUTE MYELOID LEUKEMIA. RESULTS OF AN ITALIAN MULTICENTRIC PHASE II STUDY

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From February 2003 to November 2003 we treated with imatinib 36 c-kit positive AML patients who were not amenable to conventional chemotherapy. Fifty-one percent of the patients were refractory/relapsed and 49% were previously untreated. All the patients had c-kit positive leukemic cells (median 55%, range 16 – 96%), with a high mean fluorescence index. One patient aberrantly expressed PDGF-receptor beta (PDGFR- beta) on blastic cells (46%). No patient was found to carry the bcr/abl rearrangement. The median imatinib dose was 600 mg/day (range 200 – 700), for a median of 31 days (range 2 – 311+). Non hematological toxicity was mild and not different from the one found in chronic myeloid leukemia imatinib trials. Six patients died while on therapy with imatinib (2 with multi organ failure, 2 of disease progression, and 2 of cerebral stroke). Fifteen patients died during the follow-up. Disease progression was the principal cause of death. No patient achieved a complete or a partial remission. In two patients the disease remained stable as defined by no peripheral blood or bone marrow modification. They are now continuing imatinib after 270 and 311 days. The patient with

PDGFR- beta aberrant expression had a platelet increase which lasted for one year with transfusion independence. In this meanwhile, blast cells became PDGFR- beta negative. Intriguingly, by flow cytometry we found that c-kit de-phosphorilation after imatinib administration was not related with the clinical response. We conclude that imatinib alone is not effective in c-kit positive AML. Further studies are warranted in the subset of patients with PDGFR- beta expression because positive blast cells may be more sensitive to imatinib therapy.

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COMPLEX CHROMOSOMAL ANOMALIES AND IMMUNOPHENOTYPE IN DE NOVO ADULT AML

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In adult AML the complex karyotypic alterations at the diagnosis characterize a sub-group of patients with worse prognosis. It is also known that the complete immunophenotypic characterization contributes to define the prognosis of de novo adult AML. We have observed 10 young adult de novo AML patients (median age: 42 years, r: 29-50) in the last 2 years, who presented complex chromosomal anomalies in bone marrow blood. On the basis of FAB classification the patients were considered LMA-M5 (3 pt.), LMA-M2 (2 pts.), LMA-M4 (2 pts.), LMA-M1 (2 pts.) and LMA-M0 (1 pt.). Two patients showed internal tandem duplications of FLT3; eight out of ten presented hyperleukocytosis (WBC> $40x10^9$/L). The clinical outcome was that one of a "high risk" AML; at the present only two patients are still alive in CR (+12 months and +14 months). The immunophenotype at the diagnosis showed the following common pattern: $CD34^+$, CD38+, CD33+, $CD13^-$, HLA-DR-. Several studies have shown the prognostic significance of the expression of differentiation myeloid markers at the diagnosis of adult de novo AML (Legrand O. *et al.,*, Blood, 2000, 96(3), 870-877). However, specific immunophenotype expression patterns associated with complex chromosomal anomalies are still unknown. Further studies are warranted to confirm the correlation between complex chromosomal anomalies $CD34^+$, $CD38^+$, $CD33^+$, $CD13^-$, HLA-DR- immunophenotypic pattern.

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USE OF DEFIBROTIDE IN THE PROPHYLAXIS OF VENO-OCCLUSIVE DISEASE IN 12 PATIENTS AFFECTED BY RELAPSING OR CHEMOREFRACTORY AML TREATED WITH GENTUZUMAB OZOGAMICYN (MYLOTARG)

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Gemtuzumab ozogamicin (Mylotarg) uses a recombinant humanized anti-CD33 monoclonal IgG4 antibody to deliver the potent cytotoxin, calicheamicin, into CD33 positive cells. The antigen CD33 is expressed on blast cells in 80% to 90% of acute myeloid leukemia (AML) cases but, importantly, is not expressed on pluripotent hematopoietic stem cells or on nonhematologic cells. Mylotarg is today a novel and effective regimen for refractory and relapsed AML but is associated with an incidence of approximately 20% Grade 3 or 4 hyperbilirubinemia and liver transaminitis and, was shown, to be associated with the development of potentially fatal VOD occurred when Mylotarg was used either as a single agent or when it was given with other cytotoxic agents. Hepatic veno-occclusive disease (VOD), diagnosed by Seattle and Baltimore standard criteria, include hyperbilirubinemia, painful hepatomegaly, fluid retention or sudden weigth gain. Generally the median time of occurrence of VOD is 25 days (range 10-35) after the first dose of Mylotarg. Recently, promising results in the treatment of established VOD with defibrotide were reported. Therefore, defibrotide may be used as a prophylactic regimen for hepatic VOD. Defibrotide is a polydeoxyribonucleotide-derived antiischemic drug with multiple sites of action involving both cellular and plasmatic targets. It is able to interfere with leukocyte adhesion to endothelial cells mainly in activated conditions, involving the ICAM-1/LFA-1 adhesion system. Besides it has been demonstrated to produce profibrinolytic, cytoprotective, and vaso-facilatory actions by decreasing the PAI-1 levels. Preclinical studies have also demonstrated profibrinolytic effects and inhibition of fibrin deposition with selective activity on small vessels. No significant effects on systemic coagulation have been shown in either preclinical studies or clinical trials of DF. Moreover, it increases the synthesis of PGI2, that is a strong inhibitor of platelet aggregation and an inducer of vessel dilatation, without affecting the coagulation. Therefore, it prevents ischemic and thrombotic events that are both present in VOD. We report our experience on 12 patients treated with Mylotarg regimen and defibrotide prophilaxis. In 8 successive patients 6 female and 2 male median age 66 (range 38-84) admitted between October 2003 and February 2005 because relapsed (older patients) or two lines primary resistant therapy (younger patients) CD33 + AML, received Mylotarg 6mg/sqm day 1 and 14 and Defibrotide prophylaxis, 10 mg/kg/day intravenously, from day -1 to day +30. In the other 4 patients, 2 male and 2 female, median age 74 (range 65-84) because older and frail patients affected by AML we have used Mylotarg 3 mg/sqm on day 1, 3, 5 and Defibrotide prophylaxis, 10 mg/kg/day intravenously, from day -1 to day + 14. In the last 4 patients we have used a manteinance therapy too, with GO 3 mg/sqm every 28 days for 8 months. Before GO therapy Bilirubin, AST and ALT values were in normal range. VOD was never observed in our series, but 2 patients have developed grade 2 hypertransaminasemia/hyperbilirubinemia. Another patients showed on day +13 abdominal pain and transient hypertransaminasemia/hyperbilirubinemia that disappeared after 5 days. An abdomen US demonstrated the presence of gallbladder stones. The remaining patient showed no toxicity signs. Although GO induced severe thrombocytopenia it was never observed an increase of the incidence of haemorrhage signs. On the other hand, Defi-

brotide administration was well tolerated and it was never observed any toxicity sign during the long-lasting administration. GO induced a PR in the two patients who were resistant to the previous treatments. Moreover, the other two patients achieved a CR and a PR, respectively. four patients died four and six months after therapy, for relapse. We have not found death due to therapy toxicity.

Conclusions. Defibrotide is a well tolerated agent that could be useful for the prophylaxis of VOD in patients treated with GO. Further studies are needed to confirm the effectiveness to prevent VOD.

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EFFICACY AND FEASIBILITY OF THE COMBINATION OF GEMTUZUMAB OZOGAMICIN (GO-MYLOTARG©) AND FLUDARABINE, ARA-C AND I DARUBICIN (MY-FLAI REGIMEN) IN AML ELDERLY PATIENTS

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Treatment of elderly patients with acute myeloid leukemia (AML) is characterized by a low complete remission (CR) rate of less than 50% and short remission duration with a median disease-free survival (DFS) of less than one year. Antibody-targeted therapy is a promising approach in this kind of patients. In particular gemtuzumab ozogamicin (GO), an anti-CD33 antybody linked to calicheamicin, was approved for the treatment of elderly patients with AML. It has been recently demonstrated that incubation of leukemia cells with anti-CD33 monoclonal antibody and Arabinosyl Citosine (AC) or Idarubicin (IDA) independently leads to additive antiproliferative effects and therefore enhance the cytotoxicity. Moreover IDA is showed to reduce Pgp-mediated resistance of blastic cells and Fludarabine (FLUDA) can increase the cytotoxic effect of AC. Only few data are available about the combination of GO with other conventional chemotherapeutic agents *in vivo*. We treated with My-FLAI regimen 8 elderly AML patients. They received FLUDA 25 mg/m^2, AC 1 g/m^2 and IDA 5 mg/m^2 for 3 days; on the fourth day GO was administered at 3 mg/m^2. Two cycles were planned. The median age was 70 years, the male/female rate was 5/3. Four patients had normal kariotype, 3 had a complex one, while in a patient it was not performed. In five cases leukemia was secondary to a myelodysplastic syndrome. Five of the 8 patients who received the first cycle reached the CR (63%), two patients obtained a partial remission (PR), and a patient was resistant to the treatment. Of the 6 patients that underwent the second cycle 5 resulted in CR (4 maintained from first cycle, 1 achieved with the second one). The overall CR response rate was 63% after two cycles. The most common adverse events were fever and chills during the administration of GO, infections secondary to neutropenia (66%) and transient grade I/II gastrointestinal toxicity. No hepatic veno-occlusive disease nor grade III/IV bleeding were recordered. The median time to recovery from severe neutropenia (ANC < 0.5x10^9/L) was 16 days and from severe thrombocytopenia (platelets < 20.000x10^9/L) was 14 days. We conclude that the association between GO and FLAI regimen is effective and well tolerated in AML elderly patients: in particular the CR rate appears consistent with previous studies employing conventional chemotherapy. Further studies are required to assert, after a longer follow-up, if this target-based regimen can improve DFS, which is the major problem of elderly AML patients.

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OUTCOME OF ACUTE MYELOID LEUKEMIA IN THE ELDERLY PATIENTS AFTER EITHER INDUCTION OR PALLIATIVE THERAPY

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Acute myeloid leukemia (AML) is a hematopoietic malignancy that especially affects older adults. Despite scientific advance in epidemiologic, genetic and biological features of AML, the prognosis of elderly AML remains highly unsatisfactory, due to higher rate of both biological and clinical unfavourable prognostic factors. The aim of this study was to analyze the outcome of elderly patients (pts) affected by AML receiving either induction chemotherapy or only palliative or no treatment. Patients and methods. Between January 1998 and December 2003 197 pts were admitted to our department with newly diagnosed, untreated AML. Among these, 95 (48%) were > 59 years old , median age 66 years (range 60-86), M:F = 47:48, 58 were de novo and 37 secondary AML (MDS 33, MPS 3, chemotherapy 1). According to cytogenetic, of 73 pts 2 were categorized in the intermediate, 20 in the high and the remaining 2 patients in the good risk group. Of the 95 pts, 70 were treated with induction therapy and 25 received palliative treatment. The two groups were different for risk factor distribution: the first had 64 years as median age (range 60-79), while the second showed 73 years as median age (range 63-86)and worse performance status. The median overall survival was 47 weeks in pts receiving induction therapy with schedule containing Ara-C and 9 weeks in those receiving non-curative treatment (p<0,0001). Among all 95 pts 30 were > 70 years: of them 10 with good performance status received induction chemotherapy and 20 palliative care. The median survival in the two groups was 7.7 and 7.6 weeks, respectively. Conclusion. These results indicate that there is a significant advantage in intensive chemotherapy rather than no-curative approach only for pts < 70 years. No difference has been detected regarding overall survival in pts > 70 years, so a palliative approach rather than aggressive chemotherapy seems to be more reasonable in this group of patients in order to preserve the quality of life.

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FLUDARABINE IN ADDITION TO CYTARABINE AND G-CSF IN ELDERLY PATIENTS AFFECTED BY ACUTE MYELOID LEUKEMIA

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The incidence of acute myeloid leukemia(AML)is especially high in elderly people. In this group of patients (pts) outcome is very poor with a low response rate and short overall survival. Promising results have been published in

literature using fludarabine + cytarabine + granulocyte colony stimulating factor (FLAG regimen). The rational of this therapeutic approach depends on the ability of fludarabine to increase the intracellular concentration of the active metabolite ara-C5'triphosphate (ARA-CTP) which is responsible for the cytotoxic effects in leukemic blasts. The aim of this study was to analyze the outcome of elderly pts affected by AML receiving FLAG induction chemotherapy with G-CSF and fludarabine 30 mg/mq followed after 4 hours by cytarabine 2gr/mq for 5 days. Patients and methods. Between January 1998 and December 2003 95 pts over 60 years were admitted to our Division; among these, 44 received FLAG schedule. Of pts receiving FLAG the median age was 64 years (range 60-72), M:F = 24:20, 26 having de novo AML and 18 secondary AML. Karyotype was performed in 40 pts: 25 were classified in the intermediate, 14 in the high and 1 pt in the good risk group. After one FLAG cycle, 15 pts (34%) achieved complete remission (CR) and 4 (9%) partial remission (PR), that became complete after the second course of FLAG. We observed 3 deaths for infection (7%) and 22 failure (50%). Pts in CR after induction received consolidation and in 5 cases intensification with autotransplant. The median disease free survival (DFS) of responding pts was 66 weeks and the median overall survival (OS) of all pts was 45 weeks. Univariate analysis was performed analysing the impact of leukocytosis, serum LDH, karyotype, and de novo vs secondary AML on DFS, OS and CR: no significant differences were detected in the different subgroups. All pts receiving FLAG regimen were evaluated for side-effects, duration of chemotherapy-induced cytopenia and infections. The median time to a neutrophil count > 500/mmc was 15 days (range 7-45); a platelet count > 50000/mmc was reached after a median of 17 days (range 6-48). G-CSF was administered for a median of 16 days (range 2-21). The FLAG regimen was associated with WHO grade III-IV toxicity in 18 cases (8 gastrointestinal, 8 cardiac and 2 liver toxicity). During neutropenia we recorded 7 episodes of FUO, 29 infections microbiologically documented and 14 clinically documented. We observed 3 deaths due to infections. Conclusion. The FLAG regimen, used in elderly AML pts, showed good tolerability, mild toxicity and similar efficacy to other chemotherapeutic regimens. It allowed to achieve an acceptable response rate also in pts with secondary AML. No difference was detected in response rate between pts with secondary and de novo AML.

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ACUTE MYELOID LEUKEMIA CELLS EXPAND A POPULATION OF CD4+CD25+ REGULATORY T CELLS THROUGH THE CONSTITUTIVE EXPRESSION OF THE IMMUNOREGULATORY ENZYME INDOLEAMINE 2,3-DIOXYGENASE

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The expression of the catalytic enzyme indoleamine 2,3-dyoxigenase (IDO) has been recently identified as a T-cell inhibitory effector pathway in some subsets of normal regulatory dendritic cells, bone marrow mesenchimal stem cells and solid tumors. Here, we show that human acute myeloid leukemia (AML) cells constitutively express IDO mRNA and protein and exhibit functional IDO activity by inhibiting allogeneic T-cell proliferation. Conversely, normal $CD34^+$ cells do not express IDO mRNA and they inhibit T-cell function through IDO expression only upon stimulation with IFN-gamma. We, then, cultured IDO+ AML cells with allogeneic T cells with and without the IDO-inhibitor 1-methyl tryptophan (1-MT). As compared to control T cells, cells cultured without 1-MT showed reduced proliferation and increased production of IL-10, IL-4 and TGF-beta1, whereas those cultured with 1-MT showed comparable proliferation and type 1 cytokine production. Cells cultured with IDO+ AML cells without 1-MT had increased percentage of CD4+CD25bright T cells, which expressed surface CTLA-4 and FOXP3 mRNA. Purified CD4+CD25+ T cells showed low or absent proliferation and produced high amount of IL-10 and TGF-beta1. Stimulation with IDO+ AML cells resulted in the expansion of CD4+CD25bright T cells since their absolute number significantly increased over that observed with control T cells. Moreover, CD4+CD25+ cells obtained after culture with IDO+ AML cells in absence of 1-MT markedly reduced allogeneic proliferation of naive T cells in a dose-dependant manner, whereas CD4+CD25- T cells had no significant effect. Of note, cells cultured with IDO+ AML cells in presence of 1-MT had no inhibitory capacity on allogeneic T-cell proliferation. These data suggest that IDO overexpression may act as a novel and specific mechanism of leukemia escape from immune response. Moreover, our in vitro results demonstrate that IDO-expressing tumor cells inhibit T-cell immune response through the expansion of a population of CD4+CD25+ regulatory T cells.

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FLUDARABINE-BASED REGIMENS AS FRONT-LINE THERAPY IN ACUTE MYELOID LEUKEMIA OF ELDERLY PATIENTS: COMPARISON BETWEEN TWO DIFFERENT REGIMENS

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Association between fludarabine (Fluda) and cytosine arabinoside (ARA-C) have shown in a variety of single arm studies an effective antileukemic effect with acceptable toxicity in high risk myelodisplasias and acute myeloid leukemias in elderly patients. In order to increase the synergistic effect of fluda and ARA-C further association (i.e. anthracyclines) or different schedules of administration (i.e. continous infusion) have been proposed, but different regimens have not been compared. In order to asses if there is an advantage of an association or schedule compared to another we retrospectively analyzed results from two different fludarabine-based regimens administered as front-line therapy in patients with acute myeloid leukemias aged up to 60 years. A total of 42 patients were treated with fluda 25 mg/m^2, ARA-C 1 gr/m^2 and idarubicin 5 mg/m^2 as single day administration for three days (FLAI) or fluda 20 mg/m^2 and ARA-C 1,4 mg/m^2 as continuous infusion for three days fluda and 4 days ARA-C (c.i.FLA). Total dosage of fluda was 75 mg/m^2 in FLAI and 70 mg/m^2 in c.i.FLA,

while ARA-C total dose was 3 gr/m^2 in FLAI and 4,7 gr/m^2 in c.i.FLA. Dose administered of idarubicin was 15 mg/m^2. Patients who obtained complete remission (CR) defined as less of 5% of bone marrow blasts received a second course of the same chemotherapy as consolidation. 19 patients were treated with FLAI regimen, 9 males and 10 females, mean age 69,3 years (range 61-75) and 23 received c.i.FLA regimen, 12 male and 11 female, mean age 69,4 years (range 61-84). Results were as follows: 8/19 (42%) patients obtained CR after induction with FLAI while 11/23 (48%) were in CR after first course c.i.FLA. Difference is not statistically significative. Median disease free survival (DFS) was 9,53 months for FLAI and 7,67 months for c.i.FLA (p=NS) and median overall survival (OS) in the group in CR was 10.8 in the FLAI group and 10 months in c.i.FLA group (p=NS). Induction deads were 1/19 in FLAI group (5%) and 3/23 in c.i.FLA group (13%). The global median OS, including resistant patients was 6,57 months for FLAI and 6 months for c.i.FLA (p=NS). One patient in each group underwent autologous bone marrow transplantation in complete remission. Results of the comparison of this two different regimens suggest that there is no substantial difference between single daily administration added of idarubicin and continuous infusion of fluda and ARA-C. Both cycles seem to have the same antileukemic activity with overlapping toxicity, so they can be, in our opinion, safely used as front-line therapy in elderly patients affected by acute myeloid leukemias. What is to underline in this setting of patients is the short duration of the CR. Probably some kind of maintenance or association without increasing toxicity is needed, and experience with monoclonal antibodies are an interesting way to follow.

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EVALUATION OF MINIMAL RESIDUAL DISEASE IN ACUTE PROMYELOCYTIC LEUCEMIA PATIENTS THROUGH"REAL TIME" QUANTITATIVE PCR. COMPARISION WITH CONVENTIONAL RT-PCR TECHNIQUES

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Introduction. Acute promyelocytic leukaemia (APL) is characterised by reciprocal translocation t(15; 17) involving promielocytic leukemia (PML) gene, by a transcription factor on chromosome 15q22 and by retinoic acid receptor alpha (RAR alpha) gene on chromosome 17q21. Quantitative evaluation of minimal residual disease, kind of fusion transcripts, could be useful on clinical practice. Aims of the study To compare the results obtained with quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) with those obtained whit less sensitive conventional RT-PCR standardized by BIOMED-I protocol. To verify the presence of cryptic break cluster region 2 (bcr2) positive patients in qualitative Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). To evaluate, through, QRT-PCR, minimal residual disease of APL patients in order to characterise kinetic of quantitative decrement of transcripts

Materials and methods. This research involves 11 cases, 6 male and 5 female (Mean age 46). Mean white blood count was 26.2 x10^3/microliter. All patients met morphologic criteria for the diagnosis of acute myelocytic leukemia [AML]-M3-variant (APL) according to the French – American – British classification system. Diagnosis of APL was confirmed by RT-PCR analysis for PML/RAR alpha rearrangements. Cells from bone marrow or peripheral blood were collected at exordium, induction and consolidation phase. Results In all cases avoid false-negative results, a housekeeping gene (Abelson) was assayed simultaneously during first-round PML/RAR alpha amplification. Seven cases Bcr1 positive (63%) and 4 Bcr3 positive (36%) were identifies. The "real-time" PCR did not revealed bcr2 cases cryptic among bcr1 positives cases. The pre-amplification methods by QRT-PCR reveals 1 false negative (14%) inside bcr1 negative patients respect RT-PCR method. The same procedure made for bcr3 cases, did not reveal difference. Different kinetics of decrement in the fusion transcripts have been evidence from quantitative PCR. Seven cases with Bcr1 positive isoforms were negative at first cycle of consolidation. While all cases with Bcr3 positive isoforms were negative after therapeutic induction.

Conclusions. The pre-amplification techniques by QRT-PCR reveals false negative isoforms respect RT-PCR method. The real-time PCR with pre-amplification, is a more sensible method respect conventional RT-PCR with a minor execution time and use of small reagents quantity.

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INTERNAL TANDEM DUPLICATION, D835 MUTATION IN ACUTE MYELOID AND PROMIELOCITIC LEUKEMIA

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Introduction. Biological characteristics and prognostic values of FMS-like kinasi 3 (FLT3) gene dislocated on 13q12 were studied. This protein is employed in staminal and B cell differentiation. Mutations of FLT3 are present in 35-40% of patients with acute myeloid leukaemia. These genetic alterations consist of "Internal tandem duplications" (ITDs) and substitution of aspartic acid (Asp) in the 835 positions. Aims of study The aim of this study was to individuate a diagnostic method for FLT3/ITDs and D835 mutation.

Materials and methods. From January 2004, 34 consecutive cases with de novo Acute Myeloid Leukemia (AML) have been studied. Median age was 50 years (range 25 – 80). Twenty-three case were male and 11 female. Two PCR methods have been used for FLT3/ITDs and one for D835 mutations. Mixed lineage leukemia gene (MLL) study and t(9,22)p190, t(9,22)p210, t(8;21), t(4;11) translocations analyses have been performed. Study of sequence gene were made in Leukaemia Frankfurt Institute. Were utilised a set of new primers dislocated between 10/11 and 12/13 exons of FLT3 gene

Results. In 11/34 (34,4%) cases, FLT3/ITDs transcripts were positives by RT-PCR. In only two cases, FLT3/ITDs positives was confirmed with sequence analysis. In 2/11 positive cases (18,1%) were revealed base substitutions but

not specific mutations in FLT3 gene. MLL studies and specific AML translocations were negative on all cases. D835 mutations by exon 17 were positives on 2 cases (5,8%). Conclusions The new primers set, for exon 11/12; 12/13 in FLT3/ITDs gene, had given results confirmed by sequence analysis.

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MONITORING OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA USING PERIPHERAL BLOOD AS AN ALTERNATIVE SOURCE TO BONE MARROW

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In acute myeloid leukemia (AML), the level of MRD as determined by multiparametric flow cytometry (MPFC) has been shown to impact on remission duration and survival. BM is the most common source to perform MRD assessment. We have previously showed that MRD negativity, as defined by a number of BM residual leukemic cells (BMRLC) <3.5x10^{-4} after the consolidation cycle, was associated with a significant longer disease free survival (DFS) and overall survival (OS). However, it has recently reported that PB could be used as an alternative source to BM, for MRD studies in children with T acute lymphoid leukemia. Based on this, we investigated whether PB can be used instead of BM to monitor MRD in AML patients. Thirty-four adult patients with AML were entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61 yrs) or AML13/AML15 (age>61 yrs), all consisting in intensive induction and consolidation cycles, and, for patients aged <61 years, autologous or allogeneic stem cell transplantation. Median age was 50 years (range 21-73), all FAB subtypes were represented with the exception of M3 cases. We used MPFC to compare MRD measurements in 34 and 32 pairs of BM and PB after induction and consolidation, respectively. Findings in BM e PB were completely concordant after induction and consolidation therapy. Median value of BMRLC and PB residual leukemic cells (PBRLC) after induction, were 5.1x10^{-3} (range 1x10^{-4}-1.64x10^{-1}) and 4.7x10^{-3} (range 3x10^{-4}-1.14x10^{-1}), respectively (r=0.84, p<0.001). After consolidation, the median value of BMRLC and PBRLC were 6.8x10-3 (range 1x10-4-6.6x10^{-2}) and 7.7x10^{-3}(range 3.5x10^{-5}-1.34x10^{-1}), respectively (r=0.83, p<0.001). The cut-off value of PBRLC which correlated with the clinical outcome was 1x10^{-4}; in fact, 22 of 31 (71%) patients with PBRLC >1x10^{-4} after induction (MRDInd+) had a relapse, whereas, the 3 patients with <1x10-4 did not (p=0.036). After consolidation, using the same threshold, 27 patients were considered MRDCons+, and 78% (21/27) experienced a relapse; the remaining 5 patients, who were MRDCons-, are still in complete remission (p=0.0022). The duration of DFS was significantly longer in the MRDCons- patients as compared to those MRDCons+ (median 52.6 mos, range 20–163 versus median 28 mos, range 4.4–140.6, respectively) (p=0.035); the multivariate analysis confirmed the independent prognostic role of the PB MRD status at the end of consolidation (p=0.009). In conclusion: 1) PB may be used to monitor MRD in patients with AML, allowing closer monitoring of leukemia while sparing patients the discomfort of BM aspirations; 2) the level of MRD in the PB after consolidation therapy, may provide strong prognostic informations. Our results warrant further studies in a larger group of patients recruited to different treatment protocols and monitored at different intervals.

P150

CONTINUOUS SEQUENTIAL INFUSION OF FLUDARABINE AND CYTARABINE FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA. RESULTS FROM A PHASE II STUDY ON 73 PATIENTS

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New approaches are needed for elderly patients with acute myeloid leukemia (AML). The combination of fludarabine (FAMP) with cytarabine (ARA-C) $^{+/-}$ G-CSF has been proven to be effective in poor risk AML. However, little is known as concerns continuous sequential infusion of the two drugs in newly diagnosed patients. In a phase II study, we investigated the efficacy and toxicity of a regimen including FAMP + ARA-C administered as sequential continuous infusion (CI-FLA) in a series of untreated non-M3-AML patients aged more than 60 years. FAMP at a loading dose of 10 mg/sqm over 15 min at day 0, and after three hours and half ARA-C at a loading dose of 390 mg/sqm over 3 hours were given; at the end, FAMP at 20 mg/sqm/ci/24 hours for a total of 72 hours and ARA-C at 1440 mg/sqm/ci/24 hours for a total of 96 hours were started. G-CSF was added at day +15 at a dose of 5 microg/kg. Patients achieving CR were programmed to receive an additional course of CI-FLA. After consolidation, G-CSF at 10 microg/kg was given from day 15 in order to mobilize CD34^{+} cells. Between June 2001 and January 2005, 73 patients received the treatment. Median age was 69 years (range 61-81). In 29 patients (40%) an antecedent myelodysplastic syndrome preceded overt AML. Cytogenetic analysis showed normal karyotype in 35 patients, complex karyotype or other unfavourable chromosomal abnormalities in 28 cases, no mitoses in 10 cases. Finally, 55 patients were affected by one or more concomitant diseases requiring specific treatment. Overall, 46 (63%) patients achieved CR, all but one following one course of CI-FLA. There were 15 induction deaths (20%), while 12 patients (16%) were refractory to induction treatment. The median number of days to neutrophil > 0.5x10^{9}/L and platelet > 20x10^{9}/L was 19 (7-34) and 19 (9-38), respectively. Patients needed a median of 3 platelet units (0-19) and 7 blood units (1-38), respectively. All patients required broad spectrum empiric antibiotic therapy, while 25/73 cases (18%) needed intravenous antifungal treatment. Documented infections occurred in 9 cases (12%). Thirty-seven patients out of 46 were eligible for the programmed consolidation course. Five of them died from infectious complications during subsequent pancytopenia; therefore, last 25 patients received reduced consolidation with FAMP+ARA-C at 48 and 72 hours, respectively. Thirty-one patients were monitorized for the mobilization of CD34^{+} cells, collection being successful in 25 (81%). Medi-

an number of $CD34^+$ cells/kg collected was $6.8x10^6$ (2-60.3), median number of apheresis being 2 (1-2). Overall, 18 patients have received autologous stem cell transplantation (ASCT). After a median follow-up for remitters of 10 months (2-38), 24 patients are alive: among these, 16 are in continuous CR, while seven have relapsed and one never achieved CR. In conclusion, this study demonstrates that CI-FLA is an effective and well-tolerated regimen for elderly patients with AML. Therapeutic results are extremely encouraging as to CR achievement, $CD34^+$ cell collection and ASCT feasibility. A longer follow up is needed to properly evaluate therapeutic results in terms of long-term disease free survival.

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POST-REMISSIONAL TREATMENT OF ACUTE MYELOID LEUKAEMIA: COMPLIANCE TO TRANSPLANT PROCEDURES AND LONG-TERM FOLLOW UP

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Post-remissional treatment of patients with acute myeloid leukaemia (AML) in Ist complete remission (CR) is a controversial issue as concerns the employ of allogeneic or autologous bone marrow transplantation, moreover, patients are often unable or refuse to undergo these intensive procedures. In our study we evaluated 99 AML patients consecutively enrolled in the EORTC/GIMEMA AML10 trial in our institution between 4/92 and 6/98. According to this protocol, all patients were planned to receive either allogeneic or autologous transplantation in Ist CR after a 3-drug induction and 1 consolidation course, but only 24 (group A) and 38 (group B), respectively, underwent these procedures. The remaining 37 patients (group C) did not receive any further treatment, due to refuse (9 patients), medical decision (2 patients), or concomitant severe diseases (infective complications, 15 patients, liver disease, 5 patients, heart failure, 2 patients, neurological complications 2 patients, other causes 2 patients), Among the 24 patients of group A, 4 (16,6%) died from transplant-related toxicity, and 5 relapsed after a median time of 7 months from CR (range 5-37); the remaining 15 patients are in continuous CR after a median time of 111 months (range 75-149). Among the 38 patients of group B, 5 (13%) died during the aplastic phase of autologous transplantation and 15 relapsed after a median time of 10 months (range5-84) from CR; the remaining 18 patients are still in CCR after a median time of 112 months (range 79-154). As for the 37 patients of group C, 22 relapsed after a median time of 8,5 months from CR (range 3-65), while 15 are still in CCR after a median of 132 months (range 82-153). 13-year projected probability of overall survival for the three groups of patients (A,B,C) is 62%, 49% and 48% respectively. 13-year projected probability of leukaemia-free survival is 62%, 47% and 39% respectively. In conclusion, more than one third of patients with AML in Ist CR is unable to complete the intensive post-remissional treatment with a transplant procedure; however, some of these patients may remain disease free and be possibly cured without any other treatment. These findings emphasize the value of clinical as well as biological prognostic factors at onset, in order to tailor intensive treatment and avoid unnecessary toxicity in good-risk AML patients.

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GENTUZUMAB OZOGAMICIN,CITOSINE ARABINOSIDE, G-CSF COMBINATION IN THE TREATMENT OF ELDERLY POOR PROGNOSIS ACUTE MYELOID LEUKEMIA. A MULTICENTRIC STUDY

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Gentuzumab Ozogamicin (GO) is an anti-CD33 antibody linked to the cytotoxic antibiotic calicheamycin and it is effective as single agent in the treatment of poor risk acute myeloid leukemia (AML) patients. In 3 Italian Hematology Departments from September 2003 to December 2004, we treated 26 AML patients, both untreated (12 cases) and resistant (14 cases) with the following protocol: rhG-CSF(5 µg/kg, on days 0 through 8), Aracytin as continuous perfusion (100 mg/m^2 on days 4 through 8) followed by GO (6 mg/m^2 iv over 2 hours on day 9) (G-GOA). Inclusion criteria were: 1) $CD33^+$ de novo or secondary AML (except M3 AML; 2) Primary refractory AML or relapse of AML in patients between 61 and 80 years; 3) Untreated patients >70 years or not eligible for aggressive chemotherapy. There were 13 male and 13 female with a median age of 69 years (range 58-77). FAB classification was 5 M0, 5 M1, 7 M2, 2 M4, 1 M5, 6 AML post-MDS. Ten patients presented a sAML. The median duration of first complete remission (CR) of 9 patients with relapsed AML was 48 weeks (range 8-76). Cytogenetic study was performed in all patients; karyotype was intermediate in 13 cases, unfavourable in 7 cases. In 6 patients no metaphases were observed. All patients performed the $CD33^+$ evaluation on BM, the median percentage of CD33 positive blasts was 90% (range 25%-95%). Fourteen patients (56%) achieved a CR and 1 patient had CR with delayed platelet recovery (CRp) with an overall response (OR) of 58% (7 untreated AML and 8 resistant patients). One patient obtained a partial remission with only a transient hematologic improvement, characterized by a peripheral increase of all hematological parameters and by a 50% reduction of the bone marrow blast count. Five patients (19%) resulted refractory to treatment and 5 patients died during the aplasia period post induction treatment. The most common adverse event was myelosuppression, as expected. Median durations of neutropenia and thrombocytopenia in patients reaching CR were 19 days (range 15-62) and 16 days (range 10-37) respectively. No VOD was recorded. Six patients (23%) developed documented infection (including pulmonary aspergillosis in 3 cases). Notably, in 2 cases we observed a grade III/IV bleeding consisting in CNS haemorrhage. Two patients died while in CR, 1 due to bladder cancer relapse and 1 to ischemic stroke. Seven patients (27%) relapsed; the median CR duration was 20 weeks (range 8-41+). At March 2005 10 patients (38%) are alive of whom 6 are still in CR (27%). The median overall survival was 17,5 weeks (1-60 weeks). On the basis of our results the G-GOA protocol seems to be effective, expecially in patients with poor risk and/or refractory elderly AML.

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COMBINATION WITH MEK1 INHIBITOR GREATLY ENHANCES ARSENIC TRIOXIDE APOPTOSIS IN PRIMARY ACUTE MYELOGENOUS LEUKEMIA BLASTS

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We have recently found that in NB4 acute promyelocytic and in K562 BCR/Abl+ cell lines treatment with MEK1 inhibitor greatly enhanced apoptotic cell death induced by Arsenic Trioxide (ATO) alone. Combined treatment resulted in the up-regulation of the strongly pro-apoptotic p53AIP1 protein (p53-regulated Apoptosis-Inducing Protein 1) in both cell lines via p73 activation, a p53 paralog that has been shown to regulate several p53-target genes including p21, Bax and p53AIP1 (Blood 104: 519-525, 2004). Our results prompted us to investigate whether MEK1 inhibitor PD184352 (Pfizer Global Research & Development, Ann Arbor, MI) and ATO synergize to induce apoptosis of primary acute myeloid leukemia (AML) blasts. Twenty primary AML samples with different FAB classification were analyzed at diagnosis. Primary AML samples were cultured in presence of escalating doses of PD184352 (0.1-20 microM), ATO (0.125-10 microM) or combinations of the 2 agents at a 1:1 ratio. After 48 h of treatment the cells were harvested for mitochondrial transmembrane potential, annexin V and sub-G1 DNA content detection. Combination Index (CI) plots were then generated using the Chou-Talalay method and Calcusyn software (Biosoft, Ferguson, MO). TAp73 (anti-proliferative, pro-apoptotic isoform) and DNp73 (pro-proliferative, anti-apoptotic isoform) ratio, and p53AIP1 expression were investigated. MEK1 inhibition strikingly increased the apoptosis induced by ATO in sixteen of twenty primary AML samples analyzed. Isobologram analysis confirmed the additive or synergistic nature of this interaction. Five responder patients showed additive effect, eleven synergism (one M1, three M2, three M3, two M4, two M5). In the responsive samples the combined treatment strikingly potentiated loss of mitochondrial transmembrane potential induced by ATO. Furthermore, in responsive samples TAp73/DNp73 and p53AIP1 expression were enhanced after PD184352 plus ATO treatment compared to ATO alone. Our data have demonstrated the potential therapeutic usefulness of this pre-clinical model of molecular therapy in AML.

Chronic Myeloid Leukemia I

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FREQUENCY, DISTRIBUTION AND PROGNOSTIC VALUE OF ABL MUTATIONS IN DIFFERENT SUBSETS OF IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA PATIENTS: AN ITALIAN MULTICENTER STUDY BY THE GIMEMA-CML WORKING PARTY

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Background. ABL kinase domain mutations are associated with imatinib (IM) resistance in chronic myeloid leukemia (CML) patients (pts). Aims Aims of this study in a large series of IM-resistant CML pts were: a) to assess and compare the incidence and distribution of ABL mutations in subsets of pts differing for phase of disease and for type/degree of IM-resistance; b) to evaluate the clinical/prognostic relevance of ABL mutations. Methods Using D-HPLC and sequencing, we screened for ABL mutations 165 IM-resistant CML pts. At the time IM was started at 400-600 mg/d, 143 pts (87%) were in chronic phase (CP) (27 previously untreated, 116 post-IFN failure), 4 pts (2%) were in accelerated phase (AP), and 18 pts (11%) were in blast crisis (BC). At present, clinical data are available for 119 pts. Median age at IM start was 48 years (range, 17-70). Median delay between diagnosis and IM start was 40 months (range, 0-160). Median duration of IM was 22 months (range, 9-55). Results Evaluable pts were 134/165 (81%). At the time of analysis, 91/134 (70%) pts were in CP (14 previously untreated, 77 post-IFN failure), 14 (10%) pts were in AP and 29 (22%) pts were in BC. Sixty-six pts had primary resistance to IM; 68 had acquired resistance. Sixty-one mutations were identified in 56/134 (42%) pts. In 5 pts (4 BC; 1 CP post-IFN) two mutations simultaneously occurred. Mutations mapped to 14 codons, the most frequent ones being E255K/V (12 pts), Y253F/H (8 pts), F359V/I (6 pts), M244V (6 pts), G250E (6 pts), M351T (6 pts). Three novel amino acid substitutions (F311I; E355D; F359I) and a novel mutated codon (P296H) were detected; biochemical/structural characterization will be presented. Mutations were detected in 23/91 (26%) CP pts (2/14 (14%) previously untreated, 21/77 (27%) post-IFN), 8/14 (57%) AP patients and 25/29 (83%) BC pts (CP vs. AP, p=0.02; AP vs. BC, p=0.03; CP vs. BC, p<0.0001). Mutations were associated in 14/66 (22%) pts with primary resistance (2/2 hematologic and 12/64 cytogenetic)

and in 42/68 (62%) pts with acquired resistance (5/14 pts who lost CCgR, 6/11 pts who lost HR, 31/43 pts who progressed to AP/BC)(primary vs. acquired, $p<0.0001$). Ten out of 25 pts with P-loop mutations had already progressed to AP/BC at the time of mutation detection; 9 additional pts subsequently progressed within 2 to 12 months. Three out of 4 pts with the T315I mutation had already progressed to AP/BC; one progressed after 3 months. In contrast, only four of the 27 remaining pts with mutations had progressed or subsequently progressed ($p<0.0001$). Detailed correlation analysis of mutation status according to clinical features/outcome will be reported for the whole series of patients. Conclusions We conclude that: a) there is a significantly higher probability of mutations according to disease phase (BC>AP>CP); b) there is a significantly higher probability of mutations in pts with acquired resistance vs. pts with primary resistance; c) P-loop and T315I mutations are significantly associated with disease progression and seem to confer a worse outcome. Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L. grants.

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MOLECULAR RESPONSE TO A IMATINIB PLUS ALPHA-INTERFERON THERAPY IN NEWLY DIAGNOSED, EARLY CHRONIC PHASE CHRONIC MIELOID LEUKEMIA

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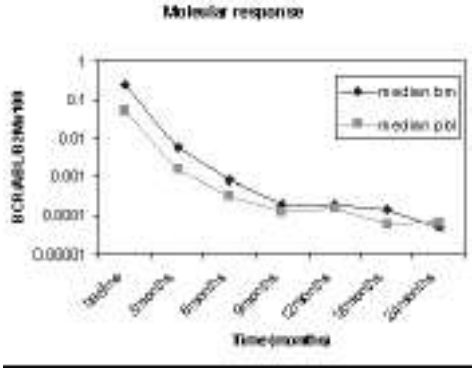
We sought to determine dynamics of BCR/ABL mRNA expression levels in 76 patients with early chronic phase Chronic Mieloid Leukemia (CML) during Imatinib and alpha-Interferon therapy. Al patients received a standard dose of Imatinib (400mg/d), while alpha-Interferon scheduled dose was 50mg/wk, 100mg/wk and 150mg/wk in the 3 different cohorts. The response was monitored by cytogenetics from bone marrow metaphases and molecular response was assessed by RT-PCR (TaqMan) from bone marrow and peripheral blood samples. We expressed molecular response as the ratio between BCR/ABL and beta2-microglobulin x 100 and the lowest level of detectability of the method was 10(-5). A complete cytogenetic response was achieved in 53 patients after a median observation time of 12 months, while in other 10 patients a partial cytogenetic response was obtained, for an overall major cytogenetic response rate of 63 of 76 (83%). After 12 months we observed a progressive decrease of the amount of the BCR/ABL transcript in the patients who achieved a complete cytogenetic response. The reduction of the BCR/ABL transcript levels that we observed in this group of patients was about 3logs. In the patients who had achieved a complete cytogenetic response, the molecular response was assessed also after 18 and 24 months. So we observed that 18 months after the first dose of Imatinib and alpha-interferon 70% of patients were still in complete cytogenetic response. At 24 months the median value of BCR/ABL transcripts continues to decrease about another 1log, as shown in figure 1. Although there had not been observed any differences between the 3 cohorts our conclusions are that treatment with Imatinib in newly diagnosed CML patients is associated with a rapid decrease of BCR/ABL levels and that the BCR/ABL transcripts continues to decrease after 2 years of Imatinib therapy. Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L.



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COMPARISON BETWEEN PATIENTS WITH PH+ CHRONIC PHASE CHRONIC MYELOID LEUKEMIA WHO OBTAINED A COMPLETE CYTOGENETIC RESPONSE WITHIN 1 YEAR OF IMATINIB THERAPY AND THOSE WHO ACHIEVED SUCH A RESPONSE AFTER 12 MONTHS OF TREATMENT

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Imatinib mesylate is a potent, specific inhibitor of BCR-ABL, the constitutively active tyrosine kinase protein critical for the pathogenesis of chronic myeloid leukemia. Real time Q-RT PCR is increasingly used to monitor responses in leukemia. It has been demonstrated that the BCR-ABL transcript level is an accurate surrogate of the contemporaneous bone marrow cytogenetic response. We reviewed 284 patients with late chronic phase Philadelphia-positive chronic myeloid leukemia treated with imatinib 400 mg daily after interferon-alpha failure. In a retrospective study

we evaluated the pattern and rapidity of the response to imatinib, comparing the cytogenetic and molecular responses in patients who obtained a complete cytogenetic response very quickly, i.e. within one year of treatment (early responders, n=114), and in patients where a complete cytogenetic response was detected only later, after 12 months of treatment (late responders, n=37). After 3 or 4 years of treatment the molecular response of the late cytogenetic responders was similar to that of the early cytogenetic responders. At 36 months of treatment the amount of residual disease measured by standardized quantitative reverse-transcriptase polymerase chain reaction was 0.00047 in late responders versus 0.00022 in early responders (median values, P value n.s.), and at 48 months it was 0.00019 versus 0.00026 (median values, P value = n.s.). We also analyzed whether there was any difference between early and late responders in terms of progression-free survival and overall survival and found no difference. Though late responders achieved complete cytogenetic remission after 1 year of imatinib therapy, they showed a progression-free survival rate and an estimated 4-year overall survival similar to or even better than those of early responders (88% versus 100% and 92% versus 100%, respectively). These results suggest that the sensitivity and the response to imatinib may require 1 year or more and that late responders are by no means rare. In conclusion, the long-term follow-up results of patients with chronic-phase CML treated with imatinib mesylate after IFN-alpha failure continue to improve in terms of the rates and durability of the complete cytogenetic response and major or complete molecular response, and in terms of improved survival compared with previous accepted treatments for CML. Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L.

P157

TREATMENT WITH IMATINIB DOES NOT IMPAIR AN EFFICIENT *IN VIVO* AND *IN VITRO* IMMUNE RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS VACCINATED WITH P210-DERIVED PEPTIDES

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Up to date imatinib mesylate is considered the "gold standard" treatment for chronic myeloid leukemia (CML) patients. Several recent reports have indicated that at least in vitro or in mouse models, this tyrosine kinase inhibitor may impair the immune system mainly through an inhibitory effect on T cells and antigen presenting cells (APCs) function. So far, no immunological response data in CML patients treated with this agent have been published. As a model for evaluating the immune response in Imatinib treated patients we studied the effect of a P210 derived peptide vaccine (CMLVAX100) associated to QS-21 and GM-CSF as adjuvants in 16 patients vaccinated 12 months or longer after being on this tyrosinase inhibitor. Thus, before and after 6 vaccinations with CMLVAX100 we monitored the immunophenotype assessment and peptide-specific delayed-type hypersensitivity (DTH) *in vivo* and peptide-specific $CD4^+$ T cells proliferations, in vitro. As concerning the analysis of peripheral blood lymphocytes subsets we observed a statistically significant increase of $CD57^+$ T cells after 6 vaccinations while no variations were documented in the other populations. After 6 vaccinations, a strongly positive peptide-specific skin reaction was measured in 12/16 patients which consisted of a local induration and erithema after 24-48 hours following a very small dose of peptides only. In addition, in 16/16 vaccinated patients CMLVAX100 induced a peptide-specific $CD4^+$ T cell proliferations as measured by standard [3H]thymidine incorporation assay with a "stimulation index"; ranging from 1.8 to 15.9. A comparable peptide specific immune response was observed in 7 CML patients vaccinated with CMLVAX100 while on Interferon-alpha (IFN-alpha). Thus, despite the fact that the peptides contained in our vaccine are poorly immunogenic self-tumor antigens, the vaccinations during treatment with Imatinib were followed by an appropriate and specific T cell response which was similar to the one observed in IFN-alpha; treated patients. Based on these findings we suggest that the recently questioned "immunosuppressive effect of Imatinib" should be reconsidered or alternatively supported by further in vitro and *in vivo* studies aimed to better establish the relationship between this novel agent and the immune system of CML patients.

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IMATINIB IN CML PATIENTS HBCAB AND/ OR HCVAB POSITIVE: REPORT OF TWO CASES

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The introduction of imatinib has dramatically changed the managment of chronic myeloid leukemia (CML).Imatinib mesylate is a specific inhibitor of the bcr-abl tyrosine-kinase with prevalent liver metabolism.Imatinib has became the gold standard of care for treatement of CML. Although it's generally well tolerated, it is not devoid of side effects. The most common reasons of discontinuation of imatinib were: skin rashes (2%) and hepatotoxicity in less then 1% of patients . We describe our experience of two patients with chronic myeloid leukemia (CML) who developed hepatotoxicity during imatinib mesylate treatment. Case 1. In March 2003 chronic phase Ph positive CML was diagnosed in a 64 year old woman, with high risk Sokal and Euro score. Serologic studies for hepatitis B and C were: HBs Ag negative, Ab anti HBs and Ab anti HBc positive, but HBV DNA negative; Ab anti HCV positive but HCV RNA nega-

tive. The hepatic function was normal. Imatinib was started at a dose of 400 mg and three months later the patient achieved a complete cytogenetic response. In November, after 9 months of treatment, she presented a grade 1 hepatotoxicity and imatinib was stopped for two weeks, with complete normalization of hepatic function. At a dose of 300 mg, imatinib was restarted but after 2 weeks severe hepatic disfunction reappared (grade 3 hepatotoxicity). The other biochemical values (colestatic and synthesis index) were normal as also the ultrasonography of abdomen. Imatinib was stopped. Serologic revaluation was negative for CMV, EBV and HBV DNA and HCV RNA were negative at different repeated checks. A percutaneous liver biopsy was performed two weeks after withdrawl of imatinib. The biopsy showed chronic active hepatitis, with ductular regeneration and parenchimal changes with steatosis, spotty and confluent necrosis and Kupffer cell activation, with many macrophages filled with ceroid pigment. At this point the patient’s CML was treated with a interferon. The hepatic function was restored to normal values in a few weeks, but six months later a cytogenetic evaluation showed a minimal cytogenetic response.(16% Ph-). Case 2 A 63 year old man was diagnosed with Ph positive CML in June 2003, with intermediate risk Sokal and Euro score. At diagnosis, hepatic function was normal. Serology for hepatic viruses was not investigated. Imatinib was started at a dose of 400 mg in July. Three months later, the patient achieved a complete cytogenetic response. In December 2003 he developed skin lesions diagnosed as liken-like manifestations of uncertain etiology at biopsy. Imatinib was lowered to a dose of 300 mg, without worsening of clinical appearence that responded to a short course of oral steroids. After nine months of treatment with imatinib a complete cytogenetic response was confirmed. In August 2004 the patient developed grade 2 hepatotoxicity. Serologic evaluation was negative for HBV but positive for HCV infection (HCV RNA 1.566.590 UI/mL, 2a/2c genotype). Imatinib was stopped and was followed by a rapid complete normalization of hepatic function. Ultrasonography of abdomen was normal. The drug was restarted at a dose of 300 mg with reapparance of hepatotoxicity grade 1 and percutaneous liver biopsy was performed without withdrawing imatinib. The liver biopsy was similar to the one previously described in the first patient. Despite histological features we decided to go on with imatinib, at a dose of 300 mg, because: a) the patient had 100% performance status, b) hepatotoxicity was only aspartate aminotransferase >1.5 the normal value and c) a complete cytogenetic response was mantained. We decided to repeate hepatic biopsy after 6 months in order to monitor hepatic damage closely and a possible change of therapy. In our experience histologic lesions : centrolobular necrosis, macrophages filles with ceroid pigment and septum fibrosis were suggestive for iatrogenic hepatotoxicity. Unlike cases reported by other authors, our patients had had a previous exposition to viral damage. In literature, serology positivity for B and/or C hepatitis is not considered an exclusion criteria for imatinib mesylate therapy. We exeperienced two hepatic toxicity in 53 patients treated with imatinib (3.7%) that it is more than reported (<1%); given the fact that patients HbcAb and or HCV Ab positive are not a rare occurrence (9.4% and 7.5% respectively). The outstanding matters remain how to deal with CML patients HBcAb and or HCV Ab positive, considering two order of problems. The first is that imatinib is the gold standard terapy but that this drug may cause liver disfunction . The second problem is that hepatitis virus infection with histological damage has a high risk of mortality in patients with hematological malignancies.For these reasons we believe that ,in this cohort of patients, liver biopsy is mandatory before starting therapy and if necessary, also during treatment. The endpoint is to monitoring the liver damage not only through the liver function tests, but also by an histological point of view: in order to prevent irreversible liver disfunction and to guarantee the best control of leukemia.

P159

SIGNIFICANT REDUCTION IN FASTING BLOOD GLUCOSE IN NONDIABETIC PH+ CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB MESYLATE

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Introduction. Imatinib mesylate is a well known selective tyrosine receptor kinase inhibitor used in several haematological and non haematological tumours, most notably Chronic Myeloid Leukemia (CML). Recent reports have revealed that imatinib may improve fasting blood glucose, increase the metabolic compensation, and decrease the dosage of anti-diabetic drugs in diabetic CML patients. A direct effect of the drug on key enzymes of glucose metabolism (hexokinase, G6PD, and transketolase) has been suggested, as well as changes in tumour glucose metabolism. However, the effects of imatinib on non diabetic patients are unknown.

Methods. We investigated the effect of imatinib in fasting blood glucose (FG) in 26 nondiabetic CML patients in chronic or accelerated phase during the first year of treatment, at baseline, 3rd , 6th, 9th , 12th month . The monitored patients were enrolled in several trials of imatinib. The dose of imatinib ranged between 400 mg and 600 mg daily.

Results. At baseline time the mean value of FG was 104 mg/dL, ±3,8 SE. After 3 months therapy the mean glucose level decreased to 98 mg/dL, ±2,7 SE (p value = 0,1); at the 6th month the FG mean value was of 94 mg/dL, ± 2,8 SE (p value = 0,02). After 9 and 12 months of imatinib therapy, the mean glucose level was settled at 96,1 mg/dL, ±2,9 SE (p value = 0,03) and 96,0 mg/dL, ±3,3 SE (P value = 0,006) respectively . These data do not consider the amount of food intake, which usually increases with imatinib treatment.

Conclusions. Our results show the presence of a small but statistically significant reduction of FG in non diabetic CML patients receiving imatinib, during the first year of treatment; these results suggest a possible role of imatinib on glucose metabolism also in a non diabetic population. It remains to be established whether the observed decrease is due to a peripheral or to a central mechanism.

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ABL INHIBITOR BMS354825 BINDING MODE IN ABELSON KINASE REVEALED BY MOLECULAR DOCKING STUDIES

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BMS354825 is a SRC-Abl dual inhibitor active against several imatinib-resistant Bcr-Abl mutants except T315I. BMS354825 has been reported as an ATP-competitive inhibitor of Abl.

Methods. To better understand imatinib and BMS354825 binding, the two molecules were docked using the FLEXX software in different available structures of Abl: closed conformation in which the activation loop is closed, and intermediate conformation where the activation loop is open.

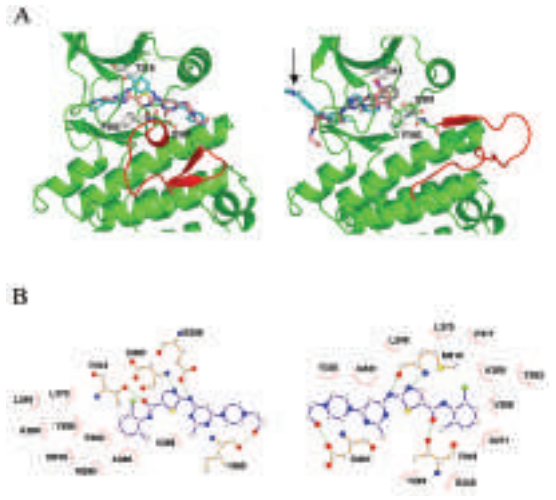

Figure 1 (A) Ribbon representation of the structure of the Abl kinase domain (green) with the activation loop (red) in the closed position (left, closed conformation) and open position (right, intermediate conformation). Thr315, Asp 381 and Phe382 of the DFG motif are shown as color coded capped stick model and labeled. On the left the experimentally determined orientation of imatinib and the docked orientation of BMS354825 are shown as color coded capped stick model with the carbon atoms in cyan and rosa respectively. On the right the experimentally determined orientation of PD173955, the docked orientation of imatinib and BMS354825 are shown as color coded capped stick representation with the carbon atoms in gray, cyan and rosa respectively. The arrow indicates the moiety of imatinib failing to interact with the protein.
(B) Schematic diagram of the interactions made by BMS354825 while docked in the Abl kinase domain with the activation loop in the closed (left, closed conformation) or open position (right, intermediate conformation). Protein residues forming hydrogen bonds are labeled and shown in ball and stick representation. Carbon atoms are colored white, nitrogen are colored blue, oxygen are colored red, chlorine atoms are colored green and sulfur atoms are colored yellow. Protein bonds are shown in brown while inhibitor bonds are in magenta. Hydrogen bonds are indicated with dotted lines and residues making van der Waals interactions with the inhibitor are semi-circled.

In both structures the highly conserved DFG motif has a similar conformation with the phenylalanine residue pointing towards the ATP binding site while the aspartic acid residue points away from it , indicating that the enzyme is still in its inactive form i.e. the one unable to perform the catalytic reaction. In addition, a model of Abl with the DFG in the productive conformation, with the aspartic acid residue pointing towards the ATP binding pocket and the activation loop in the open conformation was generated (open conformation) to study the effect of its orientation on the binding mode of these compounds.

Results. BMS354825 binds, at difference with imatinib, both conformations of Abl (closed and intermediate), although in opposite orientations. In fact, while the piperazine moiety of BMS354825 is aligned to the one of imatinib in the closed conformation, it points in the opposite direction in the intermediate conformation. Docking studies on a open conformation Abl model showed a different binding orientation and conformation of BMS354825 in the active site of Abl. While BMS354825 binds in an extended form to the closed and intermediate conformations of the protein, it adopts a bent one in the active conformation Abl model. The binding mode predicted for BMS354825 in the open conformation of Abl does not allow tight binding to the protein and a micromolar IC50 value could be expected for this conformation, in contrast to the experimentally determined nanomolar IC50 values. This suggests that the putative binding mode of BMS35425 in the active conformation should not be the pharmacologically relevant one.

Conclusions. The binding of two different conformations of Abl provides BMS354825 with a significant advantage over imatinib. These findings also indicate that BMS354825 cannot strictly be defined as true ATP-competitive inhibitor, and that it does not bind to the open conformation of Abl in a pharmacological relevant way.

P161

AUTOIMMUNE HEPATITIS PRESENTING AS IMATINIB TOXICITY: DESCRIPTION OF A CASE AND OF THERAPEUTIC ALTERNATIVES

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Introduction. Imatinib mesylate represents the treatment of choice for CML and GIST. Imatinib is generally well tolerated with few severe adverse effects. Grade III, IV hepatic involvement has been reported in less than 5% of cases and usually it requires imatinib suspension.

Patients and Methods. We report on a patient with chronic myeloid leukaemia (CML) in chronic phase (CP), intolerant to interferon (neurologic toxicity), who started imatinib, 400 mg daily, in October 2000. He developed an acute hepatitis.

Results. The patient achieved a major cytogenetic remission (MCR) after 6 months of therapy. In May 2001 due to an elevation in liver transaminases (SGPT > 11 fold x UPN, SGOT > 4 fold x UPN) the drug was interrupted and an haepatic biopsy was performed. The histological findings included wide areas of necrosis in zone 2-3 of Rappaport's

acinus and polymorphonuclear cells infiltrate, suggesting for a moderate to severe acute hepatitis. In September 2001, five months later, there was the biochemical evidence of a normalization in transaminases values. Unfortunately the patient relapsed hematologically and had to start a treatment with Hydrossiurea (HU), 400 mg/die. In November 2001 the patient was seen at our centre. A search for viral markers of viral hepatitis was negative, but the patient tested strongly positive for anti smooth muscle cells antibodies, allowing a diagnosis of non viral autoimmune hepatitis. A low dosage of imatinib (100 mg/die) resulted after a week of therapy in the rise of transaminases (SGPT> 5 fold x UPN, SGOT >2 x UPN). The patient was treated with prednisone, 50 mg/day for 2 weeks and then gradually tapered. After a week SGPT decreased to 1,6 fold x UPN and returned into a normal range 7 days later. Subsequently imatinib was resumed at a normal dosage (400 mg daily) and prednisone was gradually reduced and then stopped. After one year the dose of imatinib was increased to 800 mg per day, due to the lack of a MCR. Liver function tests remained within the normal range. At present the patient is in CCR and is continuing imatinib therapy at 800 mg daily since 27 months. *Conclusions.* As this case report shows in case of apparent liver toxicity of imatinib the presence of an underlying autoimmune hepatitis must be considered. In such a case prednisone therapy can obtain a remission of hepatitis, thus allowing treatment with imatinib to be continued.

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EVALUATION OF MORPHOLOGICAL PREDICTIVITY OF MOLECULAR REMISSION OF PATIENTS WITH CML IN CHRONIC PHASE TREATED WITH IMATINIB

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We evaluated bone marrow smears and molecular genetic status of 29 patients affected by chronic myeloid leukemia (CML), all in chronic phase, to detect any morphological feature predictive of molecular response. All patients, 13 males and 16 females with a median age of 43,5 years (19-68), were treated with Imatinib mesylate (Gleevec) at standard daily dose of 400 mg, from September 2002 to May 2004. Fourteen patients had previously received alpha-Interpheron (9 patients) or Interpheron and Aracytin (5 patients):out of these, at the start of treatment with Gleevec, seven patients showed only morphological bone marrow remission, two patients reached also molecular remission (negativity for bcr-abl rearrangement), while the remaining five patients were in chronic phase. Cellularity on bone marrow smears was estimated in a semi-quantitative mode evaluating the ratio between fat and hemopoietic compound in at least ten marrow particles; the myelogram was performed by two different hematologists, counting 500/1000 cells (according to the cellularity) in two or more different slides. We evaluated the following morphological parameters: bone marrow cellularity, myeloid/erythroid ratio, lymphocyte, basophil and eosinophil count. The group of previously treated patients didn't show any difference in the analyzed parameters except for the myeloid/erythroid ratio and the basophil count which were normal at the start of treatment with Gleevec. Bcr-abl rearrangement was detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Follow-up was performed at 6 month intervals from the start of therapy up to 30 months, with a median overall follow-up of 138 months (48-174). Bcr-abl by RT-PCR became negative in 18 patients during the period of the follow-up Patients who became negative for bcr-abl had no morphologic evidence of CML. We observed a decrease in bone marrow cellularity to normal or slight reduced levels since at the 6 month of follow-up, with a normalization of myeloid/erythroid ratio in both groups (molecular responders and non-responders). Evidence of increased lymphocytes count, more marked in the molecular responsive group has been detected. from 6 to 24 month of follow-up. Basophil count showed a normalization in the molecular responsive group while in the non-responsive group median value remained high. All molecular responsive patients showed a relevant increase of eosinophil median count at 30 month of follow-up. The simultaneous peak of eosinophils observed in all molecular responsive patients at month 30 must be investigated for a longer period to better understand its biological significance. In conclusion our data show that there is no evidence of morphological predictivity for molecular remission in patients with chronic phase of CML during a 30 months follow-up.

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LONG TERM EFFECTS OF SUSTAINED IMATINIB MESYLATE TREATMENT IN PATIENTS INTOLERANT OR REFRACTORY TO INTERFERON

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Background A number of studies have shown the ability of IM to rescue patients with chronic myeloid leukaemia (CML) not responsive or intolerant to previous recombinant alfa-interferon (IFN) treatment. While the cytogenetic response rate is lower in these patients than in patients receiving IM at diagnosis, their long term outcome is still partially known. The aim of the present study is to evaluate the long-term effects of IM in patients pretreated with IFN by comparing them to patients treated with IFN or IM only.

Patients and methods. A total of 28 patients (age range: 30-72) initially treated with IFN for chronic phase CML between September 1995 and April 2002 and subsequently receiving IM were considered. Treatment switch occurred after a median of 18 months (range: 4-73) and was determined by intolerance in 6 patients or by the lack or loss of major/complete cytogenetic response after at least 6 months of IFN treatment. Of note, IM treatment was continued irrespective of the achievement of a cytogenetic response in all patients and it was stopped only in 5, because of progression (2) or second neoplasias (3). This group of patients, called IFN>IM, was compared with a group of 30 patients treated at the same institution with IFN only, before the availability of IM (group IFN) and with

a group of 17 patients receiving, IM at diagnosis (group IM), since August 2000. The median follow-up in months of the groups was: IFN>IM 67.5 (23-161), IFN 73 (5-204), and IM 30 (2-66), respectively.

Results. Cytogenetic response occurred in 7/28 (25%) in IFN>IM group, in course of IFN therapy. In the same group the cytogenetic response improved after IM therapy (17/28 - 60.7%). Evolution to blastic phase occurred in 3 patients in group IFN>IM (10.7%) compared to 13 in group IFN (43.3%) (p=0.008) and to none in group IM (0%) (p=0.27). Death occurred in 5 patients in group IFN>IM, compared to 13 in group IFN (p=0.048) and to none in group IM (p=0.14). Interestingly, while all deaths in group IFN were CML related, 3 patients in group IFN>IM (10.7%), median age 61 (60-69), died of carcinomas (stomach, pancreas, lung), diagnosed while in chronic phase and on IM for a median of 21 months (15-37). Eleven patients in the IFN>IM group have been receiving IM for a median of 35 months (5-50) in spite of being cytogenetic unresponsive, but their actuarial leukaemia-specific survival did not differ from that of IM cytogenetic responsive patients. Group IFN>IM had a similar actuarial overall survival (median 161 months versus 111 months; log-rank analysis: p=NS) but a significantly longer leukaemia-specific survival (not reached versus 111 months; p=0.025) compared to group IFN. No statistically significant survival differences existed between group IFN>IM and group IM. *Conclusions.* Patients intolerant or refractory to IFN treatment can achieve prolonged survival with sustained IM therapy, independently from the achievement of a cytogenetic remission, a condition which should therefore not mandate the withdrawal of IM. The frequency of the development of second carcinomas is worrisome and needs further studies.

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GENE EXPRESSION PROFILE OF CHRONIC MYELOD LEUKAEMIA PATIENTS INNATELY RESISTANT TO IMATINIB MESYLATE

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Imatinib (STI571) a specific ABL tyrosine kinase inhibitor has been reported to have a significant clinic effect on chronic myeloid leukaemia (CML) in the chronic phase as well in blast crisis. However, many patients treated with Imatinib relapse at a relatively early time, suggesting that leukaemia cells tend to acquire resistance. This secondary resistance is probably due to a powerful selective pressure on rare cells that carry amplified copies of the BCR-ABL fusion oncogene or to point mutations in the BCR-ABL tyrosine kinase domain affecting the binding site of the drug to the oncoprotein. In other patients resistance appears to exist prior to drug exposure. Such innate mechanism of resistance (primary resistance) is poorly understood but some evidences suggest that activation of alternative oncogenic pathway, may confer BCR-ABL independent survival to CML cells. Comparative genome wide expression studies provided important insight in biological processes such as proliferation, differentiation, apoptosis and transformation. Only few gene expression profiling-based studies on CML and Imatinib treated patients have so far published resulting in heterogeneous conclusions. To investigate about the molecular events involved in innate Imatinib resistance in CML we compared the expression profile of "resistant" and "responsive" patients by a low density array approach. We chosen 380 genes known to be relevant in processes like haematopoietic differentiation, apoptosis, cell adhesion, cell proliferation, signal transduction, chromosome/DNA dynamics. We selected as "resistant" two patients with less than 1 log of reduction of BCR-ABL numbers of copies of transcript after six months of treatment and as "responsive" two patients who achieved a reduction greater than 3 log. The samples were tested by a Micro Fluidic Card (Applera) a low density array based on RQ-PCR that allows to obtain an accurate and validate measurement of expression of all 380 genes at the same time. The analysis of two "responsive" and two "resistant" samples showed different expression of 29 genes. The resistant cells over expressed (1,7-6 fold increase) different categories of genes. Among them BCL2, a suppressor of apoptosis, whose over-expression has been involved in a BCR-ABL independent form of Imatinib resistance in leukaemia cells. The over expression of others genes like RUNX1 and FLT3 suggests the activation in resistant cells of alternative pathways that maintain viability and growth independently of BCR-ABL kinase activity. Finally, another group of four genes (CCD2,CCD, CDK6 and TUBA2) over expressed in resistant cells is strictly related to centrosome aberrations, DNA stability and to tumour progression. The data here described were obtained from few patients and is possible that the different expression profiles may be in part due to individual variation, however can be considered preliminary for testing logical candidates as possible cause and/or marker of primary resistance., Acknowledgement: partly supported by: AIL (Associazione Italiana contro le Leucemie-Linfomi e Mieloma) and AIRC (Associazione Italiana per la Ricerca sul Cancro).

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IMPROVEMENT OF FASTING BLOOD GLUCOSE IN DIABETIC PH+ CML PATIENTS TREATED WITH IMATINIB

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Imatinib can be easily and safely administered to CML patients with type 2 diabetes mellitus. We report on 8 diabetic Ph+ CML patients, in 7 of whom an improvement of fasting glucose (FG) was observed in concomitance with obtainment of complete cytogenetic response (CCR) with consequent reduction of antidiabetic therapies. Three patients were males and 5 females, median age 66 years (range 57-70). Five patients were in chronic phase (CP) (4 were resistant to prior IFN therapy and 1 previously untreated) and were given imatinib at 400 mg/d; 3 patients were in accelerated phase (AP), all were pre-treated with IFN, and were given imatinib at 600 mg/d. Type 2 diabetes was diagnosed from 3 (1 patient) to more than 10 years

before CML. Four patients were treated with insulin and 4 with antidiabetic oral drugs. Before starting imatinib, median glucose level was 250 mg/dL (range 160-330). At 3 months, 7/8 patients obtained CCR and median FG level was 110 mg/dL (range 96-140), wich allowed reduction of antidiabetic therapy. At 12 months, all 7 patients maintained the CCR and a good glicemic control (median FG 108 mg/dL, range 89-130). In one responding patient, we also monitored the glycosilated haemoglobin (HbA1C) fraction and the plasma insuline level all along the imatinib therapy. We found a progressive reduction of HbA1C (8% at 6 months and 5% at 12 months) as compared to before starting imatinib (HbA1C 12% and plasma insulin level 25 UI) with stable values of plasma insulin dosage (24 UI). At the time of this writing all patients have reached 18 months of therapy and 7/8 still maintain CCR and a good glicemic control (median FG 105, range 90-125), while 1 AP patient, who was resistant to imatinib, never obtained blood glucose control. The mechanisms through which imatinib could improve FG in our diabetic CML patients is presently yet not understood. A direct effect of imatinib on key enzymes of glucose metabolism has been demonstrated in vitro (Boren et Al); it is conceivable that at therapeutic concentrations *in vivo* it may act not only on bcr/abl tyrosine kinase, but also on intracellular pathways involved in the action of insulin on peripheral cell receptors. Insulin resistance in type 2 diabetes is mediated by a reduction of insulin receptor substrates (IRS) or through an elevated fatty acid (FFA) concentration. It is possible that imatinib exert its effect on glucose metabolism *in vivo* by reducing FFA or by interfering with potentially disrupted molecular mechanisms downstream the IRS in the diabetic cells. In conclusion, a possible role of imatinib on glucose metabolism with potential implication in the imatinib resistance is suggested, which deserves further investigations, both in diabetic and non-diabetic CML patients.

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SUDDEN BLASTIC CRISIS IN CML PH+ PATIENTS IN COMPLETE CYTOGENETIC REMISSION INDUCED BY IMATINIB

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Only rare cases of sudden BC occurring in Ph+ CML patients while in CCR induced by imatinib have been reported. We describe here 6 cases that, while in CCR under imatinib, developed a sudden (BC) and compare the presenting features of these patients with those of patients who developed BC while in resistant disease. In a period of 4 years we treated with imatinib at the standard dose of 400 mg/d a total of 164 CP-CML patients after resistance/intolerance to IFN (100 patients) or as first line therapy (64 patients). Among these patients, 11 developed an acute transformation (6.7%); in 6 BC presented suddenly and occurred after early detection (3 months) of CCR. Five patients were males and 1 was female, median age 55 years (range 40-74). According to Sokal score, 3 patients were low and 3 patients intermediate risk. In only 1 patient karyotype analysis had revealed at diagnosis a deletion del 9q. At molecular analysis fusion transcript was b3a2 type in 5 patients and b2a2 type in 1 patient. Four patients were pretreated with IFN and hydroxyurea and in 2 patients imatinib was the first line therapy. Median duration of CP was 35.5 months (range 12-111), median duration of imatinib therapy was 9 months (range 5-15). All patients had obtained complete haematological remission between the first and the third week and CCR at 3 months. BC morphological and phenotypic characterization showed a myeloid type in 3 patients (2 had an initial extramedullary localization), B-lymphoid in 2 patients and biclonal in 1 patient. Cytogenetic analysis at the time of SBC revealed a clonal evolution in 1 patient (double Ph+), 100% Ph+ positive cells in 1 patient, a Ph+/Ph- cell mosaicism in 2 patients, a normal Ph- karyotype in 2 patients. In 1 patient a M351T mutation of the catalytic domain was detected with DHPLC analysis. At the time of this writing only 2 patients are alive (1 at 8 mo.s after a cord blood transplantation and 1 at 19 mo.s after intensive chemotherapy). As compared to presenting features of the 5 patients who developed BC after being imatinib resistant, we found that in patients with SBC showed a preponderance of male sex, of low risk score, of b3a2 fusion transcript type and of lymphoid phenotype. This finding seems to suggest the possible existence of biological and clinical heterogeneities between the two patients subgroups, which deserve to be further and in depth investigated. Moreover, in 4/6 SBC patients the first sign of evolution was a sudden drop of WBC count, in the absence of other clinical and/or haematological abnormalities. In conclusion, our observation stresses on the need for carefull monitoring of both clinical-hematological and molecular aspects in patients under imatinib treatment. The need to find strategies for eradication of leukemic residual cells is further emphasized.

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APPLICATION OF THE EBMT RISK SCORING SYSTEM AND KINETICS OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Backgroud and Aim. Allogeneic stem cell transplantation (SCT) has the potential to eradicate chronic myeloid leukaemia (CML), however only a minority of patients is eligible for this procedure due to the high risk of treatment related mortality and morbidity. The ability to identify patients at high risk of relapse is of considerable interest in the prospective of early intervention as donor leukocytes infusion or additional drugs treatment as alfa-interferon or imatinib. We analyzed the impact of the EBMT scoring system and 3 methods for MRD detection in 31 CML consecutive patients who underwent SCT at our centre.

Methods. The overall survival (OS) and the 5-years leukaemia-free survival (LFS) were estimated using the Kaplan-Meier method. For MRD evaluation karyotype, variable numbers of tandem repeats (VNTRs) and molecular analysis were performed on bone marrow samples col-

lected at intervals of 2-3 months for the first year post-SCT and every 6-12 months thereafter. Results. According to the EBMT scoring system 10 patients (32%) resulted at low-risk (0-2) and 21 (68%) resulted at high-risk (3-7). Table 1 reported patients' risk factor. A the time of analysis, 15/31 patients (48%) were alive: 12 transplanted with HLA-identical sibling and 3 with unrelated donor. Sixteen patients had died: 3 within 100 days from transplantation, 2 of relapse and 11 of non relapse-related causes. The median OS of the entire group was 38.7 months; it was 69.9 months for the low-risk group vs. 20.7 months for the high-risk group (p=0.0049 log-rank test). At 5 years post-transplantation the OS was 88.9% for the low-risk vs. 23.4% for the high-risk group. The 5-years LFS, calculated for all 31 patients as the time from transplant to death or disease progression, was 40,2% (78,7% for the low-risk group vs. 23,4% for the high-risk group). In the follow-up cytogenetic analysis was performed in 20 patients, VNTR analysis in 21 patients and molecular analysis in 28 patients. 18/20 patients (90%) reached cytogenetic remission; 18/21 (90.5%) reached complete donor chimerism; 22/28 (78.6%) obtained molecular remission. 6/28 patients failed to obtain PCR-negativity and 3/22 relapsed within the first year post transplantation, after a transitorial negativization; all of these 9 patients were at high-risk. Conclusions. As expected our data confirm the higher OS of the low-risk group of patients compared to the high-risk group. Almost 90% of the patients reached complete donor chimerism and cytogenetic remission in spite of the risk-score; in contrast, molecular negativization was observed in 100% of the low-risk group vs. 67% of the high-risk group. In our cohort of patients PCR-positivity at 6-12 months post-transplant correlates with a higher probability of relapse. The comparison of the 3 methods was performed for 20 patients and allows us to conclude that the molecular detection of the bcr-abl transcript is the only procedure of prognostic impact.

Table 1. Distribution of the patients according to EBMT risk scoring system.

Risk parameters	Donor type	Donor type		Disease stage		
	HLA-identical sibling	HLA-identical sibling	Matched unrelated	First chronic phase	Accelerated phase	Blast crisis/ >CP1
Score	0	0	1	0	1	2
N° patients	20	20	11	23	2	6

Risk parameters	Age of recipient			Sex combination	
	<20 years	20-40 years	>40 years	All	Male recipient/female donor
Score	0	1	2	0	1
N° patients	1	13	17	19	12

Risk parameters	Months from diagnosis to transplant		Score			
	<12 months	>12 months	Lowest risk	Highest risk	Low risk	High risk
Score	0	1	0	7	0-2	3-7
N° patients	18	13	0	0	11	20

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SUCCESSFUL PERIPHERAL BLOOD STEM CELL MOBILISATION WITH G-CSF IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA ACHIEVING COMPLETE CYTOGENETIC REMISSION WITH IMATINIB.

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Recent clinical studies have demonstrated the efficacy of Imatinib in the induction of hematological and cytogenetic remission (CCR). A molecular reduction and/or disappearance of the bcl-abl transcript, as shown by RT-qPCR, has been also demonstrated. Unfortunately, the optimal long-term management of patients who achieve CCR after Imatinib is unknown. It is unclear that Imatinib alone will prove to be curative and initial responders may eventually lose Imatinib responsiveness. Therefore it may be prudent to collect autologous PBSC in CCR patients treated with Imatinib with low levels of detectable leukemia analyzed by molecular tests. We evaluated PBSC mobilized after G-CSF in 18 patients who have achieved CCR with Imatinib. Our data demonstrated that the target $CD34^+$ cell yields/2.0x10^6/kg was attained at the dose of 10 mg/kg/day in 4/8 (50%) patients during uninterrupted Imatinib therapy and in 8/10 (80%) when Imatinib was temporarily interrupted. Three patients (37%) in the first group and 7 patients (70%) in the second group achieved >1x10^6/kg $CD34^+$ cell yield per apheresis. Twelve patients were evaluated on PBSC for bcr-abl by RT-qPCR. Three patients were negative and in the other 9 patients, a median of 0.20 (range, 0.02 8.6) remained detectable. These data compared favourably with a median of 0.04 (range, 0.02 0.86) of all measurements taken before mobilisation. There was no impact of G-CSF mobilisation on the CML as measured by cytogenetic and serial blood bcr-abl levels. In conclusion, PBSC mobilisation with Imatinib and G-CSF in CCR patients is feasible, $CD34^+$ cell yield is significantly better with temporary withheld of Imatinib, G-CSF did not preferentially mobilize leukemic progenitors and leukemic burden did not show significant change in the months following G-CSF mobilisation.

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IMATINIB ACHIEVES HIGH RATES OF COMPLETE CITOGENETIC REMISSION IN CML PATIENTS RELAPSED AFTER AUTOGRAFTING AND IFN-α; THERAPY

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Background. Imatinib, a selective inhibitor of the bcr-abl tyrosine kinase, induces CCR in the majority of patients with CML in first chronic phase (CP). In the present study

we addressed the question if such achievement of CCR can occur also in patients relapsed after autografting and IFN-α; therapy.

Methods. from 1991 to 2001, 50 patients with early chronic phase were treated with hydroxyurea; subsequently, when WBC count was less than $10x10^9$/L, the patients were treated with idarubicin containing regimen (ICE/mini-ICE protocols), mobilization of diploid or prevalently diploid peripheral blood stem cells (PBSC) with G-CSF during the early phase of recovery after chemotherapy and high-dose therapy followed by autologous diploid PBSC previously collected and cryopreserved. After engraftment, the patients received maintenance therapy with IFN-α; (3MU/three times weekly). From september 2000, Imatinib became available for CML patients relapsed after IFN-α; treatment. From that time all patients who relapsed after autografting and IFN-α; received rescue therapy with Imatinib at 400 mg/daily for CP (Novartis protocol CSTI-106) and 600 mg/daily for accelerated phase (AP) (Novartis protocol-Italian Cooperative Study Group CSTI-003). Imatinib was given to 23 patients: 22 patients in CP and 1 patient in accelerated phase (AP).

Results. At the time of analysis, the median treatment duration with Imatinib was 32 months (range, 1 to 47). Six patients stopped Imatinib between 9 and 12 months for no response/progression. The patient in AP died of heart failure one month after starting Imatinib; since this patient have had previous history of cardiac failure, it is not clear if this event should be correlated to Imatinib therapy. Imatinib induced major cytogenetic responses in 16/23 patients (70%) of whom 14 patients achieving CCR (61%). At a median follow up of 36 months (range, 1 to 47) PFS and OS were 87% and 91%, respectively. Grade III hematological toxicity occurred in 5 patients, which resolved with temporary discontinuation of the treatment. Conclusion: Imatinib is an effective therapy for CP-CML patients who relapsed after intensive therapy and IFN-α. The high rate of sustained haematological and cytogenetic responses achieved with Imatinib were associated to a favourable and manageable toxicity profile.

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IMATINIB VS IFN INDUCED COMPLETE MOLECULAR RESPONSES: CLINICAL-BIOLOGICAL FEATURES OF BEST RESPONDERS

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Molecular response has been accepted as a major end point in the treatment of CML, as it has been shown to be highly predictive of long term disease free survival. It can be reached in only a very small proportion of patients given IFN (5-7%), but in a significantly higher rate (4-30%) of patients treated with imatinib. With the aim of identifying prognostic predictors in these two subsets of patients we retrospectively analysed and compared clinical and biological features of our CML cases with complete molecular response (CMR) obtained after IFN or after imatinib (following IFN intolerance or as a first line). Among a total of 250 Ph^+ CML patients in chronic phase, observed in a period of ten years, 67 obtained CMR, defined as the absence of detectable BCR/ABL transcript at RT-PCR. Molecular study was performed on bone marrow (BM) cells by assaying BCR-ABL transcripts using qualitative reverse transcriptase nested polymerase chain reaction (RT-nested-PCR), in patients who were in stable complete cytogenetic remission (CCR). There were 33 males and 34 females, median age 57; of the 67 patients, 23 had reached CMR with IFN and 44 with imatinib (29 after IFN failure or intolerance and 15 in first line therapy). At presentation, of the 23 IFN treated patients, 9 were males and 14 females, median WBC count was $49x10^9$/L and median Platelet count was $347x10^9$/L; according to Sokal score, 18 were low risk and 5 intermediate risk, and according to Hasford score, 19 patients were low risk and 4 intermediate risk. Molecular analysis revealed b2a2 type of BCR/ABL transcript in 7 patients and b3a2 in 16 patients. Median time to achieve CCR was 1.2 years, and median time to achieve CMR was 6.7 years. Fourteen/23 patients definitively suspended IFN and maintained CMR at subsequent controls, a median time from suspension being 3.3 years. Of the 44 imatinib treated patients, 24 were males and 20 females, median WBC count was $72.5x10^9$/Land median platelet count $384x10^9$/L; according to Sokal score, 28 patients were low risk, 13 intermediate risk and 3 patient high risk. According to Hasford score, 26 patients were low risk, 17 patients intermediate and 1 patient high risk. b2a2 type of transcript was evidenced in 15 patients and b3a2 type in 29 patients. Median time to achieve CCR was 4.5 months and median time to achieve CMR was 1.2 years. We found significant differences between the two groups of patients: as compared to the IFN group, patients treated with imatinib at diagnosis presented with more evident epato and splenomegaly (p=0.02 and 0.03, respectively), higher WBC count (p=0.001), higher incidence of more unfavourable prognostic scores according to Sokal and Euro (p=0.05 and 0.03, respectively). Our data, while confirming the superiority of imatinib with respect to IFN in terms of rate and speedy of responses, show that the commonly used prognostic scoring systems, tend to not retain the same weight as for IFN treated patients when these are given imatinib. Thus, new variables, at clinical and possibly at molecular level, should be identified for risk models adapted to the new drug treatment.

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GENE EXPRESSION PROFILE DURING CHRONIC MYELOID LEUKEMIA PHASE PROGRESSION

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Chronic myeloid leukemia can be divided into three clinically distinct phases: an initial chronic phase (CP) followed by an accelerated phase which subsequently leads to blast crisis (BC). Fusion of the cABL and Bcr, as result of t(9;22) reciprocal chromosomal translocation, is believed to be the primary cause of the disease. However, the progression of

the disease cannot be tracked down to a single molecular event nor known single pathway can account or predict for blastic evolution. The use of cDNA array technology represents a powerful tool which enables large-scale screening of gene expression profiling. In our study we used microarray analysis to identify differences in the gene expression profile in six bone marrow samples obtained from three patients at diagnosis and three patients in blast crisis. Patients were chosen as most biologically homogenous as possible in order to reduce genetic background noise. In all cases CD34+ cells were selected and used to perform c-RNA synthesis. Samples were subjected to a prior quality control standard regarding the amount and the quality of labeled c-RNA. They were hibrydized on Affymetrix Human genome U133A GeneChip® arrays that are representative of ~13000 known human genes. Detailed analysis of genes statistically differentially expressed between CD34+ cells collected from healthy donors (n=6), chronic phase and blast crisis CD34+ cells allowed us to identify 4 genes down-regulated and 32 genes overexpressed in blast crisis. These genes are factors involved in trascription, zinc fingers, regulators of cell cycle, adhesion molecules, enzymes belonging to oncogenes pathways, receptors of ligands able to influence important cell functions such as proliferation or differentiation. Now we intend to confirm data obtained from arrays analysis in RealTime PCR to demostrate different amount of transcript of every gene between chronic phase and blast crisis. The identification and the characterization of new genes with altered expression in CML might provide insights into the molecular pathways involved in CML blastic transformation and therefore represent new molecular markers for a quick diagnosis and potential therapy.

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MOBILIZATION OF PH-NEGATIVE PERIPHERAL BLOOD STEM CELLS IN CHRONIC MYELOID LEUKAEMIA PATIENTS WITH COMPLETE CYTOGENETIC RESPONSE IMATINIB MESILATE INDUCED

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Imatinib mesilate, a tyrosine kinase inhibitor, specifically contrast the BCR/ABL product in CML cells and yelding a high rate of complete cytogenetic response in CML patients. This consolidated data have modified the management of CML but, unfortunately, the long-term effects of IM are unknown and it is uncertain how durable the CCyR will be. For this reason, we decided to mobilize CCyR CML patients in order of to obtain a sufficient quantity of Ph-negative PBSCs in case of relapse. The CCyR was confirmed by at least two consecutive negative karyotipic and FISH analysis. Furthermore, the minimal residual disease reduction was verified by the quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). After written consensus, we enrolled 18 CML patients, 13 male and 5 female; 13 of these its were pretreated with alfa-interferon. The median time from diagnosis to mobilization was 36 months (range:13-152) and the median time of imatinib treatment was 25 months (range:11-35). Imatinib was suspend, on the average,4 days before the administration of rHu-G-CSF, at median daily dose of 5 mcg/Kg. Mobilization was achieved after a mean of 5 days of G-CSF therapy. Collection was started when the circulating CD34+ cells were 10/microL or above and we conventionally established mobilization failure when the leukocytes were more than 50x10^9/L and the circulating CD34+ cells were lower than 10/microL. In our experience, only 1 out of 18 patients failed mobilization wile 17/18 patients (94%) started the apheretic procedures. All collected patients (100%) obtained an apheretic product higher than 2x10^6/Kg CD34+ cells. The cytogenetic analysis was performed for all collections and each of there was Ph-negative (100%). The RT-PCR analysis showed an increase of the BCR/ABL transcript in only one patient (6%); a molecular negative harvest was demonstrated in 9 of 17 patients (53%). We concluded that Ph-negative PBSCs collection is possible in CCyR CML patients after imatinib mesilate. So far no statistical correlation was observed between α-IFN pre-treatment and a molecular negative harvest.

See details in the following table.

UPN	α-IFN Pre-IM	CD34+ x10^6/Kg	Harvest Cytog	Harvest (bcr-abl)/abl
TM/00	Y	4:55	Neg	0,06875
OI/91	Y	3:34	Neg	Neg
VE/95	Y	3:33	Neg	Neg
SG/98	Y	3:07	Neg	Neg
RR/01	Y	4:45	Neg	0.0001519
MG/00	N	2:56	Neg	0.0002118
SC/00	N	3:20	Neg	0.001125
SC/01	Y	0,125	Neg	0.004130
CA/99	N	0,177083333	Neg	0.0006470
TG/01	Y	3:14	Neg	Neg
VM/91	Y	0,128472222	Neg	0.0004520
MM/01	Y	NA	NA	NA
MM/02	Y	0,222916667	Neg	Neg
CG/97	Y	2:34	Neg	0.002950
DL/03	N	3:54	Neg	Neg
SM/01	Y	3:02	Neg	Neg
SM/03	N	0,14375	Neg	Neg
FP/96	Y	0,177083333	Neg	Neg

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CYTOGENETIC AND MOLECULAR RESPONSE IN ELDERLY CHRONIC PHASE CML PATIENTS TREATED WITH IMATINIB

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From 7/2001 to 10/2004, 50 patients with Ph+ Chronic Myelogenous Leukemia (CML) in chronic phase (CP) aged more than 60 yrs were treated with Imatinib Mesylate at our Institution. There were 31 males and 19 females, median age 69.4 yrs (range 60.2–83.0), with 24 patients older than 70 yrs. Ten patients were untreated while 40 patients were resistant/intolerant to prior alpha-Interferon (IFN) (28 patients) or had received Hydroxyurea (HU) (12 patients), with a median interval from diagnosis of 39.8 months (range 2.8 – 146). According to Sokal score at diagnosis, 11 patients (22%) were low risk, 30 (60%) intermediate risk and 9 (18%) high risk. All patients received Imatinib at a

single oral dose of 400 mg/day. Complete Haematological Response (CHR) was obtained in all patients within the first month. Complete Cytogenetic Response (CCR) (100% Ph-negative metaphases) was achieved in 40 patients (80%), in 34 of whom within 6 months, Major Cytogenetic Response (MCR) (Ph-negative metaphases > 66%:) was achieved in 4 patients (8%). The remaining 6 patients (12%), including 2 cases who stopped Imatinib after 2 months for severe skin toxicity, resulted to be resistant. Haematological toxicity was mild: 11 patients had a transient neutropenia and/or thrombocytopenia, which required only temporary discontinuation (mean time 10 days) or dosage reduction to 300 mg daily. Severe skin reactions were the main extra-haematological toxicity and occurred in 8 patients (16%): in 2 of these permanent Imatinib discontinuation was necessary after 2 months. Five patients (10%), of whom 2 resistant and 3 responsive to Imatinib (2 MCR and 1 CCR), evolved to Blastic Crisis (BC) after 4,6,6,7 and 8 months. After a median follow-up of 25 months (range 5 – 42), 3 patients died from BC, 2 were lost to follow-up and 45 are alive (1 in BC, 4 in resistant CP, 1 in MCR and 39 in stable CCR). In 12 patients with stable CCR, molecular qualitative analysis (RT-nested-PCR) for BCR/ABL fusion transcript was performed and 6 patients (50%) resulted to be negative. Present data indicate that Imatinib is safe and effective also in elderly patients with CML; results seem at least as good as in younger patients, as concerns haematological, cytogenetic and molecular response rates. However BC incidence seems to be higher than that observed in younger patients population. Further evaluation with longer follow-up as well as larger studies are needed to better define clinical and biological features responsible for the increased risk of Imatinib resistance and/or disease progression in this subset of patients.

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PROGNOSTIC IMPACT OF SOKAL AND HASFORD SCORES IN ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB

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The increase of mean survival of the general population leads to a higher incidence of chronic myeloid leukemia (CML). In the last 5 years in our series of 260 cases from a single institution the mean age of CML patients was 61.9 years, with 40% of them over 70. In the Sokal index age is a very heavy parameter which significantly increases the prognostic risk. Imatinib (STI) has been demonstrated to exert the same efficacy in elderly as well as in young cases. The purpose of our study is to correlate efficacy and tolerability of STI to Sokal and Hasford scores. Our series consists of 31 patients over 65 yrs whose median age was 72 yrs (65-83) at the time of STI therapy. Co-morbidity included hypertension (5 cases), renal failure (2), cardiovascular diseases (4), diabetis (1), psoriasis (1), B-cell Chronic Lymphocytic Leukemia (1). All cases were BCR/ABL positive (p 210), 27 Ph 1 + and 4 Ph1 -; 8 patients were in accelerated phase (AP) and the remaining in chronic phase (CP). Before starting therapy with STI, 12 patients had received hydrossiurea, 10 interferon and 9 no previous treatment because they were at the onset of the disease. The mean interval from diagnosis to STI therapy was 38.4 months (2-244). Out of 10 patients previously treated with Interferon, 6 were resistant (1 in AP), 2 were intolerant and 2 in cytogenetic (CG) complete response. STI was administered at the dose of 400 mg/day in CP patients and 600 mg/day in AP cases. CP patients rapidly obtained the hematological response (15-21 days); only two AP cases reached the hematological response. CG response was obtained in 19 CP patients (82%), only in 2 out of 8 AP cases (1 complete and 1 major response). Quantitative molecular response was obtained in 16 out of 23 CP cases and in 1 out of 8 AP patients. Tolerability was good. In 10 CP cases STI dosage was reduced to 300 mg/day; since 50% of them lost citogenetic and molecular response the dosage was restored to 400 mg/day. WHO grade 4 toxicity was observed in 3 cases requiring STI permanent withdrawal, 1 patient because of hepatic failure, 1 subdural hemorrage needing surgery, 1pericardic effusion. The remaining relevant toxicity consisted of grade 3 cough, scrotal oedema, reversible lethargy due to cerebral oedema. Grade 2 toxicity was represented by oedema, dyscromia, fatigue, myalgia. The median weight gain was around 3 kg; no grade 3-4 hematological toxicity was reported. After a median follow-up of 25 months, among CP patients 66% (16/24) were in complete CG response and 4% (1/24) in major response, 4% (2/24) did not obtain a CG response, 4/24 showed progression to AP and in 1 case STI was stopped for toxicity. 58% (14/24) were in molecolar response. As far as AP patients are concerned, 2 are in complete CG and molecular response, 2 are alive in CP with minor CG response, 2 are alive in persistent AP and 2 died. When Sokal index was applied to the present series of elderly patients, the majority of them were stratified within the intermediate risk subset. In fact, Sokal index was low in 3 cases, intermediate in 22, and high in 2, while Hasford index was low in 15, intermediate in 12 and high in no case. Interestingly, the analysis of overall survival in CP patients did not show any difference comparing intermediate Sokal and low Hasford cases (69% vs 75% at 43.2 months, P not significant). CONCLUSION. The results of the present study suggest the possibility that Sokal index is less appropriate than Hasford score to assess the prognosis of CML patients treated with STI.

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IMPACT OF IMATINIB ON THE HEMATOPOIETIC SYSTEM OF CML PATIENTS

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The introduction of Imatinib (IM) for the treatment of Chronic Myelogenous Leukemia (CML) patients has revolutioned the therapy. This therapy has dramatically changed the percentage of hematologic and cytogenetic response. While these data have now become consistent worldwide, monitoring of minimal residual disease by real-time quantitative polymerase chain reaction (RTQ-PCR)

methods is being evaluated with the aim to predict duration of response and to establish indications for other therapeutic options. In our series, 37 CML patients in chronic phase (CP) were started on Imatinib at 400 mg/day of whom 16 at diagnosis and 21 in late CP. Eight patients had previously received an autografting with Philadelphia-negative (Ph^-) mobilized hematopoietic progenitors and all but one had residual disease at cytogenetics (at least >10% Ph+ with 3 patients 100%Ph^+) while on low-dose IFN. Marrow evaluation, including CFU-GM frequency (colonies/2x10^4), cytogenetic analysis and RTQ-PCR, were performed before starting Imatinib (time 0), every 3 months in the first semester and then every 6 months. Normalization ratios of BCR-ABL transcript levels were done by comparison with the level of ABL transcript. Dose of Imatinib was reduced or withheld for toxicities >grade 2. Only 1 patients crossed back to hydroxycarbamide for intolerance to Imatinib (muscle cramps with CPK elevation) and only 1 patient had Imatinib withheld for more than 2 weeks. Median age was 55 yrs (26-82), median interval between diagnosis and Imatinib was 14 months (1-127), median follow-up was 24 months. Grade 3-4 neutropenia and thrombocytopenia occurred in 4 (11%) and 3 patients (8%), respectively. We observed that median values of CFU-GM at time 0 was statistically different between patients at diagnosis [27 (7-61)] and the autografting group [6 (0-15)] (p=0.05). After 3 months from Imatinib treatment all CFU-GM were Ph-negative. Interestingly, the CFU-GM frequency of autografted patients came back to values of normal subject (7-59) 3 months after treatment [24 (2-39)], and were comparable to patients at diagnosis at different time points. Interestingly enough in the last 4 patients who were diagnosed very early (WBC <25.000/mmc) we found that within that $CD34^+$ derived CFU-GM more than 90% were Ph-negative indicating that in the early phase the Ph-positive cells predominate only within the mature compartment. Thirty-five patients were evaluable for cytogenetic (CyR) and molecular response (MolR). Complete CyR (CcyR) was achieved in 34 patients (97%), and only 1 patients had a transient minor response (at 3 months). Among 26 evaluable patients at 12 months, 25 (97%) were in CCyR. Major MolR (below 0.05%) has been achieved in 18/35 patients (51%) and complete MolR in 7/35 patients (20%) irrespective to the previous disease history. By stratifying patients for Sokal's risk score at time 0, low risk (LR) patients had significantly lower BCR-ABL transcripts by RTQ-PCR compared to IR (p=0.01) and HR patients (p=0.01). At 12 months , only the LR group has achieved a median value of BCR-ABL transcripts below 0.05% [3.12 (0-39)], which is roughly equivalent to a 3-log reduction. None of patients who reached a major or complete MolR showed evidence of disease recurrence by molecular and cytogenetic analysis whether treated either in early or late chronic phase. Taken together these data indicate that the Ph-negative reservoir in patients who achieve cytogenetic remission including patients autografted is well preserved and can sustain long-lasting therapy. The correlations between Sokal risk score and tumor burden by RTQ-PCR identified in this small group of patients may warrant further analysis in a larger series.

Hodgkin's Lymphoma

P176

HIGH DOSE SEQUENTIAL CHEMOTHERAPY AS SALVAGE TREATMENT IN RELAPSED AND REFRACTORY HODGKIN'S LYMPHOMA

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The optimal treatment for patients (pts) with Hodgkin's lymphoma (HD) who do not achieve a complete remission (CR) or relapse after induction therapy is still not definite. Conventional salvage therapies produce a second CR in few patients. Recently the German Hodgkin Study Group produced good results with high-dose sequential chemotherapy (HDST) and autologous stem cell transplantation (ASCT) in this subset of pts. Since March 2002 to September 2004 10 pts were enrolled in this study in our institution. The schedule was constituted by a first phase (2 cycles of DHAP), a second phase (cyclophosphamide 4g/sqm, methotrexate 8 g/sqm, etoposide 2g/sqm every 14 days) and an ASCT with BEAM conditioning. All phases but the ASCT one were delivered on outpatient basis. After the second DHAP, a CT-scan evaluation was executed as chemosensibility assessment: pts not having at least a partial response (PR) according to Cheson were considered off-study. Leukapheresis was done after the administration of cyclophosphamide and G-CSF 5 microg/Kg from day +5 until apheresis. Pts status at the enrolment were: 5 relapsed within a year after the obtainment of CR; 3 refractory to the induction therapy (ABVD); 2 refractory to a conventional salvage treatment for a relapse occurred after 9 years. Characteristics of the pts at diagnosis were: median age (range) 28y (19-42); 5 pts were female; 4 had B symptoms; ECOG performance status were 0 in 7 pts, 1 in 2 pts, 2 in a patient. All pts received the two cycles of DHAP without delays: after this phase 3 pts were excluded, one for progressive chemoresistant disease, two for not achieving a PR (late relapsed pts: both are still alive with disease). The other seven pts continued the therapy: after the treatment completion all of them were in CR with negative whole body PET scans and after a median follow up of 754 days (range 42-888) are all still in CR (a patient had a negative PET scan after the second phase). A patient delayed the methotrexate infusion because of an abdominal pain after the cyclophosphamide infusion. A CR patient developed 13 months after the ASCT a myelodisplasia (WHO: refractory cytopenia with multilineage dysplasia), subsequently evolved in an acute myeloid leukemia. HDST is an effective and low-toxic therapy for treatment of relapsed and refractory HDG pts. A longer follow-up is needed to evaluate the toxicity, especially mielotoxicity, and the real efficacy of this therapy.

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EARLY RESTAGING POSITRON EMISSION TOMOGRAPHY WITH 18F-FLUORODEOXYGLUCOSE (FDG PET) PREDICTS RESPONSE IN PATIENTS WITH HODGKIN'S DISEASE

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More than half of all patients with Hodgkin's disease (HD) are cured with standard chemotherapy. Therefore, it is important to distinguish between responders to standard treatment and non-responders who may benefit from an early change to a more effective therapy. This study was intended to assess the value of a FDG PET performed after two cycles of standard chemotherapy to predict final clinical response in patients with HD. Between June 2003 and August 2004 in our institute, twenty-two newly diagnosed patients with advanced stage (IIB-IVB) HD were consecutively treated with ABVD chemotherapy. Patients' characteristics were: sex M/F, 9/13; median age 31 years (range, 13-48); 13 NS, 1 LP, and 8 LD according the histopathology; 9 with bulky mass; and 5 with extranodal sites. All these patients underwent a specific staging and restaging (early and final): CT and PET at time 0, PET after 2 cycles, CT scan and PET after the completion of chemotherapy-radiotherapy front-line program. In four of 22 (18%) positive FDG PET results obtained after the second cycle were associated with a persistence/progression of the disease at the completion of chemotherapy treatment (p=0.0001). In these four patients the mean SUV values were: 13.2 (range, 6.5-20) at time 0; 8.1 (range, 3.7-12.9) after two cycles; 10.8 (range, 8.4-15) at the chemotherapy completion. The remaining 18 patients showed a PET negativity after two cycles and at the end of front-line treatment. Very early (after two cycles) FDG PET may be used to tailor induction chemotherapy in patients with HD.

P178

MECHANISM OF ACTION OF RITUXIMAB IN MURINE MODELS OF LYMPHOMA

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Background. Rituximab is an anti-CD20 antibody used for the treatment of B-NHL and B-CLL. Its mechanism of action *in vivo* may include complement dependent cytotoxicity (CDC), antibody dependent cytotoxicity (ADCC) and/or induction of apoptosis.

Aims. To determine the role of CDC, of different immune cell populations and of apoptosis in the mechanism of action of rituximab in two murine models of lymphoma in mice.

Methods. Tumour cell growth was analysed by PCR and by measurement of subcutaneous tumours. Complement

was depleted *in vivo* with cobra venom factor. NK cells and neutrophils were depleted by repeated treatments with the TM-ß1 and RB6-8C5 antibodies and macrophages by clodronate-liposome injections.

Results. We have set up two B lymphoma models to investigate the mechanism of action of rituximab *in vivo*. The first model was a fast growing syngeneic B lymphoma, the 38C13 cell line, homing in lymph nodes and other hematopoietic organs. The 38C13 murine B lymphoma was stably transduced with the human CD20 cDNA and gave rise to tumours in bone marrow, spleen and lymph nodes in syngeneic mice. Treatment with 250µg rituximab i.p. cured 100% of animals. Depletion studies demonstrated that complement alone, and not NK cell, PMN or macrophages, is responsible for the therapeutic activity of rituximab. Rituximab maintained efficacy when given up to 10-15 days after tumour inoculation, when tumour load was however still minimal. In order to study rituximab in a bulky lymphoma, a second tumour model was set up and characterised after subcutaneous inoculation of the BJAB human B lymphoma in athymic mice. Rituximab, given weekly after tumour had reached 250 mm^2, led to complete disappearance of the lymphoma within 2-3 weeks. Complement, as well as NK cells, PMN and macrophages were required for the therapeutic activity of rituximab in the BJAB model.

Conclusions. These data demonstrate that complement is consistently required for the therapeutic activity of rituximab *in vivo*, to cure both minimal syngeneic tumours as well as bulky slow growing xenografts, but that different tumours may vary in their requirement for immune cells mediating ADCC and phagocytosis. Differential leukocytes infiltration, susceptibility to ADCC as well as tumour bulk may contribute these differences.

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GLUTATHIONE-S-TRANSFERASE P1 GENOTYPE AS PROGNOSTIC MARKER IN HODGKIN'S LYMPHOMA

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Glutathione-S-Transferase (GST) P1 is a member of the GST enzyme superfamily which is important for the detoxification of several cytotoxic drugs and their by-products. A single nucleotide polymorphism results in the substitution of Isoleucine (Ile) to valine (Val) at codon 105 causing a metabolically less active variant of the enzyme. Recently, the GSTP1 105Val genotype has been associated with favorable prognosis following chemotherapy with drugs known to be GSTP1 substrates in a variety of malignancies, such as pediatric acute lymphoblastic leukemia, myeloma, breast and colon cancer. We assessed the impact of the GSTP1 codon 105 genotype on treatment outcome in patients with Hodgkin's lymphoma. The Ile105Val polymorphism in the GSTP1 gene was analyzed using a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique. DNA was extracted either from peripheral blood or paraffin-embedded lymph node biopsies from 180 patients with Hodgkin's lymphoma (median age 31 years, range 13-71 years; 78 females and 102 males). 168 Patients were treated with standard chemotherapy regimens: 95 patients received ABVD, 32 pts a modified Stanford V regimen (substituting 6 mg/m^2 metchloramine with 650 mg/m^2 cyclophosphamide), 24 pts MOPP (+ABVD), 17 pts BEACOPP. GSTP1 genotype was assessed in 180 patients with Hodgkin's lymphoma, of which 16 (9 %) were homozygous for the 105Val/105Val genotype, 58 (32 %) were heterozygous (105Ile/105Val) and 106 (59 %) were homozygous for 105Ile/105Ile GSTP1 genotype. The GSTP1 Ile105Val polymorphism was associated in a dose-dependent fashion with an improved failure-free survival in patients with Hodgkin's lymphoma (p=0.02). The probability of 5-year survival for patients homozygous for the 105Val/105Val GSTP1genotype was 100%, for heterozygous patients 74% (95% C.I., 54-81), and 51% (95% C.I., 39-62) for patients homozygous for the 105Ile/105Ile genotype. When the analysis was restricted to 95 patients treated with ABVD chemotherapy, essentially the same differences in failure-free survival were observed. In univariate analysis of established prognostic factors, stage proved to be of prognostic value in our patient group (limited disease in stage I-IIA vs advanced disease in stage IIB-IV, p=0.01) The Cox multivariate analysis showed that GSTP1 codon 105 genotype and stage were independent prognostic factors (p=0.028, respectively). Our study indicates that the GSTP1 genotype predicts clinical outcome in patients with Hodgkin's lymphoma and points to the importance of pharmacogenetics to eventually identify patients with altered metabolism of cytotoxic drugs modifying their response to the treatment.

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INTENSIFICATION OF SALVAGE TREATMENT WITH HIGH-DOSE SEQUENTIAL CHEMOTHERAPY FOLLOWED BY HIGH-DOSE CHEMOTHERAPY AND AUTOTRANSPLANTATION IMPROVES THE OUTCOME OF PATIENTS WITH REFRACTORY OR RELAPSED HODGKIN'S DISEASE

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Purpose. To evaluate in a single center setting wether a high-dose sequential chemotherapy (HDS) administered prior to high dose chemotherapy (HDT) and autologous transplantation (ASCT) of peripheral blood progenitor cell (PBPC) could optimize the salvage of patients with refractory or relapsed Hodgkin's disease (HD). Patients and *Methods.* Between 5/1994 and 9/2003, 49 consecutive and unselected patients (median age, 32 years, range 16-60) with HD (NLPHL=2; NSHL=43; MCHL=3; LDHL=1) received HDS as salvage therapy. Eighteen had a biopsy-confirmed refractory disease and 31 an early (<12 months) (n=13) or late (>12 months) (n=18) relapse after MOPP/ABVD (n=20) or ABVD (n=29)+ radiotherapy. HDS included the sequential delivery at 15-20 day intervals of cyclophosphamide 7g/sqm, followed by PBPC harvesting, methotrexate 8g/sqm (n=25) or Ara-C 2g/sqm b.id. for 6 days (n=24), fol-

lowed by a rescue of PBPC, and etoposide 2g/sqm. Autotransplant conditioning regimen consisted in BEAM and PBPC autografting.

Results. Forty patients (85%) responded to HDS and 43 eventually achieved a complete response (CR) after ASCT (88%). Two patients did not proceed to autotransplantation because of progression. None of 6 cases resistant to HDS attained CR. Treatment-related mortality was 2%. All 47 transplanted patients engrafted. After a median follow-up of 36 months (range 6-116) 5-year estimates of overall survival, event-free survival and disease-free survival were 79%, 68% and 77%, respectively. 5-yr EFS varied according to the state at enrollment ranging from 81% in patients in 1st relapse to 46% in refractory cases (p=0.01), without significant differences between early and late relapse. Cox multivariate analysis showed that only chemosensitivity to HDS predicted a long EFS.

Conclusions. This phase II study shows that salvage treatment using HDS had relatively low toxicity and was associated with remarkable response rates, allowing further effective therapy with high-dose autograft programmes.

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10-YEAR RESULTS WITH MOPPEBVCAD CHEMOTHERAPY AND OPTIONAL, LIMITED RADIOTHERAPY IN ADVANCED HODGKIN LYMPHOMA

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Introduction. In 1987 the MOPPEBVCAD chemotherapy regimen was one of the first efforts to reduce late toxicity and second tumor incidence while exploiting some concepts of the Goldie and Coldman theory on cell growth to increase effectiveness. The new schedule intensified and hybridized all the drugs of three previously alternating regimens (CAD, MOPP and ABV), lowered the cumulative dose of mechlorethamine and delivered irradiation to no more than 2 sites (either originally bulky or partially responding to chemotherapy).

Methods. The patients treated have been included in one open and controlled GISL study and in two randomized trials (GISL and IIL) in which MOPPEBVCAD represented one of the compared treatment arms. Staging and treatment criteria were identical in the 3 trials. Drug dosages (mg/sm) were as follows: HN2 6 i.v. d 1 (cycles 1, 3 and 5), CCNU 100 p.o. d 1 (cycles 2, 4 and 6), VDZ 3 i.v. d 1, MPH 6 p.o. d 1-3, Pred p.o. 40 d 1-14, EPI 40 i.v. d 8, VCR i.v. d 8, PCZ 100 p.o. d 8-14, VBL 6 i.v. d 15 and Bleo i.v. 10 d 15 (q 28 day for 6 cycles). Radiotherapy doses ranged from 25 to 40 Gy.

Results. A total of 307 treated patients were reviewed. With a median follow-up of 110 months, 10-year overall-, disease- and failure-free survival were 78%, 81%, 79%, respectively. Remission was complete in 290 patients (94%), partial in12 (4%) null in 5 (2%). Forty-two patients relapsed and 60 died. The causes of death were Hodgkin's lymphoma in 36 patients, second neoplasms in 12, cardiorespiratory diseases in 4, pulmonary diseases in 2, and unknown in 6. Sixteen second tumors were diagnosed, 9 myelodisplasias and/or acute leukemias, 3 secondary non-Hodgkin lymphomas and 4 solid tumors. Among these patients deaths due to second neoplasms were 8, 2 and 2, respectively. Outside this series, MOPPEBVCAD obtained complete response in 12/15 relapsed and 9/9 refractory patients previously treated with other regimens.

Conclusions. Clinical response and long-term results are very satisfactory while the second tumor incidence was reduced less than expected with respect to MOPP analogues. Due to its response / late toxicity balance MOPPEBVCAD cannot undermine the leading role of ABVD, but can be indicated as a very effective second-line conventional therapy.

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LOW-DOSE GEMCITABINE MAY BE AN EFFICACIOUS AND WELL-TOLERATED BRIDGE THERAPY IN PATIENTS AFFECTED BY HODGKIN'S LYMPHOMA RELAPSING AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION

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Background. Relapse of Hodgkin's lymphoma after autologous HSCT is a major event, jeopardizing survival. Little is known about third-line therapy, and toxicity due to previous treatment is a common problem. Patients and *Methods.* From January 2000 to March 2005 we treated with Gemcitabine 6 consecutive pts. affected by Hodgkin's lymphoma and relapsing after second line high-dose chemotherapy and autologous HSCT (6 over 20 - 30% of our casistics). In all cases the patients had already been heavily treated with multiple courses, and Gemcitabine was used as third-line therapy in 3 pts, fourth-line in 1 and fifth-line in 2. A low dose schedule was adopted, as reported by Sezer *et al.*, with Gemcitabine at 250 mg/m^2 over 4 hour iv infusion, days 1, 8, 15 every 4 weeks. Results. Drug infusion was always very well tolerated, with no immediate adverse reaction and only mild thrombocytopenia (nadir around 50.000/mmc) as side effect, even on repeated cycles (up to 5 months). In 4 cases we obtained a response on the lymphoma (3 partial and 1 complete response), usually shortly after administration (mean response time 6 weeks, range 4-8). These responses were unfortunately short-lived, lasting few months (median 4, range 2-7) despite the use of repeated courses (3 doses/month). The drug was discontinued in all cases because of disease progression and the need of further therapy. This latter was possibile in 4 out of 6 pts., and in no cases particular toxicity due to previous Gemcitabine has been observed. In three cases we used Gemcitabine again after other partially successful chemotherapy, obtaining disease control in 2 cases of second Gemcitabine courses and no response in the last one. These improvements have been lasting 1 and 1,5 months so far. At present, 3 out of 6

pts. are still alive, 1 in apparent complete remission and the other 2 with stable disease, at 30, 38 and 42 months after auto-HSCT, and 16, 17 and 30 months after relapse. Conclusion. Low-dose Gemcitabine is an efficacious and well tolerated third- or even more advanced-line therapy with little toxicity in pts. affected by Hodgkin's lymphoma relapsing after autologous HSCT. It does not prevent other therapeutic options, giving in most cases time for adeguate recovery after myeloid and systemic toxicity. Responses are generally short-lasting, even with repeated cycles, and continuation of therapy is always necessary. Gemcitabine can be used as "bridge therapy" to further intensification.

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DETECTION OF CIRCULATING CD34+ CELLS AND LEUCOCYTE ALKALINE PHOSPHATASE IN PATIENTS WITH CHRONIC MYELOPROLIFERRATIVE DISORDERS PHILADELPHIA NEGATIVE

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A recent study in the Division of Hematology Pavia, reported a cut-off ≥15x10^6/L can accurately distinguish myelofibrosis with myeloid metaplasia (MMM) from those with other Philadelphia negative chronic meloproliferative disorders (CMD). On these reported, we have detected, by flow cytometry, the absolute number of circulating CD34 positive cells in 38 patients with Ph negative chronic myeloproliferative disorders. Inside, these disorders (PV, TE) are usually associated with high levels of leucocyte alkaline phosphatase (LAP). We quantitatively assayed for LAP in chronic myeloproliferative leukocytes by flow cytometry method. The method was used for the determination "range" percentage of LAP expression in normal and CMD disorders.

Materials and methods. Of the 38 conecutive patients enrolled in this study, 10 had PV, 27 had ET and 1 MMM, utilized such as control. 10 normal subjects were studied to define a normal reference group. The diagnosis of PV was estabilished according to the modified criteria proposed by Pearson et al and that of ET according to Murphy *et al.,* Peripheral blood samples were collected into EDTA tubes for complete blood counts and enumeration of the percentage of CD34 positive cells by flow cytometry, with monoclonal antibodies CD45 and CD34. Analyses were performed using a FACScalibur flow cytometer and a double platform assay following the cell-gating guidelines recommended by ISHAGE protocol. The quantitative flow cytometric assay for LAP was used on all granuclocytes with monoclonal antibodies CD13 and anti-LAP. Results. Median numbers and ranges of circulating CD34 positive cells were 7.56/mm^3 (range 1.12-73/ mm^3) and percentage of 0.10%. 14 patients had higher circulating CD34$^+$ cells than our cut-off. Absolute number of circulating CD34$^+$ cells of 73/mm^3 (2.1%) was related to MMM, with characteristic diseases parameters such as splenomegaly and high LDH. We have enrolled a patients subset (5 in total, 4 ET + 1PV) with not only high circulating CD34 positive cells, but also showed splenomegaly and high LDH.. One patient of these (ET without treatment), has been revaluated, after two years, with bone marrow biopsy (BOM) and diagnosis was MMM. About these results, a follow up program and restaging will be activate, include BOM The quantitative flow cytometric assay for LAP showed high levels in PV than ET patients. The percentage LAP+/CD13+ was 48%-85% (median 70%) in ET patients and 60%-92% (median 77%) in PV patients. Normal subjects showed range from 20% to 50%. Conclusion. The molecular mechanism underlying the higher than normal levels of LAP observed in these myeloproliferative disorders are not yet know. Our experience confirm necessity that enumeration of CD34 positive cells and parameters clinics (splenomegaly, high level LDH) might be used to follow patients with PV or ET in order to predict diagnose evolution into MMM.

P184

RESULTS OF VBMP CHEMOTERAPY REGIMEN IN ELDERLY PATIENTS WITH HODGKIN DISEASE: THE GISL EXPERIENCE

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Background. It is well-known that elderly patients with Hodgkin disease (HD) have a less favorable prognosis than do younger ones and that a specific chemotherapy for these patients is still lacking.

Methods. Between 1991 and 2001, 19 HD patients over 65 years were staged and treated from 11 different Italian institutions according to the VbMp schedule: vinblastine 6 mg/m^2 bleomycin 10 mg/m^2 (4 mg/m^2 from the 3rd to the 6th cycles to reduce pulmonary toxicity) and methotrexate 30 mg/m^2 on days 1 and 8, prednisone 25 mg days 1–5 and 8–12. Chemotherapy (CT) was repeated every 21-28 days for a maximum of 6 cycles. At the end of the chemotherapy involved-field (IF) radiotherapy (RT) was planned. *Results*. Mean age was 72 years (range 65-83). The patients were distributed according to the stage as follows: 9 (47%) in stage I, 7 (37%) in stage II, 1 (5%) in stage III, 2 (11%) in stage IV; B symptoms were present in 4 patients (21%). The histology was nodular sclerosis in 53%, mixed cellularity in 32% and lymphocyte predominance in the remaining 15%. The median follow up was 33 months (5-103). Of the 19 patients, 14 (74%) achieved complete response (CR), 2 (11%) partial response (PR), 2 (11%) did not respond and 1 (4%) had disease progression (PD). Of the 16 patients in early stage, 15 (95%) achieved a response (CR + PR), 1 (5%) had PD; of the 3 patients in stage III-IV only one (33%) had a CR, while the remaining (67%) showed no benefit from the treatment. After chemotherapy IF RT was actually delivered to nine patients, 7 of whom were in CR at the end of CT, 2 achieved CR after RT. Adverse reactions included grade 3-4 haematological toxicity in 4 patients (21%); grade 3 non haematological toxicity included viral and/or bacterial infections in 4 patients (21%). The relative dose intensity of chemotherapy administered to the whole group was 0.84. The overall 5-years survival rate was

72.7%. Conclusions. The VBMp regimen seems to provide a safe and effective therapeutic option for elderly patients with untreated HD. The best results, as well as in young patients, seem to be gained in early-stage disease.

P185

TREATMENT RESULTS AND TOXICITY IN LATE RELAPSE HODGKIN'S DISEASE

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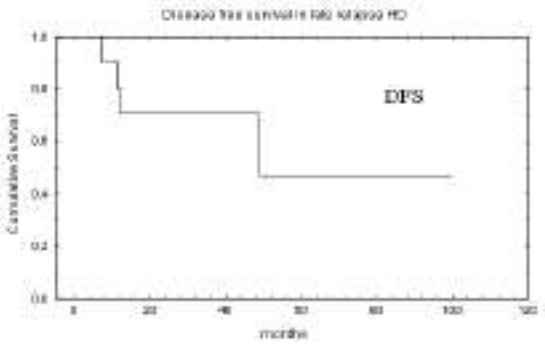
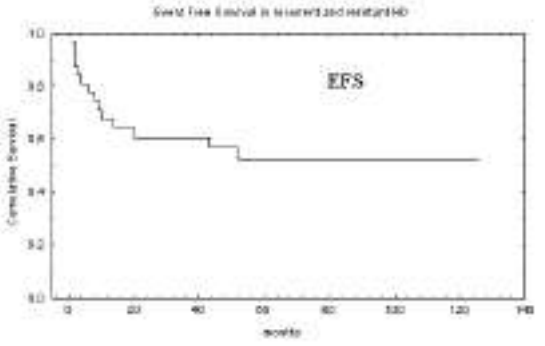
Relapsing Hodgkin's disease (HD) patients (pts) usually do so within 3 years after treatment. Recurrences of HD after 5 of complete remission are rare and pathological and clinical features in this subset of pts are to be assessed. Aim of this study is to report clinical characteristics, outcome and toxicity of pts who experienced late relapses, defined as relapses occurred 5 or more years after first complete remission. Of 532 consecutive pts with classical HD treated at our Institution from 1985 to 1999, 452 pts (85%) had complete remission, but 150 (33.2%) had a relapse (135 – 29.8%- early and 15 –3.3%- late). The histologies of the 15 pts in late relapse were mixed cellularity in 7 (46.7%) and nodular sclerosis in 8 (53.3%).





The median age was 40 yrs (16-70). 8 pts (53.3%) had stage I-II. International prognostic score were 0-1 in 9 pts (60%) and 2-3 in 6 (40%). 12 pts (80%) presented B symptoms at diagnosis, and 7 (46.7%) had bulky disease. Late relapse occurred after a median disease free interval of 7 yrs (range 5-18) and it involved sites of previous disease in 10 pts (66.6%). Salvage treatment induced a complete response in 14 pts (93.3%) and a prolonged complete remission in 11 (73.3%). At a median of 4 years after therapy for late relapse, 9 pts (60%) are still alive and free of disease and 6 (40%) died (1 of HD, 1 for cardiac disease, 1 for tromboembolic disease, 1 for HCV reactivation, 2 for bacterial infection). The probability of disease free survival at 4 years was 70%.

Conclusions. Late relapse is associated with a better survival than relapse occurring within the first 4 yrs from the time of diagnosis. The majority of deaths are related to treatment related complications while deaths for HD are unusual. Future treatment regimens for late relapse HD will be designed to minimize complications

P186

(18)F-FDG-PET AND CT-SCAN IN RESTAGING AFTER THERAPY AND FOLLOW-UP OF PATIENTS WITH HODGKIN'S LYMPHOMA: A RETROSPECTIVE ANALYSIS.

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Objectives. The evaluation of the response at the end of the therapy represents one of the biggest problems in the management of Hodgkin's lymphoma (HL) especially for the interpretation of residual masses in bulky mediastinal disease at presentation. In the past CT scan's answers to this issue were unsatisfactory. In the last years FDG-PET findings seemed to define better than CT the response to the treatment. Few data are available on the utility of FDG-PET in the follow up. We report a retrospective analysis comparing FDG-PET and CT scan in restaging after treatment and follow up.

Patients and Methods. 129 patients coming from three Hematology Department underwent 204 FDG-PET studies after the completion of the treatment and during follow-up. In the same time (no longer than 2 months from FDG-PET) all patients underwent CT scan of chest, abdomen and pelvis. PET findings were compared with CT results. If the PET was positive a confirmation biopsy was performed when a superficial lymph node was available, and in selected cases for patients with deep lymph nodes.

Results. 35 studies resulted positive to both PET and CT. Out of these only 20 relapsed. PET and CT were concordantly negative in 93 studies (only 1 relapse); 6 of which had a presentation with a mediastinal bulky mass. In 65 studies (39 with bulky in mediastinum) PET was negative while simoultaneous CT was positive: no relapse was seen in this group. Finally, 11 CT scans were negative while the equivalent PET showed a persistent or a recurrant disease: only 2 cases relapsed. On the basis of these data FDG-PET showed a sensitivity of 95,6% vs 86,9 % of CT. Specifici-

ty was 86,7% for the PET vs 55,8% of CT. Positive predicting value: PET 47,8% vs CT 20%. Negative predicting value: PET 99,3% vs CT 97,12%. Cases could be correctly classified in 59.3% and 87.8% with CT scan or PET respectively ($p<0.0001$). Probability of relapse estimated on PET/CT results was 0,3% for PET neg/CT neg, 1,1% for PET neg/CT pos, 24,7% for PET pos/CT neg and 55,1% for PET pos/CT pos.

Conclusions. Our data confirm that FDG-PET is the gold standard in the evaluation of mediastinal bulky masses after treatment. FDG-PET is more accurate than CT scan and PET positivity at restaging and during follow up is much more predictive for relapse than CT positivity alone. However the number of FDG-PET false positive is not negligible and additional investigations are recommended.

P187

COMPARISON BETWEEN 2-DEOXY-2- [18F] FLUORO-D-GLUCOSE POSITRON EMISSION AND COMPUTER TOMOGRAPHY FOR STAGING OF PATIENTS WITH HODGKIN'S LYMPHOMA

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Staging has an important role in the treatment of all malignancies but is critically important for patients (pts) with lymphoma. Accurate staging allows minimization of toxic therapies, such as extended field radiation or overly aggressive chemotherapy. Particularly in HL a tailored therapy decrease the risk of secondary malignancies which exceeds 10% in several historical series in patients with early stage disease. Anatomic imaging modalities lack sensitivity and specificity because the definition of lymph node involvement is based on size criteria. During the last decade FDG-PET has been introduced for noninvasive staging of lymphoma. Herein we propose a prospective multicentric study with the aim to assess the impact of FDG-PET on the staging of pts with diagnosis of HL. A total of 122 consecutive pts coming from five Italian hematological Institutions underwent a FDG-PET scan (equipment: C-PET, MultiringPET and CT-PET) in addition to conventional staging procedures, which include physical examination, laboratory data, bone marrow biopsy and imaging of the neck, thorax, abdomen and pelvis using CT scan. In general the adjunctive informations from PET did not influence the therapuetic options in use at a given centre at a particular time. Pts characteristics were the following: 62 male and 60 female, 97 (80%) with diagnosis of nodular sclerosis classical HL, 15 (12%) mixed cellularity classical HL, 7 (5%) lymphocyte-rich classical HL, 1 (1%) lymphocyte-depleted classical HL and 2 (2%) non specified HL. At clinical and instrumental standard staging 11 (9%) pts were stage I, 72 (59%) stage II, 23 (19%) stage III and 16 (13%) stage IV. FDG-PET and CT were concordant in 101out 122 pts (83%). FDG-PET allowed to identify in 29 out 101 concordant stage more nodal (22 pts) or extranodal (7 pts: two bone, two spleen, one lung, one liver and one liver and spleen) involvement in comparison with CT imaging. In two out 101 concordant stage CT showed one more involved site in comparison with FDG-PET (an abdominal node near the spleen which indeed was positive to both and a lung positivity in other case). FDG-PET results suggested an upstage in 18 pts (15%) and a downstage in 3 pts (2%). In these three pts FDG-PET did not confirm extranodal involvement identified by CT without histological confirmation (1 in stomach, 1 in the spleen and 1 in the liver). Eleven pts (9%) with localized disease (I-II) at standard staging changed in an advanced stage as a result of the FDG-PET scan: five pts shifted from II to IV stage and six pts from II to III stage. FDG-PET was able to identify disease sites not revealed by CT in 47 pts. We will evaluate if the information provided by FDG-PET led to a change also in the therapeutic options in particular for the extent of the radiation fields or in more prolonged chemotherapy. In conclusion our data confirm that conventional staging system has an high sensibility nevertheless in this large cohort of pts FDG-PET is a relevant noninvasive method that supplements conventional procedures and should therefore be used routinely to stage HL particularly in pts with early stage, where a change in staging will modify disease management.

P188

TREATMENT OF RELAPSED REFRACTORY HODGKIN DISEASE BY DHAP REGIMEN. A SINGLE CENTRE PILOT EXPERIENCE

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Appropriate debulky strategy for advanced relapsed/refractory Hodgkin Disease is a controversial issue. Few data are available on efficacy and safety of Cisplatinum, Ara-C and Dexametasone (DHAP) regimen in this situation. Since June 2002 to February 2005 six patients (5 male and 1 female) with relapsed/refractory Hodgkin Disease (three nodular sclerosis and 3 mixed cellularity) were treated by DHAP regimen. Median age was 31 years (range 25 – 62). All patients have been treated with classical ABVD (four to eight cycle) as first line therapy and after relapse with a minimum of one to a maximum of six lines of additional chemotherapy before the DHAP regimen. All patients were treated as in-patients and admission lasted four days in all of them. In five patients DHAP was administered twice and followed by two Ifosfamide, Etoposide, Vinorelbine (IEV) regimen as consolidation therapy. One patient had four DHAP without further therapy for clinical reasons.

Results. Haematological toxicity was limited: neutropenia WHO toxicity grade 4 for a maximum of four days in five patients and for one day in one occurred. Thrombocytopenia WHO toxicity grade 3 for a maximum of three days in three patients and grade 4 for one day in the others occurred. No patients had infections during or after DHAP treatment. Renal toxicity was limited (WHO grade 1 in five with a rapid recovery in few days). Four of six patients obtained a complete remission, one patient obtained a very good partial response (more than 50% bulky reduction). One had progression disease and subsequently died four

months later. At the time of this writing four patients are in continuous complete remission 28, 10, 8 and 6 months after therapy including the one who did not receive IEV consolidation. The patient who had obtained a very good partial remission is receiving IEV consolidation currently. Conclusion: the DHAP regimen in an efficacy and well tolerated debulky strategy for advanced relapsed refractory Hodgkin Disease. A prospective phase II trial using DHAP regimen as debulky strategy before autologous hematopoietic stem cell transplantation as second line therapy in relapsed refractory Hodgkin disease is warranted.

P189

FATAL MYASTHENIA GRAVIS AFTER CHEMOTHERAPY FOR HODGKIN'S LYMPHOMA

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We report the case of a 30 year-old woman with nodular sclerosing type Hodgkin's lymphoma, AAS IIA Bulky confined to the lower mediastinum. The patient had received 3 cycles of ABVD with an acceptable response of more than 60%, when she developed an acute respiratory failure that required urgent mechanical ventilation. Few days later the patient developed a rapid dysfunction of pharyngeal muscles with feeding and swallowing difficulties. A diagnosis of myasthenia gravis was made, based on clinical signs and compatible neurophysiologic studies, and confirmed by high acetylcholine anti-receptor titers. Thorax CT scan revealed a further reduction of the initial mass, which was actually confined to the lower mediastinum, measuring no more than 5 cm of maximum diameter. The patient never re-established her autonomous ventilation and died two days later, in spite of high-dose steroidal therapy. Myasthenia gravis is a B-cell-mediated autoimmune neuromuscular disorder characterized by weakness and fatigability of skeletal muscles. The underlying defect is an autoantibody-mediated attack on the acetylcholine receptors at the neuromuscular junction. Epidemiological similarities (genetic influences and the role of viral agents) between Hodgkin's lymphoma and myasthenia gravis, and the immune abnormalities found in these two diseases suggest that their association is not fortuitous. Few cases of such association have been reported in the literature, but none of them seemed to undergo the acute course we have observed. However, some of the described episodes have resolved after chemotherapy or auto-antibodies based therapy, confirming the relevance in the elimination of autoreactive B-cell clones.

P190

HODGKIN'S LYMPHOMA LINKED TO GAUCHER'S DISEASE

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Malignant neoplastic disorders, mainly haemathological malignencies (LLC, MM and Lymphoma), have been frequently described in patients with Gaucher's disease. A special type of this phenomenon is crystal-storing histocytosis or the so called pseudo-pseudo Gaucher cells in which crystalline protein storage in macrophages is induced by a paraproteinemia. We describe a 28-year-old woman with Hodgkin's Lymphoma nodular sclero stage II A + mediastinal Bulky. Her bone marrow biopsy evidenced a nodular and interstitial infiltration with 50% Gaucher cells. The patient's diagnosis did not show anaemia, or leucopiastrinopenia or hepatosplenomegaly, or any bone lesion compatible with Gaucher's disease. A glucocerebrosidase test has been carried out on leukocytes and a chitotrossidase test on plasma; besides glucocerebrosidase gene molecular analysis evidenced a N370S mutation in apparent homozygosis and proved the Gaucher's disease diagnosis (molecular tests on her parents are being carried out). After treatment with 4 cycles of ABVD and mediastinal radio therapy the patient showed C.R. during 6 month follow up. while no therapy for Gaucher's disease has been introduced, as she presented no symthoms. Discussion: The presence of two diseases in the same individual can be useful to make a common pathogenic hypothesis, yet a casual link cannot be excluded. Many studies explain how Gaucher's disease is linked to lymphoproliferative disorders as caused by excessive chronic antigenic stimulation on the immune system determined by a glucocerebrosidase increase for the altered lipidic metabolism. Moreover Gaucher cells cause the release of cytokines (IL1, IL6, IL10 and TNFalfa).The glucocerebrosidase gene is placed on chromosome 1 q21, like the oncogene c-ski is placed on chromosome 1 q21-23, that we know is involved in many lymphoproliferative disorders. Greater attention should be given to the research and study of Gaucher's disease supported by the modern diagnostic technologies as molecular biology, so to provide new hypothesis of study on 1) The response of chemotherapy in lymphoproliferative disorders with Gaucher cells; 2) The opportunity of an early substitutive therapy in Gaucher's disease so to prevent lymphoproliferative disorders.

P191

GONADAL FUNCTION AFTER COMBINATION CHEMOTHERAPY IN HODGKIN'S LYMPHOMAS ACHIEVING LONG LASTING COMPLETE REMISSION

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Introduction. With the increasing cure rate of patients (pts) treated for Hodgkin's lymphoma (HD, the evaluation of the late effects on gonadal function remains an important issue: sometimes in HD-survivors the price paid is ovarian failure and sterility. The incidence of Premature Ovarian Failure (POF) after polichemotherapy (CHT) was reported in 15-62% affected pts, with an increased risk over 30 years old. The gonadal function of 29 relapse-free long survivors with HD was studied. MATERIAL AND METHODS This study was carried out on the female pts, aged ranging from 15 to 35 years, affected by HD, followed-up at from january 1998 to june 2003. Histology was nodular sclerosis in 21 cases (72,4%); stage was III-IV in 16 pts (55,1%). All pts were treated according ABVD regimen at standard doses. 20 pts received sovradiaphragmatic radiotherapy, for a total dosage of 2900-6300 Gy. No pts had pelvic radiotherapy. All pts were in complete remission (>1 year) at the time of the study. Median age of pts was 24,32±6,67 years at the time of treatment and 28,55±7,38 at time of the evaluation. The control of the residual ovaric reserve has been performed by transvaginal ultrasonography to count the antral ovarian follicle (AFC) on the 2nd or 3rd day of the cycle and by evaluating, at the same time, an hormonal profiling (FSH, LH, Inhibin-B–INB, anti Mullerïan Hormon-AMH).In 14 pts (48,2%), we have attempted to minimize the gonadotoxic effect of CHT by the co-treatment with a gonadotropin-releasing hormone agonistic analog (GNRH) to induce a temporary prepubertal milieu.

Results. After the CHT 22 pts (76%) had again regular menses while 7 (24%) remained amenorrhoic. These pts had high levels of FSH (23,49±7,07 vs 9,46±7,07; p=0,5595) and LH (15,65±16,04 vs 6,67±3,27; p=0,3671) and low levels of INB (41,28±94,70 vs 65,47±58,23;p=0,0779) and AMH (2,73±5,6 vs 11±11,39; p=0,1076) and AFC (2,2±4,38 vs 5,4±3,97; p=0,05) suggesting a POF. The analysis of clinical and laboratory data of those pts treated with GNRH analogue revealed that none of them experienced amenhorrea, while 46% (7/15) of the untreated pts were amenhorroic. Chemotherapy induced amenorrhea in 1 pt, within the last two cycles of chemotherapy, the other POF pts, all aged >30 years old at the time study, developed amenorrhea after 2-5 years from the end of chemotherapy. Mean age of pts with regular menses was not significantly different from the mean age of women with amennorrhea (mean age of POF pts vs pts with regular menses: 34,5 ± 3,1 vs 28,1± 6,5; p>0,05), although it was possible to evidence a tendence to develop a premature ovarian failure by increasing the age. No pts aged less than 30 years old at the time of chemotherapy showed variation of menses. 2 pts after the end of chemotherapy had successful pregnancies and delivered normal an healthy babies. The analysis of the hormonal markers of ovarian reserve did not show any statistical significance. *Discussion.* Toxic effects of CHT on gonads have been described by several authors. Elevated levels of plasma gonadotropins and low levels of gonadal steroids, INB and AMH associated with injury of ovarian tissues were reported as consequence of a direct toxic effect of alkylating agents, such as cyclophosphamide. In our experience,the gonadal toxic effect was evidenced only after the age of 30, even if pts received chemotherapy before 30 years. Ovarian dysfunction is a common conseguence of chemotherapy. The histologic appearance of the ovaries after chemotherapeutic treatment reveals abundant primordial follicles with maturation arrest beyond the primary follicle stage. The fact that the number oocytes decreases steadily with increasing age suggests that ovarian function in the pubertal stage may be less susceptible to cytotoxic-induced damage. The major toxic damage of the ovary by increasing the age of pts be explained by the associated physiological follicular atresia and hence women over 30, already depleted in number of oocytes by chemotherapy, become at high risk to POF.

*Conclusions.*Age related follicular depletion is a cofactor inducing premature ovarian failure. Hence young pts do not seem to have immediate consequences on ovarian function and the reproductive outcome in these pts should be accomplished before 30 years old. In young female pts with HD the subject of reproduction as to be faced using strategies preserving the follicular ovarian reserve before CHT (biopsy and crio-preservation of ovary, GNRH analogue) and evaluating the gonadic damage incurred. At present the use of GNRH analogue gave contraddictory results, even if it is used in many centers (it doses not seem to be effective in saving the follicular reserve of women treated with CHT because of uncomplete suppression of hypophysis). Hormonal markers (FSH, LH) do not seems to be sensitive enough to predict the reduction of fertility. The AFC seems to be the best available test to determine the ovarian reserve. Concluding in fertile women with HD who are going to be treated with CHT the estimate of ovarian reserve should be performed by dosing INB and AMH and by counting the antral follicles. GNRH analogues seems to partially protect from the distruction of the ovarian reserve and these effects appars to be indipendent from the age of women and time elapsed between CHT and evaluation of fertility.

P192

THE IMPACT OF RELATIVE DOSE INTENSITY ON RESPONSE AND SURVIVAL IN A SERIES OF 175 NEWLY DIAGNOSED PATIENTS WITH HODGKIN'S LYMPHOMA

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The nature of the relationship between chemotherapy dose-intensity and clinical outcome, remains a subject of much controversy. Recent clinical trials support the importance of sustaining full dose-intensity in front-line chemotherapy for malignant lymphomas. There is good evidence for a threshold of relative dose-intensity (RDI) for some regimens below which patients may receive little clinical benefit. Thus substantial reductions in RDI may compromise outcomes. Hasenclever et al, have proposed

the concept of the effective dose approach, which takes into account not only the total dose of chemotherapy and RDI but also the heterogeneity of Hodgkin's Lymphoma with respect to chemosensitivity and tumour growth rate. They hypothesize that in rapidly growing lymphomas tumour re-growth between cycles is a potential problem. Applying the effective dose model MOPP/ABVD, MOPP/ABV, and BEACOPP baseline are not predicted to be notably different from ABVD in cure rates. However, the model predicted that a dose escalation of approximately 30% in conventional chemotherapy within the same treatment duration would translate into a relevant difference in freedom from progression of more than 10% in plateau. This indicates that there is a subset of patients with progressive disease, under standard treatment, that are kinetically resistant because of rapid re-growth during treatment intervals. For these patients, shortening of treatment intervals and dose-escalation of critical drugs may be an option. Retrospective studies on our series of patients with advanced Hodgkin's Lymphoma undergoing their first chemotherapy treatment, revealed that the most common factor in treatment failure was a suboptimal drug dose intensity, mainly due to delay in therapy and decreasing drug doses induced by persistence of myelosuppression at recycling. In addition, clinical heterogeneity was observed among patients: data from history, presentation, clinical and biological features, varied from indolent to rapidly growing pictures. To improve ABVD results we first developed a protocol which adds G-CSF (5mcg /Kg/daily from d8 to d11) to the standard ABVD treatment and uses more stringent criteria for dose reduction or delay in treatment together with dose compensation so as to maintain a relative dose intensity RDI near 1. From March 1997 to May 2004 70 patients with HL were treated with this dose-adjusted ABVD protocol plus primary G-CSF and 22 with a standard ABVD protocol. Recently we designed a new dose-dense and dose-intensity ABVD scheme (escABVD-21) for advanced HL: in this new schedule the adriamycin was escalated from 25 to 35 mg/m^2 (cycles 1,2,3,4) and the inter-cycle period was shortened from 28 to 21 days (for all 6 cycles); primary G-CSF (5mcg/Kg/daily) was administered from d3 to d8 and drugs were delivered at d10 and d21 of every cycle. From June 2004, eleven patients were treated with this protocol.

Administered relative dose-intensity (RDI) was calculated for any of these 103 newly diagnosed cases of HL patients treated with ABVD. The results were also compared with a historical group of 72 patients who had undergone hybrid MOPP/ABVD. In this last series we had 2 early deaths (included in the study on the basis of the intention-to-treat criteria). In Table 1 we report the presentation features of all 175 HL pts. HL patients received from 4 to 8 cycles of chemotherapy $^{+/-}$ involved fields radiotherapy (IF-RT). Data on administered RDI were available for 173 cases . Patients were divided in 3 groups according to the Administered RDI (range 0.5-1.5) . The first group included 42 (24%) pts with RDI less than 0.85; the 2nd group, 64 (37%) pts with RDI values between 0.85 and 0.99 and, finally, the 3rd group included 67 (39%) pts with RDI values of 1 or more. Patients treated with hybrid MOPP/ABVD had significantly acute toxicity compared to ABVD treatments. Patients who received ABVD with primary G-CSF showed the best toxicity profile and were able to maintain administered RDI closely to the programmed doses.

Table 1. Presentation features of 175 HL patients

n. pts	Male sex	Age >45yrs (14-77)	Early Stage	Adv. stage	E sites >1	Bulky	B sym	Stage IV	high LDH	ESR > 50	nodal Sites >3	Groin nodes	IPS 0-2	IPS 3+
175	91	42	33	142	21	78	80	31	78	100	83	42	119	56
%	52%	24%	19%	81%	12%	45%	46%	18%	45%	57%	47%	24%	68%	32%

Table 2. Response (CR) and Survival (FFP and OS) according to 3 levels of Administered. Relative Dose Intensity (RDI) in 175 newly diagnosed Patients with Hodgkin's Lymphoma

groups Range:	RDI levels Pts 0.5-1.5	% rate	CR	Fisher's Exact Test (2-sided)	FFP rate	Log-rank stat	OS rate	Log-rank stat
	overall		89%		80%		88%	
1	<0.85	24%	67%		45%		60%	
2	>0.85-0.99	37%	94%	28.912	84%	43.01	95%	35.10
3	> 1-1.5	39%	100%		97%		99%	
	significance			0.0001		0.0001		0.0001

In Table 2 we report the CR, FFP and OS rates according to the 3 levels of RDI. Figures 1 and 2 show FFP and OS curves according to Kaplan-Meyer. Response and survival rates of groups 1,2 and 3 were: 64%vs94%vs100% for CR (Fisher's Exact test 0.0001); 45%vs84%vs97% for FFP (log-rank 0.0001); and 60%vs95%vs99% for OS (log-rank 0.0001). The best progression rates of Complete Response, Freedom From Progression and Overall Survival. were seen in patients with RDI >1.

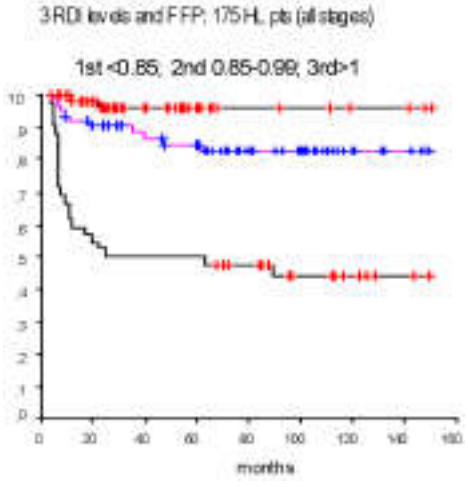

Figure 1.

In particular, the new dose-dense and dose-intensity ABVD protocol seems very promising in terms of complete response and toxicity profile: 11/11 pts (100%) obtained a CR; the dose-escalation of adriamycin and the dose-density of the overall schedule were well-tolerated; toxicity was mild. We believe that modification of dose-intensity and/or

dose density of current protocols may overcome the regrowth during treatment intervals improving the outcome of patients especially if they have a very aggressive and rapidly growing presentation picture.

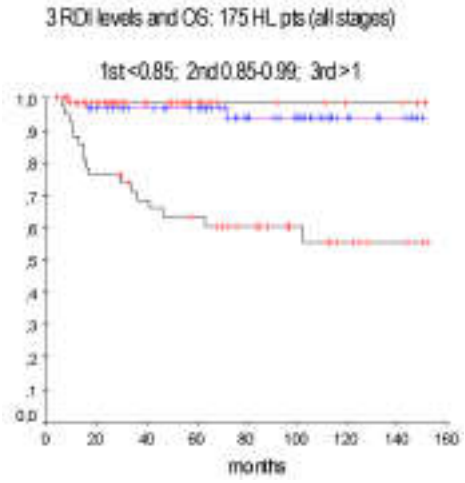

Figure 2.

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B-LYMPHOCYTE STIMULATOR SERUM LEVELS CORRELATE WITH CLINICAL FEATURES AND OUTCOME IN PATIENTS WITH HODGKIN'S LYMPHOMA

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B-Lymphocyte stimulator (BLyS) is a novel member of the Tumor Necrosis Factor (TNF) Ligand superfamily acting as a potent survival factor for B lymphocytes. BLyS, which exists either in a membrane-bound and soluble form, is specifically expressed and released by cells of myeloid origin such as macrophages, monocytes and dendritic cells. Recently, it has been demonstrated that IFN-g, IL-10 and G-CSF are able to induce BlyS gene expression influencing membrane-associated and/or soluble forms levels. Since Hodgkin and Reed-Sternberg cells (HRS cells) represent a clonal population of transformed pre-apoptotic germinal center (GC) B-lymphocytes and based on the fact that several elements composing the HL reactive infiltrate may express and release sBLyS, we hypothesized an involvement of this molecule in HL pathogenesis. To test this hypothesis we measured the levels of sBLyS in 97 serum samples collected from patients with HL at the diagnosis finding significantly elevated levels as compared to those determined in 33 normal donors. The correlation between sBLyS, clinical features and outcome are shown in the table. Soluble BLyS concentration appeared to positively correlate with patients pathologic and clinical features such as advanced stage of disease (IIB-IV), bulky mass and presence of systemic B symptoms. Moreover, we detected significantly higher levels of sBLyS in patients who experienced treatment failure as compared to those with a favourable outcome. Our results indicate BLyS as one of the TNF superfamily members participating in the complex cytokine and chemokines network influencing the pathogenesis and outcome of HL. Further studies are needed in order to establish the possible prognostic significance of this molecule in HL.

	N° of cases	BLyS pg/mL Mean ± SEM	Median (range)	p-value*
Controls	33	2687 ± 1157	2760 (871-5000)	<0.0001
HL patients	97	4428 ± 2418	4236 (756-11936)	0.48
Males	57	4592 ± 2492	4236 (756-11936)	0.14
Females	40	4196 ± 2319	4196 (840-10535)	0.003
Age ≥ 45	18	5268 ± 2642	4862 (906-11936)	0.017
Age < 45	79	4238 ± 2340	4136 (756-10800)	0.033
Stages I-IIA§	44	3517 ± 1650	3506 (1018-7896)	0.38
Stages IIB-IV	48	5098 ± 2674	4777 (756-11936)	0.046
A§	54	3874 ± 2059	3717 (1018-10800)	
B	41	5153 ± 2723	5183 (756-11936)	
Bulky§	29	5081 ± 2266	5060 (1465-8756)	
Non Bulky	65	4108 ± 2472	3829 (756-11936)	
NS§	79	4367 ± 2391	4217 (756-11936)	
MC	9	4707 ± 3135	4924 (840-8756)	
LP	5	3342 ± 1444	3578 (1804-5484)	
CCR§	76	4368 ± 2502	4160 (756-11936)	
Progression relapse	15	5440 ± 1812	4924 (2370-7920)	

**p value refers to Mann-Whitney U and Kruskal-Wallis tests. § not available for all cases.*

Non-Hodgkin's Lymphoma I

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THE REPLACEMENT OF CONVENTIONAL DOXORUBICIN WITH CAELYX IN CHOP-RITUXIMAB POLICHEMOTHERAPY SEEMS TO IMPROVE THE SAFETY PROFILE IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background. At present CHOP plus rituximab (CHOP-R) appears to be the golden standard treatment for elderly patients with diffuse large B-cell lymphoma (B-DLCL) with 76% CR rate and 60% 2 years EFS. Treatment related toxicity, in particular infectious complications and cardiac toxicity, however, still represent a major cause of morbidity and mortality. Previous *in vivo* studies have shown the good therapeutic activity and favourable safety profile of pegylated liposomal doxorubicin (Caelyx). Here we report the preliminary results of a pilot study performed to evaluate the efficacy and tolerability of a Caelyx-modified CHOP-R regimen (COP-Caelyx-R).

Methods. Thirty consecutive untreated patients, 60 years or older, with CD20 positive B-DLCL, stage II-IV or bulky (lymphoma masses greater than 10 cm)stage I, were enrolled in the study. Patients with a previous history of cardiac disease were excluded from the study. Caelyx 30 mg/m^2 was given on day 1 in combination with standard dosage of Prednisone, Vincristine, Cyclophosphamide (according to CHOP regimen) every 21 days for 6 courses. Rituximab 375 mg/m^2 was given on day 0. G-CSF was administered as appropriate. Patients with initial bulky disease or localized residual disease received consolidation-involved field radiotherapy. The procedures planned for cardiac toxicity evaluation were: 1) echography with ejection fraction (EF) at baseline, before the fourth cycle and then 2, 6 and 12 months after the end of therapy; 2) serum troponin dosage at time 0 and the 1 h, 24 h and 48 h after Caelyx administration. Hematological and extra-hematological toxic effects were graded according to the WHO classification. Results. Patients' median age was 69 (range 60-75 years); the distribution according to International Prognostic Index (IPI) was: low 14%, low-intermediate 24%, high-intermediate 38%, high 24%. Twenty-nine patients are valuable for response and toxicity (1 patient abandoned the study after the second course while in major response). Overall response (OR) and CR rate are 76% and 59%. The projected one year EFS and OS are 65% and 79%. No treatment-related mortality was documented. WHO grade III-IV neutropenia and thrombocytopenia were 86% and 3%. Extra-hematological III-IV toxicity was represented respectively by a single case of infection, mucositis and bleeding. One patient with a previous history of atrial fibrillation experienced a single episode of arrhythmia. None of the patients had other clinical, instrumental and laboratory signs or symptoms of cardiac toxicity; none developed palmar-plantar erythrodysesthesia. Conclusions. COP-Caelyx-R regimen is an active regimen for the treatment of elderly B-DLCL. The replacement of conventional doxorubicin with Caelyx seems to reduce the incidence of extra-hematological toxicity, in particular cardiac and infectious complications.

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FOLLICULAR DENDRITIC CELL SARCOMA. REPORT OF TWO CASES TREATED BY SURGICAL EXCISION AND COP PLUS PEGYLATED LIPOSOMAL DOXORUBICIN

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Background. Follicular dendritic cell (FDC) sarcoma is a rare neoplasm arising in lymph nodes but also in extranodal sites from accessory cells of the immune system that are essential for the function of antigen presentation and germinal center reaction regulation. Recently there has been an increasing interest in this tumor due to availability of specific antibodies to confirm FDC lineage. In vitro studies have suggested that dendritic cells are sensitive to doxorubicin. PEG-coated liposomes prolong circulation time of doxorubicin and may enhance its localization to tumors. PEG liposomal doxorubicin has already shown activity in aggressive NHLs and related Kaposi's sarcoma; we have tested its activity in combination with COP in FDC sarcoma.

Methods. We report the cases of two 49 and 39 year-old females that showed a common histologic pattern with neoplastic cells consisted of oval to spindle cells with elongated nuclei, vesicular or stippled chromatin and eosinophillic cytoplasm with indistinct border. The FDC nature of the tumor was in both cases confirmed by positive staining with CD 21, S-100 protein, vimentin and negative staining for cytokeratin. Case #1 presented in November 2003 with an enlarged (2 cm) lymph node of the right cervical area; the patient underwent a right neck dissection with excision of lymph node. Case #2 presented in July 2004 with an abdominal mass of 16 x 12 cm. The tumor was removed together with the involved jejunal loop and successfully detached from the near organs. FDC sarcoma has a significant recurrent and metastatic potential and it is reasonable that resected localized disease may be prevented from recurrence by adjuvant radiotherapy or chemotherapy. Our patients after surgical excision received COP plus Caelyx: case #1 was treated with five and case #2 with six courses of Cyclophosphamide 750 mg/m^2 i.v. and Vincristine 1.4 mg/m^2 (day 1), PEG-liposomal doxorubicin (Caelyx) 40 mg/m^2 i.v. (day 1) and Prednisone 100 mg p.o. days 1-5, every 3 weeks. Results. In case #1 WHO grade 3 skin toxicity (erytrodysesthesia) was observed and consequently we reduced at 70% the dose of Caelyx at the fifth course of treatment. The patient at 16 months after the diagnosis is in CR confirmed by CT scan and 18-F-FDG PET. In case #2 WHO grade 3 leucopenia was reported, but no skin toxicity was registered. The patient is disease

free at 8 months after the diagnosis and both CT scan and 18-FDG PET are negative for residual disease.

Conclusions. The optimal combination treatment for FDC sarcoma has yet to be defined. As our knowledge these are the first reports of FDC sarcoma treated with adjuvant chemotherapy including PEG-liposomal doxorubicin. Although based on the description of only two cases, that need to be validated in a larger series of patients with a longer follow up, in our opinion this adjuvant combination treatment may be useful in the management of FDC sarcoma, specially for large or deep lesions, once identified and well characterized by the appropriate application of immunohistochemically staining.

P196

GEMCITABINE AS FRONT-LINE TREATMENT FOR CUTANEOUS T-CELL LYMPHOMAS: PHASE II STUDY ON 32 PATIENTS

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On the basis of gemcitabine activity in heavily pretreated cutaneous T-cell lymphoma (CTCL) patients the objective of this study was to determine the role of Gemcitabine in advanced untreated CTCL. Between June 2002 and February 2004, 32 untreated patients with mycosis fungoides (MF) (26 patients), peripheral T-cell lymphoma unspecified (PTCLU) with exclusive skin involvement (5 patients) and Sezary Syndrome (SS) (1 patient) were enrolled in a 7-institution, phase II trial and treated with gemcitabine. This drug was given on days 1, 8, 15 of a 28-day schedule at a dose of 1200 mg/m^2 intravenously over 30 minutes for a total of six cycles. Of the 32 patients, 7 (22%) achieved complete response (CR), 17 (53%) partial response (PR), while the remaining 8 showed no benefit from the treatment. Five of the CRs were histologically confrmed. The CR and PR rates were the for MF and PTCLU patients, respectively. The median duration of CR patients was 10 months (range, 4 to 22 months). Treatment was well tolerated; hematologic toxicity was mild and no nausea/vomiting or organ toxicity was recorded. The results of the present phase II study show activity of gemcitabine as a single agent in untreated CTCL patients. Further studies using gemcitabine in combination, contemporary or sequentially, with other drugs in advanced stage untreated CTCL patients are needed.

P197

TEMOZOLOMIDE TREATMENT IN PRETREATED MYCOSIS FUNGOIDES: EXPERIENCE IN 9 PATIENTS

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To evaluate the efficacy and toxicity of temozolomide, an oral alkylating agent of the imidazotetrazine derivates with a low-toxicity profile and activity in solid tumors, in patients with relapsed or refractory mycosis fungoides (MF). Between July 2003 and March 2004 in our institute, 9 previously treated patients with MF were enrolled into a phase II trial and treated with temozolomide. This drug was given orally every four weeks for a total of three cycles at the following doses: for the first cycle at a dose of 150 mg/m^2/day per os for 5 consecutive days, and then for the second and the third course at a dose of 200 mg/m^2/day for 5 consecutive days. Of the 9 patients, one (11%) achieved complete response (CR), 2 (22%) partial responses (PR), 3 (33%) stable disease, and the remaining 3 showed no benefit from the treatment. The duration of response was 4 months (for CR patient) and 3 and 6 months (for PR patients, respectively). Treatment was well tolerated: hematologic toxicity was mild; nausea/vomiting was mild and no organ toxicity was recorded.

The results of the present phase II study show activity of temozolomide as a single agent in patients with heavily pretreated MF. Further studies that use temozolomide alone or in combination with other drugs in earlier stages of the disease are needed.

P198

ROLE OF ANEMIA IN SURVIVAL OF PATIENTS WITH ELDERLY AGGRESSIVE NON-HODGKIN'S LYMPHOMA AFTER CHEMOTHERAPY

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Baseline anemia is a relevant prognostic factor in the overall population of non-Hodgkin's lymphoma (NHL) patients, and studies focusing on elderly NHL are awaited. We conducted a pooled analysis of a cohort of comparable patients enrolled (1993–2001) in three multicenter clinical trials on use of a MACOP-B-like regimen (VNCOP-B) for front-line treatment of elderly aggressive NHL. Models for of Cox's proportional hazards regression analysis of prognostic value of pre-/post-treatment hemoglobin values in terms of 3-yr overall survival included age, sex, initial tumor staging and response to treatment.

Of the 168 patients screened, 16 were excluded due to missing data or lack of 3-yr follow-up. In addition to achievement of complete/partial remission (adjusted relative risk [RR], 0.215; p=0.0001) and advanced stage (II-IV vs. I–II; adjusted RR, 1.55; p=0.0023), post-treatment hemoglobin values were an independent predictor of survival (adjusted RR per 1-g/dL increment, 0.76; p= 0.0041). In the present analysis, pretreatment hemoglobin values were associated with only marginal risk reduction (adjusted RR per 1-g/dL increment, 0.985; p=0.049).

Post-treatment hemoglobin values appear to provide a strong independent predictor of 3-yr survival in elderly aggressive NHL, supporting the potential role of anemia correction in this group of patients.

P199

PROGNOSTIC FACTORS IN PRIMARY CUTANEOUS B-CELL LYMPHOMA: A 542 PATIENTS SERIES. THE ITALIAN STUDY GROUP FOR CUTANEOUS LYMPHOMAS

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Primary cutaneous B-cell lymphomas (PCBCL) are a distinct group of diseases in the general scenario of the extranodal non-Hodgkin's lymphoma and, particularly, in the cutaneous lymphoma subset. In the literature, conflicting data exist regarding the prognostic factors and these data were evaluated on little numbers of patients. In the present retrospective study, the prognostic factors and follow-up data of a large group of PCBCL patients are reported. The study group included 542 patients with PCBCL referred, treated and followed in 11 italian centers (the Italian Study Group for Cutaneous Lymphomas, GILC) during a period of 24 years (1980-2003). There were four different histologic subtypes: marginal zone lymphoma (MZL), large B-cell lymphoma (LBCL), large B-cell lymphoma of the leg (leg-type LBCL), and follicular center lymphoma (FCL). The univariate analysis for overall survival (OS) identified age older than 55 years ($p<0.0001$), presence of regional or disseminated cutaneous lesions ($p=0.0089$), a lower limb localisation ($p<0.0001$), a histologic diagnosis of LBCL, and a first-line chemotherapy treatment. The univariate analysis for disease-free survival (DFS) showed only the single lesion as statistically significant factor ($p=0.001$). Multivariate analysis identified all LBCL, others and leg-type ($p=0.039$ and $p<0.0001$, respectively) for OS and the single cutaneous lesion ($p=0.003$) for DFS. The 10-year DFS for patients with leg-type LBCL was 21.5%, compared to 44% for the remainder cases. The global 10-year OS rate of all 542 study patients was 83% and the global 10-year DFS of all 483 CRs was 44%. The results of this large retrospective PCBCL study suggest that patients with LBCL histology (paricularly, the leg-type one) and/or the presence of a disseminated disease have a more unfavorable prognosis. In terms of outcome, about 50% of the PCBCL patients are cured.

P200

IFOSFAMIDE, EPIRUBICIN, ETOPOSIDE (IEV) AND AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANT: A FEASIBLE AND EFFECTIVE SALVAGE TREATMENT FOR LYMPHOID MALIGNANCIES

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Background. The combination of ifosfamide, epirubicin, and etoposide (IEV) has shown good tolerability and marked therapeutic activity against HLs and aggressive NHLs and excellent efficacy in mobilising PBSCs.

Patients and methods. The IEV schedule consisted of epirubicin 100 mg/m^2 over 30' on day 1, etoposide 150 mg/m^2 over 1 hour on days 1-3, and ifosfamide 2.5 g/m^2 over 1 hour on days 1-3 together with MESNA. Granulocyte colony stimulating factor was given when clinically required, or from day + 5 after the end of chemotherapy, until completion of PBSC harvest. Patients who proceeded to HDT received conditioning therapy with BCNU, etoposide, Ara-C and melphalan (BEAM, in HLs and NHLs), or melphalan 100 mg/sqm and mitoxantrone (MM patients). The present study currently includes 65 patients with a median age of 53 years: 27 with aggressive NHL, 20 with HL, 7 with indolent NHL, and 11 with MM. Fifty-five patients received IEV for disease that was refractory to conventional induction regimens or in first or second relapse; 4 patients with high risk NHL and 4 with MM were treated with IEV while in CR after chemotherapy in order to mobilise PBSCs .

Results. Ninety per cent of patients with HL responded to IEV, and 85% of them achieved CR. Both aggressive and indolent NHLs were less responsive (ORR 55% and 33%, respectively; CRR 45% and 16.5%, respectively). MM patients showed intermediate responsiveness (ORR 50%, CRR 30%). Among 16 HLs and NHLs treated for chemorefractory disease, only 2 responded to treatment (1 CR, 1 PR). In our series, more than 90% of patients with HL and NHL who received IEV while in PR following the previous therapy responded to our salvage therapy (CR rate of 82% and 80%, respectively). Similar response rates were observed among patients treated in relapse (CR rate of 83% and 67% in patients with HL and aggressive NHL, respectively). IEV was well tolerated in most patients. Haematological toxicity was not negligible, and 37% and 30% of patients received RBC and platelet transfusions , respectively. Twenty-six per cent of patients had fever of unknown origin, but no life threatening infections were recorded. No severe heart complications were observed. PBSC mobilisation was successful in 37 out of 39 patients (95%) and led to the collection of a median of 16, 12, and 13.7x10 ^{6} CD34$^+$ cells / Kg in patients with HL, NHL, and MM, respectively. All 37 patients actually underwent autologous stem cell transplant, following a 1 to 2 month interval after the end of IEV. Two patients were submitted to allogeneic transplant. Median overall survival in HL, aggressive NHL and indolent NHL have been 32 (5-60), 16 (2-46), and 14 (4-42) months, respectively. Median EFS have been 31 (5-60) , 7 (2-46), and 7,5 (4-42) months, respectively.

Conclusions. In conclusion, our study confirms that IEV ± HDT is a well tolerated and effective salvage treatment for lymphoid malignancies, and that IEV acts as an excellent stem cell mobiliser.

P201

ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA AND EBV-ASSOCIATED B-CELL LYMPHOMA

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Angioimmunoblastic T-cell lymphoma (AITL) is classified by the WHO as a peripheral T-cell lymphoma. B-cell

proliferations, particularly those related to Epstein-Barr virus (EBV), are thought to be secondary to immune dysregulation produced by AITL. To note, EBV plays an important role in the pathogenesis of different lymphoproliferative disorders, in particular in the post-transplant setting (PTLD). The diagnosis of AITL is often difficult because of a varying clinical and pathological picture. Regarding prognosis only a quarter of patients experience long-term remissions even after multi-agent chemoterapy. However, virtually all patients relapse, and despite some promising reports on the role of high dose chemotherapy with autologous bone marrow support, AITL seems to be an high grade lymphoma with the worst prognosis.

We report on an immunocompetent woman in whom AITL occurrence and degree of lymph node enlargement strongly correlated with EBV load in serum (EBVL). The biopsy of the cervical node showed typical features of AILT. Flow cytometric immunophenotyping identified an aberrant CD4+ T-cell population that lacked surface CD3. Polymerase chain reaction analysis of the T-cell gamma receptor gene revealed a clonal rearrangement. In addition to the AITL, the lymph node showed partial involvement by a B-cell lymphoma. The B lymphoma cells and admixed immunoblasts and Reed-Sternberg-like B cells in the AITL were positive for Epstein–Barr virus(EBV) by in situ hybridization. Our findings raise the possibility that EBV-associated B-cell lymphoma was a secondary event in AITL via EBV infection or reactivation followed by clonal expansion of an immortalized EBV-infected B cell clone. Treatment with Valacyclovir at the early stage resulted in a drastic more than 3 log10 decrease of EBVL and a remission. Since antiviral therapy was incapable of achieving long-term control, chemoterapy including anti-CD20-antibody therapy had administrated but resulted only a partial remission. The lymphoma progressed and 33 month after it was diagnosticated the patient died.

In conclusion, this case describes an EBV-associated lymphoma and demonstrates that antiviral treatment with valacycovir exhibited a remarkable regression at an early stage. Although in vitro studies do not support valacyclovir activity against EBV, this compound ought to be futher investigated in the treatment of EBV-associated AITL, PTLD or other EBV-associated lymphomagenesis, such as,and in combination with antineoplastic drugs.

P202

ABERRANT SOMATIC HYPERMUTATION IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: IMPLICATIONS FOR DISEASE PATHOGENESIS AND COMPARISON WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction. Primary mediastinal large B-cell lymphoma (PMLBCL) is a subtype of diffuse large B-cell lymphoma (DLBCL) arising in the mediastinum. Compared to DLBCL, PMLBCL displays specific clinical, morphological and molecular features suggesting that PMLBCL may represent a distinct clinico-pathologic entity. Aberrant somatic hypermutation of multiple proto-oncogenes, namely PIM-1, PAX-5, RhoH/TTF and c-MYC has been recognised as a molecular feature distinctive of DLBCL.

Methods. To investigate whether PMLBCL is associated with aberrant somatic hypermutation, we performed mutational analysis of PIM-1, PAX-5, RhoH/TTF and c-MYC in a panel of 19 PMLBCL. For comparison, 19 DLBCL were also analysed. For each gene, a region spanning up to 1.5 Kb from the transcription start site was analysed by PCR amplification and DNA direct sequencing.

Results. Overall, the prevalence of mutated cases was similar among DLBCL and PMLBCL. Mutations targeting at least one of the 4 genes were found in 14/19 (73.6%) PMLBCL and 13/19 (68.4%) DLBCL, while mutations in more than one gene were found in 7/19 (36.8%) PMLBCL and 9/19 (47.3%) DLBCL. Among the four genes, the prevalence of mutated cases and the mutation frequency was also superimposable between PMLBCL and DLBCL. In fact, PAX-5 was mutated in 9/19 (47.3%) PMLBCL with a mean mutation frequency of 0.20 x 10-2/bp and in 7/19 (36.8%) DLBCL with a mean mutation frequency of 0.18 x 10-2/bp. RhoH/TTF was mutated in 6/19 (31.5%) PMLBCL with a mean mutation frequency of 0.08 x 10-2/bp and in 8/19 (42.1%) DLBCL with a mean mutation frequency of 0.27 x 10-2/bp. PIM-1 was mutated in 3/19 (15.7%) PMLBCL with a mean mutation frequency of 0.09 x 10-2/bp and in 7/19 (36.8%) DLBCL with a mean mutation frequency of 0.11 x 10-2/bp. c-MYC was mutated in 6/19 (31.5%) PMLBCL with a mean mutation frequency of 0.23 x 10-2/bp and in 5/19 (26.3%) DLBCL with a mean mutation frequency of 0.11 x 10-2. The mutation pattern was also similar between PMLBCL and DLBCL and was consistent with the physiological somatic hypermutation process. A total of 74 mutational events were detected in PMLBCL. The majority of the mutations were represented by single base-pair substitution (n=66), whereas only 8 deletions of a short DNA stretch were observed. Of the 66 single base-pair substitutions, 41 were transitions and 25 were transversions, with a transition/transversion ratio of 1.64 (expected 0.5; p=0.0001) and a G+C/A+T ratio of 3.33 (expected 1.28; p=0.009). The frequency of mutations targeting RGYW/WRCY was significantly higher than that occurring outside RGYW/WRCY (2.3% versus 1.3%; p=0.03). Among DLBCL, a total of 87 mutational events were detected. Mutations were preferentially represented by single base-pair substitutions (n=81), whereas only 4 deletions and 2 insertions of a short DNA stretch were observed. Of the 81 single base-pair substitutions, 42 were transitions and 39 were transversions, with a transition/transversion ratio of 1.07 (expected 0.5; p=0.02) and a G+C/A+T ratio of 1.89 (expected 1.28; p=n.s.). The frequency of mutations targeting RGYW/WRCY was significantly higher than that occurring outside RGYW/WRCY (5.0% versus 1.7%; p<0.0001.). Intraclonal heterogeneity as a marker of ongoing mutation activity was identified in 1/4 PMLBCL analysed for the PAX-5 gene. Among PMLBCL, one missense mutation affected PIM-1 exon 2 causing the substitution of Ala for Gly at position 28 and three missense mutations in c-MYC exon 2 leading to the substitution of Ser for Pro at position 59, Glu for Gly

at position 91 and Lys for Ser at position 64, with potential functional consequences.

Conclusions. The implications of our results are twofold. First, aberrant somatic hypermutation is involved in the pathogenesis of PMLBCL by affecting regulatory and coding sequences of proto-oncogenes potentially relevant for transformation. Second, our results indicate that aberrant somatic hypermutation targets both PMLBCL and DLBCL with similar prevalence, distribution and mutational pattern. Since aberrant somatic hypermutation has been advocated as a molecular marker of DLBCL, our results corroborate the notion that PMLBCL represents a subtype of DLBCL rather than a distinct clinico-pathologic entity.

P203

PHYSIOLOGICAL AND ABERRANT SOMATIC HYPERMUTATION IN PRIMARY BREAST LYMPHOMA: IMPLICATION FOR HISTOGENESIS AND PATHOGENESIS

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Introduction. Primary breast lymphoma is a rare clinical entity accounting for <2% of extranodal non-Hodgkin's lymphoma. Almost all cases of primary breast lymphoma display morphology consistent with diffuse large B-cell lymphoma (DLBCL). With respect to nodal DLBCL or DLBCL arising in other extranodal sites, primary breast lymphoma seems to display peculiar clinical features, namely a poorer outcome and a tendency to spread to the CNS. The pathogenesis and histogenesis of primary breast lymphoma are poorly investigated and represented the aim of this study.

Methods. Thirteen cases of primary breast DLBCL were analyzed for physiological somatic hypermutation (SHM) of IgV and BCL-6 genes, as well as for aberrant SHM of PAX-5, PIM-1, RhoH/TTF and c-MYC genes.

Results. A functional IgVH rearrangement was identified in 8/13 (61.5%) primary breast lymphomas. All IgVH genes displayed SHM, with a mutation frequency ranging from 4.0% to 25.9%, suggesting a derivation from germinal center experienced B-cells. The IgVH gene families utilized by primary breast lymphoma included VH4 (4/8 cases), VH3 (3/8 cases) and VH2 (1/8 case). Three cases utilized the same VH 4.30.1/4-31 gene, without homology in the CDR3. Mutations of BCL-6 were detected in 7/13 (53.8%) cases, corroborating their origin from germinal center-experienced B cells. Analysis of aberrant SHM of proto-oncogenes was performed on selected regions known to contain >90% of mutations found in lymphoma. Overall, mutations in at least one of the four proto-oncogenes targeted by aberrant SHM were found in 9/13 (69%) cases, whereas mutations in more than one gene were found in 4/9 (44%) cases. Each of the four proto-oncogenes was altered in a significant fraction of primary breast lymphomas (PAX-5 in 4/9 cases; RhoH/TTF in 5/9 cases; PIM-1 in 5/9 cases and c-MYC in 2/9 cases). The mutation pattern was consistent with the SHM process. The overwhelming majority of mutations was represented by single base-pair substitutions (n=36), whereas in only one instance a deletion of a short DNA stretch was observed. Among the 36 single base-pair substitutions, the transition/transversion ratio was 1.76 (expected 0.5; $p<0.01$; Chi square test). Three primary breast lymphomas displayed four missense mutations localized within the serine-threonine kinase domain of PIM-1, leading to aminoacid substitution with potential functional consequence.

Conclusions. The implications of our results are twofold. First, the molecular profile of primary breast lymphoma is characterized by mutations of IgV and BCL-6 genes, documenting a derivation from germinal centre experienced B-cells. Second, aberrant somatic hypermutation of PAX-5, PIM-1, RhoH/TTF and c-MYC is involved in the pathogenesis of primary breast lymphoma by affecting regulatory and coding sequences of proto-oncogenes potentially relevant for transformation.

P204

NEWLY DIAGNOSED CASES OF HAEMATOLOGICAL MALIGNANCIES IN ITALY IN THE EARLY 2000S: AN ESTIMATION OF THEIR NUMBER AND OF AGE DISTRIBUTION ON THE BASIS OF AN EPIDEMIOLOGIC SURVEY IN SARDINIA (1974-1993)

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In a previous report[1] we described age and sex distributions and temporal changes in incidence of Haematological Malignancies(HM) in the island of Sardinia during the years 1974-1993. Cases (in total 7.264) were collected by direct manual consultation of registries of all pathology and clinical institutions active in Sardinia during that period; diagnoses were validated by consultation of clinical records, possible in 95% of cases. In the present report we estimate the number of cases of Haematological Malignancies expected to be newly diagnosed in the resident population of Italy during the year 2001, and we classify the predicted cases according to disease and age. Estimation of the cases has been performed by applying the HM specific incidence rates calculated from the survey mentioned above to the resident population of Italy at 2001 census.[2] Details of the procedure applied are described in the legend of Table 1.

Based on our analysis, a minimum of 21.385 new HM cases would be expected to be diagnosed across the whole Italian population (56.995.744 at 2001 census) in the year 2001. Subdivision of this figure by disease and by two particular age classes(i.e.<15 and >65 years of age respectively) is illustrated in Table 1. The estimated number of patients under the age of 15 is 674. Patients over 65 years of age are expected to represent more than 50% of the total HM cases (11.765/21.385). Co-morbidity typical of this age group further increases their burden on health care system. Our estimates, although based on a reliable survey, that attained a good completeness of cases ascertainment and an accurate diagnoses validation, must be considered with caution. Indeed, they have inherent, important,

defects: 1) they apply to Italian population the rates observed in Sardinian population, that is 1/36 smaller; moreover, Sardinian rates, being calculated from relatively small numbers of cases, have quite large C.I.; 2) possible geographic differences in incidence are not taken into account; 3) NHL and MDS cases are likely higher, because we applied the incidence rates of the last five surveyed years, that were still increasing; 4) cases of AML should be higher, due to the new diagnostic criteria required by WHO for these diseases.[3]

Table 1. Hematological Malignancies In Italy: Expected Cases, 2001.

	Total cases	>65 y	<15 y
Acute Myeloid Leukemia	1.838 *(1.300-2.636)*	949 *(711-1.260)*	84 *(47-161)*
Acute Lymphoblastic Leukemia	831 *(572-1.226)*	198 *(123-317)*	294 *(232-373)*
Myelodysplasia	1.814 *(1.253- 2.741)*	1.458 *(1.060-2.013)*	n.a.
Chronic Myeloid Leukemia	959 *(683-1.364)*	421 *(307-581)*	3 *(1-13)*
Polycytemia Vera	414 *(221-805)*	215 *(119-394)*	2 *(1-13)*
Essential Thrombocytemia	826 *(434-1.605)*	429 *(242-773)*	n.a.
Myelofibrosis	355 *(189-698)*	258 *(149-451)*	n.a.
Chronic Lymphocytic Leukemia	2.566 *(2.011-3.314)*	1.705 *(1.370-2.123)*	n.a.
Multiple Myeloma	2.525 *(1.970-3.272)*	1.633 *(1.306-2.043)*	n.a.
Hodgkin's disease	1.559 *(1.192-2.057)*	268 *(180-401)*	42 *(27-70)*
Non Hodgkin Lymphomas	6.701 *(5.235-8.678)*	3.650 *(2.970-4.487)*	80 *(32-214)*
Hairy Cell Leukemia	238 *(117-530)*	93 *(44-203)*	n.a.
Other Hematological Malignancies *	759 *(524-1.132)*	488 (362-560)	17 *(6-50)*
TOTAL CASES	**21385**	**11765**	**522**

** Other Hematologic Malignancies : Solitary plasmacitoma, Waldenstrom Macroglobulinemia, Acute Leukemia unknown type.*

Table 2 : Estimates Comparison

	Present estimates	Globocan 2002[4] estimates
Hodgkin's Lymphomas	1.559 (1.192-2.057)	1.184
Non-Hodgkin's Lymphomas	6.701 (5.235-8.678)	10.281
Multiple Myeloma	2.525 (1.970-3.272)	4.413

In table 2 we make a comparison(only possible for HD, MM, NHL) of ours with Globocan 2002 estimates, that are calculated from data of Italian Cancer Registries and from mortality data. This comparison shows that, while estimates concerning Hodgkin's disease are quite similar, those of Globocan 2002 for NHL and MM are much higher. Our lower value of NHL cases is likely due to the fact that, as previously exposed, our estimates were based on the still increasing rates of the last five surveyed years. We cannot explain the difference in MM estimates: indeed, the rates we applied concerned a period with a quite stable incidence of this disease. Our estimates give only a rough knowledge of the burden of newly diagnosed cases in Italy: nevertheless, we hope that, waiting for more accurate data, they can be useful for implementation of an adequate medical care for these diseases.

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TREATMENT OF HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA WITH INTENSIFIED INDUCTION THERAPY AND HIGH DOSE SEQUENTIAL THERAPY

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Patients (pts) with diagnosis of DLBCL and IPI age adjusted 2 or 3 present a poor prognosis. The results reported by Gianni *et al.,* with high dose sequential therapy (HDST) showed a significant improvement of Overall Survival (OS) and Event Free Survial (EFS) in comparison with a standard chemotherapy. Considering this experience, we decided to use this therapy adding an intensified scheme in the debulking phase. The first phase was constitued by an intensified CHOP (MegaCHOP): cyclophosphamide 3 g/sqm; doxorubicin 75 mg/sqm; oncovin 1,4 mg/sqm on day 1 and prednisone 100 mg for 5 days. Stem cells collection was planned after the third cycle. The second phase was: cyclophosphamide 4 g/sqm, methotrexate 8 g/sqm and VP16 2 g/sqm. The third phase was the peripheral blood stem cell transplantation (PBSCT) with melphalan 180 mg/sqm and mitoxantrone 60 mg/sqm as conditioning regimen. Inclusion criteria were: large B-cell lymphoma, age less than 50 years and intermediate-high with bulky or high risk IPI.

From March 2002 until February 2004 we enrolled 11 pts. Median age was 39 years (range 25-49), 8 pts were stage III-IV (73%), 7 presented bulky disease (64%), 9 pts had a WHO performance status 0-1 (82%), all but one had an abnormal LDH value. Four pts performed the first cycle with MegaCHOP as inpatients. All other cycles and the phase II were performed on outpatient basis. At the end of planned therapy 9 pts obtained a complete remission (CR) (82%), a patient after radiotherapy was alive with disease and one died of progressive disease during phase I. After first phase six pts obtained a very good partial remission (PR), after phase II six were in CR unconfirmed and 4 were in PR. After PBSCT 8 pts were in CR and 2 were in PR, one of this obtained a CR after mediastinal radiotherapy. After a median follow-up of 21 months (range 6-34) OS was 81%. Two pts died: one for progressive disease and one after relapse. With a median follow-up of 18 months 88% of complete remission pts were free from disease and only one patient relapsed after 2 months from CR. We did not

find grade III or IV WHO extrahaematological toxicity.

We can conclude that this protocol is feasible as outpatient basis in phases I and II. Moreover, this therapy was highly effective (81% OS) in a subset of pts with very high risk characteristics at diagnosis. Rituximab could further improve these results and could also be used in pts with involvement of bone marrow at diagnosis.

P206

HIGH RATE OF CLINICAL AND MOLECULAR REMISSIONS IN FOLLICULAR LYMPHOMA PATIENTS RECEIVING TOTAL SEQUENTIAL TREATMENT AND AUTOGRAFTING AT DIAGNOSIS

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Previous reports have presented that intensified treatments with autologous transplantation are a promising therapeutical approach for patients with high-risk follicular lymphoma (FCL) at diagnosis. This study was intended to assess the results of a phase II monocenter study of a total sequential chemotherapy schedule with peripheral blood progenitor (PBPC) autografting. Bcl-2 was used as lymphoma-specific marker.

Between June 2002 and February 2004 in our institute, 15 newly diagnosed patients with advanced-stage FLIPI-2 FCL were consecutively treated with a total sequential treatment (TST) including 4 different phases: 1) CHOP-R x four cycles; 2) high-dose cyclophosphamide (7 g/m^2) with further PBPC mobilization and two Rituximab administrations at day +3 and +11 for *in vivo* purging; 3) FM (Fludarabine, Mitoxantrone) regimen x four cycles; 4) high-dose chemotherapy program followed by PBPC autografting. The collection of PCR-negative cells and the achievement of posttransplantation molecular remission was observed in 11/15 (66%) and in 13/15 (87%) patients, respectively. All patients obtained a clinical complete response after the TST. With a median follow-up of 20 months, clinical relapse was observed in 3 patients: at +6, +8, and +15 months. Two of these patients presented clinical and molecular relapse.

Our preliminary results indicate that in high-risk FCL patients the TST could be an interesting sequential approach in terms of clinical and molecular response.

P207

PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA WITH SCLEROSIS: A CLINICAL STUDY OF 42 PATIENTS TREATED WITH MACOP-B CHEMOTHERAPY PLUS RADIATION THERAPY

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Primary mediastinal large B-cell lymphoma (PMLBCL) with sclerosis has recently been recognized as a specific clinical and pathologic entity for which the best therapeutic approach seems to be a combination of chemotherapy and radiotherapy. Between 1992 and 2003 in our institute, 42 previously untreated patients with PMLBCL with sclerosis were treated with a combination of a third-generation chemotherapy regimen (MACOP-B) and mediastinal radiation therapy. Thirty-five (83%) patients achieved a complete response (CR) and five (12%) obtained a partial response after MACOP-B regimen and radiation therapy. Among the 35 patients who obtained a CR there were 5 (14%) relapses after 3, 4, 6, 6, and 7 months respectively. All the remaining 30 patients are currently in continuous CR with a median follow-up of 57 months (range, 15-131 months). Projected overall survival curve was 72% at 10 years and the relapse-free survival curve of the 35 patients who achieved CR was 82% at 10 years. In patients with PMLBCL with sclerosis combined treatment utilizing MACOP-B chemotherapy regimen plus radiotherapy induces a good remission rate with the possibility to cure two third of the patients.

P208

THE TRANSACTIVATING ISOFORMS OF P63 ARE OVER-EXPRESSED IN HIGH-GRADE FOLLICULAR LYMPHOMAS INDEPENDENT OF THE OCCURENCE OF P63 GENE AMPLIFICATION

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p63 is a p53-related gene mapping to 3q28, that codes for multiple mRNA transcripts with (TA-p63) or without (DN-p63) transactivating properties on genes promoting cell differentiation and apoptosis. We analyzed p63 alterations by immunohistochemistry, quantitative real time RT-PCR and FISH in a series of follicular lymphomas (FL). None of the cases showed immunoreactivity (IR) for the p40 antibody, that recognizes only the truncated isoforms of p63, or DN-p63 mRNA expression. IR for the 4A4 antibody, recognizing both the transactivating and the truncated p63 isoforms, was found in 5±5.5%, 6.85±4.88%, and 33.2±22.31% of grade I, II and III FL cells, respectively (p<0.0001). Quantitative RT-PCR analysis showed that all the cases but one had TA-p63 mRNA levels higher than non-neoplastic lymphocytes, and that TA-p63 mRNA expression was significantly (r=0.9194, p<0.0001) correlated with the prevalence of p63 IR. p63 gene extra-signals were found in seven (23.3%) of the 30 cases analyzed (0/6 grade I, 2/15 grade II and 5/9 grade III, p=0.01937). Further hybridizations showed a pattern highly suggestive for a chromosome 3 polysomy in six cases. One of these cases also bore p63 and bcl-6 gene extra-copies. Co-localization of p63 and IgH signals was found in one case. No association between the prevalence of p63 IR and p63 gene extra-signals was detectable when the cases were dichotomized according to a threshold of 10% of p63 IR. Our data suggest that TA-p63 is over-expressed in high-grade FL, possibly independent of the occurrence of gene abnormalities, and it may contribute in modulating cell apoptosis.

P209

GENE EXPRESSION ANALYSIS OF PERIPHERAL T-CELL LYMPHOMA NOT OTHERWISE SPECIFIED REVEALS THE EXISTANCE OF TWO SUBGROUPS RELATED TO DIFFERENT CELLULAR COUNTERPARTS

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Peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) is the commonest non Hodgkin lymphoma of T-cell derivation. It is characterized by heterogeneous morphologic and phenotypic features, aggressive clinical behavior with frequent extra-nodal diffusion, scarce response to conventional treatments and dismal prognosis. In order to investigate the nature of the PTCL-NOS phenotype and its relationship to normal T-cells as well as the molecular basis of the disease, we studied the gene expression profile (GEP) of 17 PTCL-NOS patients and a series of purified normal peripheral T-cell subsets, using the Affymetrix DNA microarray system (U133 2.0 plus representing approximately the whole genome).

We show here that PTCL-NOS displays a relatively homogeneous pattern of gene expression, which is clearly distinct from that of normal T-cells and other lymphoid malignancies. Comparison with the gene expression profiles of purified normal T-cell subpopulations, including CD4+, CD8+, naïve (HLA-DR-), and activated (HLA-DR+) T cells, shows that PTCL-NOS cells are more related to activated T cells either CD4+ or CD8+, suggesting a derivation from these T-cell populations. Notably, immunohistochemistry by specific anti-CD4 and CD8 antibodies does not allow to surrogate the global molecular profiles defined by GEP. In addition, PTCL-NOS cells display a remarkable deregulation of cell programs often involved in tumorigenesis, such as apoptosis, proliferation, cell adhesion, drug resistance and matrix remodeling. The latter analyses identify several genes that are specifically expressed in PTCL-NOS and whose expression is confirmed at the protein level by immunohistochemical analysis of tissue microarrays including 209 primary PTCL cases. Special interest merits the aberrant expression of both CYR61 (a molecule involved in drug resistance) and PDGFRalpha (a tyrosine kinase targeted by imatinib mesylate). These results can have biological implications relevant to the pathogenesis of the tumour as well as clinical implications for its clinical management.

P210

EFFICACY OF A MONTHLY MAINTENANCE FOR ONE YEAR WITH RITUXIMAB IN PATIENTS WITH INDOLENT LYMPHOMA

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Background. The therapeutic choices after an obtaining clinical response post conventional chemotherapy for lymphomas are matter of debate. Since a relatively short time we know that rituximab in maintenance can addict a certain vantage in term of duration of response and event free survival; but at the moment little is established about the best administration schedule. On the basis that prolonged exposure to the drug could improve the response to rituximab we are administering one monthly dose for twelve months.

Methods. Here we report eight cases of indolent lymphoma who obtained response af-ter induction with conventional chemotherapy plus rituximab and were maintained with a monthly rituximab schedule for twelve months. Only in two cases induction therapy and successive maintenance with rituximab were administered respectively as second and third therapeutic line.

At diagnosis the characteristics of the patients were: median age 51; male/female ratio 6/2; histology follicular I and II grade respectively in 3 and 1 patients, marginal zone in 3 patients and lymphoplasmacytic in 1 patient; BM involvement 3/8 pts; stage II in 3 pts, stage III in 2 pts and stage IV in 3 pts; elevated LDH in 1 case; in two single cases respectively previous chemotherapy and rituximab therapy. Induction therapy includes: FCR in 3, R-CEOP in 2, R-CHOP, CEOP and PROMACE-CYTABOM respectively in 3 patients. The patients, who failed previous therapeutic lines, had received as first line CEOP plus Inter-feron as maintenance.

Results. After induction 7/8 and 1/8 achieved respectively complete and partial remission. After the maintenance the patient with partial remission improved obtaining a complete remission, while the others continued to stay in complete remission. At the moment all the observed patients are still in complete remission. At a follow-up median of 32 months (range 17-95), the median event free survival from the initiation of maintenance was 24 months (range 9-38). In one patients we have considered the mid - treatment response (post six months), because he hasn't finished the whole maintenance yet. Adverse effects include bronchopneumonia in 2 cases, reactivation of HCV and HBV infections in two cases. The patient HbsAg positive reactivated HBV infection after 8 months from the start of maintenance and died after 4 months from reactivation under lamivudine therapy in complete remission for lymphoma.

Conclusion. Here we have shown : all patients remained in complete remission with a median EFS of 24 months from the beginning of the maintenance; one patient obtained a complete response during the maintenance; the two patients, who re-achieved a complete remission after a successive induction chemotherapy plus rituximab, have maintained their response. In conclusion we take these data to indicate that a monthly rituximab schedule for one

year is effective in the control of the disease and in the prevention of relapse for its continuative anti-tumour effect. Nevertheless the number of patients is too small and these data need to be confirmed in a prospective trial with a longer follow up.

P211

RITUXIMAB PLUS IFOSFAMIDE BASED REGIMENS: EFFECTIVE SECOND LINE APPROACH IN RELAPSED OR REFRACTORY NON HODGKIN LYMPHOMA

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High dose therapy with peripheral blood stem cell (PBSC) rescue is the gold standard therapy in relapsed NHL: consistent data outline that remission status at transplantation significantly influences disease outcome. Patients undergo transplantation in complete response have better progression free survival with respect those in partial response. Second-line therapy should to re-induce response and to mobilize PBSC. In this last years ifosfamide-based regimens have been largely employed in relapsed NHL patients inducing objective responses and PBSC mobilization. While Rituximab+chemotherapy is became the approach of choice in 1st line-therapy, in 2nd line this type of schedule has not yet been largely applied. In the aim to test the efficacy of combined immuno-chemotherapy in 2nd line treatment we designed a protocol including Rituximab plus IEV. The planned treatment consists of 3 cycles of IEV or MINE (>65 y patients), plus Rituximab (375 mg/sqm) given before (day 1) and after (day 7) each cycle (Rituximab total dose 6). Granulocyte-colony-stimulating factor (G-CSF) was administered from day 7 until haematological recovery and, after the third cycle, was continued until end of leukapheresis.

From April 2002 to September 2003 11 consecutive patients – 6 males, 5 females, median age 57 years (min. 21 max. 73 years) with refractory (7) and relapsed (4) Diffuse Large B Cell Lymphoma (DLBCL) entered in this study. At time of treatment 6 patients were classified, according to IPI score, as having intermediate- high grade NHL, 7 had extranodal disease, and finally 4 were hepatitis C virus positive. Nine patients completed treatment, 7 R-IEV and 2 R-MINE, in 2 cases therapy was discontinued because of disease progression (1) and lost to follow up (1). Eigth patients were responders, 3 CR, 5 PR, while 1 had progressive disease (PD) and went off-study. The main toxicity was haematological: WHO grade > 3 neutropenia, anemia and thrombocytopenia were recorded in 5, 1and 2 pts, respectively. Six patients underwent transplant, 4 autologous and 2 (>60 y) reduced intensity allogeneic SCT. Among the responders the remaining 2 went off study because of no CD 34 harvesting (1) and disease progression (1). Of transplanted patients 2 (1 in CR post Mini Allo, 1 in progression post Auto) died 5 and 14 months after transplant. To date 7 patients are alive: 3 CR, 1 PR, 3 PD, respectively. Overall median survival from diagnosis was 18 months (range 11-42). Our experience, if limited to a small series of patients, shows that the addition of Rituximab to second line chemotherapy is able to increase the second line response rate even in adverse IPI score cases, to obtain a good CD 34 harvest and to act as a effective *in vivo* purging agent. Further studies are needed to define which chemotherapeutic regimen to choose in association to Rituximab to enhance tumor sensitivity and to ameliorate the remission status at transplantation.

P212

MOLECULAR ANALYSIS OF POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS OF DONOR ORIGIN OCCURING IN LIVER TRANSPLANT PATIENTS

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Posttransplant lymphoproliferative disorders (PTLD) represent a frequent source of morbidity and mortality among solid organ transplant recipients receiving immunosuppression therapy. Most PTLD occurring in solid organ patients arise from recipient cells, whereas only a fraction of cases derive from donor transplanted lymphocytes. Donor-derived PTLD usually have a predilection for the allograft tissue and are particularly frequent following liver transplant. The factors that determine the outgrowth of donor B cells and their peculiar intragraft location are still unknown. To clarify the histogenesis and the pathogenesis of donor-derived PTLD, we investigated a panel of 11 monoclonal PTLD occurring in liver transplant patients, including 6 cases arising from recipient cells and 5 cases arising from donor cells. Several phenotypic and genotypic markers of B-cell histogenesis were analyzed. Phenotypic markers of histogenesis included expression of BCL6, MUM1 and CD138, which segregate the germinal center (GC) stage of B-cell differentiation (BCL6+/MUM1$^{-/+}$/CD138-) from later stages of maturation (BCL-6-/MUM1+/CD138$^{+/-}$). Genotypic markers of histogenesis were represented by somatic hypermutation of immunoglobulin variable (IGV) genes, a phenomenon experienced by B-cells during the GC reaction. To ascertain the role of antigen in the pathogenesis of the disease, we also analyzed the usage and the mutational profile of clonal IGV heavy (IGHV) and light (IGLV) chain gene rearrangements. All PTLD or donor origin were EBV-infected lymphoproliferations morphologically classified as polymorphic PTLD (P-PTLD). PTLD arising from recipient cells were classified as diffuse large B-cell lymphomas (5 cases) and P-PTLD (1 case); EBV infection was restricted to 2/6 cases (1 P-PTLD and 1 DLBCL) of recipient-derived PTLD. Analysis of phenotypic markers of B-cell histogenesis showed expression of the phenotypic profile BCL6+/MUM1-/CD138- in 3/6 DLBCL with centroblastic features, all arising from recipient cells. The phenotypic profile BCL-6-/MUM1+/CD138$^{+/-}$, consistent with a post-GC stage of pre-terminal B-cell differentiation, was detected in 8/11 PTLD, including 5/5 donor-derived PTLD and 3/6 recipient-derived PTLD. A clonal IGHV rearrangement was identified in 11/11 cases. Analysis of somatic hyper-

mutation showed the presence of somatically hypermutated IGHV genes in 6/11 PTLD. Unmutated IGHV rearrangement were identified in 3/5 donor-derived PTLD and in 2/6 recipient-derived PTLD. Mutated cases showed highly mutated IGHV genes (frequency of mutation >6%), a condition that, in normal B-cell, results in lower affinity for antigen and consequent induction of apoptosis. Analysis of intraclonal heterogeneity showed the presence of ongoing mutations in 1 donor-derived PTLD. In all the other cases intraclonal heterogeneity was absent. Analysis of the distribution of individual IGHV families and genes disclosed differences between donor-derived and recipient-derived PTLD and between normal repertoire and donor-derived PTLD. In particular, donor-derived PTLD preferentially rearranged IGHV3 (2/5 cases) and IGHV4 (3/5 cases) family genes, whereas recipient-derived PTLD rearranged virtually all IGVH families. The IGHV4-39 gene was the most frequently rearranged IGHV gene in donor-derived PTLD (3/5 cases); conversely, the IGHV4-39 gene was absent in recipient-derived PTLD and relatively rare in the non-neoplastic B-cell repertoire. Despite extensive investigation by multiple PCR strategies, a functional IGV light chain rearrangement was found in only 4/11 PTLD. Two recipient-derived PTLD harbored IGLV rearrangement, whereas 2 donor-derived PTLD harbored a functional IGKV rearrangement. In 4 recipient-derived and in 2 donor-derived PTLD, we identified only non-functional IGV light chain rearrangements. In conclusion, our data suggest that both donor-derived and recipient-derived PTLD occurring in liver transplant patients arise from a B-cell subset phenotypically mimicking post-GC, pre-terminal differentiatiated B-cells. Lack of IGV mutations, however, suggests that a fraction of cases failed to perform a proper GC reaction. The biased usage of the IGHV4-39 gene suggests that antigen stimulation and selection might have a role in the pathogenesis of donor-derived PTLD.

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MOLECULAR ANALYSIS OF IMMUNOGLOBULIN VARIABLE GENES IN AIDS-RELATED NON HODGKIN LYMPHOMA: IMPLICATIONS FOR DISEASE PATHOGENESIS AND HISTOGENESIS

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Non-Hodgkin's lymphomas (NHL) represent a frequent complication of HIV infection and a major source of morbidity and mortality among patient affected by AIDS. Although the incidence of NHL in AIDS patients has diminished since the introduction of highly active antiretroviral therapy, NHL constitute an increasing proportion of the AIDS-defining events diagnosed in recent years. Molecular and immunohistochemical studies have demonstrated that AIDS-related NHL (AIDS-NHL) derive from mature B-cells and are phenotypically and histogenetically related to germinal center (GC) or post-GC B-cells. AIDS-NHL are a suitable model to study lymphomagenesis in a context of disrupted immunosurveillance and the molecular analysis of the immunoglobulin variable region (IGV) genes can provide insights into the nature of the cell of origin and its clonal history following neoplastic transformation. Using multiple PCR approaches, we investigated a panel of 67 AIDS-NHL, including 30 AIDS-diffuse large B cell lymphomas (DLBCL), 21 AIDS-Burkitt lymphoma/Burkitt-like lymphomas (BL/BLL), 6 AIDS-primary effusion lymphomas (PEL) and 10 AIDS-plasmablastic lymphomas (PBL) for usage, mutation frequency and intratumoral heterogeneity of clonal IGV gene rearrangements. Moreover, to ascertain the role of antigens and/or superantigens in AIDS-NHL pathogenesis, we analyzed the mutational profile and CDR3 structure of IGHV, IGKV and IGLV genes. Results where compared to a database of 200 IGV rearrangements from DLBCL of immunocompetent hosts as well as to the normal B-cell repertoire. We identified a total of 65 IGHV and 56 IGV light chain rearrangements in AIDS-NHL. A functional IGHV rearrangement was found in 60/67 (90%) cases, a functional IGKV chain rearrangement was identified in 17/38 (44.7%) cases and a functional IGLV rearrangement in 21/38 cases (55.3%). Fifty-three out of 60 AIDS-NHL (88.3%) showed somatic hypermutation in IGHV and/or IGV light chain genes. The average mutation frequency was 9.42% (median 7.50%, range 2.04%-23.3%) for IGHV genes and 5.42% (median 4.20%, range 2.01%-12.5%) for IGV light chain genes. IGV germline rearrangements selectively associated with AIDS-PBL ($p<0.001$). Among mutated cases, average mutation frequencies did not differ among AIDS-NHL groups. AIDS-NHL showed a significant overrepresentation of the IGHV4 family (28/60; 46.6%) and a significant underrepresentation of IGHV3 family (18/60, 30.0%) compared to aggressive lymphomas of immunocompetent hosts ($p<0.05$) and to normal B-cells ($p<0.05$). IGHV4-34 was the IGHV gene most frequently rearranged (17/60; 28.3%) and was overrepresented in AIDS-NHL versus aggressive lymphoma of immunocom-

petent hosts (17%; $p<0.03$) and normal B-cells (4%; $p<0.001$). Moreover, IGHV4-34 expressing cells preferentially associated with lambda chain rearrangements (70%). The IGKV4-1 gene was the IGKV segment most frequently rearranged (6/17; 35.3%) and its usage was biased in AIDS-NHL compared to normal B-cells (5.30; $p<0.001$). The single IGLV gene most frequently encountered was IGLV1-44 (6/17; 35,3%). Analysis of distribution of replacement and silent mutations in IGHV sequences showed tendency to conserve FR sequences and maintain antigen binding in 34/52 (65.4%) cases. A higher than expected number of CDR replacement mutations, suggesting selection for high affinity antigen binding, occurred in 17/52 (32.7%) cases. Analysis of intraclonal heterogeneity showed the presence of ongoing mutations in only 3 out of 15 AIDS-NHL (1 AIDS-BLL and 2 AIDS-DLBCL). In 12 out of 15 cases intraclonal heterogeneity was absent. Implications of these data are multifold. First, most AIDS-NHL categories derive from B-cells persistently subjected to GC reaction, suggesting a potential role for antigen stimulation in the pathogenesis of these lymphomas. This hypothesis is supported by the finding of antigen binding preservation in the majority of AIDS-NHL and selection for high affinity antigen binding in a fraction of cases. The absence of IGV genes mutations in a fraction of AIDS-PBL suggests a different histogenetic and pathogenetic pathway for these lymphomas. Second, the preferential usage of IGHV4-34 and IGKV4-1 genes in a fraction of AIDS-NHL may suggests a role for a superantigen stimulation of pre-neoplastic B-cells with polyreactive and/or autoreactive activity. Finally, at variance with NHL of immucompetent host, the presence of intraclonal heterogeneity is a rare finding in AIDS-NHL, suggesting a derivation from B-cells that have concluded the GC-reaction.

Non-Hodgkin's Lymphoma II

P214

ROLE OF RITUXIMAB IN INDOLENT HCV-RELATED LYMPHOPROLIFERATIVE DISORDERS ASSOCIATED WITH SYSTEMIC AUTOIMMUNE DISEASES

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The evidence of an increased frequency of B-Non Hodgkin's Lymphomas (NHL) in patients with HCV and systemic autoimmune diseases suggests a relationship between infection, autoimmunity and cancer. Choosing the best therapy for patients affected either by HCV-related lymphoma or autoimmune disorders is not easy; in fact, some treatments may be accompanied by an excessive hepatic toxicity and may be followed by a reactivation of hepatitis. There is growing interest in the search for an ideal therapy for this setting of patient. Thanks to its mechanism of action and good toxicity profile, Rituximab could prove to be an attractive therapeutic option: it has been reported to be highly active in low-grade NHLs and has been proposed for the management of autoimmune diseases. We evaluate the role of anti-CD20 monoclonal antibody in mono-therapy in ten patients with either indolent HCV-related lymphoma or autoimmune disease.

Four of ten patients had been previously treated (two with oral cyclophosphamide orally, one with clorambucil and one with CHOP regimen). All cases presented bone marrow involvement (Stage IV); splenomegaly was present in four patients and lymphoadenopathies in one. All patients showed positivity for IgH rearrangement at molecular assay before treatment.

All cases were also affected by autoimmune disease (six by rheumatoid arthritis (RA), three by mixed cryoglobulinemia (MC), and one by both RA and MC).

Patients , treated with Rituximab (375 mg/m^2 once a week, for a total of four doses), were evaluated for response 1 month after the end of treatment, every 3 months during the first 2 years and then every 6 months.

A very high rate of response, of both NHL and of the associated autoimmune disease, was observed (100% of clinical response), with no significant hepatic and extrahepatic toxicity. In fact, five of ten patients (50%) achieved a complete hematological response (CR) and the remaining ones showed a partial response (PR). Molecular analysis after treatment was performed in 8/10 patients. Four of these (50%) achieved IgH-negativity.

After a median follow-up of 14 months (range, 10-33) all patients survived with a median progression-free survival (PFS) of 12 months (range, 8-31).

Interestingly, the same patients who had achieved hematological CR (50% of cases), also achieved the complete clinical and laboratory disappearance of concomitant autoimmune disorders; the remaining 50% showed a clinical and laboratory improvement of the autoimmune disease. No further treatment for autoimmune symptoms was administered during the following 12 months.

Our data, although the number of patients was small, suggest an interesting role of anti-CD20 in patients with HCV-related lymphomas and concomitant autoimmune syndromes.

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THE PATTERN OF IMMUNOGLOBULIN VARIABLE GENES INDICATES THAT MANY POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS DERIVE FROM B-CELLS THAT HAVE FAILED THE GERMINAL CENTER REACTION

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Posttransplant lymphoproliferative disorders (PTLD) represent a major complication of solid organ transplantation and are related to the chronic administration of immunosuppressive therapy. PTLD are generally of B-cell origin and comprise a histologic spectrum ranging from polyclonal hyperplasia to overt lymphoma. Based on WHO classification, PTLD are classified in early reactive lesions, polymorphic PTLD (P-PTLD) and monomorphic PTLD, comprising diffuse large B-cell lymphoma (DLBCL) and Burkitt/Burkitt-like lymphoma (BL/BLL). Although molecular and immunohistochemical studies have recently shown that the majority of PTLD derive from mature, antigen-experienced B-cells, a detailed molecular characterization of immunoglobulin variable (IGV) genes in these disorders is currently lacking. We investigated 64 PTLD (16 P-PTLD, 42 DLBCL and 6 BL/BLL) for usage and mutational profile of clonal IGV rearrangements. IGV rearrangements were amplified by multiple PCR approaches and sequences were analyzed by comparison to the V-BASE sequence directory (MRC Centre for Protein Engineering, Cambridge, UK; http://vbase.mrc-cpe.cam.ac.uk/). A functional IGV heavy chain (IGHV) rearrangement was identified in 61/64 (95.3%) cases. In 10 PTLD only non-functional rearrangements were identified. In 7 cases, the only rearrangement obtain was non-functional because of crippling mutations. Three cases showed hybrid IGV-D-J rearrangements: two cases with a V-V fusion rearrangement and one case with a J-J fusion rearrangement, suggesting a failed attempt of heavy chain receptor revision in the germinal center (GC). Despite extensive investigation by multiple PCR strategies, a functional IGV light chain rearrangement was found in only 39/64 (60.9%) cases. The high rate of failure in amplifying functional IGV rearrangements prompted immunohistochemical analysis of the expression of immunoglobulin light chains in representative cases. Overall, by combining molecular analysis and immunohistochemical studies, only 33/64 (51.6%) PTLD expressed a potentially functional B-cell receptor, with the majority of cases rearranging lambda chain genes (22/33; 66.7%). Analysis of somatic hypermutation showed the presence of somatically hypermutated IGV genes in 56/64 PTLD (87.5%). Conversely, IGV rearrangements of 8/64 (12.5%) PTLD were unmutated, suggesting a derivation from B-cells that have failed to perform a proper germinal center reaction. Among mutated cases, the average mutation frequency was 8.04% (median 7.62%, range 2.20%-23.5%) for IGHV, 4.61% (median 4.40%, range 2.30%-8.05%) for IGV kappa and 6.59% (median 6.55%, range 2.25%-14.1%) for IGV lambda genes. Twenty-nine cases (61.7%) showed highly mutated (mutation frequency >6%) IGV genes, a condition that, in normal B-cell, results in lower affinity for antigen and apoptosis. Analysis of the distribution of replacement and silent mutations in functional IGV heavy and/or light chain sequences showed tendency to conserve FR sequences and maintain antigen binding in 15/28 (53.8%) cases. Selection for high affinity antigen binding occurred in 9/28 (32.1%) cases. Analysis of IGV gene usage showed a preferential rearrangement of the IGHV3-15 gene in PTLD (6/54; 11.1%), compared to the normal mature B-cell repertoire ($p<0.05$). Overall, our data suggest that most PTLD arise from B-cells that have experienced the GC-reaction and frequently display impaired B-cell receptors. Since a functional receptor is required for normal B-cell survival during GC transit, PTLD development may implicate rescue from apoptosis and expansion of B-cells that have failed the GC-reaction. The high frequency of IGV loci inactivation by crippling mutations and hybrid rearrangements is a peculiar feature of PTLD, among immunodeficiency-associated lymphoproliferations. Among lymphoma arising in immnocompetent host, the high frequency of IGV loci inactivation showed by PTLD is also shared by Hodgkin lymphoma. Future studies are required to define whether PTLD and Hodgkin lymphoma utilize similar mechanisms for apoptotic rescue.

P216

FND REGIMEN (FLUDARABINE, MITOXANTRONE AND DEXAMETASONE) IS AN EFFECTIVE COMBINATION FOR FIRST LINE FOLLICULAR NON-HODGKIN LYMPHOMA

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The combination of Fludarabina, Mitoxantrone and Dexamethasone (FND regimen) constitutes an important advance in the treatment for first line of patients with follicular lymphomas (FL) including grade I, II and III (WHO classification). Seventy patients with newly diagnosed Ann Arbor stage II bulky to IV FL, 30 males and 40 females, median age 54 years (range 28-78) received 6 courses of FND regimen (Fludarabine 25 mg/mqs i.v. days 1-3, Mitoxantrone 10 mg/mqs i.v. day 1, Dexamethasone 20 mg i.v.days 1-3). All patients received antibiotic oral prophylaxis during treatment and growth factors (G-CSF) when grade III granulocytopenia occurred. Of 53 patients with bone marrow involvement, bcl-2 translocation was positive in 36 cases (68%). At the end of treatment, 24 of these patients had CR and 20 converted to polymerase chain reaction (PCR) negativity. The overall response rate was 95%: 62 (88%) patients achieved complete response (CR), 5 (7%) patients a partial

response and 3 (5%) were non-responders. Hematologic grade III or IV toxicity, associated with modest infectious complications was seen in 14 patients (20%). No death related to the administration of FND regimen was observed. After a median follow-up of 36 months (range 10-92) 67 patients are alive. Of these 58 (82%) patients are in CR and 9 (13%) relapsed. The 3 (5%) refractory patients died for progression disease. In our hands, FND regimen has been a well tolerated and effective treatment for follicular NHL patients, resulting in a high percentage of complete haematological and molecular remissions. When associated with prophylaxis for Pneumocystis Carinii, no severe opportunistic infection has been observed.

P217

L-VAMP REGIMEN PLUS RADIOTHERAPY IN PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Primary Central Nervous System non-Hodgkyn's Lymphoma (PCNSL) represents a rare disease in immune-competent patients (less than 1% out of all NHLs). The prognosis is poor and less than 20% of patients are alive at 5 years.

We report our experience on 15 consecutive patients with PCNSL (7 females and 8 males; median age 55 years, range 27-72) observed in the last 5 years. All patients had diffuse, large-cell histology with B (14 cases) and T-cell phenotype (1 case). Six patients had poor performance status (3-4 ECOG). The therapy at diagnosis: L-VAMP protocol plus radiotherapy. The schedule included a single day administration of Vincristine (VCR) 1,5 mg/mqs, Cytosine-Arabinoside (ARA-C) 500 mg/mqs, Methotrexate (MTX) 1000 mg/mqs, followed by foline acid rescue 30mg/mqs after 24 hours. Concomitantly, MTX 15 mg and Prednisone 40 mg were administered intrathecally. The treatment was given every two weeks for a total of six cours. Radiotherapy (40-45 Gy whole brain plus a 20-25 Gy boost for single lesion) was carried out at the end of chemotherapy. Two patients died for progression disease during chemotherapy. Thirteen patients completed the planed combined treatment and achieved complete remission (CR). Of these, 11 patients are alive and in CR at +13,+18,+22,+23,+23,+24,+25,+30,+33,+40,+55 months, respectively. Two patients relapsed: one at +7 months and he is still alive after salvage chemotherapy by using CIOP protocol (containing Idarubicin); the other patients newly underwent L-VAMP schedule. On the whole, the treatment was well tolerated and did not produce significant haematological toxicity or neurological sequelae. In conclusion L-VAMP protocol followed by brain radiotherapy is an feasible and effective regimen for PCNSL. In particular, our results compare favourably, in terms of serious side effects and quality of life, with other more aggressive schemes including higher doses of MTX, ARA-C and Idarubicin plus Ifosfamide. However, a higher number of patients and longer follow-up are needed to draw any firm conclusion.

P218

(18F)FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY VS COMPUTED TOMOGRAPHY SCAN IN THE STAGING OF PATIENTS WITH UNTREATED FOLLICULAR LYMPHOMA

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Background. FL is the second most frequent lymphoma after diffuse large B-cell lymphomas representing 20%-25% of all lymphomas. It is characterized by widespread disease at diagnosis. Despite the usefulness of whole-body FDG-PET in staging Hodgkin's disease and aggressive non-Hodgkin's lymphoma, its role in FL has not yet been established.

Aim of study. The purpose of this single-institution study was to compare FDG-PET with conventional imaging CT scan in the staging of previously untreated FL pts.

Patients and methods. Fifty-eight newly diagnosed FL pts, according to WHO criteria, who underwent FDG-PET scanning and CT scan, between January 2002 and December 2004, were identified. All staging FDG-PET was performed before bone marrow biopsy and evaluators were blinded to the results of CT-scan and other diagnostic procedures. The characteristics of pts were: median age 54 years (range 27-82 yrs), CS I-II vs III-IV (28% vs 72%), G1 (30%), G2 (46%), G3 (24%), BM+ vs BM- (37% vs 63%), FL International Prognostic Index (FLIPI) score: low risk, 42%; intermediate risk, 24%; and poor risk, 34%.

Results. Overall FDG-avid disease sites were detected in 92% of cases, as compared with a detection rate of 94% for CT scans of neck, chest, abdomen and pelvis, while the remaining 8% of cases were found PET negative. FDG-PET detected 16% more abnormal peripheral or thoracic nodal sites as compared to CT, while the two imaging techniques appeared comparable as to the detection of extranodal disease sites. In 8% of cases PET identified "putative" disease sites unconfirmed by CT, MRI and/or ultrasonography, being mostly related to skeletal sites and soft tissues. Conversely, PET sensitivity remained low for the detection of bone marrow infiltration. Interestingly, FDG-PET positivity was independent from histological grading (G1, 50%; G2, 59%; G3, 43%) and Ki-67 staining. Finally, the rate of FDG uptake appeared to progressively increase among pts with higher FLIPI risk (low, 45%; intermediate, 54%; high, 57%).

Conclusion. FDG-PET represents a useful technique for evaluation of FL pts independently from histologic grading. FDG uptake, as opposed to CT scanning, shows a higher detection rate of superficial and thoracic nodal involvement and may correlate with unfavourable prognostic features in FL. Prospective studies are required to evaluate the impact of these discrepancies on the clinical management of FL pts.

P219

WATCH AND WAIT AS INITIAL CHOICE FOR THE MANAGEMENT OF PATIENTS WITH INDOLENT NON FOLLICULAR NON HODGKIN LYMPHOMA; A PROSPECTIVE STUDY PERFORMED BY THE GISL

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Introduction. Indolent non follicular non Hodgkin Lymphoma (INFL), originally included in Working Formulation (WF) group A, according to REAL and WHO classifications represent a heterogeneous group of entities including small lymphocytic (SL), lymphoplasmocytic (LP), splenic marginal zone (SMZ), nodal marginal zone (NMZ), mucosa-associated lymphoid tissue (MALT) NHL. The Gruppo Italiano per lo Studio dei Linfomi (GISL) designed a prospective study with the aim of evaluating the clinical outcome of patients with INFL without signs of active diseases in this NHL subset prognostic criteria directed to identify patients with an indolent non progressive clinical course eligible for a watch and wait policy.

Methods. Patients with a diagnosis of INFL, not previously treated, could be enrolled in this prospective trial; patients shouldn't present any of the following features defining active disease: B symptoms, bulky disease (>5 cm), Hb level < 10 g/dL, platelet count < $100X10^9$/L, diffuse pattern of neoplastic infiltration at bone marrow biopsy and lymphocytes' or nodes' doubling time shorter than 1 year. The principal endpoint was Freedom from Treatment (FFT).

Results. Starting from 1993, 111 patients have been enrolled in this trial. Sixty-four percent were older than 60 years, 62% were males, 96% had stage IV disease. After a median follow-up period of 38 months, 5 years FFT was 73%. In fact, 23 (23%) patients progressed and required therapy for their Lymphoma. No difference in terms of FFT were observed among the different histologic subtypes. The main reason for requiring treatment was the appearance of new sites of disease or the enlargment of existing sites; in one patient treatment was started due to anemia and in one patient due to the appearance of a cioglobulinemia. Univariate and multivariate analysis are currently running in order to identify additional parameters which will allow a better identification of those patients with INFL which are at higher risk of progression.

Conclusions. From this preliminary analysis GISL criteria for definition of indolent non follicular lymphoma are able to identify most cases with a truly indolent disease eligible for a watch and wait policy.

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FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINATION IN THE TREATMENT OF PATIENTS WITH INDOLENT NON-FOLLICULAR B-CELL NON-HODGKIN'S LYMPHOMA. RESULTS OF A PHASE II TRIAL BY THE "GRUPPO ITALIANO PER LO STUDIO DEI LINFOMI (GISL)"

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Introduction. Indolent non follicular lymphomas (INFL), comprise a rather heterogeneous subgroup of lymphomas, including small lymphocytic lymphoma (SLL), Immunocytoma (IC) and marginal zone lymphomas (MZL). With available treatments a median survival of 5 to 10 years is warranted. Typical initial treatment strategies vary from the use of single agents to high doses therapies but alkyilator single agents monochemotherapy can still be considered as the standard treatment. In April 2002 GISL started a phase II trial with the aim of verifying the efficacy of Fludarabine and Cyclophosphamide (Flu-Cy) combination in this subset of NHL, in terms of response, survival and safety.

Patients and Methods. To be included in the trial patients should have a diagnosis of SLL, IC, MZL or CD5-ve mature B cell Leukemia (MBCL), supported by morphologic, phenotyipic and molecular data; patients should also have had no previous treatement for lymphoma and have active disease defined by the presence of anemia (Hhb> 11 g/dL) or thrombocytopenia (Plt <$10x10^9$/l.000/mmc) or bulky disease or rapidly increasing lymphocytosis or enlarging masses. Treatment consisted of Fludarabine 25mg/sqm iv day 1-3 and cyclophosphamide 300mg/sqm IV days 1 to 3, to be repeated every 28days for 6 cycles; an intermediate evaluation of response after 3 cycles was planned and an adequate anti-infective prophyifilaxis was mandatory: the use of G-CSF was not mandatory.

Results. As of March 2005, 54 patients have been registered into the trial; two patients were excluded due to incorrect histology. Median age of the remaining 52 patients was 63 years (range 49-74), M/F ratio was 1,7. The diagnosis was SLL in 16 patients, IC in 6, MZL in 20 and MBCL in 5. All patients had stage IV disease; 19% presented with B symptoms. Anemia (Hb <11 g/dL) was present in 29%, elevated β-2-microglobulin in 63%, abnormal LDH in 32%. Three patient didn't complete treatment due to severe hematologic toxicity; 3 patients had fatal bacterial or fungal infection during treatment. At the time of the present analysis 44 patients completed the treatment and 48% achieved a complete remission (CR) with an Overall Response Rate (ORR) of 95%. Overall, grade III or IV hematologic toxicity was observed in one third of the cases mainly represented by neutropenia (38%); severe infections occurred in 12%. After a median follow up of 16 months, 2-yrs PFS was 75% and 2-yrs OS was 79%.

Conclusion. The results of our study demonstrate that FC combination is effective in the treatment of patient with

INFL. Due to its relevant toxicity profile FC combination should be administered with caution and always in combination with adequate antifungal and antibacterial profilaxis.

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MACOP-B PLUS RADIOTHERAPY AS FIRST LINE THERAPY FOR PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA WITH SCLEROSIS: A CLINICAL STUDY OF 92 PATIENTS

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Introduction. Primary mediastinal large B cell lymphoma (PMLBCL) represents a distinct clinicopathological entity of large B cell lymphoma occuring preferentially in young females with a bulky mediastinal mass.

Methods. Between 1991 and April 2004, 92 consecutive untreated patients (pts) with PMLBCL were diagnosed and treated at our institution. The median age was 33 years (range 15-61), 68/92 (74%) were females,72 pts had stage II and 20 stage IIE, 43(47%) presented B symptoms, LDH was increased in 68(74%), 81(88%) had a bulky mass and 47(51%) had a superior vena cava syndrome. According to age-adjusted IPI score 52 pts had an IPI= 0-1 and 40 pts IPI= 2-3. All pts were treated with standard MACOP-B chemotherapy (CHT) and 86 pts underwent mediastinal radiation therapy (RT) at dose of 30-36 Gy. Six (7%) pts did not receive RT (refusal=3, progression=2, death =1). The response was evaluated in all pts after CHT and at the end of RT.

Results. After MACOP-B regimen the response rate was: CR/CRu=72(78%), PR=18 (20%), NR=1(1%), toxic death =1(1%). Six (6%) PR pts underwent to intensification therapy with high dose therapy and ASCT. After RT pts achieved CR/CRu= 78 (91%), PR= 3 (3%), NR=5(6%). After CHT 67Gallium scan was positive in 51/60 (85%) while after RT was positive in 12/53(23%) $p<0.0001$. After a median follow-up of 58 months (1-165)relapse was observed in 9 pts. Five of the 9 relapsed pts are alive in CR after second line therapy with ASCT. Five not responding pts had progressive disease and died. To date 82 (90%) pts are currently in continuous CR. Projected 5-years OS and PFS are 88% and 84%, respectively. The 5 years OS was better for pts with IPI=0-1 compared to IPI= 2-3 (96% vs 76% $p=0.006$).

Conclusions. Combined modality treatment using the MACOP-B regimen and mediastinal RT induces high response and survival rates. Radiation therapy plays an important role in the achievement of these results.

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COMBINED WHOLE GENOME AND EXPRESSION MICROARRAYS TO IDENTIFY NEW GENES IN MANTLE CELL LYMPHOMAS

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Introduction. Mantle cell lymphomas (MCL) prognosis is the worst among all B cell lymphomas. Since there is no standard treatment for MCL, new therapeutic targets based upon the biology are needed. We combined array-CGH and gene expression profiling (GEP) to identify new possible targets.

Methods. MCL cell lines and clinical samples were analysed. GEP and arrayCGH were performed using Affymetrix HU133 set and Affymetrix GeneChip Mapping 10k, respectively. Recurrent genomic amplifications and deletions have been identified using Affymetrix CCNT and in-house algorithms developed on the R statistical package. GEP data were analysed using the Bioconductor package. To validate the arrayCGH technique and analysis, the cell lines were studied with karyotype analysis, with CNIO OncoChip microarray and with FISH using 12 Vysis probes.

Results. Twenty-two MCL patients and four cell lines have been studied. Recurrent losses were in 13q and 17p13.3-p12 (frequency 35%), 9p23-p21.3 and 11p11.12 (31%). Recurrent gains/amplifications were 9p24.3 (frequency 61%), 6q21 (46%), 18p11.32 (38%), 8q22.3-q23.1 (35%), 4q26, 8q24.3 and 14q12 (31%). Already known amplifications of cMYC and BCL2 or losses of TP53 and CDKN2A were clearly identified by the combination of arrayCGH and GEP.

Conclusions. The combination of whole genome profiling combined to gene expression is a promising approach to study MCL pathogenesis.

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T/NK "NASAL TYPE" LYMPHOMAS: AN ITALIAN COOPERATIVE SURVEY

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T/NK "nasal type" lymphoma is a neoplasm that shows a peculiar geographical distribution. In fact most of the reported cases come from Far Eastern countries such as Hong Kong, and Central America; conversely this kind of lymphoma occurs rarely in western countries.

Between 1997 and 2005, 26 new cases of T/NK "nasal type" lymphoma were diagnosed in 10 Italian Hematology Divisions. Their characteristics were as follows: all Caucasian, 19 men and 7 women; median age 50 years (range 20-80). The presentation at the onset was in the nasal cavity or adjacent structures in 22 cases, who presented symptoms like nasal obstruction, dysphagia, orbital edema; in 2 cases skin lesions were the first sign of disease, follow by oro-pharingeal involvement; in 1 case, the patient was studied because of migrating bone pain, and in the last patient lymphoma presented in lymphnodes and bone marrow. Considering the stage of disease, 12 patients presented a I stage (in 11 cases IA,1 IE); 6 patients were in stage II (2 II A, 2 II B, 2 II EB); 8 patients presented an advanced stage disease (6 IVA, 2 IVB). Thus, early stage patients were 18, while advanced stages were 8.

The median time from the onset of signs, as skin lesions, or symptoms, as nasal obstruction to diagnosis was 3.5 months, ranging between 1 and 30 (assessed on 24 patients). Diagnosis was based on the finding of a T/NK phenotype by the histological examination of oropharingeal or cutaneous lesions in 24 cases, and by a bone marrow biopsy in 2 patients. All patients but one were treated with chemotherapy, alone (in 11 cases), or associated to radiotherapy (13 cases); 1 patient underwent chemotherapy, radiotherapy and surgery, and the last patient was treated with surgery alone. Chemotherapy regimens usually adopted contained anthracyclines (18 cases); in the other cases patients were treated with DHAP (3 cases), CVP (2 cases), low dosages of cyclophosphamide (1), cyclophosphamide-vinblastine-mithoxantrone-bleomicine (1). Radiation dosages ranged between 27.5 and 47.5 Gy, with a median dosage of 40 Gy. Nine patients reached a clinical improvement (34%), while 17 patients resulted refractory or presented a limited response to therapy. The overall median survival time was 6 months (range from 1 to 144); 19 patients died within 23 months from diagnosis. At last follow-up (June 2004) 7 patients are still alive, at a time from diagnosis ranging between 2 and 144 months. Of note both patients treated with surgery presented a prolonged follow-up. The preliminary results of this retrospective survey confirmed that T/NK "nasal type" is a very rare lymphoma in Italian population, and it is characterized by a very bad prognosis. The rarity of this disease causes lacking of a standardized therapeutic approach. More data are needed to know the epidemiology of this kind of lymphoma in Europe.

P224

MITOXANTRONE, CARBOPLATINUM, CYTOSINE-ARABINOSIDE AND METHYLPREDNISOLONE FOLLOWED BY AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION: A SALVAGE REGIMEN FOR REFRACTORY OR RELAPSED NON HODGKIN'S LYMPHOMA

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The prognosis of relapsing or refractory aggressive NHL is very poor. Historically a second line chemotherapy with cisplatinum, cytosine arabinoside and dexametasone rescued a significant proportion of patients and high dose chemotherapy followed by autologous bone marrow transplantation showed better results compared to chemotherapy only. From September 1991 to August 2002 in our Institution patients affected by NHL, submitted to CHOP or CHOP-derived regimen, were treated using a regimen, MiCMA (mitoxantrone 10 mg/m^2/d day 1, carboplatinum 100 mg/m^2/d days 1-4, cytosine arabinoside 2 g/m^2/d day 5 and methylprednisolone 500 mg/m^2/d days 1-5) planned every 28 days. During the second or third course, G-CSF was added to collect peripheral blood stem cells (PBSC). Ninety-four consecutive patients, 45 female, 49 male, median age 43 years (range 16-60), 65% affected by high grade NHL, 29% by low grade NHL and 6% by mantle cell NHL, received a median of 3 courses of MiCMA. Disease status before MiCMA was partial remission (PR) 41%, progressive disease (PD) 35% and relapse 24%.

Ninety-one patients were evaluable for response to MiCMA, 85 for survival analysis. The regimen was well tolerated and no grade III or IV non hematologic toxicity was observed. All but one patients yielded an adequate stem cell collection. Total response rate was 70%: (26% complete remission (CR) and 44% PR). Response rate was significantly (p<0.0001) influenced by disease status before MiCMA: (PR or relapse had a response rate of 90% while in PD had a response rate of 34%). Fifty-four out of 62 patients responsive to MiCMA and 8 no responsive or PD after MiCMA proceeded to aPBSCT. Overall response rate was 82%: 74% CR and 8% PR. At the time of overall survival (OS) analysis, 55% of patients are alive at a median follow up of 58 months (range 10-149); the OS rate at 5 years is 55%. Considering only patients undergoing PBSCT 63% are alive with a median follow up of 56 months (range 18-144) and the median OS rate at 5 years are 62%. OS rates were significantly (p<0.0001) affected by the disease status before MiCMA (68% at 5 years in relapse or PR and 31% in PD or resistant disease), by the response to MiCMA (71% at 5 years for CR or PR and 15% for PD), by the response to aPBSCT (74% at 5 years in CR or PR after transplant and 9% in PD). Multivariate analysis confirmed that disease status before MICMA and response to MiC-

MA were statistically significant factors influencing OS. The median time to progression calculated in all patients was 36 months; estimated freedom from progression (FFP) rate at 4 years was 46%. The overall FFP rates were significantly (p<0.0001) affected by the disease status before MiCMA: 63% after 3 years, in PR or in relapse and 21% in PD. In patients undergoing aPBSCT FFP rate was not influenced by the disease status before MiCMA (48% after 5 years for relapse or PR and 33 % in PD or resistant disease). Disease status at transplant significantly influenced FFP and PD patients were not rescued by transplant, their FFP was significantly poorer compared to patients of those obtaining response (CR, PR) (p=0.0001). Multivariate analysis showed that chemo-sensitivity is major prognostic factor influencing OS and FFP as already reported in literature. Patients not responding to this second-line therapy will not be rescued by transplant. The addition of rituximab to MiCMA which in an ongoing pilot study proved to be safe might probably improve these results.

P225

ACOP-B REGIMEN FOLLOWED BY INVOLVED FIELD RADIOTHERAPY IN LIMITED STAGE AGGRESSIVE NON-HODGKIN LYMPHOMA. MULTICENTRIC ANALYSES OF TOXICITY, EFFICACY AND LONG TERM FOLLOW UP

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Background. Several short time-out patients chemo-radio therapy regimens have been used for limited-stage diffuse large cell lymphoma but large series with extensive follow up are uncommon. Here we review the Multiregional group experience in limited stage non-Hodgkin lymphoma in order to assess efficacy, toxicity and long term follow up, including secondary malignancy, in a large population of patients.

Patients and methods. One hundred and eighty-six consecutive patients (median age of 57, range 18-85) with limited stage diffuse large cell lymphoma diagnosed and treated between January 1993 and December 2004 were reviewed. Only patients with nodal or extra nodal stage I A (137 = 74%) or II A (49 = 26%), according to Ann Arbor staging system, were included. Extranodal sites of involvement were observed in 108 (58%) patients. All patients received first line therapy with ACOP-B regimen (Adryamicin 50 mg/mq day 1, 15, 29, Cyclophosphamide 350 mg/mq day 1, 15, 29, Vincristine 1.4 mg/mq day 8, 22, 36, Bleomicine 10 mg/mq day 8, 22, 36, Prednisone 50 mg/die day 1 to 42), followed by involved field radiotherapy (36 Gy).Patients with sinus, orbital, nasal cavity or epidural involvement received prophylactic intrathecal chemotherapy.

Results. treatment was well tolerated, with limited haematological toxicity (grade 3-4 WHO) in 19 (10 %) patients. Extrahematologic toxicity consisted of alopecia and grade 2-3 mucositis after radiotherapy of Waldeyer'ring in 12 (6 %) patients.

Complete remission was achieved in 180 patients (97 %) after combined treatment, no differences between stage I and II and between age group were observed. There were 22 deaths, 11 by progressive disease and 11 due to other unrelated causes. After a median follow-up of 5 years (range 0.5 –11.5 years), Kaplan-Meier probability of overall survival and disease free survival were 0.81 % (95% C.I.: 0.72-0.91) and 0.75 % (95%C.I.: 0.68-0.82), respectively. Six secondary malignancies were observed (0.6 cases /100.000/year)

Conclusions. These results indicate the feasibility and the high cure rate of ACOP-B regimen followed by involved field radiotherapy for patients affected by limited stage non Hodgkin lymphoma independently from stage and age. In addition a secondary tumour incidence comparable to the expected rate was observed.

P226

RITUXIMAB IN WALDENSTROM'S MACROGLUBULINEMIA: A SINGLE CENTER EXPERIENCE

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Background. The WHO's and REAL's lymphoplasmacytic lymphoma named Waldestrom's macroglubilinemia is a rare lymphoproliferative disorder that affects older persons and is characterized by bone marrow infiltration of a CD20 expressing malignant B-cell secreting IgM. The Current treatment strategies for symptomatic patients are plasmapheresis, which reduces the amount of circulating IgM; chemotherapy including alkylating agents especially chlorambucil ,and nucleoside analogs like fludarabine and cladribine that inhibits tumor growth. A novel treatment approach is the administration of the anti CD20 chimeric antibody named rituximab. We performed a single institution study using Rituximab in Waldestrom's macroglobulinemia to evaluate efficacy and toxicity.

Patients and Methods. 22 consecutive patients with lymphoproliferative disorders have been examinated (M/F ratio 12/10). All patient have clinical indications to starting Rituximab treatment. Of the original 22 6(27%,4 males and 2 females) were affected by Waldenstrom's Macroglobulinemia. Median age 58 (range 35-72);1 HCV+, 5 HCV-. All patients were pretreated before rituximab treatment 2 with one line of therapy (ifn) 2 with two lines of therapy (IFN-chlb, 2-cda-CHLB) 2 with 4 lines of therapy (COP-VACOP-B-IFN-CHLB)(IFN-CHLB-CNOP-2-Cda). All patients have been treated with 4 doses of Rituximab 375 mg/m^2 B.S, once a week for 4 consecutive weeks.

Results. Rituximab response was obtained in 4 of 6 patients (CR:33%, PR:17%, MR 17%, NC 17%, P 17%.) Actual survival 3/6 patients. Median time to progressin 12 months; Median follow up 16 months .Median survival from diagnosis 86 months. Treatment was well tolerated with intolerance (fever anc chills) during firts dose in all patients. Only in 1 patient toxicity was of grade IV (allergic) and therapy was stopped.

Conclusions. We conlude that rituximab is an effective treatment in at least 40-50% of patients,is well tolerated even in pretreated and elderly patients.

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LOW DOSE RITUXIMAB IN MAINTENANCE THERAPY IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA: A SINGLE CENTRE EXPERIENCE

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Non-Hodgkin Lymphoma (NHL) is composed of many histologically and biologically distinct lymphoid malignancies. Many therapeutic options are available but clinical outcomes and cure are far to be satisfactory. Rituximab, a chimeric mouse/human anti CD-20 monoclonal antibody, is an active agent against B-cell malignancies.

We report a single Centre experience of low dose Rituximab associated to Cyclophosphomide, Interferon Alfa and Dexamethasone in maintenance schedule in very poor prognosis NHL patients.

From June 2003 to December 2004, 11 patients (7 males and 4 females) affected by NHL were submitted to maintenance schedule including Rituximab (100 mg, day 1), Cyclophosphamide (100 mg, day 8), Interferon Alfa (1.500.000 U at day 15, 18, 22 and 25) associated to Dexamethasone (4mg/iv twice/week). This course was repeated every 28 days for a total of 12 courses. At diagnosis median age was 65,5 yrs (range 45-73). Six patients were affected by diffuse large cell lymphoma (stage II: 1; stage III: 1; stage IV: 4), 4 patients by indolent lymphoma (stage III: 1; stage IV: 3) and one patient was affected by a malt lymphoma (stage IV). Ten patients showed IPI >= 3 and one, affected by follicular lymphoma with diffuse growth pattern (grade 3/3), IPI:1. Previous induction treatments including Rituximab(5), Antracyclines(6) and local radiotherapy(4). Seven patients resulted responders to a first line therapy, the others were responding to a further line therapy because of refractory (1) or in progression (3).

At time of starting maintenance therapy, six patients were in complete response and 5 resulted partial responders. All patients were treated like outpatients. The median number of courses is 7,5 (3-12), five patients completed treatment plan. Rituximab was well tolerated in all patients. No haematological toxicity was observed. Only one patient had discontinued therapy because of psychological event that not required hospitalisation, after 3 courses. No progression was observed during maintenance. As follow-up the five off-therapy patients resulted responders from 3, 3, 3 ,8 and 9 months, respectively

The median survival from diagnosis and from starting maintenance was 27,6 months (range 8-50) and 15,4 months (range 3-21) respectively.

In conclusion, low dose Rituximab associated to Cyclophosphamide, Interferon-alpha and Dexamethasone can be administered as maintenance therapy, is well tolerated and can maintain response in poor prognosis NHL patients. The feasibility and toxicity profile seem acceptable since no patients required hospitalisations. Further studies are required to investigate the effectiveness of low but prolonged dose Rituximab in NHL.

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

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Purpose. The standard treatment for patients with aggressive B-cell lymphoma, in particular diffuse large-B-cell lymphoma [DLBCL), is cyclophosphamide, doxorubicin, vincristine and prednisone [CHOP) plus rituximab, a chimeric monoclonal antibody against the CD20 antigen. However, some very elderly patients are not fit enough to tolerate CHOP or they have a comorbidity that exclude antracyclin in the regimen. The overall survival in this subset of patients is very poor. Pegylated liposomal doxorubicin is associated with a lower risk of cardiotoxocity than conventional formulations of doxorubicin, allowing the use of higher cumulative doses The aim of this single institution study was to investigate the outcome of pegylated liposomal plus cyclophosphamide (Cae-CY) and rituximab (R) regimen in patients >/= 75 years old, with diagnosis of DLBCL and cardiological comorbidity.

Patients and Methods. in this study, 9 patients aged over 75 years (median 79, range 75-87 years) with aggressive non-Hodgkin's lymphoma (NHL) (International Prognostic Index (IPI) -2, 2 (22%); IPI-3, 4 (44%); IPI-4, 3 (33%)) received pegylated liposomal doxorubicin (25mg/m^2/day 1), cyclophosphamide (300 mg/m^2/day 1) and rituximab (375mg/m^2/day 2), q28.

Results. All completed 6 treatment cycles and were evaluable for efficacy and safety. A complete response was achieved in 7 (75%) patients and a partial response in 2 (25%) patients, with an estimated median time to progression of 12 months. The major toxicity was haematologic: grade 2 leukocytopenia occurred in 4 patients, grade 3 thrombocytopenia in 3 patients, but no grade IV toxicity occurred. There were no episodes of clinically significant bleeding. One only patient developed febrile neutropenia. No significant decrease in LVEF or clinical evidence of congestive heart failure was observed during the treatment or in follow-up.

Pegylated liposomal doxorubicin plus cyclophosphamide is an effective and well-tolerated regimen for the treatment of aggressive NHL in elderly people.

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BORTEZOMIB (VELCADE) OUTSIDE THE SETTING OF MULTIPLE MYELOMA: PRELIMINARY EXPERIENCES WITH MANTLE CELL LYMPHOMA, HODGKIN’S DISEASE, AMYLOIDOSIS AND MYELODYSPLASTIC SYNDROMES

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Bortezomib (VELCADE, formerly PS-341) is a proteasome inhibitor with documented antitumor activity in multiple myeloma. Recent clinical trials have demonstrated that bortezomib could be also effective in other malignancies. Here we report our preliminary data about the use of bortezomib in seven patients with non-myelomatous hematological tumors. Three males and four females, aged 29 to 77, respectively affected by mantle cell lymphoma (MCL) (n. 2), Hodgkin's disease (HD, n. 2), AL amyloidosis (n. 1), and myelodysplastic syndromes (1 RAEB type-1 and 1 RCMD, according to WHO classification) were evaluated. All of them have previously received at least two lines of treatment, including fludarabine and rituximab for MCL, autologous stem cell transplantation (followed by non-myeloablative allogeneneic transplant in one case) for HD, melphalan plus prednisone and thalidomide for amyloidosis, r-EPO and thalidomide for MDS. All patients had progressive disease at the time of the treatment. Bortezomib was given on an out-patient, compassionate basis, at the standard dose of 1.3 mg/sqm body surface i.v. twice weekly for two weeks (one cycle), followed by 10-12 days without treatment. The patient with amyloidosis also received dexamethasone (20 mg/d for two consecutive days after every administration of bortezomib). All patients were required to sign an informed consent. Doses and intervals were modulated according to the manufacturer instructions. So far, a total number of 16 cycles has been administered. The drug was generally well tolerated. Grade I-III toxicities (mainly thrombocytopenia, neuropathy and gastro-intestinal symptoms) occurred in three patients, but none of them interrupted the treatment due to adverse events. One female patient with MCL achieved a very good, stable partial remission at re-staging after two cycles. She currently continues to receive bortezomib (four cycles performed). The second patient with MCL rapidly progressed after a transient response achieved at second cycle. Progressive disease was also observed in patients with HD, where bortezomib was stopped after 2 cycles. The female patient with amyloidosis experienced an initial improvement of general conditions after the first cycle and she is now receiving the second cycle. Both patients with MDS received two cycles with bortezomib: a moderate improvement of Hb levels was observed in one case. These two patients still remain on therapy.

These data confirm that bortezomib may be effective in MCL. No response was observed in our patients with advanced HD. Updated results in patients with MCL, amyloidosis and MDS, as well as those obtained in some further patients who are starting bortezomib at the time of this abstract, will be presented at the Meeting.

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HIGH EFFICACY AND SAFETY OF LOW-DOSE ORAL FLUDARABINE PLUS CYCLOPHOSPHAMIDE AS FIRST-LINE TREATMENT IN ELDERLY PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMA

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Fludarabine (Flu) containing regimens have been reported to be highly effective in the treatment of indolent lymphoid malignancies, however an excessive toxicity has been documented, particularly when used in elderly patients. Flu plus cyclophosphamide (Cy) represents one of most widely investigated combination and several attempts have been made in order to reduce its toxicity. We showed that both intravenous and oral "low-dose" regimens resulted in a mild toxicity still allowing a high response rate. Based on these experiences we started a pivotal trial to assess efficacy and safety of low-dose oral Flu+Cy as first-line treatment. Twelve elderly patients (7 males and 5 females) with untreated B-cell indolent non-Hodgkin lymphomas with a median age of 73 years were enrolled in this study. Treatment schedule consisted of oral Flu 25 mg/m^2/day (40 mg total dose) and Cy 150mg/m^2/day, both for 4 consecutive days. Treatment was repeated every 28 days for 4 cycles. Eight patients completed the planned treatment and responses were assessed after 28 days from the last cycle. All patients were responsive. In particular, CR were obtained in 7/12 (58%) patients (5 MZL, 1 FL, 1 SLL) and PR were obtained in 5/12(42%) patients (3 MZL,1 FL, 1 SLL). Hematological toxicity was low (3 pts with grade-2 and only a patient with grade-3 toxicity); also extra-hematological toxicity was mild (grade-1/2 nausea/vomiting in 5 patients, grade-3 neurological toxicity in one patient; grade-1 treatment-induced fever in a patient). Two patients died because of infection, the first during a grade 3 febrile neutropenia occurred after the third cycle and the other due to pneumonia occurred 3 months after the completion of the treatment. This oral regimen confirms its efficacy and safety in elderly patients with treatment-requiring indolent lymphomas. The schedule is easy to administer on an outpatient-basis and its tolerability is good. In particular, compared to our previous experience conducted in mostly pretreated patients, we achieved in untreated patients a significantly higher percentage of CR. Based on these encouraging preliminary results, this regimen could be proposed as first-line treatment. Further issues and larger series of patients are needed to confirm these data and better clarify the role of this combination.

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IS ENDOSCOPIC ULTRASOUND USEFUL IN FOLLOW UP EVALUATION OF GASTRIC LYMPHOMA?

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Endoscopic Ultrasound (EUS) is considered the best technique for locoregional staging at diagnosis but its role in the follow up of patients with gastric lymphoma after organ conserving strategies has not been established. We conducted a retrospective study in order to evaluate if EUS is a reliable tool in the follow up of patients with gastric lymphoma treated with a stomach conservative approach. We retrospectively studied 23 patients (12 males and 11 females), aged 24 to 79 years (median 59 years), with primary gastric lymphoma: 16 pts with Mucosa Associated Lymphoid Tissue (MALT) Lymphoma and the others 7 pts with Diffuse Large B Cell Lymphoma (DLBCL). Five patients were treated with H. Pylori eradication therapy alone (omeprazole + Amoxicillin + clarithromycin); 9 patients received a treatment including HP eradication and chemotherapy and the remaining 9 patients were treated with chemotherapy alone. At the end of initial treatment a CR was documented in 15 (65%) patients by using endoscopy with biopsy (E-Bx). At the same time only 2 patient showed normalization of EUS. Patients were then evaluated with EBs and EUS every 3/6 months and at the last evaluation, the number of patients in CR by E-Bx has increased to 21 (91%). At the same time, although EUS showed a reduction of median value of thickness of gastric wall from 1 to 0.6 cm (p= 0.0031), only 7 patients (30%) had a normal EUS. A total of 98 evaluation with both EUS and EBs were evaluated and we found concordance between the two methods in only 33 cases (34%). No significant difference was recorded between MALT and DLBCL (see Table).

N° of patients	Type of disease	N° of examinations	EUS + E-Bx +	EUS - E-Bx -	Total (%)	EUS + E-Bx -	EUS – E-Bx +	Total (%)
7	DLBCL	24	4	0	4 (17)	20	0	20 (83)
16	MALT	74	9	20	29 (39)	43	2	45 (61)
23	TOTAL	98	13	20	33 (34)	63	2	65 (66)

After a mean follow up of 48 months we have observed only 1 relapse in 16 pts with a positive EUS but normal histology. Although the length of follow up cannot exclude late relapse, we think that in gastric lymphoma EUS tends to over-stage residual disease. Therefore, in these patients conventional gastroscopy with biopsy remains the gold standard for follow up.

P232

IFOSFAMIDE, CARBOPLATIN AND ETOPOSIDE AS CONDITIONING REGIMEN FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN'S LYMPHOMAS

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Objective. Several drug combinations are currently used as conditioning regimens for autologous stem cell transplantation (ASCT) in non-Hodgkin's lymphomas (NHL). One of the most used is the combination of ifosfamide, carboplatin and etoposide (ICE): we analysed our NHL patients (pts) who underwent ASCT with ICE as conditioning regimen.

Methods. We here analyse 81 consecutive autologous transplantation in NHL patients performed since March 1994 in our institution using ICE as conditioning regimen. 41 pts were male, 40 female. A high-grade histology was diagnosed in 55 pts (67,9%), a low-grade one in 26 pts (32,1%). Ann Arbor stage at diagnosis was I and II in 27 pts (33,3%) and III and IV 54 pts (66,7%); 30 pts (37%) suffered B-symptoms; bulky disease was present in 32 pts (39,5%); performance status was 0 in 63%, 1 in 23,3%, 2 in 11%, 3 in 2,7%. LDH value at diagnosis was evaluable only in 57 pts; 43,9% of them had a pathologic LDH value. In the evaluable subset IPI at diagnosis was 0 in 11 pts; 1 in 23; 2 in 19; 3 in 4. First-line therapy was a CHOP-like regimen in 33,5%, a third generation regimen in 64,9%; radiotherapy and high-dose sequential therapy were used in one pts respectively. The median age at ASCT was 43 years (range 18-63); the median interval between diagnosis and ASCT was 14,4 months (range 4,5-133,2). Status at ASCT was first complete remission (CR) in 52,5%; more than first CR in 24,1%; partial remission (PR) in 18,8%; refractory disease (RD) in 5%. The source of stem cells was peripheral blood in 53 pts; bone marrow in 8; both in 20. ICE regimen included ifosfamide 3 g/sqm on days –6 to –3, carboplatin 500 mg/sqm and etoposide 300 mg/sqm X 2 on days –6 to –4 . Bacterial and fungal prophylaxis was routinely performed with ciprofloxacin 500 mg bid and itraconazole 100 mg bid.

Results. ICE regimen was well tolerated by most pts without transplantation related mortality. All of them had nausea and emesis, but only 7 needed total parenteral nutrition. The median time to engrafment was 11 (range 9-23) days for absolute neutrophil count (>500), 12 (range 9-37) days for platelets (20.000). The median hospitalisation was 21 (range 18-27) days. 80% had fever during aplasia (median 1 day; range 1-8 days). The actuarial median time to treatment failure (TTF) was not reached after 5 years (58%). The actuarial overall survival (OS) after 5 years was 81%. At the univariate analysis the presence of B-symptoms and the status at ASCT were statistically associated with overall survival. Considering the subset of patients with diffuse large B-cell lymphoma, overall survival was 85% after 5 years and TTF was 77% after 5 years: IPI and status at transplantation were statistically associate with overall survival and TTF.

Conclusions. These data show a low toxicity and a good efficacy in term of TTF. We can conclude that ICE is a safe and effective conditioning regimen for ASCT in non-Hodgkin's lymphomas.

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REINFUSION OF AUTOLOGOUS LYMPHOCYTES WITH GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR INDUCES RAPID RECOVERY OF CD4+ AND CD8+ T CELLS AFTER A DOUBLE HIGH-DOSE CHEMOTHERAPY FOR MULTIPLE MYELOMA PATIENTS

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High-dose chemotherapy (HDCT) followed by autologous peripheral-blood progenitor cell transplantation (APBPCT) induces CR rates in approximately 50% of newly diagnosed Multiple Myeloma (MM) patients. Several observations support the role for the immune system in tumor control after transplant. We investigated, in a prospective phase II study, whether autologous lymphocytes, infused after HDCT, could induce a more rapid recovery of T cells. 9 MM patients (stage II-III) followed a chemotherapy consisted of three course of VAD and a course of cyclophosphamide prior to stem cell harvest, and a double HDCT with melphalan (Mel) 200 mg/m^2 and APBPCT. The first VAD course was given followed by GM-CSF (molgramostim) 2.5 mg/kg for 12 days and, in the last 7 days, low-dose interleukin-2 (IL-2) 2 MIU/m^2 subcutaneously. The second day after stopping GM-CSF and IL-2, peripheral-blood mononuclear cells (PBMC) were harvested, to be thawed and infused after the first and second HDCT. After the CTX and granulocyte colony-stimulating factor (G-CSF; filgrastim), peripheral-blood progenitor cells (PBPC) were harvested to support two cycles of HDCT. These patients received stem cells, GM-CSF, and lymphocytes after the first and second HDCT. Toxicity of concurrent GM-CSF and low-dose IL-2 consisted of mild fever (1 patient) and a slight pain (1 patient). 1.6 to 4.8 x10^{10} lymphocytes were harvested through two to three aphereses. Our preliminary data show that lymphocyte infusion had a significant effect on the recovery of lymphocytes, T cells, and CD8$^+$ T cells (normalized on day 25). Recovery of CD4$^+$ T cells was significantly accelerated by lymphocyte reinfusion and GM-CSF, leading to counts of 500/mL at 25 days. NK cell recovery was adequate after 2 months and only B-cell recovery was slow. In conclusion, in our small series lymphocyte reinfusion with GM-CSF had a significant effect on the recovery of CD8$^+$ and CD4$^+$ T cells, whether the rapid recovery of CD4$^+$ and CD8$^+$ T cells prevents or delays relapse of the disease should be further investigated.

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A CONSOLIDATION THERAPY WITH LOW DOSE THALIDOMIDE AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANT CAN IMPROVE THE OUTCOME OF MULTIPLE MYELOMA PATIENTS BUT IS ASSOCIATED WITH HIGH TOXICITY

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The outcome of patients (pts) with multiple myeloma (MM) following a conventional treatment (CT) is unsatisfactory. A double high-dose therapy (HDT) followed by autologous peripheral blood stem cell transplant (APBSCT) achieves a greater tumor reduction with longer disease-free (DFS) and overall survival (OS). Despite high response rates fewer than 30% of pts remain in remission 3 to 7 years later. We evaluated tolerability and efficacy of a treatment with low dose Thalidomide (Thal) as maintenance therapy after a double course of HDT in a group of MM patients (stage II-III).

Patients and Methods. 17 pts (11 males and 6 females), median age 56 years old (range 41-63) received induction chemotherapy including 3 cycles of VAD and a course of cyclophosphamide. 10 pts were in partial remission (PR), 4 in stable disease (SD) and 3 in complete remission (CR) on entry into the transplant phase of therapy. All pts received intravenous melphalan 200 mg/m^2 as their conditioning regimen for the first and second HDT. 13 of 17 pts attained a PR post-transplant, and 4 a CR. Thal was started at a median of 3.9 months (mo) post-transplant (range 1.0-11.3), with a median duration of treatment of 7.1 mo (range 0.6-26.1). Pts received Thal orally as monotherapy at a dose of 100 mg daily. 10 pts failed to tolerate thalidomide due to: peripheral neuropathy in 5, severe fatigue in 2, neutropenia in 1 and severe infectious in 2. The most common adverse effects were constipation (5), rash (4), dry skin (3) and dizziness (3). After a median follow-up of 25.2 mo (range 5.6-32.0) from the start of treatment with Thal, 7 pts attained a CR (with a conversion rate from 23.5 % to 41.1%) and 2 had a progression of disease. Conclusion: Low dose Thal appears to be an efficacy drug in the post-transplant setting, but toxicity is high and not negligible and could prevent a lengthy use of this antineoplastic agent.

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SERUM FREE LIGHT CHAINS: A NEW TOOL FOR DIAGNOSIS AND MANAGEMENT OF MULTIPLE MYELOMA

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Free monoclonal immunoglobulin light chains (FLC) are found in serum and urine of patients with a number of B-cell lymphoproliferative disorders, including multiple myeloma (MM) and Bence Jones MM. Recently, a sensi-

tive, latex-enhanced, immunonephelometric assay for the detection of serum FLC on the Beckman IMMAGE(e)™ nephelometer, has been developed. Briefly, a noncompetitive, near-infrared, particle enhanced immunoassay, has been used. A six-point calibration curve with a third-order polynomial curve fit was employed. The assay components were as follows: 15 µL of serum sample for kappa and 5 µL for lambda, 60 µL of latex reagent, 5 microlitres of 120 grams/litre polyethylene glycol 6000 (final concentration, 2 grams/litre) and 195 µL of phosphate-buffer saline. The reaction time for each assay was 10 min with a serum dilution 1:10. This test could detect as little as 0.1-0.2 mL/dL of FLC. Reference values we used, were: free kappa 0.33-1.94 mL/dL; free lambda 0.57-2.63 mL/dL and kappa/lambda ratio (KLR) 0.26-1.26. We tried to determine whether serum levels of FLC could be used to monitor disease evolution in MM, could be used as an alternative to measuring urine monoclonal paraproteins in Bence Jones MM and as a marker for the diagnosis and monitoring of non-secretory MM. Eleven patients were studied, 4 patients had MM secreting intact monoclonal paraproteins, 4 had Bence Jones MM with FLC detectable in the urine by standard methods; the remaining 3 patients had non-secretory MM. Preliminary data showed that serum FLC concentrations and ratios at diagnosis were within reference values in 1 patient and abnormal in the other 3 MM secreting intact monoclonal paraproteins. In all responsive patients, FLC concentrations fell more rapidly than intact immunoglobulin. The serum half-life of FLC was much shorter than that of IgG (2-6 h versus 20 days) and of IgA (6 days). Thus, changes in FLC concentrations could provide an earlier indicator of response to therapy and remission. In all 4 patients with Bence Jones MM, the presence of monoclonal paraproteins could also be detected in serum at diagnosis by measuring serum FLC. Follow-up evaluation also revealed a remarkable correlation between serum FLC and daily urinary excretion of paraproteins. In 1 out of 3 patients with non-secretory MM, increased concentrations of serum FLC and abnormal KLR were detected during the disease follow up. This patient could be classified as oligo-secretory MM. The other 2 patients had normal or increased FLC concentrations with normal KLR. Studies on a larger series of patients are currently in progress to optimise this assay that could eventually be adopted for routine clinical use in the diagnosis and management of MM.

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IGM-MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND IGG-/IGA-MGUS: COMPARISON OF THE PROBABILITY OF EVOLUTION INTO MALIGNANT LYMPHOPROLIFERATIVE DISEASE

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Introduction. IgM-MGUS has been recently redefined as an any size of IgM monoclonal component (MC) without histopathological evidence of bone marrow (BM) lymphoplasmacytic non-Hodgkin's lymphoma. Whether according to the aforementioned criteria an IgM-MGUS has the same probability of evolution and the same risk factors of an IgG-/IgA-MGUS is largely unknown.

Patients and Methods. 163 patients with IgM-MGUS and 1,012 patients with IgG-MGUS (n=877) or IgA-MGUS (n=135) (all with BM histopathology available), diagnosed from July 1975 to May 2003, were included in the study. Cumulative probability of transformation into malignant lymphoproliferative disease (MLPD) was calculated by means of the Kaplan Meier estimator. Survival curves were compared by means of the log-rank test. Univariate and multivariate Cox models were used to identify risk factors for evolution.

Results. Cumulative probability of transformation at 5 and 10 years was 4% (95% CI, 2-10%) and 18% (95% CI, 11-29%), respectively, in IgM-MGUS; it was 6% (95% CI, 2-4%) and 18% (95% CI, 13-24%), respectively, in IgG-/IgA-MGUS (p=0.88). Indeed, the proportion of IgM-MGUS presenting evolution to MLPD (9.2%, at a median follow-up 74 months, range 12-291) did not differ significantly from that of IgG-/IgA-MGUS (6.1% at a median follow-up of 76 months, range 12-204)(p=0.2). In IgM-MGUS, at univariate analysis hemoglobin (Hb) was the only parameter significantly associated with evolution probability (p=0.02), while MC level (p=0.06), Bence Jones proteinuria (p=0.06), erythrocyte sedimentation rate (ESR) level (p=0.07) and absolute lymphocyte counts > $4x10^9$/L (p=0.1) were associated with a trend for increased risk of transformation. In IgG-/IgA-MGUS, MC level (p=0.001), high ESR (p=0.02), and reduced normal Ig levels (p=0.0001) were significantly associated with the probability of evolution, while IgA isotype (p=0.1), and BM plasma cell infiltration (p=0.1) were associated with only a trend for increased risk of transformation. Hb was not detected to significantly influence evolution probability (p=0.12). At multivariate analysis, reduction of normal Ig levels (p=0.0001) and high ESR levels (p=0.001) independently predicted malignant transformation.

Conclusions. Isotype class does not significantly influence the probability of evolution of MGUS. Whether the different prognostic value of anemia in IgM-MGUS as compared to IgG-/IgA-MGUS actually reflects a biological distinction between the two subgroups needs to be confirmed with further investigation.

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CRYSTAL-STORING HISTIOCYTOSIS" WITH MONOCLONAL GAMMOPATHY: A CASE REPORT

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"Crystal-storing histiocytosis" (CSH) is a rare event in disorders associated with monoclonal gammopathy. The disorder is accompanied by the expression of k light chains. Some data suggest that CSH results from storage of crystals produced by plasma tumor cell that either overproduce k light chains or express a structurally aberrant molecule. We report a case of a 75-year-old man who was admitted to the hospital because of fever, weight loss and fatigue. He reported to have a IgG K gammopathy (MGUS) and thrombocytopenia diagnosticated two years before. Physical examination showed pale face, light jaundice, submaxillary lymphonode 1 centimeter diameter, hepatosplenomegaly and few crackles in the bilateral lower lobes. Complete blood count revealed: white blood cell count of 4600 leukocytes per millimeter cubic with neutrophlis 63 per cent, platelets count 69000 per millimeter cubic, reticulocyte count 12 per thousand; there was a mild reduction of hemoglobin level (10 grams per deciliter). Blood chemistries were as follows: serum creatinine level 1.75 milligrams per deciliter, bilirubin 3.07 milligrams per deciliter, alkaline phosphatase 108 units per liter, gammaglutamyltransferase 76 unit per liter; serum aspartate aminotransferase 66 unit per liter, serum alanine aminotransferase 41 unit per liter and serum lactate dehydrogenase 388 unit per liter. Erythrocyte sedimentation rate was 74 millimeter first hour, reactive C-protein was 4,97 milligrams per deciliter, beta 2 microglobulin was 4,55 milligrams per deciliter, ferritin was 556 nanograms/milliliter. Prothrombin activity was 65 per cent (INR 1.46); activated partial thromboplastin time was 35 seconds; serum fibrinogen was 258 milligrams per deciliter. Serum immunoelectrophoresis showed an IgG k paraproteinemia and nephelometric analysis resulted in a serum concentration of 2050 mg/dL IgG and of 542 mg/dL k light chain. Quantitative urine analisys resulted negative for k light chain. Anti-nuclear antibodies (ANA) and anti-double stranded DNA antibodies (anti-ds DNA) were negative such as hepatitis A, B, C, HIV. Chest x-ray revealed bronchopneumonia in the lower lobe of the right lung such as toracoabdominal computed tomography revealed a moderate hepatomegaly with portal congestion and splenomegaly (15 centimetrs). Radiographic examinations of the skull, entire spine, pelvis and left and right humerus and femur indicated no lytic bone lesions or diffuse osteopenia. Bone marrow biopsy showed the accumulation of enlarged macrophages with cytoplasmatic crystalline material in a proportion of 35% and 40% of the total cell volume. The crystal-storing histiocytes reacted positively with the anti-CD68 antibody KP1, antibodies against CD20 (L26) gave a negative reaction in these cells. Immunohistochemically, the crystal-storing histiocytes were positive for IgG and in particular heavy chains k. Congo red was negative. The patient refused liver biopsy and steroid therapy.

Eight months after diagnosis of CSH the patient is alive and his lifestyle is normal.

Conclusions. CSH is an uncommon phenomenon in disorders associated with expression of monoconal immunoglobulins; it is presumed to be an accumulation of secreted paraproteins or immunoglobulins which aggregate in crystals. However, the mechanism has not been clarified. The storage and analysis of serum, urine, and tissue samples from such patients is important to understand and study the pathogenesis of CSH.

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THE NEW HISTONE DEACETYLASE INHIBITOR ITF2357 IS A STRONG INDUCER OF APOPTOSIS IN MULTIPLE MYELOMA CELLS

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Background. Multiple myeloma (MM) is an incurable disease characterised by neoplastic proliferation of plasma cells and drug resistence to conventional therapies. Several studies demonstrate that aberrant acetylation/deacetylation of histone proteins is an integral part of growth arrest, differentiation and apoptosis in many tumours. Various agents such as the Histone Deacetylase Inhibitors (HDACIs) modify histone status and are being examined for their potential therapeutic use in the treatment of numerous malignancies and in particular of MM.

Aims. To determine the cytotoxic and anti-proliferative activity in vitro of a novel hydroxamic acid-based HDACI, ITF2357 (Italfarmaco, Cinisello Balsamo, Italia), in multiple myeloma in comparison to SAHA, the prototypic hydroxamic HDACI.

Methods. The cytotoxic effect of ITF2357 and SAHA was assessed on 9 human myeloma cell lines and 6 freshly isolated MM samples using the Alamar Blue dye, annexin-propidium iodide staining and FACS analysis and standard cell cycle analysis. Patients' MM cells were purified by positive selection using anti-CD138 antibody and magnetic beads (Miltenyi). Clonogenic assays were performed by plating cells in methylcellulose and counting colonies after 21 days.

Results. ITF2357 had a strong cytotoxic activity in 7/9 MM cell lines, with IC50 ranging from 0.1 to 0.2 microM. Only two cell lines were somewhat more resistant but still responded with an IC50 of about 1 microM. SAHA tested in parallel on the same cell lines showed an IC50 of 1 microM or above in all cases. Apoptosis induced by ITF2357 started to be observed at 24 hours but was best measured at 48 hours. Similarly to MM cell lines, ITF2357 had also more potent cytotoxic activity compared to SAHA against freshly isolated purified MM samples at both 24 or 48 hours of culture, with an IC50 ranging from 0.1 to 0.8 microM. Clonogenic assays on human myeloma cell lines further confirmed the capacity of ITF2357 to completely abolish colony growth at lower concentrations than SAHA. Cell cycle analyses are being performed at different time points to determine whether a cell cycle block occurs before induction of apoptosis.

Conclusions. These results demonstrate the efficacy of

ITF2357 in inducing apoptosis of both freshly isolated MM samples and cultured MM cell lines, with a 2-10 fold higher potency compared to SAHA. They provide the framework for future phase I studies of ITF2357 in relapsed MM patients.

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ANALYSIS OF MTHFR POLYMORPHISMS AND P16 METHYLATION AND THEIR CORRELATION WITH CLINICAL-BIOLOGICAL FEATURES OF MULTIPLE MYELOMA

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Low folate intake and changes in folate metabolism due to polymorphisms in the methylentetrahydrofolate reductase (MTHFR) gene were associated with myelomagenesis. However, controversial data have been published regarding a protective role of variant alleles of MTHFR on multiple myeloma (MM). In order to investigate the influence of two common polymorphisms of MTHFR C677T and A1298C on the risk of MM we performed a matched case-control study. Moreover we analyzed the methylation status pattern of p16.

The frequency of 677CC, 677CT, 677TT were 31%, 44%, 25% respectively whereas the frequency of 1298AA, AC, CC were 48%, 44%, 8% in MM patients. No significant association between susceptibility to MM and 677 and 1298 MTHFR variants was detected. Regarding p16 methylation we confirmed a high prevalence of p16 methylation (40%) in patients affected by MM and demonstrated that MTHFR 677CC is associated with a higher prevalence of p16 hypermethylation. Although previous reports showed decreased risk of MM development associated with the MTHFR polymorphisms, our data demonstrated that variant alleles did not play a key role neither in protection or increased risk suggesting that the effect of both MTHFR on folate metabolism might be modified by diet intake. Moreover our findings demonstrated that p16 hypermethylation might be a frequent genetic aberration in MM and may contribute with other molecular aberrations in the pathogenesis of this malignant disorder.

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TRANSCRIPTION REPRESSION ACTIVITY IS ASSOCIATED WITH THE TYPE I ISOFORM OF THE MMSET GENE INVOLVED IN THE T(4;14) IN MULTIPLE MYELOMA

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The WHSC1/MMSET gene is deregulated by the t(4;14)(p16.3;q32) translocation occurring in approximately 15% of multiple myelomas. As a result of alternative splicing or transcription initiation events, it normally encodes several putative isoforms thought to play a role in transcription regulation. Here, we investigated the transcriptional activity of MMSET isoforms MMSET I, MMSET II and RE-IIBP and their biological effects on proliferation in transfected cells. Both MMSET I and MMSET II were localized in the nucleus, while RE-IIBP isoform showed cytoplasmic and nucleolar staining. MMSET I was able to repress the transcriptional activity of a TK promoter in a dose-dependent manner, while both the MMSET II and RE-IIBP isoforms had no effect in our system. Interestingly, trichostatin A was able to reduce the activity of MMSET I; furthermore co-immunoprecipitation analyses in vitro indicated that MMSET I recruits specifically HDAC1 and mSin3b, whereas no interaction with HDAC2 or HDAC4 was observed. Notably, neither MMSET II nor RE-IIBP diplayed histone methyltransferase activity. Finally, MMSET isoforms did not affect growth or apoptosis rate in transfected 293T cells. Overall, our experimental data support the general hypothesis that MMSET may act as a transcription regulator and suggest that different functional activities could be associated with distinct isoforms.

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11Q13 POLISOMY AND ABSENCE OF IGH TRANSLOCATIONS AND CHROMOSOME 13Q14 DELETION CHARACTERIZE A DISTINCT SUBGROUP OF MULTIPLE MYELOMA PATIENTS EXPRESSING CCND

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Cytogenetic and molecular studies have provided evidence that multiple myeloma (MM) is characterized by markedly heterogeneous chromosomal aberrations, in particular translocations involving the immunoglobulin heavy-chain (IGH) locus at 14q32. A recently proposed TC classification grouped MM patients into five classes on the basis of their cyclins D expression profiles and the presence of the main translocations involving the immunoglobulin heavy-chain (IGH) locus at 14q32 (Hideshima, T. *et al.,* Blood 104:607-618, 2004). TC1 is characterized by the t(11;14) or t(6;14) translocation, with the consequent overexpression of CCND1 or CCND3; TC2 shows low to moderate levels of the CCND1 gene in the absence of any primary IGH translocation; TC3 includes tumors that do not fall into any of the other groups, most of which express CCND2; TC4 shows high CCND2 levels and the presence of the t(4;14) translocation and TC5 expresses the highest levels of CCND2 in association with either the t(14;16) or t(14;20) translocation. CCND1 overexpression, investigated by real-time quantitative PCR (Q-RT-PCR), has been

found in 25-50% of MM patients, suggesting the involvement of mechanisms other than t(11;14) (15% of MM cases), such as gene amplification or polysomy. CCND2 is a transcriptional target of the MAF proteins and we and others have shown that it is deregulated in MM cases with t(4;14), t(14;16) and t(14;20). In order to provide insights into the potential role of cyclin D loci amplification and genes deregulation in the molecular classification of MM, fluorescence in-situ hybridization (FISH) was used to investigate the cyclin D loci arrangements and the chromosome 13q deletion in a panel of 50 MM cases. The cases were stratified into the 5 TC classes on the basis of the main IGH translocations and the cyclin D expression levels obtained by high-density oligonucleotide microarray analysis of purified plasma cells. Cyclins D expression was also evaluated by Q-RT-PCR: a statistically significant correlation of the expression levels obtained by the two different methods has been verified. Q-RT-PCR data indicated that TC2 cases showed lower CCND1 expression levels than the t(11;14) cases (TC1, p=0.000003), but significantly higher CCND1 expression levels than the TC3, TC4 and TC5 groups. Interestingly, the TC2 group showed extra copies of the CCND1 locus, absence of CCND2 polysomy, and neither IGH translocations nor the chromosome 13q deletion, whereas TC1 group did not show any CCND1 polysomy. CCND2 overexpression was found in TC3, TC4 and TC5, whereas neither any of the 50 patients showed structural alterations in the CCND2 locus nor any group was characterized by CCND2 extracopies or chromosome 12q polysomy. Moderate CCND3 expression levels could be detected in all of the MM tumors and could not be correlated with extracopies of the gene; a marked overexpression was found in the t(6;14) patient. Chromosome 13q deletion was more frequent in the TC4 group than in all of the other MM samples (p=0.0220), whereas its absence significantly correlated with the TC2 group in comparison with all of the other MM cases (p=0.0017). Our results indicate that the TC2 group could represent a distinct molecular entity within the MM patients and could provide important contributions to the understanding of the molecular and biological features of distinct MM subtypes associated with different prognoses or treatment responses.

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MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA: A DISTINCT TRANSCRIPTIONAL PROFILE CHARACTERIZES PATIENTS EXPRESSING CCND1 AND NEGATIVE FOR 14Q32 TRANSLOCATIONS

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The deregulation of CCND1, CCND2 and CCND3 genes represents a common event in multiple myeloma (MM), being at least one of them deregulated in almost all MM tumors. A recently proposed TC classification grouped MM patients into five classes on the basis of their cyclins D expression profiles and the presence of the main translocations involving the immunoglobulin heavy-chain (IGH) locus at 14q32. (Hideshima, T. *et al.*, Blood 104:607-618, 2004). TC1 is characterized by the t(11;14) or t(6;14) translocation, with the consequent overexpression of CCND1 or CCND3; TC2 shows low to moderate levels of the CCND1 gene in the absence of any primary IGH translocation; TC3 includes tumors that do not fall into any of the other groups, most of which express CCND2; TC4 shows high CCND2 levels and the presence of the t(4;14) translocation and TC5 expresses the highest levels of CCND2 in association with either the t(14;16) or t(14;20) translocation. The aim of our study was to identify the putative transcriptional fingerprints associated with the deregulation of the different D-type cyclins and the presence of IGH translocations. The cyclin D expression levels obtained by high-density oligonucleotide microarray analysis of purified plasma cells from 50 MM cases were used to stratify the samples into the five TC classes, along with the molecular characteristics determined by fuorescence in-situ hybridization. A multi-class classification analysis was performed on the gene expression data and used to identify the transcriptional fingerprints of the 5 TC groups. 112 probe sets were selected as characterizing the TC1, TC2, TC4 and TC5 groups, whereas the TC3 samples showed heterogeneous phenotypes and no marker genes. In particular, the TC1, TC4 and TC5 groups were characterized by the molecular signatures associated with the primary IGH translocations target genes. The TC2 group, which showed extra copies of the CCND1 locus and absence of chromosome 13q deletions, was characterized by the overexpression of 30 genes, mainly involved in protein biosynthesis at translational level. Among the most specifically modulated transcripts in the TC2 group we identified a novel gene containing a BTB/POZ domain, typical of many zinc finger transcription factors and associated with transcriptional repression activity. A meta-analysis performed on two publicly available MM datasets, containing almost 250 cases, validated the identified gene expression signatures with a global classification rate of 86% and 90%, respectively. Our data contribute to the

understanding of the molecular and biological features of distinct MM subtypes; the identification of a distinctive gene expression pattern in TC2 patients may improve risk stratification and indicate novel therapeutic targets.

P243

EVALUATION OF THE EFFICACY AND CARDIOTOXICITY OF LIPOSOMAL ANTHRACYCLINES IN ONCO-HAEMATOLOGICAL DISEASES: OUR EXPERIENCE

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Background. Onco-haematological diseases are treated with international protocols that differ according to aetiology and severity of the disease, clinical conditions and patient's age. In order to reduce the systemic toxicity of anthracyclines, in Our Department, we treated patients with Multiple Myeloma (MM) and Non-Hodgkin Lymphoma (NHL) with therapeutical regimens replacing conventional anthracyclines with non-pegylated liposomal doxorubicin (Myocet), that has a better pharmacokinetic profile with much lower gastroenterological and cardiological toxicity.

Methods. Between October 2003 and February 2005, patients with MM, both at first line approach (n=4) and resistant or with relapse (n=17) were referred to Our Department. Diagnosis at admission included patients with IgG Myeloma (n=12), IgA Myeloma (n=8) and micro-molecular Myeloma (n=1).

All patients were treated with one or more polichemotherapeutic agent cycles according to CNR/Myeloma scheme: VCR 1 mg iv for 1 day + Myocet 25mg/sm for 2 days + CTX 100 mg/sm/day for 1-4 days + PDN 60 mg/sm/day for 1-4 days.

In all patients, Monoclonal Component (MC) and Left Ventricular Ejection Fraction (LVEF) were assessed in order to evaluate treatment efficacy and cardiotoxicity of the liposomal anthracycline administered.

Results. We followed-up 21 patients (11 M, 10 F), mean age 70.7±8.5 years, mean baseline MC 1762±735, and mean baseline LVEF 55±5 %.

Myocet was administered at mean dosage of 41.1±6.9 mg/sm, with a mean of 8±3 chemotherapeutic cycles, equal to a total administered dosage of 367.6±191.0 mg/sm.

Evaluation of treatment efficacy was made in 18 patients: MC was significantly reduced from 1762±735 to 1425±668 (p<0.001). The possible Myocet cardiotoxicity was evaluated by echocardiographic assessment of LVEF (n=19): before chemotherapy, the mean value was 55±5% and slightly increased to 57±5% (p = ns) as a sign of cardiac performance maintenance.

We also found similar results in our patients population [9 patients (8 M, 1 F) with NHL treated according to CHOP protocol where conventional doxorubicin was replaced with Myocet (CTX 750 mg/sm iv + Myocet 30 mg/sm iv + VCR 1,4 mg/sm iv for 1 day + PDN 100 mg/sm orally for 1-5 days)]: although their follow up is still on-going, LVEF assessed at the beginning of chemotherapy (58±5%) seems to remain stable (56±3 %) even if they were treated with higher unit dose (80±15 mg/sm).

Conclusions. Our findings show that treatment with liposomal anthracyclines (Myocet) statistically significantly decreases MC in patients with MM. Despite the large number of treatment cycles in some patients (range 2-16), cardiac function remains preserved. We expect similar observations in patients NHL.

P244

EVALUATION OF DISEASE EXTENT WITH PET-TAC AT DIAGNOSIS AND AFTER TREATMENT IN MULTIPLE MYELOMA

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Very little information is available regarding the diagnostic utility of whole body positron emission thomography (PET) of fluorinated deoxyglucose FDG) combined with CT scan (PET-CT) in multiple myeloma (MM) patients. The objective of this study is to define the role PET-CT in assessing the extent of active disease at the time of initial presentation and in evaluating treatment response in MM .

We studied 16 previously untreated MM patients at diagnosis, after induction therapy and 3 months after autologous stem cell transplantation (ASCT) . The results of PET-CT scans were compared with standard whole skeletal survey and whole spine Magnetic Resonance (MR) at diagnosis and with clinical response to treatment at restaging . Nine patients were male, 7 female, median age was 61 years (range 49-69), 7 had IgG and 8 IgA monoclonal protein, one had a Bence Jones MM. Three patients had stage IA MM, 4 had stage IIA and 9 stage IIIA MM according with Durie and Salmon classification. The patients underwent induction therapy with thalidomide 200 mg daily associated with monthly cycles of high-dose dexamethasone for 4 months, then proceeded to collection of PBSC mobilized by 4 g/m^2 Cyclophosphamide + G-CSF and myeloablative treatment with 200 mg/ m^2 Melphalan.

At diagnosis PET-CT scans were negative in 9 patients (56%) and positive in 7 patients (44%). PET-CT scans and skeletal radiographic findings were concordant in 12/16 patients (75%). In 4/16 cases (25%) PET-CT scans detected more disease sites than skeletal survey (3 bony and one soft-.tissue). Moreover, whole spine MR showed a focal pattern of bone marrow involvement of a few vertebras in 4 patients, who had both negative radiographic and PET-TAC findings. . Eight patients had a restaging after induction chemotherapy (6) or after ASCT (2). Out of 7 responsive patients, 5 had positive PET-CT scans at diagnosis : 3 showed a significant reduction of the number and the extension of the sites of abnormal uptake after therapy and 2 patients , who obtained a complete remission after ASCT, achieved a negative PET-CT scan.

We conclude that in our series of newly diagnosed MM

PET-CT could identify additional sites of diseases to skeletal survey in 4/16 (25%) of the patients. In other 4/16 patients (25%) whole spine MR could identify minimal involvement of single vertebras, that were not recognized by PET-CT and skeletal survey. Moreover, all the 5 patients with pre-treatment positive PET –CT scans showed a reduction (3) or a disappearance (2) of the sites of abnormal uptake after therapy , that correlated with clinical response.

P245

ZOLEDRONIC ACID IN PATIENTS WITH STAGE I MULTIPLE MYELOMA

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In clinical practice patients with stage I multiple myeloma (MM) are not treated by chemotherapy, nevertheless the potential evolution is difficult to know preventively. Zoledronic acid is a highly potent bisphosphonate that has anti-tumor potential activity. Starting from these considerations we treated 18 patients in stage I MM with zoledronic acid at a dose of 4 mg by intravenous infusion every month. Patients' characteristics: 8 men, 10 women, mean age 68 years (range 54-87), IG isotype IgG 15, IgA 3; 5 pretreated with pamidronate, mean serum beta2-microglobulin 2347 ng/mL (range 1093-6439; normal value 1200-2500), mean serum creatinine 0.7 mg/dL (range 0.4-1.2; normal value 0.5-0.9), mean serum calcium 9.2 mg/dL (range 8.6-9.8; normal value 8.6-10.2). After a mean time treatment of 21 months (range 8-32) and mean follow-up of 31 months (range 10-59) we observed 8 stable diseases with mean monoclonal component variation between –23% and +15% (median +7.5%), 10 progressive diseases: 5 skeletal events (stage III) 14,14,16,23,53 months after diagnosis, respectively; 3 monoclonal component progressions (stage II); 2 haemoglobin reductions (stage II). Zoledronic acid infusions were well tolerated. Toxicity, according to NCI common toxicity criteria, was mild: grade 1 hypocalcemia was observed in 19/359 (5.2%) of total administrations, grade 2 fever with flu-like syndrome in 21/359 (5.8%). Mean values after treatment were: beta2-microglobulin 3257 ng/mL (range 1447-14891), serum calcium 9.3 mg/dL (range 8.7-10.4), serum creatinine 0.9 mg/dL (range 0.5-2.07). One patient had osteonecrosis of the jaw after a tooth extraction; this process is a rare complication, possibly drug-induced avascular bone necrosis. Our preliminary results are not conclusive; higher number of patients should be studied to validate the use of zoledronic acid in stage I MM.

P246

INTRAVENOUS NERIDRONATE FOR TREATMENT OF SKELETAL INVOLVEMENT IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Aim. To compare the efficacy of Neridronate (NER) and Zoledronate (ZOL) in reducing skeletal morbidity in patients with multiple myeloma.

Patients and study design. The study population includes 14 patients (7 males and 7 females; age range 53-72 yrs) with multiple myeloma. Type of myeloma: IgG (n 8), IgA (n 4), light chain (n 2). Clinical staging: IA (n 1), IIA (n 3), IIIA (n 8), IIIB (n 2). When enrolled in the study, all patients had evidence of osteolytic lesions/pathologic fractures and/or secondary osteopenia (-2.5 S.D. < T-Score < - 1 S.D.)or osteoporosis (T-Score <-2.5 S.D.), in the assence of metabolic bone disorders. Patients received a double (n 10) or a single (n 4) autologous peripheral cell transplantation. Patients were randomly allocated to receive monthly ZOL (4 mg via 15-min infusion) or NER (100 mg via 150-min infusion). In the two groups there are no major differences with respect to mean age, performance status, disease type and stage, median time from diagnosis, renal functioning, severity of bone involvement and extent of antineoplastic therapy. Bone Mineral Density (BMD)has been assessed at baseline and after a 12-month follow up. Serum calcium, phosphor and alkaline phosphatase levels have been assessed monthly.

Results. Lumbar spine (L1-L3)/hip BMD and serum alkaline phosphatase % changes after 12 months of treatment are shown below: NER group: Mean BMD % change, Spine (+/- S.D.): 3.5 (4.37); Mean BMD % change, Hip (+/- S.D.): 1.4 (3.16); Mean % change, serum alkaline phosphatase: -50.25. ZOL group: Mean BMD % change, Spine (+/- S.D.): 6.21 (4.69); Mean BMD % change, Hip (+/- S.D.): 2.38 (3.05); Mean % change, serum alkaline phosphatase: -28.25.

Conclusion. Although wider trials are needed to assess its efficacy in reducing the incidence of skeletal events such as pathologic fractures, spinal cord compression, radiation therapy or surgery to bone, these data suggest that - at the tested doses - NER might be comparable to ZOL in improving BMD and bone metabolic activity in patients with myeloma-induced skeletal damage.

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MYELOMA CELLS INHIBIT HUMAN OSTEOBLAST PROLIFERATION AND INDUCE OSTEOBLAST APOPTOSIS: POTENTIAL INVOLVEMENT OF FAS/FAS LIGAND SYSTEM IN MYELOMA PAZIENTS

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Multiple myeloma (MM) patients with high plasma cell infiltrate are characterized by a lower number of osteoblasts and a decreased bone formation. The mecha-

nisms by which myeloma cells reduce bone formation are not completely identified. In this study we have investigated the effect of human MM cells on proliferation and survival on human osteoblastic cells. We found that conditioned media of MM cells significantly reduced the number osteoblastic cells in culture and suppressed osteoblast proliferation. In a co-culture system we found that MM cells are able to induce apoptosis of human osteoblast-like cells (MG-63) either in presence or absence of a transwell insert even if the cell-to-cell contact condition was more effective. Any significant difference in the number of apoptotic osteoblastic cells was observed between $CD56^+$ and $CD56^-$ MM cells. On the other hand we found that CD95/FAS+ osteoblastic cells, are more sensitive to MM cells apoptosis. Consistently the presence of blocking anti-FAS ligand Ab in the co-cultures reduced the pro-apoptotic effect of FAS ligand positive MM cells on osteoblasts. The potential expression of apoptotic molecule TRAIL by MM cells and its role in myeloma-induced osteoblast apoptosis have been also investigated demonstrating that blocking anti-TRAIL Ab reduced osteoblast apoptosis but did not completely blunted the pro-apoptotic effect of TRAIL positive MM cells. Our in vitro data was supported *in vivo* in a cohort of 32 MM patients by the finding of higher FAS ligand expression by CD138+ cells in MM patients with osteolytic bone lesions as compared to those without bone lesions (median % of expression: 68% vs. 40%, p=0.02). Moreover a significant reduction in the number of osteoblastic cells was observed in osteolytic MM patients with high FAS ligand expression by CD138+ cells as compared to those with lower FAS ligand % of expression.

In conclusion our data indicate that myeloma cells inhibit osteoblast proliferation and osteoblast apoptosis with the potential involvement of FAS/FAS ligand system.

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BORTEZOMIB TREATMENT IN REFRACTORY/RELAPSED MULTIPLE MYELOMA: A PILOT STUDY

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Background. In the last few years, the introduction of high dose of chemotherapy with PBSCT and new drugs as thalidomide, IMIDs and proteosome's inibithors (bortezomib), has improved the outcome of multiple myeloma (MM). We report our experience with bortezomib in a group of patients with refractory/relapsed MM.

Casistic and Treatment. In our pilot study we enrolled 8 patients (all female, aged from 42 to 74 yrs) with advanced MM. All patients have been pretreated: 2 with 2 lines of chemotherapy, 3 with 3 lines and 3 with more than 3 lines. All patients received thalidomide before this programme. Schedule of treatment was the standard dose of 1.3 mg/m^2 d 1, 4, 8 and 11 with recicle at 21 for a total of 6 – 8 cicles or at least 2 cicles after the best response. Patients characteristics are reported in Table:

Case	Monoclonal Comp. (gr%)	Ig Dosage (mg%)	Follow-up (months)	Therapeutic Response	Survival (Months)
1) M.I.	3,50	2660	4	No Responder	4
2) M.M.	2,70	2500	7	PR	7+
3) C.M.	2,30	2940	6	MR	6+
4) M.M.	4,00	2850	3	PR	3+
5) T.P.	0,60	?	1	Progr	1
6) C.A.	3,80	3340	7	RC	7+
7) C.L.	1,20	1000	1	Progr	1
8) A.E.	2,4	1940	1	NV	1+

Results and Conclusions. Treatment has been well tolerated but all patients presented a moderate astenia; a severe piastrinopenia associated with peripheral neurophaty was observed only in one patient with HCV liver cyrrosis, but was reversible after withdrawal of bortezomib. In our study, 3/8 patients (37.5%) obtained 2 PR and 1 CR (EBMT response criteria). Our casistic and follow-up is to small but some problems should be better understood in routine use of bortezomib: the optimal dosage, the best terapeutic schedule and, particulary, the time and association of this molecule in the history of MM.

P249

OSTEONECROSIS OF THE JAWS DURING BISPHOSPHONATES TREATMENT FOR MULTIPLE MYELOMA

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Background. BFs are universally employed in preventing pathological fractures during solid and hematological malignances with bone involvement. In the last two years some cases of osteonecrosis have been described. We report the experience of our study group.

Cases. Between 54 patients treated with BFs, we observed six patients with problems of the jaws. All patients (41F, 13M) have been treated with pamidronate (Pam) or/and zolendronate (Zol): 4 smoldering myeloma, 36 symptomatic myeloma, 8 severe osteoporosis, 4 iperparathyroidism and 2 others. Treatment with BFs was administrated one time for month (Pam 60-90 mg and Zol 4 mg) until disease progression. All six patients (5 MM and 1 WM with severe osteoporosis) with jaw's problems are female with a median time of treatment of 51 m (range 29 - 75).

Case	Monoclonal Comp. (gr%)	Ig Dosage (mg%)	Follow-up (months)	Therapeutic Response	Survival (Months)
1) M.I.	3,50	2660	4	No Responder	4
2) M.M.	2,70	2500	7	PR	7+
3) C.M.	2,30	2940	6	MR	6+
4) M.M.	4,00	2850	3	PR	3+
5) T.P.	0,60	?	1	Progr	1
6) C.A.	3,80	3340	7+	RC	7+
7) C.L.	1,20	1000	1	Progr	1
8) A.E.			1	NV	1+

The typical presenting symptoms were pain and exposed bone at the site of a previou tooth extraction. Fistula, as clinical presentation, was observed in 5/6 patients and bone exposition in 3/6. In five patients lesion was localized in mandible, and one in maxilla. Osteonecrosis appared after a previous tooth extraction in 4/6 patients and fixture application in 2/6. Diagnosis of osteonecrosis was made with oral inspection, X-ray, TC scan and MRI. Histology examination and bacteriological study were performed if necessary. All patients required antibiotic treatment, three in combination with hyperbaric oxygen therapy and two in association with sequestrectomy.

Discussion and Conclusion. Clinical introduction of BFs has reduced bone complications in MM patients and solid tumors. Some side-effects related to anti-angiogenetic effect has been reported, need more attention in the clinical use. BFs treatment in non neoplastic disease, in smoldering myeloma and in MM stable disease should be better evaluated, particulary the schedule of administration.

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NOT PUBLISHED

Myeloma and Plasma Cell Dyscrasias II

P251

COMBINED ADMINISTRATION OF DARBEPOETIN AND ZOLEDRONIC ACID IN THE TREATMENT OF REFRACTORY MULTIPLE MYELOMA

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Multiple myeloma (MM) is a neoplastic disease, that affects especially the elderly, even if in recent years it has also been observed in young patients. Painful osteolytic bone destruction is a frequent complication of MM. Bisphosphonate therapy has been shown to reduce complications of bone lesions in MM and in other malignancies. In details, it seems that zoledronate, a new generation aminobisphosphonate, also exerts antitumor effects on myeloma cells; this drug has cytotoxic activity because it causes apoptosis and block of the proliferation. Another frequent issue in patients with MM is anemia that is due both to disease progression and chemotherapy. In our institution we are following 20 patients with stage II/III MM and 15 out of 20 are currently treated, independently from the adopted chemotherapy, with zoledronate because they had osteolytic bone lesions at the diagnosis. At 6 months from the beginning of the treatment (4mg i.v. every 28 days) all the 15 patients had a partial or complete regression of bone lesions. However, five out of 15 patients (4 F and 1 M, median age: 65 years, r.: 62-77 years),suspended chemotherapy after 12 cycles of Melphalan and Prednisone regimen for excessive toxicity even if they presented steady disease (SD) at clinical re-staging performed with cytological examination of bone marrow blood and of serum markers. On the basis of the anemia recorded in these patients, (median Hb: 8.2 g/dL, r. 7.8 – 9.2) they underwent a treatment with 150micrograms s.c. darbopoetin once a week together with 4 mg i.v. zoledronate every 28 days. After 6 weeks all the 5 patients had an increase of haemoglobin (median: +1.5g/dL, r.: +1.2 – 2). Interestingly, at a clinical re-staging performed after three months from the beginning of Zoledronate-Darbepoietin combined administration a partial remission was recorded in 4 out of 5 patients while the remaining was in SD. It is well known that myeloma cells compete with normal progenitors, and above all with erythroid precursors, for the same marrow microenvironment. The stimulation of erythroid precursors with growth factors in addition to the concomitant anti-proliferative effects exherted by zoledronate on myeloma cells likely induce the anti-tumour effects observed in these patients. Further studies, supported by A.I.L. (Associazione Italiana contro le Leucemie – linfomi e mieloma), are in progress in order to investigate the activity of the combination on the quality of life and survival in this subset of patients.

P252

CD20 EXPRESSION AND CLINICAL OUTCOME IN PATIENTS AFFECTED BY MULTIPLE MYELOMA

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The survival of patients affected by MM is variable depending upon the tumour mass at the diagnosis and by the intrinsic biological characteristics of tumour cells. Flow cytometry and immunological methods have allowed the characterization of a series of surface antigenic molecules expressed on either MM or normal cells. With this technique several molecules differentially expressed on normal and MM cells and correlated with the prognosis of MM patients have been identified. In details B-associated antigens, growth factor receptors, myeloid antigens and adhesion molecules can be found on pathological plasma cells. At this regard CD19 e/o CD20 B markers are generally associated to normal plasma cells (a peculiar feature of reactive plasmocytosis and of MGUS), while MM cells are often CD19 e CD20 negative. However, recent studies (Lin P. *et al.*, Am. J. Clin. Pathos., 2004, 121(4), 482-488) have demonstrated that in about 10% MM patients the plasma cells express CD20, while CD19 expression is rare (<1%). We have analyzed the bone marrow blood of 40 patients affected by MM. Twenty-six out of 40 presented a IgG component and the remaining 14 patients were IgA. On the basis of the staging criteria (Durie e Salmon), 23/40 pts. were in stage II and 17/40 in stage III; the clinical stage (remission, progression or stable disease) was defined with clinical re-evaluation after chemotherapy and/or re-staging at 6 months from diagnosis. The immunophenotype of bone marrow plasma cells demonstrated the expression of CD38 (very bright) and of CD138 while CD19 was absent; 38/40 were CD56+ and 6/40 CD20+(dim). Six patients were CD20+ and were all in stage III with an unfavourable clinical outcome: 5/6 and 4/6 had a DFS and an OS, respectively, lower than the remaining patients. The possible prognostic role of CD20 in MM warrants further clinical investigation on a larger series of patients even on the basis of future therapeutic strategies (i.e.: use of anti-CD20 monoclonal antibodies combined with first-line chemotherapy).

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ACUTE PROMYELOCYTIC LEUKEMIA IN A PATIENT WITH MULTIPLE MYELOMA PREVIOUSLY TREATED WITH MELPHALAN REGIMEN

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Secondary acute leukemias are growing events in the last years due to leukemogen effect of several anti-cancer agents. A 65 year woman affected by IgGk Multiple myeloma came at our observation 4 years ago. The patient was treated with the first-line chemotherapy regimen VMCP (Vincristine, Melphalan, Cyclofosfamide, Prednisone) for 7 cycles and subsequently, subjected to 16 cycles of MP (Melphalan, Prednisone) chemotherapy regimen in combination with i.v. administered aminobisphosphonates. In June 2004 the patient developed a cytopenia characterized, above all, by thrombocytopenia (Platelets $30x10^9$/L) e severe neutropenia (Neutrophils: $300x10^6$/L) concomitantly to the presence of multiple myeloma signs (as demonstrated by the increase in serum immunoglobulins an by the presence of ostheolytic lesions). The objective examination was normal and the cytological examination of the peripheral blood did not show any morphologic anomalies, but the presence of a cytopenia. Rapidly, the patient developed a series of symptoms that suggested the onset of an intra vessel disseminated coagulation (DIC) with important cutaneous and, then, cerebral signs. A bone marrow aspirated showed the presence of a hypercellularity characterized by a high number of promyelocytic elements with Auer bodies and by blasts from an acute promyelocytic leukemia that was confirmed by the presence of the characteristic rearrangement PML/RARα as detected with molecular biology analysis. The patient, who was already treated for the DIC, was subjected to the GIMEMA AIDA/P 2000 protochol with ATRA and idarubicina. The patient deceased after few days from the diagnosis of APL.

The development of secondary APL due to chemotherapy is an event described, above all, in patients subjected to chemotherapy for solid tumours. At our knowledge, it is still reported one case of secondary APL in patients treated with conventional drugs for multiple myeloma (i.e. melphalan and prednisone) (Puerto I.M. *et al,* Haema, 2003; 6(3), 404-406). It is known that alkylating agents can induce leukemias (i.e. busulphan); the long-term treatment with drugs such as melphalan and cyclophosphamide, even if administered at low doses, can be a risk factor for the development of hematological neoplasms such as APL.

P254

INTERLEUKIN-3 IS OVEREXPRESSED IN BONE MARROW CD3+ CELLS AND INHIBITS OSTEOBLAST FORMATION IN MULTIPLE MYELOMA PATIENTS

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Bone destruction in multiple myeloma (MM) is characterized both by markedly increased osteoclastic bone destruction and severely impaired osteoblast activity. It has been reported that IL-3 levels are increased in bone marrow plasma of myeloma patients compared and that IL-3 can stimulate osteoclast formation. However, the potential source of IL-3 in MM patients and the effects of IL-3 on osteoblastic cells are unknown. First we have tested the potential IL-3 and IL-3 receptor mRNA expression by seven human myeloma cell lines (HMCLs) and by purified bone marrow (BM) CD138+, CD14+ and CD3+ cells obtained from MM patients (n°12) at the diagnosis in comparison with MGUS patients (n°10). We found that both

HMCLs and fresh purified MM cells are negative for IL-3 mRNA expression. On the other hand we found that CD3+ cells obtained from MM patients overexpress IL-3 mRNA as compared to MGUS subjects. High IL-3 receptor level expression has been found in CD14+ cells and in osteoblast/osteoblast progenitors but not in MM cells. Therefore, to determine if IL-3 inhibits osteoblast growth and differentiation, we treated human BM marrow stromal cells with IL-3 and assessed osteoblast differentiation. IL-3 inhibited basal and BMP-2 stimulated osteoblast formation in a dose-dependent manner. Importantly, marrow plasma from MM patients with high IL-3 levels inhibited osteoblast differentiation which could be blocked by anti-IL-3. IL-3 had not any effect on the osteoblats regulating gene Runx2 espression and activity and on WNT/beta2-catenin signaling. On the other hand, IL-3 increased the number of CD45+ hematopoietic cells in stromal cell cultures. Depletion of the CD45+ cells abolished the inhibitory effects of IL-3 on osteoblasts, and reconstitution of the cultures with CD45+ cells restored the capacity of IL-3 to inhibit osteoblast differentiation. These data suggest that IL-3 is overexpressed by BM CD3+ cells in MM patients and it contributes indirectly to inhibit osteoblast formation in MM

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IL-3 IS OVEREXPRESSED IN BONE MARROW CD3+ CELLS AND INHIBITS OSTEOBLAST DIFFERENTATION IN MM PATIENTS

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Bone destruction in multiple myeloma (MM) is characterized both by markedly increased osteoclastic bone destruction and severely impaired osteoblast activity. It has been reported that IL-3 levels are increased in bone marrow plasma of myeloma patients compared and that IL-3 can stimulate osteoclast formation. However, the potential source of IL-3 in MM patients and the effects of IL-3 on osteoblastic cells are unknown. First we have tested the potential IL-3 and IL-3 receptor mRNA expression by seven human myeloma cell lines (HMCLs) and by purified bone marrow (BM) CD138+, CD14+ and CD3+ cells obtained from MM patients (n°12) at the diagnosis in comparison with MGUS patients (n°10). We found that both HMCLs and fresh purified MM cells are negative for IL-3 mRNA expression. On the other hand we found that CD3+ cells obtained from MM patients overexpress IL-3 mRNA as compared to MGUS subjects. High IL-3 receptor level expression has been found in CD14+ cells and in osteoblast/osteoblast progenitors but not in MM cells. Therefore, to determine if IL-3 inhibits osteoblast growth and differentiation, we treated human BM marrow stromal cells with IL-3 and assessed osteoblast differentiation. IL-3 inhibited basal and BMP-2 stimulated osteoblast formation in a dose-dependent manner. Importantly, marrow plasma from MM patients with high IL-3 levels inhibited osteoblast differentiation which could be blocked by anti-IL-3. IL-3 had not any effect on the osteoblats regulating gene Runx2 espression and activity and on WNT/beta2-catenin signaling. On the other hand, IL-3 increased the number of CD45+ hematopoietic cells in stromal cell cultures. Depletion of the CD45+ cells abolished the inhibitory effects of IL-3 on osteoblasts, and reconstitution of the cultures with CD45+ cells restored the capacity of IL-3 to inhibit osteoblast differentiation. These data suggest that IL-3 is overexpressed by BM CD3+ cells in MM patients and it contributes indirectly to inhibit osteoblast formation in MM.

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THE NEW DCEP-SHORT IS A FEASIBLE AND EFFECTIVE MOBILIZING REGIMEN WITH A LIMITED TOXICITY SIMILAR TO THE INFUSIONAL SCHEDULE

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Background. Autologous transplantation with peripheral blood stem cell rescue represents today the standard therapy for young patients with multiple myeloma (MM) at the onset. DCEP (Dexamethasone, Cyclophosphamide, Etoposide, and cisPlatin) has proven to be an effective regimen for peripheral blood stem cell (PBSC) mobilization in MM patients with low hematological and extraematological toxicity. We conducted a study on a new short schedule of DCEP comparing the efficacy in mobilizing stem cells and the toxicity with those of the infusional version of DCEP.

Patients and Methods. From 2000 to 2004, 152 consecutive patients aged less than 65 years, with comparable characteristics, enrolled in a high-dose program, were treated with two different schedules of the DCEP scheme. From January 2000 to December 2003, 106 patients (group I) were mobilized with the infusional-DCEP (Dexamethasone 40 mg/day i.v. in days 1-4; and 4 days continuous infusion of daily doses of Cyclophosphamide 400 mg/m^2; Etoposide 40 mg/m^2; cisPlatin 10 mg/m^2). From January 2004, 46 patients (group II) were mobilized with a short version of DCEP (DCEP-short) which consists of oral Dexamethasone 40 mg/day on days 1-4, Cyclophosphamide 700 mg/m^2/day e.v., Etoposide 100 mg/m^2/day e.v., cisPlatin 25 mg/m^2/day in a total 8 hours infusion, for 2 days.

Results. The median number of CD34$^+$ cells was higher in group II (6.98 x10^6 cells/kg) with respect to group I (5.29 x10^6 cells/kg) but the difference was not significant (p=NS). Few patients in both groups failed mobilization, with no statistical difference: 7 patients (6.9%) after infusional-DCEP vs 3 patients (6.5%) after DCEP-short (p=NS). Thus most of the patients in the two groups achieved an adequate number of CD34$^+$ cells for the autotransplant procedure (more than 4 x 10^6 cells/Kg): 76.4% after infusional-DCEP and 79% after DCEP-short (p=NS). Toxicity was limited with both schedules. In fact, even though grade III neutropenia was more frequent in group II than in group I, without statistical significance (12 group I patients, 11%, vs 11 group II patients, 25%), the number of patients requir-

ing hospitalization for severe infection was low in both groups (5 group II patients, 4.7%, vs 2 group I patients, 4.5%). In both groups there were no hemorrhagic events and no need for platelet support, even if the incidence of grade II thrombocytopenia was significantly higher in II group (7 group II patients, 6%, vs 14 group I patients, 30%; p=0.009).

Conclusions. DCEP-short maintains the same efficacy of the infusional-DCEP in mobilizing stem cells with a comparable low toxicity but with the clear advantage of an easier management of the patients .

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COMBINATION OF THALIDOMIDE, DEXAMETHASONE AND PEGYLATED LIPOSOMAL DOXORUBICIN (THADD) IN ELDERLY PATIENTS WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA

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New treatment options are needed to improve the outcome of elderly patients with newly diagnosed multiple myeloma (MM). I an attempt to explore strategies aimed at achieving this, we combined thalidomide with dexamethasone and pegylated liposomal doxorubicin since this latter agent has shown to exert cytotoxic and antiangiogenetic activities with manageable toxicity in MM.

Within a prospective, multicentric study, newly diagnosed MM patients older than 65 years received thalidomide 100 mg/day (continous), dexamethasone 40 mg days 1-4 and 9-12 of each month and pegylated liposomal doxorubicin 40 mg/sqm on day 1. ThaDD combination was administered every 4 weeks for 4-6 cycles and for eligible patients transplantation was planned. All patients received antithrombotic and antimicrobial prophylaxis with warfarin (0.25 mg/day) and ciprofloxacin, respectively. Toxicity was graded according to WHO criteria.

At February 2005, 37 patients have been enrolled on the study and 32 (19 male, 13 female) are valuable for response and toxicity. Median age was 72 years (range 66-78) and 21 patients (65%) were older than 70 years. The myeloma subtypes included 18 IgG (56%), 10 IgA (31%), 2 light chain only (6%) and 2 non-secretory (6%). Twenty-five patients (78%) had stage III disease while 7 patients (22%) showed PS more than 2. Twenty-three patients (72%) had intermediate-high risk disease under the IPI-MM and one third had unfavourable cytogenetics. According to Bladè response criteria, 9 patients (28%) achieved a CR, 3 (9%) nCR, 4 (12.5%) a VGPR, 12 (37.5%) a PR and 2 (6%) a MR resulting in an overall response rate of 93%. Only one patient had progressive disease and another one died after the first course of ThaDD. Six patients (18%) set going to high-dose therapy and autotransplant. After a median follow-up of 12 months (range 4-24), 5 patients had disease progression and 4 died. Projected 2-years PFS, EFS and OS were 70%, 67.5% and 75%, respectively. Overall, we administered 124 courses of ThaDD regimen. No patients required a treatment reduction but 5 (15.5%) delayed therapy because of the occurrence of infections in 4 patients and of palmar-plantar erythrodysesthesia (PPE) in another one. One patient who developed pulmonary embolism chose to discontinue therapy. The most common more than grade 2 adverse events were constipation (6%), fatigue (3%) and tremors (3%). PPE occurred in 5 patients (16%; 3 grade 2 and 2 grade 1) and 3 patients (9%) had DVT. No patients developed more than grade 1 peripheral neuropathy and no patients had grade 3-4 alopecia. More than grade 2 neutropenia was observed in 3 patients (9%) whereas no patients experienced more than grade 2 thrombocytopenia. Twelve (10%) of 124 courses of ThaDD administered were complicated by more than grade 2 infections but no patients died from it.

In conclusion, these preliminary results suggest that ThaDD combination is feasible and highly effective in elderly patients with untreated multiple myeloma.

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TECHNETIUM-99M SESTAMIBI SCAN MAY IMPROVE DISEASE ACTIVITY ASSESSMENT IN TREATED AND UNTREATED MYELOMA PATIENTS

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Technetium-99m sestamibi scan has been reported to identify site of occult disease in multiple myeloma. To better assess the extent of disease in patients of our institution, we decided to perform technetium-99m sestamibi either in new cases of multiple myeloma either in treated ones. Since January 2005 we studied 17 patients, 9 untreated and 8 treated. 740 MBq of MIBI were i.v. injected and total body scan was acquired 30 minutes later. We graded the bone marrow MIBI uptake accordingly to a 4-point scale: 0=absent; 1=mild uptake in the axial skeleton; 2=moderate uptake in the axial skeleton; 3=intense uptake both in the axial skeleton and proximal femur and homerus. Moreover focal areas of uptake were recorded.

Treated patients: in three patients the clinical steady state was confirmed by an uptake of 1; in 1 and 2 patients the clinical disease progression was confirmed by an uptake of 3 and 2, respectively; a patient giving a pre-transplant debulking recorded a 2 point uptake; a patient in clinical steady state after autologous transplant, with many lytic areas irradiated previously, recorded a 0 point uptake.

Untreated patients: a 3 point and a 2 point uptake was registered by 3 patients and 1 patient, respectively, affected by stage III disease. In 1 patient in stage II disease a 1 point uptake was recorded. 1 patient in steady state was found to have a 2 point uptake. 3 smouldering myeloma showed a 1 point uptake.

In conclusion, MIBI is a useful additional diagnostic tool for assessing the extent of disease at diagnosis; moreover,

in treated patients it is helpful for evaluating the presence of ongoing disease activity in irradiated sites remaining abnormal on skeletal survey following treatment.

Focal areas have been detected either in treated or in untreated patients and they seem to correlate with sites of disease activity rather than with lytic lesions.

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CNS INVOLVEMENT IN MULTIPLE MYELOMA: A CASE REPORT

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In August 2003 a female of 62 years-old (A.R.) come to our observation for collapse of various vertebral bodies (L1-L3-D9-D10) accompanied with a monoclonal protein (M protein) in the serum - IgG lambda (18 g/L) - and a positive Bence-Jones protein. At the beginning there was no neurological involvement. The bone marrow aspiration showed an infiltration of plasma-cells of 50%. We make the diagnosis of multiple myeloma IgG lambda stage IIIA. She underwent polychemiotherapy with VAD regimen.

A MRI confirmed lytic bone lesions of D8-9-10-L1-3. After the third VAD regimen, the serum monoclonal protein appeared to be stabilized. The patient was, then, admitted to the hospital for acute mental confusion. At the cerebral MRI: presence of a lesion of brain origin in the right upper frontal district, which was outside the brain but pressing brain tissue; a myeloma-like lesion; scattered leptomeningeal abnormalities like mielomatous meningitis; general alteration of the skul and of anterior basis of the head. Few days later the patient died.

Conclusions. the brain involvement in multiple myeloma is relatively rare. There are only few reports in international literature which describe a similar situation.

In our case report the progression of CNS involvement was together with a small or totally absent positive reaction to polychemiotherapy. It seemed to us a very interesting clinical history for the peculiarity of radiological imaging (MRI), which shows the CNS involvement.

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PEGFILGRASTIM COMBINED WITH STANDARD CHEMOTHERAPY EFFECTIVELY MOBILIZES PBSC IN A POOR MOBILIZER MULTIPLE MYELOMA PATIENT

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A recently polyethylene glycol (PEG)-coniugated form of G-CSF has been introduced and studies performed on mice and healthy human volunters have shown that a single dose of pegfilgrastim is effective in stimulating the mobilisation of PBSC. Moreover, the peak of peripheral $CD34^+$ cells appeared 1-2 days earlier and the peak in PBSC was three fold greater than that attained with daily filgrastim. This prompted us to try pegfilgrastim stimulation in a patient previously non mobilizing with the combination chemotherapy plus filgrastim. In December 2002, a 65-year-old man was diagnosed as having stage III A IgG/k multiple myeloma. He received three couses of polichemotherapy (DC-IE) obtaining a stable response. Afterwards, the patient was treated with 7g/mq cyclophosphamide plus daily 10 micrograms/kilogram G-CSF in order to mobilize PBSC, without success. The maximum number of $CD34^+$ cells in the peripheral blood was 8/microlitre, observed on day + 13 after the end of chemotherapy. After two months off therapy, the disease progressed and the patient received alternate cycles VAD/high dose dexamethasone. A second attempt to mobilize PBSC, using daily 10micrograms/kilogram G-CSF after the second and third VAD cycle failed; the maximum number of $CD34^+$ cells was 10/microlitre and 8/microlitre after the II and III VAD, respectively. In a further attempt to mobilize PBSC, we administered a single dose of 12 mg pegfilgrastim on day 5 after a fourth VAD course. Daily evaluation of circulatory $CD34^+$ cells was started from day 8 after the end of chemotherapy. On day + 10 post chemotherapy the $CD34^+$ cell count was 24/microlitre and two apheresis were performed, harvesting 1,6x106 and $0,89x10^6$ $CD34^+$ cell/kilogram, respectively; (total $2,49x10^6$ cells/kilogram). The only side effect was moderate skeletal pain.

In conclusion, a single dose of pegfilgrastim, administered even after a VAD chemotherapy cycle is able to mobilize a sufficient number of $CD34^+$ in multiple myeloma patient, previously not responsive to high dose cyclophosphamide followed by filgrastim.

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IGM MGUS AND INDOLENT WALDENTROM MACROGLOBULINEMIA RECOGNISE THE SAME DETERMINANTS OF EVOLUTION INTO SYMPTOMATIC LYMPHOID DISRDERS. PROPOSAL FOR A COMMON PROGNOSTIC SCORING SYSTEM

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We have evaluated which clinico-hematological variables at diagnosis were prognostically related to neoplastic progression in patients with IgM monoclonal gam-

mopathies of undetermined significance (MGUS), and indolent Waldenström macroglobulinemia (IWM); we have also individuated a scoring system able to identify subsets of patients at different risk of evolution. We analysed 217 patients with IgM MGUS (serum MC and bone marrow infiltration less than 3 g/dL and 10%) and 201 with IWM (serum MC > /= 3 g/dL and/or bone marrow infiltration >/= 10%): male/female 131/86 and 117/84, mean age 63.7 and 63.6 years, respectively.. After a median follow-up of 56.1 and 60.2 months, 15/217 MGUS and 45/201 IWM patients required chemotherapy for symptomatic WM (13 and 36), non-Hodgkin lymphoma (2 and 6) and amyloidosis (0 and 3). Median Time To Evolution was not reached for MGUS and was 141.5 months for IWM. The variables adversely related to evolution were qualitatively the same in both groups: MC levels, Hb concentrations and gender. Males with MGUS and IWM respectively had a 4.182 and 2.061 RR of evolution (vs females); any unit decrease in serum haemoglobin multiplied the risk of evolution by a very similar factor (0.725 in MGUS patients and 0.685 in IWM patients). Serum MC concentration had different prognostic power in the two groups (1 g/dL increase was associated with a RR of 3.995 in MGUS and 1.340 in IWM). A scoring system based on these parameters identified three risk groups with highly significant differences in TTE in both groups (p<0.0001). In conclusion MGUS and IWM identify disease entities with different propensities for symptomatic neoplastic evolution. Both have the same prognostic determinants of progression; we here propose a practical scoring system able to identify different risks of malignant evolution and that may allow an individualised clinical approach.

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NOT PUBLISHED

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LOW-DOSE VELCADE,THALIDOMIDE AND DEXAMETHASONE; AN EFFECTIVE REGIMEN FOR RELAPSED AND REFRACTORY MYELOMA

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Despite high dose chemotherapy and bone marrow transplantation had improved response and survival, relapse is still virtually universal and MM remains incurable. Proteasome inhibition can potentially target multiple signalling pathways that are critical for tumour cell growth and survival. The SUMMIT and CREST phase II studies established that bortezomib (VELCADE, PS-341), is effective and well tolerated in patients with relapsed and refractory MM. Since pre-clinical and clinical data suggest combination regimens could be pursued given the different mechanisms of action of bortezomid and thalidomide and their non-overlapping toxicity, from January 2004 R/R MM patients referred to our Institution were designed to receive a combination therapy with VELCADE and thalidomide plus dexamethasone. Patients, irrespective of age, PS and life expectancy, were enrolled in the study once they had a measurable disease. Treatment: VELCADE 1.0 mg/m^2 i.v. twice weekly for 2 weeks of a 28-d cycle for up to six cycles, oral dexamethasone 24 mg on the day of and the day following each VELCADE dose and thalidomide 100 mg each evening. Responding patients received two more cycle as consolidation. Deep vein thrombosis prophylaxis with coumadin to maintain international normalized ratio between 2.0 to 3.0 was planned in all patients if not contraindicated.

Results as of 10 March 2005, 22 were the treated patients. Median age 65 years, median time from diagnosis 6 years, a median of 4 previous therapy lines and they all had received thalidomide. 13 (59%) were the patients who never achieved an objective response with previous therapies and were thus considered as primary refractory. 9 (41%) were those relapsed and refractory to the last therapy; 5 of them relapsed after a bone marrow transplant A median of 6 courses (range 2-8) were delivered. One patient, with a severe pre-existing cardiac impairment, died during the first course by heart failure and was thus invaluable for response. Among 21 valuable patients, 12 (57%) were the responders: 2 CR, 8 PR, 2 MR. Six patients showed a stable disease after 1-3 cycles and 3 patients had a progressive disease. The median time to best response was 2 months (range 1-4). After a median follow-up of 9 months (range 2-14), 17 patients are alive and 4 were died (2 disease progression,1 heart failure,1 gastrointestinal bleeding). Haematological toxicity was negligible. The occurrence of cytopenia was transient, with recovery within the rest period. Except for those patients accrued with low haemoglobin and platelet levels, none of the others needed red blood cells or platelet transfusions. Overall, the most commonly reported adverse events were fatigue (50%) and nausea (22%). A clinical progression of neuropathy during therapy was recorded in one patient but resolved once thalidomide was withheld. A significant improvement of PS was registered in responding patients. Except for the first cycle, delivered in hospital, subsequent cycles were delivered on outpatient basis. Our regimen with lower doses of VELCADE in respect to those usually applied, plus low dose thalidomide and dexamethasone appears efficacious. The ORR of 57% we registered, with 2 patients achieving a CR, is superior in respect to an ORR of 35% reported in the SUMMIT study and compare favourably with those registered in the CREST study within a more favourable subset of patients.

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LONG-TERM RESULTS FOLLOWING HIGH-DOSE CHEMOTHERAPY AND PERIPHERAL STEM CELL AUTOTRANSPLANT IN A PROSPECTIVE COHORT OF PATIENTS WITH MULTIPLE MYELOMA

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High dose chemotherapy (HDT) with peripheral stem cell support is presently considered the best treatment for patients with symptomatic multiple myeloma (MM). To optimise the selection of patients who can benefit of this procedure, predictors of response should be identified. The

aim of this study was to evaluate the long-term outcome of MM patients according to a number of clinical and laboratory pre-autotransplant (autoTx) variables, with particular focus on disease status.

We retrospectively evaluated the overall (OS) and event-free (EFS) survivals, and prognostic factors of 110 newly diagnosed symptomatic MM patients who entered a program of HDT with tandem autoTx (TTI). Ninety-six patients (87%) received the first autoTx, and 79 (72%) completed the program. Three patients (2.7%) died during induction therapy and 11 were shifted to other programs. Patients were followed-up for 2-98 months (median, 44) from the beginning of treatment. Twenty-nine (26%) patients were in complete remission (CR) or very good partial remission (VGPR) at the end of induction therapy. The rate of CR/VGPR increased to 73% and 87% after the first and the second autoTx, respectively. Overall median OS was 62 months and median EFS was 40 months. By univariate analysis, performance of the first autoTx and patient's status at the end of induction therapy were the major determinants of EFS and OS. By multivariate analysis. The majority of patients performed at least one autoTx and achieved CR/VGPR after the first and the second autoTx with acceptable toxicity. However, only patients in CR/VGPR before the first autoTx enjoyed significantly prolonged EFS and OS. This suggests the need to improve the induction therapy to increase the rate of early response.

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THALIDOMIDE AND BRADYCARDIA: A LOW INCIDENCE BUT AN IMPORTANT ADVERSE EFFECT

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Thalidomide is widely used in relapsed and refractory Multiple Myeloma, and has been recently proposed in first line therapy. Its most frequent toxic effects are neurotoxicity and deep vein thrombosis, while cardiac toxicity has been less commonly reported. We describe a patient with symptomatic bradycardia during thalidomide therapy for Multiple Myeloma, who was able to continue treatment after dose reducing and pace-maker implant.

Case Summary. A 61 years old woman with a history of relapsed Multiple Myeloma after autologous peripheral blood stem cell transplantation, without other comorbidities or concurrent medications that decrease heart rate, was treated with THALI-DEX schedule for five months with a very good partial response. The dose of thalidomide was 200 mg daily.

During the treatment, at the end of the fifth month, her heart rate decreased from a base line of 70 beats/min to as low as 32 beats/min with syncopes and positive electrocardiogram findings of sinus bradycardia. Thalidomide was stopped and a 24/hour Holter monitoring was performed. The results confirmed sinus bradycardia (lower registred heart rate was 23beat/min during the night) without underlying cardiac disease. After thalidomide discontinuation and a short pharmacological therapy, stopped because of patient intolerance, her bradycardia had a partial and transitory improvement (heart rate 57 beats/min). Because of good therapy response obtained and lack of other therapeutic choices (failure of new stem cell collection attempt), thalidomide was restarted at 100 mg daily after a single-lead pace-maker implant; the patient is still receiving the drug without cardiac toxic effect and with a very good haematological partial response maintenance.

Discussion and Conclusions. Clinical trials suggest that bradycardia, during thalidomide treatment, occurs less commonly than other adverse effects. Despite of low incidence and the mild entity generally reported, this collateral effect may be, sometimes, as in our case, very severe, even in young patients and without concurrent cardiac diseases or medications affecting the heart rate. Moreover it seems to be cumulative dose independent. Therefore, it should be recommended closely cardiological monitoring not only in elderly or in patient with cardiac comorbidities, but in all subjects during thalidomide administration, also in young ones, in which the drug is actually used more and more as front line therapy. In case of symptomatic marked bradycardia, if it is closely necessary to continue thalidomide therapy, it can be considered pacemaker implanting.

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INFLUENCE OF ZOLEDRONIC ACID ON CYTOKINES BEHAVIOUR IN MULTIPLE MYELOMA

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One of the most common features affecting the quality of life in multiple mieloma (MM) is the presence of bone lesions due to an uncoupling of bone resorption from bone formation so that resorption predominates leading to a constant progression of the osteolytic lesions. MM plasmacells, in the bone marrow, are in close contact with stromal elements and induce the release of several cytokines such as IL 6, TNFα, and Insulin like growth factor (IGF). These mediators modify the bone marrow microenvironment, upregulating RANKL expression and secretion by both stroma and osteoblasts. Destruction of bone matrix induced by MM and its cellular interactions is accompanied by further release of IL 6 and IGF. Biphosphonates selectively concentrate at the interface of the active osteoclasts and the bone resorption surface inhibiting osteoclasts activity. Recently, in solid tumors, Santini *et al.,* (Clinical Cancer Research, 2003, 9:2893) demonstrated that zoledronic acid could have an *in vivo* antiangiogenic property through a significant and long-lasting reduction in serum VEGF levels. Taking into account that angiogenesis play an important role in MM progression we have investigated the behaviour of several cytokines (IL6, PDGF, IGF, TNF alpha and VEGF) in patients with MM treated with zoledronic acid. Twenty-eight consecutive patients (13 female and 16 males) with a diagnosis of multiple myeloma and lytic bone lesions, were included in this study. All patients

received 4 mg of Zoledronic acid (Zometa, Novartis) in 250 ml of 0.9% saline over a period of 15 minute as in i.v. infusion. Venous blood for cytokine assessment was drawn into a serum tube just before the beginning of drug infusion and again at 1,2,7,21 days after the Zoledronic acid infusion . PDGF, VEGF, IL6, TNFα, and IGF-I were assayed with the R & D quantitative kits according to the manufacturer's instructions (R & D Systems, Minneapolis, USA). Basal cytokine levels were compared with the values observed at 1, 2, 7 and 21 days after zoledronic acid infusion using the Wilcoxon's test for nonparametric-dependent continuous variables. Differently from what observed by Santini et al in solid tumors, serum VEGF median levels significantly increased at days 7 (p=0.0047). Moreover, IL 6 and TNF alpha levels significantly increased after 1 (p=0.0000 and p=0.0001 respectevely) and 2 (p=0.0000 and p=0.0021 respectevely) days. Futhermore, PDGF significantly decreased (p=0.005) after 2 days following zoledronic acid infusion .Among these pts, 8 (28.5%) were receiving also Interferon; no statistically significant differences in the levels of VEGF, IL 6, TNF alpha and IGF between the two groups were observed . Only the PDGF levels were statistically reduced in the interferon group at 0, 1, 2, 7, 21 days. (p<0.05). In conclusion, in MM, the administration of zoledronic acid, differently from what observed in solid tumors, did not decrease the VEGF levels. This behaviour may partially explain why zoledronic acid has no effect on MM progression as originally postulated.

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GRAFT VERSUS MYELOMA EFFECT AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A CASE REPORT

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Allogeneic stem cell transplantation (SCT) is a therapeutic option for young patients affected by multiple myeloma (MM); however, its role in the therapeutic strategy of these patients is still controversial. Its potential superiority to other high-dose approaches lies on a putative graft versus myeloma effect. We report the case of a 37 year old man with an aggressive form of micromolecular MM (urinary kappa chain 572 mg/L, massive marrow infiltration by plasma cells), presenting with multiple vertebral fractures and presence of neoplastic tissue compressing the spinal cord and leading to paraplegia. He was initially treated with polichemotherapy including vincristine, adriamicine and dexamethasone, achieving a partial response. After mobilization by high-dose cyclophosphamide, he underwent to autologous stem cell transplantation, without reaching complete remission. After six months he started a salvage treatment with thalidomide for disease progression (urinary kappa chain 874 mg/L, 20% marrow plasma cells), and the search for an unrelated donor through the IBMDR was activated. Three-years after diagnosis of MM he received a SCT from a matched unrelated donor with a conditioning regimen with busulfan, melphalan and antithymocyte globulin. GvHD prophylaxis consisted of short course methotrexate and cyclosporine A (CsA); the engraftment was achieved at day +28 from transplant, with no major complications. Evaluation at +2 months showed complete remission of MM (undosable urinary kappa chain, 5% marrow plasma cells), while grade I cutaneous acute GvHD appeared. Immunosuppressive therapy was adjusted targeting a blood concentration of polyclonal CsA above 300 ng/mL. At +6 months from transplant the patient showed a progressive increase of the urinary paraprotein; CsA therapy was immediately stopped. In the following weeks the patient developed moderate chronic GvHD with skin and liver involvement, with alteration of hepatic parameters; at the same time a relative lymphocytosis was observed. Urinary paraprotein decreased in concomitance with the clinical GvHD, suggesting a close relationship among the allo-response and tumor activity. CsA was reintroduced at very low dose (blood concentration below 100 ng/mL) to control GvHD symptoms; liver parameters as well as skin lesions normalized in a few weeks. At two years from SCT, the patient is still in complete remission of his MM, without GvHD or other SCT complications, free from any treatment since 1 year. This patient seems a clear demonstration that allogeneic SCT may rescue refractory MM patients through a graft versus myeloma effect. Even if this effect is closely related to a more general GvH reaction, accurate management of immunosuppressive therapy may consent a balance between GvH and graft versus tumor effect. We are now studying the biological features of immune response after SCT at the level of T cell receptor, aiming to biologically discriminate between these closely related allo-responses for selective adoptive immunotherapy purposes.

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COMBINATION OF THALIDOMIDE, CYCLOPHOSPHAMIDE AND DEXAMETASONE IN ELDERLY PATIENTS WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA

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We and others have previously demonstrated the efficacy of a combination including thalidomide (200 mg/die, with a starting dose of 100 mg), low dose of cyclophosphamide (100 mg/day) both continuously and pulse dexametasone (40 mg/day for 4 days every month) in relapsed/refractory Multiple Myeloma patients. We therefore applied this scheme to 16 elderly previously untreated patients (10 females and 6 males). Median age was 75 years (range 60-81), median Hb 11.2 gr/dL, median albumin 3.1 g/dL and median B2 microglobulin 4 mg/L. Treatment was scheduled to be continued for 12 months. However, in only two patients the dose of thalidomide was increased to 200 mg and a total of 8 patients (50%) discontinued thalidomide prematurely. Four patients refused thalidomide due to intolerance (dizziness, confusion, abdominal pain, depression, neurotoxicity, tremor) after 1 month (3 patients) and after 3 months (1 patient) respectively. In 4 patients thalidomide was stopped after 4-6 months due to ipoacusia (1 patient), skin toxicity (1 patient) and deep vein thrombosis (2 patients). In all of these patients cyclophosphamide and dexamethasone were maintained as sched-

uled with some adjustments according to blood cell count. After a median follow up of 16 months, 3 patients (19%) achieved a CR (one IF negative), 8 patients (50%) a PR with a reduction of the monoclonal component (MC) between 52 and 87 %, 2 patients a reduction of MC < 50 %, 2 patients a stable disease and 1 patient progressed while on treatment. Although the sample is too small for a statistical analysis, we observed that a major response (CR + PR>50%) was observed in only 4/8 of patients that discontinued thalidomide vs 7/8 patients treated with thalidomide for the entire course. In this study we have observed a rate of thalidomide discontinuation higher than that we have recorded with the same schema in previously treated myeloma patients. Therefore, after the first 3 patients, we decided to withheld thalidomide for the first two months and to add this drug only at the third month of treatment. However, this measure was not sufficient to reduce the high rate of thalidomide dropout. We conclude that in elderly myeloma patients at diagnosis thalidomide is effective but not very well tolerated. It is possible that doses as low as 50 mg/day might be more appropriate for the use in combination with chemotherapy in a high proportion of this setting of patients.

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ANTIVIRAL PROPHILAXIS IN RELAPSED MULTIPLE MYELOMA PATIENTS RECEIVING BORTEZOMIB: SINGLE INSTITUTION EXPERIENCE

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Background Bortezomib. The first-in-class proteasome inhibitor, has been recently approved for the treatment of patients with refractory or relapsed Multiple Myeloma: it inhibits the activation of NF-kappaB, which controls the genes encoding IL-6, TNF-α; and other cytokines and growth factors.

Methods. In our experience 5 patients (2 F, 3 M; median age 61 yr) with relapsed Multiple Myeloma after conventional chemotherapy and autologous PBSC transplantation were treated with BORTEZOMIB. BORTEZOMIB was administered by IV push at a dose of 1,3 mg/m^2 on days 1, 4, 8 and 11 of a 21-day cycle, for a maximum of eight cycles.

Results. Four out of five patients completed the scheduled treatment and were evaluated for response; one patient did not complete the therapy because of progression disease. Three patients achieved a partial response and 1 patient a minimal response according to the EBMT/IBMTR criteria. In our experience 2/4 patients presented severe neutropenia which required the use of G-CSF; only 1 patient was hospitalizated for septicaemia. In 3/4 patients platelet infusions were performed for severe thrombocytopenia. But, the most clinically relevant adverse event (3/4 patients) was 3° WHO disseminated Herpes zoster; 1 patient developed viral infection 16 days after the end of treatment while in 2 patients Herpes zoster showed during treatment; in these two cases BORTEZOMIB was momentarily discontinued. Valaciclovir (1000 mg three times daily for 7 days orally) was given as therapy against viral infections. The clinical presentations resolved completely after antiviral therapy. In our experience no CMV reactivation was found. All our patients, evaluated before treatment with BORTEZOMID, showed a deep depletion of CD4+ T-cells (median 150 cells/μL [reference values 500 – 1300/μL]) for immunosuppressive properties of previous treatments; but, despite an increased risk of developing opportunistic infections, during the long-term follow-up, no evidence of viral infections was detected.

Discussions. Our experience confirms the recent data about the increased incidence of Herpes zoster in relapsed Multiple Myeloma patients receiving BORTEZOMIB. These clinical observations are of particular relevance. In our patients the previous regimens together with the high-dose myeloablative chemotherapy for autologous PBSC transplantation were certainly responsible of T-helper depletion; nevertheless, the decreased levels of CD4$^+$ T-cells were never associated with severe viral infections. The subsequent treatment with BORTEZOMIB did not contribute a further depletion of the total number of CD4$^+$ cells. Some authors have demonstrated that BORTEZOMIB suppresses *in vitro* T-cell responses, inhibits cytokine production; studies conducted in non-human primates have indicated that in WBCs 1 h after BORTEZOMIB treatment proteasome activity decreases significantly. Thus, CD4+ T-cells of our patients might be dysfunctional; their anergy might be the consequence of the inhibition of the ubiquity proteasome system. Further studies of BORTEZOMIB and additional clinical observations are now needed to establish and explore its full spectrum of activity. *Conclusions.* In our experience, the high incidence of Herpes zoster, despite the small number of patients, suggests that viral infections might be probably related to an intrinsic defect of memory CD4+ T-cells specific for VZV, induced by BORTEZOMIB. Because of an increased risk of developing opportunistic infections, we recommend anti-infective prophylaxis with antiviral drugs and trimethoprim-sulfamethoxazole all therapy long and until at least three months after drug discontinuation as well as monitoring CD4+ cell levels.

Platelet Disorders

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A CASE OF REFRACTORY CHRONIC AUTOIMMUNE THROMBOCYTOPENIC PURPURA TREATED WITH T DEPLETED AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background. myelo- and immunosuppressive chemotherapy followed by autologous peripheral blood stem cell (PBSC) transplantation is a sperimental approach for severe chronic autoimmune thrombocytopenic purpura (AITP) refractory to conventional treatments. T-cells depletion of the graft needs to reduce autoreactive clones. Infact T lymphocytes may initiate and maintain immune recognition of autologous platelets (PLT) and stimulate B lymphocytes to produce anti-PLT antibodies. Theoretically, autoreactive T lymphocytes collected in PBSC graft could re-establish autoimmunity, so immunologic purging of T-cells is as a fundamental condition for the favourable outcome of transplantation.

We report a 60 years old female patient affected by AITP, refractory to conventional therapy: steroids, intravenous immunoglobulins, splenectomy and immunosuppressive agents as cyclophosphamide, cyclosporine, vincristine, azathioprine. Therefore she was submitted to T-cell-depleted autologous PBSC transplantation two years after the onset of the disease.

Methods. PBSC were mobilised with cyclophosphamide (2,5 g/m^2) and G-CSF (10 micrograms/Kg/die), and collected by a single leukapheresis. CD34$^+$ positive immunomagnetic selection (CliniMACS, Miltenyi) allowed to select stem cells and to remove T-cells from the graft. The final dose of CD34$^+$ cells was 7.2x10^6/Kg, and the residual dose of CD3+ cells in the graft was 1x10^5. Conditioning regimen was Melphalan 100 mg/m^2.

Results. Neither major adverse events nor infection were observed during and following the transplant phase. Mild epistaxis and mouth bleeding were controlled by PLT transfusions during pre-engraftment phase. Neutrophil recovery (>500/mm^3 was observed from day +9 and PLT recovery (>20000/mm^3) from day +18. Asymptomatic CMV reactivation was documented only on day +29 (CMV antigenemia: 12/200.000 cells) and it was not confirmed during successive weekly controls.

Antiviral (Acyclovir) and anti-P. Carinii (Trimetoprim-Sulfametoxazole) profilaxis was continued for 6 months. After a follow-up of 7 months the patient shows a good response with a stable PLT count around 100.000/mm^3 despite an initial reduction immediately after the engraftment.

Conclusion. T cell-depleted autologous PBSC transplantation may be considered a feasible therapeutic option in refractory severe AITP, so as for other refractory autoimmune disorders, but larger studies are still required to demonstrate the percentage and the durability of the remission and the real risk/benefit profile.

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RITUXIMAB MAY ALLOW TO ACHIEVE LONG-LASTING REMISSION WITH FAVOURABLE TOXIC PROFILE IN ADULT PATIENTS WITH AUTOIMMUNE THROMBOCYTOPENIAS

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Background. Previous reports highlighted the potential mid-term therapeutic activity and safety of Rituximab in adult patients with autoimmune thrombocytopenias. Objective of this study was to evaluate the long-term efficacy and toxicity profile.

Patients and methods. From October 1999 to December 2002, 32 adults patients with active and symptomatic autoimmune thrombocytopenias that had relapsed or were refractory at least to a full course of steroid therapy (25 idiopathic thrombocytopenic purpura, 1 idiopathic thrombocytopenia and neutropenia, 4 thrombocytopenia and concomitant undifferentiated connective tissue disease, and 2 thrombocytopenia and concomitant B-cell lymphoprolipherative disorders) received weekly infusions of Rituximab 375 mg/m^2 for 4 weeks. Only treatment with steroid, if strictly necessary to maintain a safe number of platelets, was allowed during the period of Rituximab administration. A complete and partial response (CR, PR) was defined as a platelet level higher than 100.000/mmc and 50.000/mmc, respectively. Patients who necessitated of steroid administration to maintain a safe number of platelets during Rituximab therapy were considered responders only if a steroid discontinuation (previously not possible) was achieved.

Results. Patients' median age was 56 years (range 16-76 years), the median period from diagnosis to Rituximab was 24 months (1-264 months) and the platelets median count before Rituximab was 19.000/mmc (range 4.000-57.000/mmc). All patients completed the therapeutic program as planned, without occurrence of grade III-IV acute toxic events. Overall, CR and PR were 23/32 (72%), 20/32 (63%), 3/32 (9%), respectively. The median time of observation, in responding patients, is 21 months (range 4-45 months). Five patients (22%) (4 in CR and 1 in PR) relapsed after 4, 4, 6, 8, 16 months. Globally, after Rituximab therapy, 18/32 (56%) patients achieved and maintained a sustained response without need of further therapy. During the period of observation no infectious or other major toxic complication were documented.

Conclusions. Rituximab administration may allow to achieve long-lasting remission in nearly 50% of patients suffering from autoimmune thrombocytopenias without occurrence of toxic events.

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APPROPRIATENESS OF ITP HOSPITAL MANAGEMENT IN ADULT MEN AND NON-PREGNANT WOMEN: INITIAL VALUATION AT A SINGLE INSTITUTION FINALIZED TO A RETROSPECTIVE MULTICENTRIC STUDY

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Background. ITP is a rare clinical condition whose incidence, without a very different distribution between the two genders, is 3 per 100,000 per year in children under 16 years of age and 1.6 - 2.68 per 100,000 in adults. Few doctors, such as paediatricians, haematologists, or obstetricians, see many patients with very low platelet counts and this fact may produce divergence in practice from guidelines, as an audit on the management of acute ITP in childhood reveals.

Aim of the study: (i) To test whether the management of adult men and non-pregnant women hospitalized due to ITP at a single institution was in accordance with available guidelines. (ii) to report the results of the study to doctors engaged in the treatment of these patients; (iii) to verify the practicability of available guidelines in adults out of pregnancy; (iv) to point out suggestions for new practicable guidelines.

Materials and Methods. Initial data were obtained from the computerized discharge diagnosis records of a two and a half year period (January 1st 2000 – June 30th 2002) at Niguarda Ca' Granda Hospital, Milan. All coded 287.3 (ICD-9-CM: idiopathic thrombocytopenic purpura) hospital discharge diagnoses were collected. We examined in the first 48 coded 287.3 hospital charts of adult people out of pregnancy the appropriateness of ITP diagnosis and treatment according to available American and British guidelines.

Results. A total of 85 hospitalizations with 287.3 code (92,191 the total number of hospitalizations) took place at Niguarda Ca' Granda Hospital, Milan in the considered period. The ITP diagnosis appeared to be verified in only 56.2% of the first 48 coded 287.3 hospital charts of adult people out of pregnancy that we examined. In 27 patients in which ITP diagnosis was verified, the causes of hospitalization were: (i) need of hospital diagnosis or observation (1/27; 3.7%); (ii) medical treatment that could not be deferred (13/27; 48.1%); (iii) elective splenectomy (7/27; 25.9%); medical or surgical treatment of associated disease (6/27; 22.2%). According to ASH panel opinion regarding initial ITP treatment options in adult men and non-pregnant women, hospitalization was appropriate in patients who received medical treatment (7/27; 25.9%) and in those that underwent elective splenectomy (7/27; 25.9%) or were treated because of medical or surgical associated diseases (6/27; 22.2%), while the appropriateness of hospitalization was uncertain in 7/27 cases (25.9%). During hospitalization, observation without treatment was produced (for one day) only in one of 27 cases (3.7%) and it was inappropriate. Glucocorticoid treatment was appropriate in all 14 of 27 patients who needed medical treatment for ITP. However, one of these patients (7.1%) received higher than standard steroid doses and this treatment could be considered inappropriate. IVIg, as medical treatment for ITP, was appropriate in 6 of 8 patients (75%), while its appropriateness was uncertain in 2 of them (25%). Platelet transfusions, as medical treatment for ITP, were used in only 2 patients. One of them received platelet transfusions at two different times of the same hospitalization and once this use was inappropriate. Platelet transfusions were inappropriate also in the second patient who received them. The indication for splenectomy was appropriate in all 7 patients who underwent it electively. However, prophylaxis against bleeding before splenectomy and prophylaxis reducing bacterial infection risk after splenectomy were inappropriate in respectively 1/7 (14.3%) and all 7 cases.

Considerations. Present data on ITP hospital management, retrospectively obtained from a single institution, are very limited to consent definitive conclusions. More complete data can help doctors engaged in the treatment of these patients to verify their adhesion to available guidelines. Suggestions useful to formulate new guidelines can be obtained from a multicentric study.

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RETROSPECTIVE STUDY OF 165 PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA. THE ROLE OF SPLENECTOMY

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We retrospectively analyzed the data on 165 pts with immune thrombocytopenic purpura (ITP). The median platelet count at diagnosis was 42 (range 0-130). The median age at diagnosis was 48.3 (range 4-87). 21 pts were positive for HBV and 12 for HCV. 29 pts were positive for antiplatelet-autoantibody, 12 for LAC, 27 for ANA, 4 for ACA, 4 for ASMA, 4 for ENA, 3 for APCA, 4 for antiphospholipid autoantibody, 3 for thyreoglobuline autoantibody. 3 pts were positive for Helicobacter pylori: antibiotic therapy didn't increase the platelet count. 59 Pts were observed because the platelet count was greater than $30x10^9$/L without bleeding symptoms. 106 Pts were treated; their mean platelet count at diagnosis was 26 (range 0-118); their mean age was 47 (range 8-87). 48 pts had a platelet count < $10x10^9$/L and significant bleeding symptoms. All pts received steroids as the initial treatment. 92 pts were treated within 3 months from diagnosis, while 14 were treated 4-156 months after diagnosis. After steroid therapy, 45 pts (43%) achieved a complete remission, 45 pts (43%) a partial remission, while 12 pts (11%) were refractory; 4 pts were not evaluable. 49 pts among the 106 treated pts (46%) received a second-line therapy; mean distance from first to second therapy was 34.5 months (range 1-225). Their mean age was 47.5 (range 8-85). Second-line therapies were:

splenectomy (13), azathioprine (4), vinca alkaloids (10), cyclophosphamide (2), alfa-IFN (2), steroid (13) and high dose immunoglobulin alone (5); 5 more pts received high dose immunoglobulin associated with other therapies. 23 Pts were splenectomized after one (15 Pts) or more (8 Pts) medical treatments. At splenectomy the median age was 37.5 (range 11-76); mean platelet count at diagnosis in this group of pts was 23.6 (0-118). Mean time form diagnosis to splenectomy was 22.1 months (range 1-95). 15 pts (65.2%) achieved a CR, 7 (30.4%) a PR, 1 (4%) was refractory. One patient developed deep thrombosis after surgery. We conclude that steroid therapy is effective as the initial treatment in most pts, while splenectomy was effective in most pts and could be performed without significant toxicity.

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VIRULENCE PROFILE OF HELICOBACTER PYLORI STRAINS IN INFECTED PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA

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Recent data have shown that the eradication of Helicobacter pylori (HP) may persistently increase the platelet count in a fraction of infected patients with chronic immune thrombocytopenic purpura (ITP), strongly suggesting that HP may be one of the causes of ITP, at least in some countries (Italy and Japan); instead poor platelet response rates have been reported in two American and Spanish cohorts. The pathogenetic mechanisms of HP-associated ITP have still to be elucidated. To investigate the possible prevalence of some HP virulence factors in ITP patients compared with those non-ITP, with gastro-duodenal disease. Also to study the possible gastric B-cell clonality. In the last two years we enrolled 32 consecutive patients with chronic ITP, Caucasian from Northern Italy, 15 males, 18 females, median age 54 years (range 26-83). All patients were tested for HP infection by 13C Urea Breath Test (UBT) and, when infected, eradicated by a triple therapy. Platelets were counted before and at least 1 and 6 months after eradication treatment. Eight ITP patients HP-positive and eight non ITP patients with gastric disease HP-positive, as control, underwent gastric biopsy for biological studies. To determine the gastric B-cell clonality, we analyzed immunoglobulin heavy chain gene rearrangement using genomic DNA extracted from biopsy of the gastric mucosa (corpus and antrum) by semi-nested PCR amplification. The same extracted DNA was also used for the PCR amplification of HP virulence genes such as cagA, vacA, iceA1, iceA2 and babA2. At UBT, 14 patients (44%) were found positive for HP infection, 13 of infected (92%) were eradicated and 11 of eradicated (79%) showed marked increase of platelet count. The responsive by intention to treat patients were=34%. The median follow-up was 19 months. At hystologic examination, all 8 infected ITP patients were found to be suffering from asymptomatic gastritis, while all controls were suffering from symptomatic gastritis. The B-cell clonality study showed no differences between ITP patients and controls; 5 ITP patients and 5 controls were mono- or oligoclonal, while the remaining ones were policlonal. Concerning the virulence profile, 5 ITP patients showed the expression of at least three genes, cagA, vacA s1/m^2 and ice A1 ("triple positive" strains) in comparison with only 1 control. Only 1 ITP patient with triple positive strains, also expressed the babA2 gene, in respect to 3 controls (1 with and 2 without triple positive strains). The only ITP patient expressing babA2 gene was non responsive to HP eradication treatment.

Conclusions. Our data suggest that infected ITP patients have a gastritis, even if asymptomatic and confirm that a significant proportion of ITP show a sustainable recovery in platelet count after eradication of the bacterium. In respect to virulence profile, considering that HP strains are highly diverse with a variable host immune response; which awareness that the data have to be confirmed in larger series of patients, our findings seem, anyway, provide a useful insight into the pathogenesis of ITP HP-related.

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PATHOGENETIC MECHANISMS OF HEMATOLOGICAL ALTERATIONS OF PATIENTS WITH MYH9 MUTATIONS

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The term MYH9-related disease (MYH9-RD) defines a spectrum of autosomal dominant thrombocytopenias with giant platelets: May-Hegglin Anomaly (MHA, OMIM 155100), Sebastian (SBS, OMIM 605249), Fechtner (FTNS, OMIM 153640), and Epstein syndrome (EPTS, OMIM 153650), caused by mutations in the MYH9 gene, encoding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). Affected patients present since birth macrothrombocytopenia and cytoplasmic aggregates of NMMHC-IIA in granulocytes recognizable by specific antibodies. These aggregates are in most cases also evident on May-Grünwald-Giemsa (MGG)-stained blood films as characteristic "Döhle-like" inclusions. Moreover, patients present the risk of developing in childhood or adult life the additional clinical features of sensorineural hearing loss, cataracts, and/or a glomerulonephritis that can lead to end stage renal failure. A recent re-evaluation of a large case series failed to identify correlations between specific MYH9 mutations and the clinical phenotypes of 40 patients from 19 unrelated families, thus suggesting that MHA, SBS, FTNS and EPTS are not allelic forms, but rather represent a unique disease with clinical symptoms variably expressed. The pathogenetic mechanisms underlying MYH9-RD defects are either completely unknown or controversial. In particular, it is a matter of debate whether haploinsufficiency or a dominant-negative effect of mutant allele is responsible for hematological abnormalities. We

investigated 11 patients from 6 pedigrees with different MYH9 mutations. We evaluated NMMHC-IIA levels in platelets and granulocytes isolated from peripheral blood, and in megakaryocytes cultured from circulating progenitors. NMMHC-IIA distribution in megakaryocytes and granulocytes was also assessed. We demonstrated that all the investigated patients presented a 50% reduction of NMMHC-IIA expression in platelets, and that a similar defect was present also in patients' megakaryocytes. In subjects with R1933X and E1945X mutations the whole NMMHC-IIA detectable in platelets and megakaryocytes was a wild-type protein. No NMMHC-IIA inclusions were observed at any time of megakaryocyte maturation. In patients' granulocytes we measured lower NMMHC-IIA than in platelets ($p<0.01$) and megakaryocytes ($p<0.01$), and we found that the wild-type protein was sequestered within most of the NMMHC-IIA inclusions. Altogether these results indicate that haploinsufficiency of NMMHC-IIA in megakaryocytic lineage is the mechanism of macrothrombocytopenia consequent to MYH9 mutations. On the contrary, we obtained strong evidences that in granulocytes a dominant-negative effect of the mutant allele is involved in formation of inclusion bodies and it is responsible for a severely reduced amount of NMMHC-IIA normally distributed in the cytoplasm.

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RITUXIMAB AS TREATMENT FOR REFRACTORY THROMBOTIC THROMBOCYTOPENIC PURPURA

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Intensive plasma manipulation, including plasma exchange (PE) or plasma infusion (PI), is the mainstay of treatment for patients with thrombotic thrombocytopenic purpura (TTP), resulting in a response rate of 70–90%. However, if patients do not respond within the first 5–7 days, alternative therapeutic strategies should be tried. In addition, recurrence of TTP episodes may occur after variable intervals in a considerable number of patients after discontinuation of plasma-based treatment with a relapse rate ranging from 20 to 35%. Different immunosuppressive approaches, including high-dose immunoglobulin, splenectomy, combination chemotherapy, and vincristine (VCR), have been reported as effective salvage treatment for patients with refractory TTP. More recently, in patients with recurrent or refractory TTP favourable results have been reported with rituximab (RTX), a chimeric anti-CD20 antibody that eliminates both malignant and normal B cells and is currently used for the treatment of clonal B-cell malignancies. In this study we report treatment results from two patients with relapsed TTP refractory to plasma exchange, who were given RTX as salvage treatment. Two male patients, aged 31 and 38 years respectively, received RTX in second relapse after 20 and 12 months from remission achievement, respectively. Severe thrombocytopenia, elevated serum LDH and neurological symptoms were present in both cases. RTX was given weekly at a dose of 375 mg/m^2 for a total of 4 administrations on a outpatient basis. A rapid increase of platelet value was observed in both patients after the second administration of RTX, while serum LDH returned to normal value within 39 and 15 days, respectively. Neither early or late adverse effects were observed due to RTX administration. Platelet and LDH kinetics of patient #2 are shown in the figure. No consolidation or maintenance therapy was given. After 6 and 5 months respectively from the last administration of RTX, both patients are alive with normal values of blood counts and serum LDH, in absence of any sign or symptom related to the disease. We conclude that RTX is an effective treatment for refractory TTP and could be evaluated in the context of a clinical trial in early phase of disease. In our experience, the toxicity of this approach appears to be inferior to other immunosuppressive approaches such as vincristine or splenectomy.

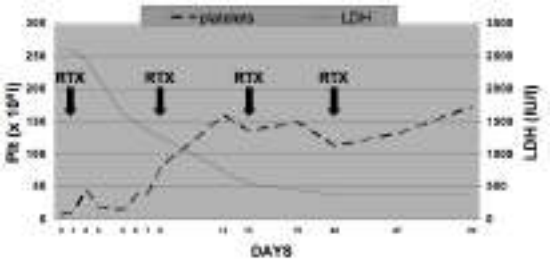

Figure 1. Platelets and LDH kinetics in patient #2 during and after rituximab administration.

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ALLELE FREQUENCIES OF HUMAN PLATELET ANTIGEN IN PATIENTS WITH CHRONIC REFRACTORY AUTOIMMUNE THROMBOCYTOPENIA

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Chronic autoimmune thrombocytopenia (AITP) is an autoimmune disorder due to specific auto antibodies against platelet glycoproteins (GP). Serum or platelet-associated antibodies are not easy to be detected; furthermore, their binding upon platelet surface may not be specific. Nevertheless, in thrombocytopenic phases, IgG or IgM antibodies with specificity against platelet antigens can be measured. It is known that the Human Platelet Alloantigenic (HPA) systems are located on platelet membrane glycoproteins (GPs), and that these GPs are polymorphic. We have examined the possible association among HPA polymorphism and development of chronic refractory AITP. The screening for auto antibodies was carried out by a solid phase system (CAPTURE P SCREEN), and the identification by ELISA system (GTI PAK PLUS). Human leukocyte DNA was isolated by the salting out method and the platelet genotype was determined by sequence-specific primer PCR (PCR-SSP). We screened 32 unrelated adult

patients affected by chronic refractory autoimmune thrombocytopenia. Direct or indirect tests for platelets autoantibodies were positive in 10 patients (31%); the presence of autoantibodies against erythrocytes was also detected in 6 non anemic patients (TCD positive). HPA 1, 2, 3, 4, 5 and 6 systems were analysed in all patients; one hundred random blood donors were used as control, in order to compare their allele frequencies with those of the patients. HPA 1, 3, 4, 5 and 6 allele frequencies in patients versus the control group were identical, while a statistically significant difference was found for the HPA 2 allele (HPA 2a frequency was 100% in AITP affected patients versus 87% in healthy controls). A previous German study performed in 1999 on a lower number of patients was in agreement with our data. The study, still in course, underlines that platelet GPs play an important role in platelet immunology both as specific immune receptors and/or as highly immunogenic targets, and also suggests that the HPA-2a allele compared to its allelic pair HPA-2b may be a preferential target of autoimmune attack and can be involved in the genesis of AITP as a specific autoepitope.

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RITUXIMAB IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. Rituximab is a chimeric anti-CD20 monoclonal antibody active against normal and malignant B cells. The effect of B cell depletion could be useful in autoimmune disorders in order to interfere with the production of pathologic antibodies.

Patients and methods. In our Institute, we have treated 6 patients (males 2, females 4, median age 43 years, range 27-63), 5 affected by ITP and 1 affected by ITP in APA-Syndrome, with Rituximab at a dosage of 375 mg/m^2, 4 doses in 5 cases and 3 doses in 1 case, once weekly. Median time between diagnosis and start of Rituximab therapy was 12 years (range 0.2-33). All patients had already received at least three lines of therapy (median 3.5; range 3-6): prednisone or prednisolone at conventional dosages, pulsed high-dose dexamethasone, azathioprine, immunoglobulin, interferon and splenectomy. At Rituximab therapy start, platelet count was $\leq 20x10^9$/L (median 7 x 10^9/L; range 3-20 x 10^9/L) in all patients. Complete response (CR) was defined as a platelet count > 150 x 10^9/L, partial response (PR) as >50 $\leq$150x10^9/L, minimal response (MR) as > 30 $\leq$50x10^9/L, no response (NR) $\leq$30x10^9/L. Patients were evaluated at the 1st month, the 3rd month and then every three months after therapy stop. Median follow-up after the end of therapy is 12 months (range 1-18).

Results. At the 1st month after therapy stop, 4 patients were no responders, 1 patient achieved CR and 1 patient achieved PR. At the 12th month only 3/6 patients were evaluable (the others had shorter follow-up): 2 were still no responders and 1 was still in PR. This last patient is still being treated with prednisone at dosages lower than before Rituximab therapy (30 mg/week vs 150 mg/week). Before starting therapy only 5/6 patients were evaluable with flow-cytometry studies. Peripheral blood B cells, evaluated by flow-cytometry as CD19+ cells, had a median baseline count of 137x10^6/L (range 58-371x10^6/L). At first evaluation, 4 patients showed absence of CD19+ cells and 2 patients showed a count of 9 and 4.4x10^6/L CD19+ cells, respectively. Between the 9th and 12th month after therapy stop, 4/6 patients were studied by flow-cytometry (2 patients had a shorter follow-up) and showed a B cell count of 4.2, 4.7, 70, 286x10^6/L, respectively. Considering the baseline count, three patients, all no responders, did not recover the CD19+ cells, while one patient in PR recovered and showed an increased value of CD19+ cells (CD19+ cells baseline level: 87x10^6/L; CD 19+ cells at the 12th month after therapy stop: 286x10^6/L). One patient experienced a papulosquamous dermatitis at the 2nd month after therapy stop. At the 9th month, 1 of the initially no responder patients achieved CR after splenectomy.

Conclusions. Two/6 patients had an early response, maintained in 1 case after 12 months from the therapy stop. No late responses were observed. The response was independent from the count of CD 19+ cells. In fact the CD 19+ cells count, evaluated at the first month after therapy stop, decreased with treatment to subnormal levels in all patients, and only one patient in PR recovered the CD 19+ cells in the late follow-up. No serious infections were observed in patients during follow-up. No patient had to stop therapy because of severe side effects.

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IMMUNOSUPPRESSIVE STRATEGIES IN RESISTANT/RELAPSED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background. Thrombotic Thrombocytopenic Purpura (TTP) is a microangiopatic disease characterized by schistocytic haemolytic anemia, thrombocytopenia, neurologic defects, fever and renal failure. Early relapse while patients continue to receive daily plasma-exchange (PEX) is frequent. Relapse occurs in more than one third of the patients (pts) who achieve remission. Recent studies have shown that deficiency in the von Willebrand factor cleaving protease ADAMTS 13, due to genetic mutations or autoimmune inhibitors, cause TTP. In patients refractory to PEX, immunosuppressive therapy could be useful in the majority of cases.

Patients. from January 1980 to February 2005 43 TTP pts were admitted to our Department, 13 males, 30 females, median age 36 yrs (r:21-78).

Treatment and Results. all pts were treated with steroids. Fresh frozen plasma FFP, 3U/day) only was performed in 6 advanced neoplastic pts. The other 37 were divided in responders (22) e non-responders (15) to PEX (median 15, range 3-108). Five pts died in the first days of PEX. In the other 10 non-responders Vincristine 1-2 mg iv/week x 4-6 times was added to PEX (responses 7/10). After failure to Vincristine, HDIg 400 mg/Kg/day x 5 days were performed

(response 1/9). In the 2 pts resistant to previous treatment the addition of cyclosporine A 5 mg/Kg/day induced complete hematologic response. Eleven pts relapsed, 4 with multiple relapses (2, 2, 4 and 8 respectively); four pts responded to steroid treatment and FFP, 2 responded to steroid and PEX, 1 only to PEX, 1 to Vincristine and PEX, and the multiple relapsing pts to different therapies in different episodes (only steroids, only PEX, HDIg, Vincristine).

Conclusions. 1) Deaths occurred at the beginning of the disease and in the first days; 2) in pts non-responsive to PEX immunosuppressive treatment is often necessary to obtain response; 3) relapses can be of different severity and each episode can respond to different treatments; 4) cyclosporine seems a good immunosuppressive alternative to Vincristine therapy after failure.

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ANTI-CD20 MONOCLONAL ANTIBODY IN THE TREATMENT OF REFRACTORY AUTOIMMUNE CYTOPENIAS IN ADULTS

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Unresponsive autoimmune cytopenias may require therapy with second-line drugs. There is no consensus that any one of these agents is more effective than another. Rituximab therapy had a variable but valuable effect in the treatment of patients with chronic refractory immune thrombocytopenia IT and refractory/ relapsed AIHA Three mechanisms are involved; complement dependent cytotoxicity, antibody dependent cellular citotoxicity and the induction of apoptosis. We report 12 patients,6 with chronic refractory autoimmune hemolytic anemia, (AIHA) and 6 with immune thrombocytopenia (IT) who each received 4 cycles of rituximab 375mg/m^2 weekly. 4 out of 6 AIHA patients (3 idiopathic,1 secondary to a lymphoproliferative disorder) had warm antibody type),1 patient had cold agglutinin disease and 1 patient had Coombs negative hemolytic anemia secondary to a myeloproliferative disorder.5 out of 6 patients with IT had idiopathic thrombocytopenic purpura [ITP],1 was CLL-associated.All patient with documented diagnosis of autoimmune cytopenias were receiving corticosteroid-based treatment either alone or combined with other immunosuppressive therapy at the time they received rituximab and were non- splenectomized because of comorbidity. Median age at first diagnosis, 57 years; range, 38-79 years;male-female ratio 1:1Duration of follow up ranged from 2 months to 32 months (average 18 mos).

Results. Complete remission (platelets counts >130 x 10^3/microL) occurred in 4 (65%) of 6 patients with IT and in 1 (16%) of 5 patients with AIHA (Hct > 38%). Only one patient converted to Coombs negative after Rx.Median time to maximum response (TMR) in responders was 9 weeks(range 6 - 18 weeks) Complete response was often durable in ITP.One patient in this subset, relapsed 12 months later and received a second course of 4 standard doses of antibody that resulted in consolidation of his platelet counts around 100x10^3/microL. No clinical or laboratory parameters were found to predict response, although there was a suggestion that patients with a shorter history of disease have a better chance of response.

Conclusions. Our findings, considered with the results of other studies, suggest that rituximab has an important role as salvage therapy for immune thrombocytopenia that are refractory to both corticosteroid treatment and are not suitable for splenectomy. On the other hand the treatment of auto-immune haemolytic anaemia (AIHA) remains unsatisfactory in those refractory to first-line management showing a very low percentage of complete responses (16%)

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THROMBOTIC THROMBOCYTOPENIC PURPURA AND ADAMTS-13 ACTIVITY MEASUREMENT: EXPERIENCE AT A SINGLE CENTRE

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The normal size distribution of VWF multimers in plasma is maintained by a metalloprotease of the ADAMTS family, the VWF-cleaving protease (ADAMTS-13). Mutations in the ADAMTS-13 gene or the presence of inhibitory auto-antibodies to ADAMTS-13 can result in a severe deficiency of the activity of this protease (i.e. <5% of that found in normal plasma) and is associated to TTP. In this study we prospectively followed the levels of ADAMTS-13 activity and inhibitors in a group of 21 consecutive TTP patients admitted to our Institution between Jan. 2001 to March 2005. ADAMTS-13 activity (by Gerritsen's method), inhibitors (by mixing studies), and anti-ADAMTS-13 antibodies (by ELISA) were measured in plasma samples of 21 TTP consecutive patients (14 in the acute phase, 7 in complete remission) upon admission and at different time points during the follow-up. The patients were classified as: idiopathic TTP (n = 15; 9 acute, 6 in remission); drug-associated TTP (n=3; 2 thienopyridine-associated and 1 fludarabine-associated TTP, acute), bone marrow transplantation (BMT)-associated TTP (n=2, acute), and pregnancy-associated TTP (n=1, in remission). All patients were treated with plasmapheresis and plasma infusion, and, if refractory, with immunosuppressive therapy. All the acute patients were followed with serial blood sampling until the clinical and haematological remission. We found a prevalence of complete ADAMTS-13 activity deficiency in 100% of the acute idiopathic TTP group (n=9). An inhibitory activity against ADAMTS-13 was found in 8/9 patients and was associated to high anti-ADAMTS-13 antibodies levels (86+60 U/mL) compared to healthy controls (7+5 U/mL). One patient was identified as a possible carrier of a true constitutive ADAMTS-13 deficiency; indeed no plasma inhibitory activity and anti-ADAMTS-13 antibodies were found, and the ADAMTS-13 activity was detectable only during plasmapheresis/infusion, but not on clinical remissions and upon relapses (n= 4). Differently, in the remaining 8 patients, ADAMTS-13 activity was severely reduced in the acute phase with a significant inhibitory activity, and variable behaviour on remission (i.e.: normal activity [> 50%] = 4 cases; moderate deficiency [20-50%] = 1 case; severe deficiency [5-20%] = 1 case; complete deficiency [<5%] = 2 cases). The 4 patients showing normal ADAMTS-13 activity on remission had no detectable anti-ADAMTS-13 antibody levels. The 2 patients with BMT-associated TTP

showed moderate reduction in plasma ADAMTS-13 activity, not associated with measurable anti-ADAMTS-13 antibodies. Differently, all the 3 patients with drug-associated TTP, showed severe ADAMTS-13 deficiency in the acute phase with anti-ADAMTS-13 antibody positivity. These three patients have normal ADAMTS-13 activity on remission. Finally, plasma ADAMTS-13 activity was normal in the patient with pregnancy-associated TTP in complete remission. In our experience, the measurement of ADAMTS-13 during the acute phase could not discriminate idiopathic TTP from drug-induced TTP, but distinguished the BMT-associated TTP. The patient with congenital deficiency was the most refractory to therapies. Interestingly, at remission, in 53% of idiopathic TTP patients, the resolution of disease manifestations (i.e.: microangiopathy, haemolytic anaemia and thrombocytopenia) was not associated with ADAMTS-13 level normalization.

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TREATMENT OF RESISTANT THROMBOTIC THROMBOCYTOPENIC PURPURA WITH MYCOPHENOLATE MOFETIL. A CASE REPORT

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Several reports have defined non familial thrombotic thrombocytopenic purpura (TTP) as an autoimmune disorder caused by antibodies to von Willebrand's factor-cleaving protease (vWF-CP). Mycophenolate mofetil (MMF) is an immunosuppressive agent originally used to prevent acute rejection of solid organ transplants and used more recently in the management of auto-immune conditions. We report its use in a case of resistant thrombotic thrombocytopenic purpura. A 29-year-old man was hospitalized in September 2004 with micro-angiopathic hemolytic anemia (MAHA), thrombocytopenia and hematuria He had been healthy, and no previous symptoms of autoimmune disease.The platelet count was $20x10^9$ cells/L, the haemoglobin level was 8.6 g/L. The serum lactate dehydrogenase level was 1969 U/L The fibrinogen level was normal. Prothrombin time and partial thromboplastin time were normal. Results of direct and indirect antiglobulin tests were negative. The peripheral smear contained more than 10 schistocytes per highpower field. Thrombotic thrombocytopenic purpura was diagnosed, and plasma exchange (1.5 volumes daily) was begun togheter with Prednisone (1 mg/kg of body weight per day) and aspirin (325 mg/d) The platelet count normalized but decreased to $34x10^9$ cells/L by day 22. Four intravenous doses of vincristine, 1 mg each every 4 days, were given without sustained benefit. Plasma exchange was resumed and mycophenolate mofetil (MMF, CellCept) with a dose of 1 g/day twice per day, was started. The platelet count increased,patient achieved complete response and plasma exchange was tapered over 3 weeks. During the next 4 months, blood counts were stable and analysis of vWF-CP activity demonstrated increased activity without detectable inhibitors.

Conclusion. Based on the our results we conclude that immunosuppressive therapy with mycophenolate mofetil can effectively treat TTP.To the moment this is the first report of MMF use in patients with a TTP refractory disease.

Allogeneic Transplantation I

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ROLE FOR IFN-GAMMA IN THE IMMUNOMODULATORY ACTIVITY OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) inhibit the proliferation of HLA-unrelated T lymphocytes to allogeneic stimulation, but the mechanisms involved are not fully understood. However, this effect is operational *in vivo*, as it has been shown recently that third party haploidentical MSCs can be safely infused to treat severe acute GvHD refractory to conventional immunosuppressive therapy. We have studied which lymphocyte subsets may be affected by human MSCs and which mechanisms may be involved in the onset of MSC inhibitory effect. MSCs have been generated from bone marrow aspirates of healthy donors, recruited after informed consent, and expanded in complete DMEM medium (15% FCS). MSCs have been characterized by immunophenotype and in vitro multilineage differentiation. The inhibitory effect of MSCs has been studied on CD4+, CD8+, CD4+ CRTH2+ T cells, NK cells, and B cells isolated from peripheral blood, using assays of proliferation, CFSE labelling and BrdU uptake, Transwell® experiments, evaluation of apoptosis by annexin V, flow-cytometry analysis of cytokine synthesis at intracellular level, blocking antibodies against IFN-gamma receptor and Fas Ligand, and competitive inhibitors of the IDO pathway. MSCs suppress the proliferation of both CD4+ and CD8+ T lymphocytes, as well as of NK cells, while they do not have effect on the proliferation of B lymphocytes. MSCs inhibit the capacity of T lymphocytes and NK cells to proliferate, without affecting the expression of cell activation markers, inducing cell apoptosis or mimicking or enhancing the activity of T regulatory cells. The suppressive activity of MSCs is not contact-dependent and requires the presence of IFN-gamma produced by activated T cells and NK cells. Accordingly, even activated B cells become susceptible to the suppressive activity of MSCs in the presence of exogenously added IFN-gamma. IFN-gamma produced by activated T cells or NK cells induces MSCs to suppress in turn their proliferation by enhancing their indoleamine 2,3-diooxygenase activity. The suppressive activity of MSCs is completely abrogated by the addition of an anti-IFN-gamma receptor (IFN-gR) mAb. These findings allow to suggest that some disorders which are primarily mediated by T helper 1 cells, such as GvHD, might improve with the *in vivo* infusion of MSCs because of the activation mediated by T cell-derived-IFN-gamma of the immunomodulatory properties of MSCs, which in turn inhibit T- and NK cell proliferation. In addition MSCs could have a role in controlling IFN-gamma-dependent B cell disorders such as some autoimmune diseases.

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FOUR VNTR PANEL METHOD IS USEFUL IN ANALYZING CHIMERISM STATUS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Chimerism status (CS) analysis is useful for evaluation of donor cells engraftment after allogeneic haematopoietic stem cell transplantation (HSCT). At present, standardized method is not available, nevertheless many laboratory use PCR based amplification of highly polymorphic DNA regions as VNTR or STR. VNTRs are 15-50 nucleotide long tandem repetitive DNA sequences, STRs are shorter repetitive sequences being only 2-6 nucleotides long. The number of repeats varies between different individuals and determines the VNTR/STR polymorphism. Different VNTR/STR polymorphism between donor (D) and recipient (R) allows to determine CS after HSCT. We tested a semiquantitavive method, based on PCR amplification of a panel of 4 VNTR loci, in order to evaluate its informativity in CS monitoring.

Methods. CS was analysed in 21 patients submitted to HSCT. Peripheral blood samples were collected for DNA extraction from D and R before HSCT and from R only at 30, 60, 90 and 120 days after HSCT. A panel of 4 paired primers for VNTR loci (YNZ22, APO-C, MCT118, PAH) was used in the initial screening to identify the most informative markers between D and R. PCR amplification with every VNTR specific primers and subsequent electrophoretic run on agarose gel were used to visualize different D and R alleles. VNTRs were considered informative markers when at least one allele was different. In post-HSCT R samples, PCR analysis was carried out using only informative VNTRs from each D/R pair. Patients who showed no evidence of R cells were considered to have a complete chimerism (CC), patients who presented each D and R alleles were defined as mixed chimerism (MC). In order to quantify D/R ratio in MC patients, a dilution curve was constructed employing different concentration of amplified D-DNA. The comparison between chimeric sample electrophoresis and diluited DNA electrophoresis gives a quantitative estimation of D/R ratio.

Results. our panel resulted informative in 20/21 HSCT patients. Informative VNTR were: 1 in 7 D/R pair, 2 in 12 D/R pairs, 3 in 1 D/R pair and 0 in 1 D/R pair. By analysis of the 75 samples after HSCT, 56 (75%) presented CC, 17 (23 %) MC ranging from 10 to 60% and 2 complete recipient reconstitution. The sensitivity of this method was 10^{-2}.

Conclusion. PCR amplification of a panel of 4 VNTR loci is a sensitive and informative method to evaluate CS in most patients after HSCT. This technique allows to quantify MC, also if a better evaluation of CS could be obtained with quantitative assays.

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NON MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH RISK LYMPHOMA: A SINGLE CENTRE EXPERIENCE

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From December 2000 to January 2005, 22 patients (11 females and 11 males) median age 40,2 years (range 18-66) underwent NST because of high risk Hodgkin Disease (HD, 8 cases), non Hodgkin Lymphoma (NHL,13 cases) and Chronic Lymphocytic Leukaemia (CLL,). Disease status at transplant was as follow: 4 in complete remission (CR, 2 NHL, 2 HD) 14 in partial remission (PR, 8 NHL, 5 HD, 1 CLL) and 4 (3 NHL, 1 HD) in progression. In 20 cases, grafts were mobilized from HLA identical sibling donors, while in 2 cases stem cells were obtained by a matched unrelated donor (MUD) bone marrow. Conditioning regimens consisted of Fludarabine, Thiotepa and Cyclophosfamide (FTHC) in 16 cases, while in the remaining , Fludarabine and Cyclophosfamide (FC) in 2 cases, Fludarabine, Melphalan, Thiotepa and ATG (FLTA) in 1 case, Fludarabine and Thiotepa (FT) in 1 case, Campath-1, Fludarabine, Melphalan and TBI (CLFT) were employed in 2 cases. Cyclosporine–A (CyA) and Methotrexate (MTX) were used as Graft Versus Host Disease (GvHD) prophilaxys in all cases but two, where Campath-1 and Moftil micofenolate were combined. A mean number of 5.8x10E6/Kg $CD34^+$ cells (range 4.2 –7.7) were infused. Patients received a mean of 5.5(range 0-22) irradiated and filtered packed red blood cells, while platelet support was guaranteed by either apheresis (median 3.0, range 0-8) or randomly obtained platelet concentrates (median 4,5, range 0-32). 4 patients experienced mucositis WHO grade 3-4, 6 patients WHO grade 2, 1 patient W.H.O. grade 1, while the remaining 11 patients did not show any sign of mucositis. 14 patients had fever (7 FUO and 7 microbiologically documented). All patients but one engrafted at median day +11 (range 6-19) for PMN > 100/mmc and +21 (range 12-39) for PLT> 30.000/mmc, respectively. One patient failed to engraft and was reinfused with autologous back up. The mean WBC count at nadir was $0.1x10^6$/dL(range 10-500). 3 patients developed a WHO grade 1 cutaneous acute GvHD, a WHO grade 2 cutaneous chronic GvHD was seen in 2 patients, while a W.H.O. grade 4 liver acute GVHD, was seen in 1 patient. After a median follow up of 14,1 months (range 1-36), 16 patients are still alive (12 in CR,1 in PR and 3 in relapse). Three transplant related deaths were documented, one for acute respiratory distress syndrome at 2 months from transplant, one for acute thrombotic thrombocytopenic purpura, at 18 months from transplant and one patient died for liver aGVHD at 3 months from transplant,

Two patients died for disease recurrence at 10 and 17 months post-transplant respectively, despite repeated infusion of donors lymphocytes. One patient died for Lymphoma EBV related at 11 months from transplant. Chimerism study showed a full donor situation in 10 cases and a mixed chimerism in the remaining patients. In conclusion, NST is feasable and should be considered for high risk lymphoma patients.

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ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IS EFFECTIVE CURATIVE TREATMENT IN PAEDIATRIC REFRACTORY/ AGGRESSIVE LANGERHANS CELL HISTIOCYTOSIS

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Langerhans cell histiocytosis is a clonal proliferative disease of unclear etiology variously classified as reactive disorder, a neoplastic process or an aberrant immune response. Although the new strategies concerning the management of LCH have been made a considerable advances on the outcome of pediatric patients, the best therapeutical approach for aggressive/refractory multi-system LCH patients remains still controversial. Antiproliferative and immunosuppressive therapy in combination with HSCT was proposed as the appropriate treatment for these poor-prognostic patients because this procedure may offer an opportunity to induce long-term remission and disease free survival. Because the appreciable morbidity and mortality of allogeneic HSCT, this strategy has therefore been reserved for the few LCH patients with a very poor prognosis. In this report we describe 4 children (14,27,96,48 months respectively old) with refractory aggressive MS-LCH, treated with allogeneic HSCT in our Institution between 2001 and 2003. Disease activity score proposed by Akkari V. *et al* (2003) was retrospectively calculated for all included patients at diagnosis and before HSCT as well as the Karnofsky status.All patients presented MSD progression despite chemotherapy with single or multiple agents or immunosuppressive therapy. Allogeneic HSCT was performed 10-22-72 and 47 months respectively from onset. Patient 1 and 3 received Umbelical Cord Blood Transplantation (UCBT) from HLA 4/6 and 5/6 mis-matched unrelated donor. Patient 2 and 4 received Bone Marrow Transplant (BMT) from their related HLA-identical donors. Conditioning regimen including Busulfan 4 mg/kg and Fludarabin 30 mg/m^2 from day –7 to day – 4, Thiotepa 10 mg/kg on day – 3 was used as preparative regimen. All patients received hors ATG 15 mg/Kg from day –6 to day –2 Cyclosporine A 3mg/Kg from day –1 to day + 180 and PDN 1 mg/kg until day + 30. After HSCT all patients are alive with a median follow-up of 30 months (51,39,19,11) . Conditioning regimen was well tolerated without major complications. Donor engrafment was demostrated for all by PCR DNA analysis and progressive improvement of disease symptoms were observed after 18,6,8,and 6 months respectively. Patient 1 ad 2 are disease free, patient 3 present chronic extensive GVHD ,reduction of esophtalmous and skin lesions,bone lesions are stable as well as for patient 4. Disease progression was evident for any of these patients. We conclude that HSCT is a good courative treatment for these poor-prognostic patients. Progressive clinical Improvements were evident long time after HSCT. Selection of patients by early response to conventionalchemotherapy and timing of HSCT remains controversial.

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UNRELATED UMBELICAL CORD BLOOD TRASPLANTATION OR CHEDIAK HIGASHI SYNDROME: A CASE REPORT

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CHS is a rare autosomal recessive disease characterized by severe immunodeficiency partial oculocutaneous albinism, progressive neurologic defects. The hallmark of CHS is the presence of giant cytoplasmic granules in circulating granulocytes. 85% of patients present an accelerated phase characterized by infiltration of activated T lymphocytes and macrophages in liver, spleen, limphnodes and bone marrow, Althought accelerated phase is cured with etoposide combined with steroids and intrathecal methotrexate, remission is transient. Bone marrow transplantation (BMT) can be considered a curative treatment for CHS. We describe a case of a 2 mouth old male of non-consanguineous parents transplanted with allogeneic UCBT. Diagnosis of CHS was based on evidences of Bone Marrow giant granules in myeloid cells, macula and hair hypopigmentation absence of NK activity.He presented an accelerated phase with persistent fever, opisthotonous, neck rigor, hepato-splenomegaly, pancytopenia, hypertriglyceridemia, hypofibrinogenaemia, hyposodiaemia, hyperferritinaemia and evidences of hemophagocytosis on bone marrow and liquor. Etoposide, intrathecal methotrexate and steroids were started inducing, after 6 cycles, a biological and clinical partial remission.

An unrelated HLA 5/6 mached cord blood unit (UCB) with a difference on DR locus and AB0 group was identified for Hematopoietic stem cell transplantation (HSCT). Conditioning regimen consisted in high dose of etoposide, busulfan and cyclophosphamide. Graft versus host desease (GVHD)profilaxis consisted of ATG15 mg/kg from day -6 to day -2 Cyclosporine (CSA) 3 mg/kg/die from day – 1 until day +180 and PDN 1mg/kg/day from day –6 e to day+ 30. He received 14.8x10^7 NC/Kg and 1.67x10^6 CD34$^+$/Kg Engrafment was achieved on day +19 (PMN> 500/mmq); on day +10 the patient presented a grade IV of acute GVHD with cutaneus, entheric and hepatic involvement and severe hyperbilirubinemia (40 mg/dL) treatement consisted in steroids (2-5 mg/kg/die), anti-tumor necrosis factor alfa (TNF-alpha) monoclonal antibody finally with e.v. Mycophenolate (MMF). Severe CMV and adenovirus infection were also evident; six mounths after transplant the patient is in complete remission, full donor leukocyte chimerism was rapidlly obtined and complete control of GVHD, CMV and adenovirus infections were achived.We conclude that unrelated UCBT is a possible therapeutical option for CHS even if only partial remission was obtined before HSCT.

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INCIDENCE AND OUTCOME OF CYTOMEGALOVIRUS INFECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING

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Objective. Cytomegalovirus (CMV) infection remains one of the most important complications for patients (pts) undergone allogeneic stem cell transplantation (alloSCT). We evaluated incidence and outcome of CMV infection after alloSCT with reduced-intensity conditioning (RIC).

Patients and Methods. 25 consecutive pts (male: female = 14:11) aged from 38 to 66 years (median: 56) were allografted with bone marrow (3) or peripheral blood stem cells (22) from HLA-identical sibling donors from 2000 to 2004. The underlying hematologic malignancies were: acute myeloid leukemia (AML 7), myelodysplastic syndrome (MDS 5), non-Hodgkin lymphoma (NHL 5), multiple myeloma (MM 5), chronic lymphocytic leukemia (CLL 2) and chronic myeloid leukemia (CML 1). RIC regimens performed were: TT-EDX (13), FLU-TT-EDX (6), TBI 200 cGy (5), and FLU-TBI (1). The following CMV donor/recipient status could be found: positive/positive (22), positive/negative (2), negative/positive (1), negative/negative (0). All pts were weekly evaluated with CMV pp65 antigenemia assay for first month; thereafter antigenemia was determined only when a CMV infection was suspected due to clinical or biochemical features. The decision to switch from prophylaxis to preemptive therapy was made on the basis of two consecutive positivity or the first positivity > 5/200.000 cells. Acyclovir was given for CMV prophylaxis, preemptive therapy was performed using ganciclovir, valganciclovir, foscarnet or cidofovir.

Results. A positive CMV antigenemia was detected in 10 pts; all of them were seropositive for CMV before allo SCT. 4 pts had AML, 2 NHL, 1 CLL, 1 MMM, 2 MDS. The incidence of CMV infection was 10/25 (40%). 7 pts presented only one episode of CMV reactivation, 1 patient two episodes and 2 pts three episodes. Median time of first CMV positive antigenemia was 52 days after allo SCT (range: 22-356), particularly 8 pts had CMV reactivation before 100 days post SCT. Median positive cells at the first appearance of antigenemia was 3/200.000 cells (range 1-14). Overall, we reported 15 episodes of CMV reactivation, 9 before and 6 after 100 days post allo SCT. These last pts who developed late CMV reactivation showed contemporaneously chronic GVHD or disease relapse. Only 8/15 (53%) episodes were treated. Anti-CMV drugs employed had similar effectiveness with a median time of negativization of 15 days. None developed CMV disease or died for CMV infection. Five/ten pts who suffered CMV infection are still alive; while 5/10 died for disease progression (4) or GVHD (1). 10/15 pts without CMV infection are still alive. In the same years, 25 pts received a myeloablative alloSCT from HLA identical donor: among them 6 pts developed a CMV infection for an incidence of 24%.

Conclusions. in our group of pts transplanted with RIC allo SCT, the incidence of CMV infection was 40%. Preemptive therapy was effective and no patient developed CMV disease.

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PROGNOSTIC VALUE OF HEMATOPOIETIC CHIMERISM IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES DURING THE EARLY PERIOD AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Hematopoietic chimerism is a prognostic factor for relapse of hematological malignancies after allogeneic stem cells transplantation (alloSCT). It is well known that a part of patients (pts) submitted to alloSCT shows evidence of recovery of recipient hematopoiesis after transplant. This so called mixed chimerism (MC) is suggestive for immune tolerance but also is considered a marker of early relapse after transplantation especially in CML. We undertook a study since February to September 2004 enrolling 12 pts submitted to alloSCT. Monthly assays of hematopoietic chimerism were performed from BM samples by PCR amplification of short tandem repeats or amelogenin loci. Patients characteristics were as follow: 5 patients with AML, 2 with ALL, 1 acute biphenotipic leukemia, 1 blastic phase of CML, 1 MM and 2 NHL/CLL. Median age at transplant was 48y(20-57); M/F: 9/3. Three pts received stem cells from unrelated donors, the remaining pts from related donors. Only 3 transplant were sex-matched. Nine pts were submitted to conventional conditioning regimen while 3 to nonmyeloablative-conditioning. Disease status at transplant was: 3 pts were at diagnosis (1 CML blastic phase), 2 in 1st CR, 1 in 2nd CR, 1 in PR, 1 in 1st relapse, 1 in resistant phase and 3 pts in PD. The median follow-up was 120 days (34-270). After transplant 3 pts did not develop aGVHD, 4 pts developed grade I aGVHD, 4 II grade and 1 patient developed grade IV aGVHD with fatal exitus. Actually 7 pts are in CR and 2 pts in relapse after respectively 2 and 3 months from obtaning CR. Two pts died in PD at 1 and 4 months after transplant and the last one patient for relapse after 2 months from transplant. The frequency of MC was 75% (3/4), 36% (4/11), 33% (3/9) and 29% (2/7) at 15 days, 1, 2 and 3 months post transplant respectively. The frequency of MC decreased over time. MC was observed at least once in 58% pts (7/12). When we compared clinical characteristics of pts with chimerism status (CS) in the group of pts with CC 2 out 7 pts (28%)developed relapse at 1 month after transplant, 0/6 at 2 months and 1/5 (20%) at 3 months. Patients with persistent CC after a median follow-up of 135 days (52-257) are in CCR. In the group of pts with persistent MC, all but one patient developed relapse or PD. These results show a statistically significant correlation between CS and disease progression or relapse (p=0.028). Comparing chimerism with OS 14% of pts with CC died while 67% of pts with MC (p=0.068)

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THE COMBINATION OF CONTINUOUS BLADDER IRRIGATION + MESNA + HYPERHYDRATION IS MORE EFFECTIVE THAN MESNA + HYPERHYDRATION FOR THE PREVENTION OF HAEMORRHAGIC CYSTITIS AFTER ALLOGENEIC HSC TRANSPLANTS

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Haemorrhagic Cystitis (HC) is a frequent complication after Allogeneic BMT. Various prophylactic measures have been used to prevent HC, but their effectiveness is limited. We studied the combination of CONTINUOUS BLADDER IRRIGATION (CBI) + MESNA + HYPERHYDRATION during conditioning containing High Dose CTX (BU-CY or TT+CTX) as mean to prevent HC after Allogeneic HST Transplant . A group of 43 consecutive patients who received allogeneic HSC transplantation for various onco-haematological diseases was prospectively studied. 25 patients received standard prophylaxis with MESNA + HYPERHYDRATION while 18 patients received prophylaxis with CBI + MESNA + HYPERHYDRATION . All patients received a conditioning containing high dose CTX (50-60 mg/Kg). Prophylaxis with MESNA was at 100% dose of CTX, MESNA was infused intravenously over 24 h and continued for 24 h after completion of the CTX; HYPERHYDRATION fluids were NS and D5% (3 liter/mq). 26 patients received HLA identical transplant from SIBLING donor and 17 from MUD. 32% of all patients had diagnosis of HC during the period of observation. Haemorrhagic cystitis was significantly more frequent in patients receiving transplant from MUD donor in respect to patients receiving transplant from HLA Id Sibling donor (52% vs 19% p=0,02). Haemorrhagic cystitis was significantly less frequent in group receiving prophylaxis with CBI + MESNA + HYPERHYDRATION in respect to group receiving standard prophylaxis with MESNA + HYPERHYDRATION (16% vs 44% p=0,059). These two groups of patients were comparable in respect of percentage of MUD transplants (p=0.5). Severe HC (grade IV) developed in 2 patients: both these patients had received standard prophylaxis with MESNA + HYPERHYDRATION. In multivariate analysis (logistic regression), use of CBI prophylaxis (p=0,03) as well as HLA Id Sib transplantation (p=0,01) were independently associated with a lower risk of developing Haemorrhagic Cystitis.

Although HC can be secondary to a variety of factors (drugs, radiation, infections), HC in BMT setting is usually either related to the use of CY or, later in the course of the transplant, to viral infections and development of severe GVHD. Our study demonstrated that use of CONTINUOUS BLADDER IRRIGATION during conditioning in combination with MESNA + HYPERHYDRATION is more effective than MESNA + HYPERHYDRATION in the prevention of Haemorrhagic Cystitis after Allogeneic HSC transplantation.

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UNEXPLAINED BONE PAINS AFTER ALLOGENEIC HSC TRANSPLANTATION ARE FREQUENT AND LIKELY RELATED TO HIGH CSA LEVEL

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After renal transplantation a bone pain syndrome associated to calcineurin inhibitor has been reported (Lucas 1991), however only cases reports have been reported after allogeneic Hematopoietic Stem Cell (HSC) transplantation (Kida 2004) and the real incidence of pain syndrome associated to calcineurin inhibitor in this setting is unknown.

In order to assess the frequency of unexplained bone pain after allogeneic HSC transplantation we have revised clinical data in a consecutive series of patients who were transplanted in our Institution. The presence of unexplained bone pain during the first 100 days after transplant was recorded together with the following laboratory data:

CSA plasmatic value, transplant related complications, overall survival. Pains arising during GVHD in active phase, during fever and during relapse phase were excluded. Forty patients were studied, 57% were female and 43% male, median age was 41 years, 65% had received Tx from a HLA-ID family donors while 35% from a VUD. GVDH prophylaxis was in all cases using CSA (1-3 mg/Kg) and short course MTX. CSA was targeted to 200-400 ng/mL. In the conditioning ATG was employed in 35% of cases while no patients received ATG after Tx to treat acute GVHD. 67% (27/40) of all patients complained about unexplained bone pains, pain was localized mainly to lower limbs and in 10% of all patients (4/40) the pain required treatment with morphine derivate.

Median time of appearance of symptoms was day +49, in 75% of all patients pain arised between day + 29 and + 89. In the group of patients experiencing pain a previous corticosteroids therapy had been used in 92% of cases while in the group of patients not reporting pains, corticosteroids had been used in 84% of all cases (chi test: p=0.4). Median plasmatic CSA level in the group of patients experiencing symptoms at time of pain onset was 330 ng/mL, moreover in this group of patients a CSA overdosage (>400 ng/mL) preceding pain was recorded in 80% of cases. TRM at 100 days for patients experiencing pains was 7.5 % versus 0% (p=0.4) in the group who did not report unexplained bone pains. With a median follow-up time of 13 months the Overall Survival was in these groups, respectively, 83% and 72% (log rank: p=06).

In conclusion unexplained bone pains after HSC transplantation are frequent and represent an important cause of morbidity, it seems associated to high level of CSA, prognosis of patients experiencing unexplained bone pain is not different from that of other patients.

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DECISION MAKING IN HIGH RISK ADULT THALASSEMIA PATIENTS UNDERGOING UNRELATED BONE MARROW TRANSPLANTATION: QUALITY OF LIFE, MOTIVATION AND COMMUNICATION FACTORS, ETHICAL ISSUES

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Introduction. Allogenic bone marrow transplantation (BMT) remains the only potentially curative treatment of adult high risk thalassemia patients without an HLA-identical donor within the family, but the candidates and their families are faced with a dramatic decision. They can either continue traditional transfusion and chelation therapy, with no chance of complete recovery, or, they can accept the risk of BMT in the hope of obtaining a definitive cure for the disease. The patients and their families need to have a realistic picture of all aspects related to transplantation outcome, the recovery of health and the global quality of life in terms of physical and psycho-social well-being. We therefore retrospectively analyzed the results of unrelated transplantation performed in 27 adult high risk thalassemia patients. We also investigated the motivation factors, the communication strategies, the post-transplantation QoL and discussed the ethical issues of this therapeutical approach.

Methods. Fifteen males and 12 females, mean 22 years, were transplanted. Twenty patients (74%) are alive after a median follow up of 41 months. Seven patients (26%) died from transplant-related causes. The 20 surviving patients were given two questionnaires. The first contained 11 items and was developed in collaboration with an expert psychologist to investigate communication and motivation factors and the global satisfaction felt for the decision of BMT, and the second questionnaire (EORTC QLQ-C30) assessed the global QoL.

Results. All patients were strongly motivated and satisfied with the communication modalities of physicians before. None of the patients regretted their choice and would make the same decision if reliving the experience. The information received had been clearly presented particularly that concerning the quality of life expectancy and the cure rate. However, in 15% of patients some information had been perceived as not completely exhaustive and another 15% of patients felt that they had not been able to ask questions about their transplant. These data suggest that communication between the physician and patient-relatives could be improved. Particular attention should be paid to the patient's level of understanding and preferences. The mean global QoL was good (mean 76.4). Thirteen of the 20 patients (65%) enjoyed a very good global QoL (score 81-100), 2 (10%) had a good score (61-80) and 4 (20%) had an intermediate score (41-60). Only one patient had a poor global score. Very good scores were obtained for the physical, emotional, cognitive, role and social function scales. Patients reported minimal distress symptoms such as fatigue, nausea/vomiting, pain, dyspnea, constipation, diarrhea, appetite loss, insomnia and financial difficulties.

We also discussed the central ethical issue of BMT in a non malignant disease, that can be framed in terms of a conflict between two fundamental principles of medical ethics, i.e., non-maleficence and beneficence.

Conclusion. The multi-disciplinary approach used in this study may offer a valuable contribution to the decision-making that surrounds the choice of treatment with a high mortality risk in a chronic non malignant disease.

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AUTOANTIBODIES IN CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Chronic graft-versus-host disease (cGVHD), the most common non-relapse problem affecting long-term survivors of allogeneic haematopoietic stem cell transplantation (HSCT), has certain similarities with autoimmune diseases and is associated with the development of various autoantibodies in some patients.

In this retrospective study we analyzed the occurrence of autoantibodies in 63 patients surviving longer than 3 months after an allogeneic HSCT for a diagnosis of malignant haematological disease, with the aim of detecting a possible association between occurrence of the autoantibodies and the development of cGVHD and with the immune recovery after HSCT. The patients were screened every 3 months for the occurrence of the following autoantibodies: anti-nuclear (ANA), anti-mitocondrial (AMA), anti-smooth muscle (ASMA), anti-cardiolipin (ACA), anti-liver-kidney microsomal (LKM), anti-DNA, anti-neutrophil cytoplasmatic (ANCA), anti-thyroid myeloperoxidase and microsomal antibodies. Peripheral blood immunophenotyping with CD3, CD4, CD8, CD19, CD20, CD16 and CD56 antibodies was evaluated at the same intervals.

Autoantibodies were never found in 18 patients (29%) (group I, negative) , at least in one screening in 29 patients (46%) (group II, partially positive) , in all the evaluations in 16 patients ((25%) (group III, positive). ANA were found in 35/45 (78%), AMA in 4/45 (9%) , ASMA in 4/45 (9%), ANCA in 5/45 (11%), ACLA in 1 (2%), anti-tyroid antibodies in 3/45 (8%) and anti-DNA in 2/45 (4%) patients. Median time between HSCT and the occurrence of autoantibodies was 272 days (range 38-950) The 3 groups were statistically not different concerning age (group I: median 43 years, range 28-64; group II: median 44, range 24-62; group III: median 50, range 18-65) , sex, underlying disease, source of haematopoietic stem cells (PBSC or BM), conditioning regimen (myeloablative or reduced intensity regimen), donor (familiar or unrelated). The incidence and the degree of acute GVHD were not different among the 3 groups. However, only 10 out the 18 negative patients (55%) developed cGVHD (8 limited, 2 extensive), where-

as 43 out 45 partially positive or positive patients (95%) developed c GVHD (21 limited, 22 extensive) (p= 0.05). An isolated antibody (mainly ANA) was recognized in 17/21 (81%) limited cGVHD, whereas 12/22 (54%) extensive cGVHD presented more than one antibody (p= 0.04) Median value of CD19 and CD20 lymphocytes of group II and III were higher than group I patients at the 3th and 12th month. No difference in the immune recovery of CD3, CD8 and CD4 lymphocytes was recognized; moreover, median value of CD 16 and CD56 lymphocytes of group III patients was significantly lower than negative patients at the 6th, 9th and 12th month. We conclude that the occurrence of autoantibodies was significantly associated with the development of cGVHD. The number of the detected autoantibodies correlated with the involvement of more organs. The increase of B- lymphocytes subsets in patients developing autoantibodies could suggest that B-cell depletion by using an anti-CD20 monoclonal antibody could be efficacious in the treatment of cGVHD in these patients. .

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RHEUMATOLOGIC DISORDER AS A MANIFESTATION OF CHRONIC GRAFT VERSUS HOST DISEASE AND THEIR MANAGEMENT: DESCRIPTION OF TWO CASES

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Chronic graft versus host disease (cGVHD) remains a major complication of allogeneic hematopoietic stem cell transplantation (HSCT). It is the result of a later phase of alloreactivity where clinical features can be similar to several autoimmune diseases. The common target organs of cGVHD are skin (80%), liver (75%), mouth (70%) and eyes (50%) but many other organs can be involved. Here we report on two patients who underwent allogeneic HSCT and developed articular disorders as manifestations of cGVHD, responsive to immunosuppressive therapies commonly used for Rheumatoid Arthritis (RA). The first case was a 46 years old male diagnosed with an Acute Lymphoblastic Leukemia who received an allogeneic HSCT from an identical HLA ABDR sibling after a conventional conditioning regimen containing TBI and Cyclofosfamide. Even though he received a GVHD prophylaxis with short course Methotrexate (MTX) plus CyclosporineA (CyA), he developed an acute GVHD of the skin (grade II) completely resolved with steroid therapy. After nine months from allogeneic HSCT, during the tapering of prednisone and CyA therapy, he started to complain a difficult and painful deambulation. Serum evaluation of the rheumatoid factor, Waaler Rose, complement, antibodies antiDNA, ANA and ENA were negative, while the nuclear magnetic resonance (NMR) of sacroiliac joints showed erosive lesions of bone and therefore a seronegative arthritis was diagnosed as manifestation of cGVHD. After two months intramuscular administration of MTX (15 mg/weekly), without any modification of prednisone and CyA therapy, the patient showed a complete resolution of clinical symptoms and normalization of NMR images.

The second case was a 40 years old female who received a reduced intensity allogeneic HSCT from an identical HLA ABDR sibling for Chronic Lymphocitic Leukemia. She developed a severe acute GVHD (grade III) involving skin and gastrointestinal tract, resolved with prednisone (2 mg/Kg) plus CyA. After six months from allogeneic HSCT she developed an extensive cGVHD (mouth and skin) responsive to extracorporeal photopheresis associated to prednisone and CyA. However, for the appearance of widespread osteoarticular pains, serum tests (rheumatoid factor, Waaler Rose, complement, antibodies antiDNA, ANA, ENA) and radiological evaluations (X-ray and ultrasounds) were performed and a diagnosis of tenosynovitis was made. A weekly intramuscular low doses of MTX (15 mg) was started but she experienced only a partial remission of osteoarticular pains. A subsequent treatment with a subcutaneous anti-TNF(etanercept) twice a week lead to a complete regression of symptoms.

In conclusion, rheumatologic manifestations of cGVHD are rare but not unfrequent. They can appear as single manifestation or associated to other organ involvement and seem to respond to immunosuppressive treatment commonly used for rheumatologic diseases.

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ALLOGENIC BONE MARROW TRANSPLANTATION IN A PNH PATIENT: IMMUNOLOGICAL AND MOLECULAR CHARACTERIZATION

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Paroxysmal Nocturnal Hemoglobinuria (PNH) is a clonal disorder of hematopoietic stem cell characterised by bone marrow failure and increased susceptibility of erythrocytes to the lytic activity of complement caused by somatic mutations of the phosphatidylinositolglycan-class A (PIG-A) gene, resulting in the absence or decreased surface expression of GPI–anchored proteins (GPI-AP).

The aim of the study was to describe the clinical, immunological and molecular characteristics of a 39 yrs-old male with 8 yrs story of PNH who underwent BMT from identical sibling donor. The patient was pancytopenic and transfusion-dependent and had suffered from Budd-Chiari syndrome. The conditioning regimen was busulfan and cyclophosphamide and the clinical course of BMT was uncomplicated. We investigated clonogenic activity of the patient before BMT and at one, six and twelve months after BMT and we performed the molecular analysis of the PIG-A gene on every single colony. We evaluated the production of IFN-gamma, TNF-α and TGF-beta by bone marrow mononuclear cells (BMMC). The results showed that at one year after the transplant the patient is well, with Hb levels of 16 g/dL, but with low WBC ($1.9x10^9$/L) and platelets ($70x10^9$/L). In addition, the patient shows persistent lymphocyte (CD3) mixed chimerism in PBMC (40% donor), in spite of 3 lymphocyte donor infusions (2 of $0.5x10^7$, and 1 of $1x10^7$ CD3/kg). The number of CFU-GM and BFU-E in bone marrow were clearly reduced before

BMT (see table), and 95% of colonies displayed the Del T774 mutation, as detected by PCR and sequencing of the PIG-A gene on every single colony. The number of BFU-E clearly increased after BMT, reaching normal ranges, and none of the CFU-GM and BFU-E showed the original PIG-A gene mutation. Likewise, studies on PB granulocytes showed that the PIG-A mutation was present in virtually all cells before BMT, and in none of them at 14 days and 1 year after BMT. As regards immunological studies, production of cytokines by BMMC before BMT showed reduced TNF-α and IFN-γ and increased TGF-beta, compared with controls. After BMT, TNF-α and IFN-γ production increased and were comparable to controls, and TGF-beta decreased, although did not reach normal values. Regarding PB cultures, cytokine production before BMT showed higher TNF-α , IFN-γ and TGF-beta levels than controls. After BMT, TNF-α and TGF-β decreased reaching normal values, whereas IFN-gamma production was unchanged (data not shown). These data suggest the existence of a compartimentalization of cytotoxic cells before BMT, possibly involved in PNH bone marrow failure. After BMT, cytokine production showed a trend towards normality both in BM and PB suggesting the correction of the hypothesized compartimentalization.

	Patient before BMT	1 month after BMT	6 months after BMT	12 months after BMT	Controls (mean±SE)
CFU-GM (n° of colonies)	7	12	22	587	22927
BFU-E (n° of colonies)	10	23	nd	nd	nd
TNF-α (pg/mL)	7	46	22	474	2803
I FN-γ (pg/mL)	10	108	488	1182	1780
TGF-β (pg/mL)	21nd±9	62+18	377±185	1207±400	1361±391

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A CASE OF HUMAN HERPES VIRUS-6 INFECTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION MIMICKING A VARICELLA ZOSTER MANIFESTATION

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Human herpes virus-6 (HHV-6) has been identified in 1986 and it is the sixth member of the herpesvirus family that includes Herpes Simplex 1 and 2, Cytomegalovirus, Varicella-Zoster virus and Ebstein Barr virus. HHV-6 preferentially infects CD4+ T lymphocytes and the infection is followed by persistence and latency in different cells and organs including monocytes/macrophages, salivary glands, brain and kidneys. Studies indicate that HHV-6 infects 90 per cent by two years of age and the most frequent clinical manifestations consist of fever, fussiness, diarrhea, roseola (macupapular skin rash) and, in some cases, febrile seizures. Reactivation of HHV-6 infection is frequent in immunocompromised patients, specially in allogeneic hematopoietic stem cell transplantation (HSCT), and may result in different clinical syndromes. Clinical manifestations associated with HHV-6 reactivation in allogeneic HSCT patients include fever and skin rash, pneumonia, hepatitis, encephalitis and bone marrow suppression. Here we report an unusual Varicella-Zoster-like HHV-6 reactivation in a patient undergone allogeneic HSCT. He was a 51 years old male who received a reduced intensity allogeneic HSCT for multiple myeloma. Despite an acute graft versus host disease (GVHD) prophylaxis with short course methotrexate and ciclosporine A (CyA), after 80 days from allogeneic HSCT he developed an acute GVHD grade II (skin) completely resolved with prednisone and mofetilmicofenolate. After 6 months from allogeneic HSCT, during the tapering of immunosuppressive treatment, he showed the appearance of a painful macupapular rash followed by vescicle eruption involving the left side of the face such as cutaneous Varicella-Zoster reactivation. Antiviral therapy with acyclovir was started and serological antibodies with PCR viral DNA research were performed. The results showed a normal IgM Varicella-Zoster titers, while IgM HHV-6 titers were high and PCR HHV-6 DNA research was positive. For this reason, since the patient didn't respond to acyclovir therapy, we started gancyclovir antiviral treatment followed by complete resolution of clinical manifestations, even if a painful neuritis persisted.

In conclusion, this case remarks that the potential role of HHV-6 should be always considered in every differential diagnosis with Cytomegalovirus, Herpes Simplex and Varicella-Zoster before starting the right treatment in allogeneic HSCT recipients.

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BONE MARROW TRANSPLANTATION FROM UNRELATED DONORS IN CLASS 3 ADULT THALASSEMIC PATIENTS

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Allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling is the only treatment available to cure patients with thalassemia major, despite the improvements made over the last two decades in survival and quality of life. However the probability of finding an HLA-identical donor within the family is less than 30%. In all other cases it is necessary to search a voluntary donor among the bone marrow donor registries worldwide. During recent years with the increased life expectancy of thalassemia patients, the majority of request for the activation of voluntary donor search are received from adult patients. For this reason we decide to evaluate whether BMT using unrelated voluntary donors selected by high-resolution typing for the HLA class I and II alleles in high risk (class 3) adult thalassemia patients can offer a probability of cure

comparable to that obtained when the donor is a compatible sibling. Twenty-seven class 3 adult thalassemia patients (mean age 22 years, range 17 - 37) were submitted to unrelated BMT. The conditioning regimen consisted of Busulphan (BU) 14mg/Kg, Cyclophosphamide (CY) 120 or 160/mg/Kg in 11 cases and BU 14 mg/Kg, Thiotepa 10mg/Kg and CY 120 or 160mg/Kg in the remaining 16 cases. Cyclosporine and short term Methotrexate were used for GVHD prophylaxis. Twenty patients (74%) are alive and transfusion independent after a median follow up of 42 months (range 12 - 137). Nine patients (36%) developed acute GvHD and 8 (36,4%) chronic GvHD. Seven patients (26%) died from transplant-related causes. These results suggest that unrelated BMT in adult class 3 thalassemia patients may be a feasible therapeutical approach when the donor is selected by high-resolution molecular typing.

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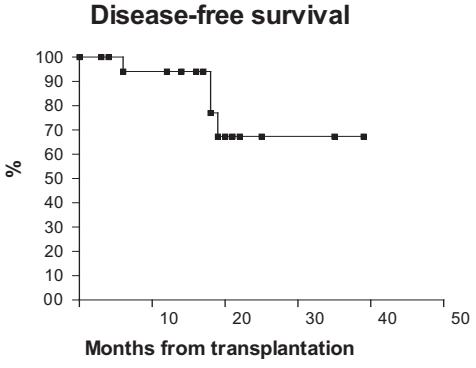
RESULTS OF ALLOGENEIC TRANSPLANTATION IN 50 MYELOMA PATIENTS WITH A REDUCED-INTENSITY CONDITIONING CONTAINING THIOTEPA, FLUDARABINE AND MELPHALAN

Majolino I, Riccardi M, Locasciulli A, Carnevalli E, Bacigalupo A, Di Bartolomeo P, Scimè R, Olivieri A, Narni F, Bregni M, De Fabritiis P, Musso M, Selleri M, Pogliani L, Corradini P, On Behalf Of The Gitmo

Ospedale S.Camillo, Roma; Ospedale S.Martino, Genova; Ospedale Civile, Pescara; Ospedale Cervello, Palermo; Ospedale Torrette, Ancona; Policlinico Univeritario, Modena; HSR, Milano; Ospedale S.Eugenio, Roma, Clinica La Maddalena, Palermo; II^Policlinico, Napoli; Ospedale S.Gerardo, Monza; INT, Milano, Italy

We have demonstrated that allogeneic transplantation is able to induce clinical and molecular remission in a high proportion of multiple myeloma (MM) patients (BMT 2003; 31:767-773). However, standard-dose conditioning still results in a high TRM. To reduce mortality, a low-intensity conditioning of fludarabine 3x30 mg/sqm, thiotepa 10 mg/kg and melphalan 80 mg/sqm with HLA-identical sibling stem cell transplantation was designed. GVHD prophylaxis is low-dose MTX plus CSA, but the latter is rapidly tapered following transplantation. PCR analysis for IgH gene rearrangement with patients specific oligonucleotides is employed for MRD assessment.



Up to now, 50 patients (38-68 y, median 53) have been allografted, at 3-123 months from diagnosis (median 12), 39 of them as a late treatment option after multiple therapy lines (including single, double or triple autograft in 32) and 11 as part of their front-line treatment following a debulking with only 3-4 courses of VAD. They received 5.53x 10^6/Kg (median) $CD34^+$ cells (range 1.5-10.6), and 2.8 x 10^8/Kg CD3+ cells (range 0.3-9.8) from BM or G-CSF-primed PB. Engraftment occurred in all, with 14 days to >0.5x10^9/L granulocytes (range 10-21) and 12 days to >20 x 10^9/L platelets (range 4-29). aGVHD >grade I developed in 46% evaluable pts, but it was > grade II in only one case. cGVHD developed in 64%. Transplant response was assessed at day +90. 20 out of the 34 (58%) evaluable pts were in CR. Only 6 pts died for transplant-related causes (TRM=11%). Four patients relapsed after achieving CR and 6 are in progression after partial remission. Sixteen patients are currently alive in continuous CR at a median FU of 17 months. OS is 80%, PFS is 55% and DFS 65% at two years. Eight CR patients were analyzed for chimerism and MRD at regular time points following transplantation. Despite the early establishement of complete donor chimerism in all, none showed a PCR-negativity for IgH-gene rearrangement on bone marrow samples 3-30 months following allograft. Reduced-intensity conditioning with fludarabine, thiotepa and melphalan is well tolerated even in patients with previous autograft(s) and ensures clinical remission in the majority. Preliminary PCR data of MRD however appear in contrast with clinical results.

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SAFETY AND EFFICACY OF LOW DOSES OF DLI IN PATIENTS WITH RELAPSE OF CHRONIC MYELOID LEUKEMIA AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Long-term survival after stem cell transplantation in patients with chronic myeloid leukemia (CML) requires adequate control of graft-versus-host disease (GVHD) and disease recurrence. The patients relapsing respond to donor lymphocyte infusion (DLI) but develop life-threatening complications. The use of DLI early at relapse is important since responses to DLI are less likely in the face of bulky or blast-phase disease; in fact, technical improvements in molecular methods of detection of the BCR-ABL transcripts permit the prediction of relapse with increased sensitivity and reproducibility. In our hand, in attempt to improve the outcome of this setting of patients with CML relapsed after allogeneic bone marrow transplant, we infused low doses of DLI after early relapse. Ten patients (7 male and 3 female) with median age at the transplant of 31,4yy(r.16-45yy) were treated. Minimal residual disease of p210 BCR-ABL detected was tested by RT-PCR nested after allogeneic transplant; in particular, during the first year, peripheral blood or marrow samples were collected and evaluated at

intervals monthly; during the second year every 3 months and at 6 months intervals thereafter. The median time between DLIs was 6 weeks. The time from transplant to relapse was 17 months and from relapse to first DLI was 2 months. The median number of infusions was 7 (range 2-16). The total median number of CD3+cells infused to reach complete remission was 37,4x106/kg(range 7,7-65). One patient developed acute GVHD. Chronic GVHD was observed in 1 patient. Three patients developed cytopenia after a median time of 30 days. Nine patients achieved complete molecular remission. The median time to obtain a complete response was 12,5 months (range 3-24).Out 4 of 10 patients had a second relapse and were infused a total median number of CD3+cells of 24,5x10^6/kg(range 3-48). All patients, but one, reached stable molecular CR. Three different patients that relapsed in Chronic Phase received a very high number of CD3+cells in a single infusion, 48, 59 and 65x106/kg, respectively. One died for brain bleeding, two had severe, prolonged and reversible cytopenia. In conclusion, the infusion of low doses of CD3+cells is a procedure advisable with minimum risk for the patients. The low doses of DLI result in CR in patients with relapse of CML. The anti-leukemia effect is not correlated with grading of GVHD. The single infusion of high dose of CD3+cells is not recommended for unacceptable toxicity.

P300

INCIDENCE AND OUTCOME OF INVASIVE FUNGAL INFECTIONS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: 12 YEARS EXPERIENCE AT A SINGLE INSTITUTION.

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Invasive fungal infections (IFI) are the most severe infectious complications in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Candida sp. and angioinvasive moulds (mainly Aspergillus) are the pathogens involved in the majority of cases. Despite recent advances in prophylaxis and therapy, the mortality of IFI remains extremely high, both in the pre-engraftment phase and in the late post-engraftment, especially when chronic graft-versus-host disease (cGVHD) is present.

We retrospectively reviewed the incidence and outcome of IFI in 246 consecutive patients who underwent allogeneic HSCT, both myeloablative (193, 78%) and non-myeloablative (53, 22%), at our Institution over a 12 years period (June 1992 – July 2004). Characteristics of the patients are summarized in Table 1. 150 patients (61%) were transplanted from a sibling donor, 96 (39%) from an HLA-matched unrelated donor (MUD). All the patients received antifungal prophylaxis, with itraconazole or fluconazole. For the definition of IFI, either proven, probable or possible, the EORTC guidelines were used. We documented 31 IFI, with a total incidence of 13%. The incidence was the same in the myeloablative (24/193, 12%) and non-myeloablative (7/53, 13%) setting. The IFI was proven in 20/31 cases (65%), probable in 7 (23%) and possible in 4 (12%). A significant higher number of cases was observed in the MUD transplants (20% vs 8%, p=0.006). The majority of IFI (26/31, 84%) developed in patients with advanced disease at time of transplantation, and 61% (19/31) had an acute or chronic GVHD at onset of infection. Moreover, 32% of IFI (10/31) occurred in patients with a fungal infection before HSCT, while only 5% of the non-IFI patients (11/215) had had a pre-transplant mycosis. Comparing the patients with or without IFI, the former were older (50 vs 39 years), with an advanced disease at HSCT (68% vs 41%) and with a MUD donor (61% vs 38%). In the myeloablative transplant the higher percentage of cases (46%) occurred in late post-engraftment (>100 days), while in the non-myeloablative setting in the pre-engraftment period (<30 days, 5/7 cases, 72%). The most common agents of IFI were Aspergillus sp. (70%) and Candida sp. (20%). The lung was the most common localization (81%) while a multi-organ involvement was present in 5 cases (16%). Only in 5/31 cases the IFI was cured, while 26 patients died of infection, with an overall survival at 30 and 100 days of 37% and 20% respectively.

TABLE 1. CLINICAL CHARACTERISTICS

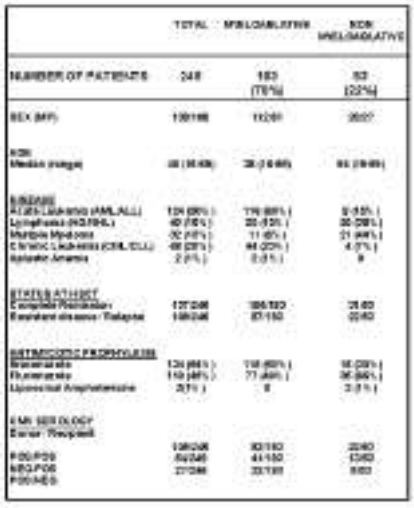
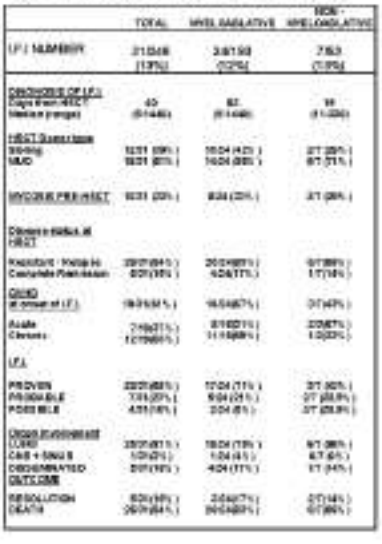


TABLE 2. CHARACTERISTICS OF I.F.I. AFTER ALLO-HSCT



P301

THE INFLUENCE OF KIR AND HLA-C GENOTYPES ON THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THALASSEMIA PATIENTS

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Killer immunoglobulin-like receptors (KIRs) mediate the activity of natural killer (NK) cells through recognition of HLA class I molecules HLA-C is the predominant ligand for the 2DL1, 2DL2 and 2DL3 inhibitory KIRs and the 2DS1 and 2DS2 activatory KIRs.

The replacement of an amino acid residue at positions 77 and 80 of the HLA-C α-helix makes it possible to divide HLA-C molecules into 2 groups (C1 and C2), each one selectively recognized by a specific pair of activatory/inhibitory KIRs.

Although numerous studies have investigated the impact of KIRs and their ligands on the outcome of hematopoietic stem cell transplantation (HSCT) for myeloid and acute lymphoblastic leukemia, the results remain controversial. The large amount of variables arising from the conditioning regimens and the clinical and immunologic factors associated with transplantation in oncohematologic disorders is probably the major drawback. For this reason, HSCT for thalassemia represents an ideal study model.

Forty-two thalassemia patients transplanted from an unrelated donor were analyzed. Twenty-eight donor/recipient pairs matched for HLA-A, B, Cw, DRB1 (B3, B4, B5), DQA1 and DQB1 were enrolled. All donor/recipient pairs were typed for the 14 KIR loci: 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1. The conditioning regimen was the same in all patients. No association was observed between transplant outcome and the number or types of activatory (2DS/3DS) and inhibitory (2DL/3DL) KIRs in donor and recipient pairs. Acute graft versus host disease and rejection were not significantly influenced by interaction of donor inhibitory and activatory KIRs with recipient Cw ligands. Interestingly, none of the 8 patients homozygous for C1 developed grade II-IV acute GvHD compared to 37% (7/28) of the patients with C1/C2 and C2/C2 genotypes. Conversely, rejection had a higher frequency in C1 homozygotes.

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SCLERODERMATOUS CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION: INCIDENCE, OUTCOME AND RISK FACTORS

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Introduction. Sclerodermatous chronic graft-versus-host disease (cGVHD) is often refractory to standard immunosuppressive therapy and life-threatening, especially in an advanced phase of disease. We evaluated its incidence, clinical characteristics, outcome and risk factors in 174 patients who underwent allogeneic haematopoietic stem cell transplantation (HSCT) from related and unrelated donors.

Patients and methods. The incidence of chronic sclerodermatous GVHD was evaluated in the 133 patients surviving at least 4 months after transplantation. Median follow-up was 33 months (range 5-155). Skin was evaluated by the modified scleroderma Rodnan skin score. Sclerodermatous cGVHD was defined as generalized when more than 2 anatomic sites were involved, localized in the remaining cases.

Results. Fourteen (10.5%) of the 133 patients analysed, showed sclerodermatous features at a median of 15 months after transplantation (range, 5-54). Previously, 11 (79%) of them had extensive cGVHD, 10 (71%) with skin involvement (hypo-hyperpigmentation/leopard skin eruption or lichenoid lesions).Nine patients (64%) presented symptoms 1-3 months before the onset of scleroderma (arthro-myalgias, leopard skin eruption or limbs edema); scleroderma was generalized in 7 cases and the median skin score was 12 (3-49). Eight patients (57%) had a complete or partial remission after first or second line therapy, which included methotrexate. Of the 6 patients nonresponsive, 4 died (3 because of progression of scleroderma, directly and/or through pulmonary infection). Eosinophilia 1-6 months before the onset of scleroderma, presence of autoimmune markers and previous skin involvement by chronic GVHD with hypo-hyperpigmentation and/or leopard skin eruption were significatively associated with an increased probability of developing scleroderma in univariate and multivariate analysis. Early onset time, generalized skin sclerosis and higher skin score could be associated with the resistance to immunosuppressive therapy and poor outcome.

Conclusions. In our patients, the incidence and the outcome of sclerodermatous chronic GVHD was about the same as that reported on previous papers. A better definition of risk factors for sclerodermatous chronic GVHD, also based on the knowledge about immunopathogenesis, should allow early diagnosis. Further investigation in a larger number of patients could allow a more precise evaluation of treatment outcome.

P303

ZOLEDRONIC ACID TREATMENT INCREASES MESENCHYMAL-DERIVED MARROW OSTEOGENIC PROGENITORS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Bone loss is recognized as one of the most frequent complications in long-term survivors after allogeneic stem cell transplantation (allo-SCT). We have recently documented that bisphoshonates increased both lumbar and femoral bone mineral density (BMD) in transplanted patients who had showed persistent bone loss. Although the specific mechanisms whereby bisphosphonates exert their beneficial osteotropic effect are not yet completely clarified, it is generally accepted that they have a direct effect on osteoclasts, inducing their apoptosis, thus inhibiting their bone-resorbing activity and blocking their precursor proliferation. In fifteen patients with osteoporosis (n=7) or rapidly progressing osteopenia (bone loss >5%/year) following allo-SCT, we investigated the 12-month effect of zoledronic acid given as three monthly doses of 4 mg on mesenchymal-derived marrow colony-forming units-fibroblast (CFU-F) cells belonging to the osteogenic stromal lineage. This group of patients was compared to a cohort of fifteen untreated patients. Enriched mesenchymal stem cells were used to obtain CFU-F cells. Mesenchymal stem cells were enriched by incubating whole bone marrow with a cocktail of antibodies against glycophoryn A, CD3, CD14, CD19, CD38 and CD66b, according to the manufacturer's instructions (Rosette-Sep, StemCell Technologies Inc., Vancouver, Canada). Unagglutinated cells, following density gradient centrifugation using lymphocyte separation, were then plated at a concentration of 1x 10^6/mL in mesencult medium containing McCoy's 5A modified medium, 10% fetal bovine serum with L-glutamine and 10-8 M dexamethasone (Sigma-Aldrich) allowing the recruitment of bone marrow mesenchymal cells to the osteoblastic lineage.

At baseline investigation, the marrow compartment of CFU-F cells as measured by in vitro testing resulted 3- to 4-fold reduced in transplanted patients compared with normal donors [mean CFU-F/106 cells plated ± SEM: 9.9±2.9 (range: 0-46) (n=19) vs 46.8±4.4 (range: 29-59) (n=15); p=0.000009].

Zoledronic acid treatment significantly improved in vitro growth of marrow mesenchymal cell-derived CFU-F cells in the whole group of transplanted patients (mean CFU-F/106 cells plated ± SEM: 8.7±1.7 vs 21.9±3.5 before and after treatment, respectively; p=0.002). These CFU-F cells clearly showed osteoblastic differentiation as documented by positivity to alkaline phosphatase staining after their replating in mesencult medium supplemented with 10-8 M dexamethasone, 0.2 mM ascorbic acid and 10 mM beta-glycerol phosphate (Sigma-Aldrich) and re-adhesion to plastic ware. By contrast, only a marginal increase in CFU-F cells was detected in untreated patients who were evaluated after an interval of time similar to that of the zoledronic acid-treated cohort (12±2 and 11±2 months in the untreated and treated cohorts, respectively): mean CFU-F/106 cells plated ± SEM: 7.1±1.6 vs 8.9±2.0, respectively; p=0.48. The beneficial effects of zoledronic acid treatment on in vitro growth of osteogenic progenitors was associated with a significant improvement of lumbar and femoral BMD in our transplanted patients.

In conclusion, short-term zoledronic acid treatment consistently improved both lumbar spine and femoral neck bone mineral density in transplanted patients who were at high risk for fast and/or persistent bone loss, partly by increasing the osteogenic progenitors in the stromal cell compartment.

P304

NEW PREDICTIVE FACTORS FOR LONG-LASTING BONE LOSS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Previously, in a cross-sectional study, we described a persistent decrease in bone mineral density (BMD) after allogeneic stem cell transplant (allo-SCT). Bone loss at lumbar spine was principally related to the transplant procedure, gonadal failure and immunosuppression, while reduced proliferative and functional capacity of osteoblast precursors influenced both lumbar and femoral BMD. However, transplanted patients may develop multiple chronic endocrine disorders and chronic graft versus host disease (cGVHD) potentially influencing cortical bone. Recently, we have also documented that increased serum leptin concentrations may contribute to T-cell activation during development of cGVHD. Although the bone effects of leptin are still controversial, it has been identified as a powerful inhibitor of bone formation through the hypothalamic system. Osteoprotegerin (OPG) is a recently identified cytokine with an important role in bone remodeling that neutralizes the effect of the receptor activator of NF-kappaB ligand (RANKL) on differentiation and activation of osteoclasts. Beside the effects of gonadal function, corticosteroid and cyclosporine A treatments, in this longitudinal study we have investigated possible influence of marrow osteogenic progenitors, circulating leptin, osteoprotegerin (OPG) and interferon-gamma (IFN-gamma) on BMD. Thirty two patients (15 female; age: 34.7 years; body mass index: 25±2.4 kg/m^2; time from transplant: 18.1 months) were enrolled and compared to 32 controls matched for age, gender and body mass index. BMD was measured at the lumbar spine and femoral neck. By using enriched mesenchymal stem cells in the colony-forming-unit fibroblast (CFU-F) assay, the osteogenic stromal lineage was evaluated. Bone densitometry and CFU-F assay were performed at the study entry and after 12 months of follow-up. Leptin (27.2±13 vs 7.3±1.8 ng/L; p<0.05), IFN-gamma (12.3±5.2 vs 6.5±5.4 mcg/L; p<0.05), and OPG (5.9±2.4 vs 1.8±1 pmol/L; p<0.05) were significantly higher in patients than controls. Lumbar and femoral BMD were lower in patients than in controls (0.9±0.16 vs. 1.04±0.08 and 0.8±0.1 vs.

0.9±0.1 g/cm^2; p<0.01). Number of colonies in the CFU-F assay (10±2.9 vs. 47±4) were significantly lower (p<0.01), too. The average 12-month lumbar and femoral BMD decrease was 3.4% and 3.8%, respectively. No significant change occurred in the CFU-F colony number. However, the amount of observed femoral BMD decrease was significantly correlated with the baseline CFU-F colony number (r=0.45; p=0.02) and inversely with leptin values (r=-0.37; p<0.01). Patients' IFN-gamma levels correlated with those of leptin (r=0.4; p<0.05) and OPG (r=0.54; p=0.04). There was no correlation between OPG and densitometric values.

Our finding suggests that persistent decrease of femoral BMD after allogeneic transplant could be at least in part regarded as a complication stemming from impairment of marrow osteogenic progenitor growth and from an immune system derangemet, both directly related to the transplant procedure.

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EFFECTS OF DIFFERENT ANTI-REABSORPTIVE TREATMENTS ON BONE MINERAL DENSITY IN YOUNG HYPOGONADAL WOMEN AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Ovarian failure is the most frequent complication after allogeneic stem cell transplant (allo-SCT) for hematological malignancies, and represents an important risk factor for the development of osteoporosis. The role of anti-reabsorptive treatment has not yet been systematically investigated in transplanted hypogonadal women. We investigate the effects of different antiresorptive treatments in females with ovarian failure long-term surviving after allo-SCT. Sixty women (age, 26.3±9 years; time from transplant, 8±4.2 months) with osteoporosis or osteopenia were divided into four groups of 15 women each. Group 1 was treated with Calcium and Vitamin D supplement alone; group 2 received the same treatment in combination with hormone replacement therapy (HRT: estradiol 2 mg daily plus dihydroprogesterone 10 mg for 14 days a month, for 12 consecutive cycles); group 3 received risedronate (35 mg weekly); group 4 received zoledronic acid (4 mg i.v. every 28 days for 3 months). All groups were similar for age, BMI, underlying disease and time elapsed from transplant. Lumbar and femoral bone mineral density (BMD) were measured at time 0 and at 12 months, while serum osteocalcin and urinary hydroxyproline were determined at 0, 3, 6 and 12 months. A significant decrease in lumbar and femoral BMD was observed in group 1 (-4.3±2.3% and –4.2±1.6%, p<0.05). A milder and not significant BMD decrease was found in group 2 (-3.1±1.4% and -3.3±1.5%). Risedronate treatment significantly increased lumbar BMD (5.8± 2.1%; p<0.05) and prevented bone loss at femoral neck (1.3±1.2%; p=NS vs. baseline). Zoledronic acid increased significantly both lumbar and femoral BMD (8.6±7%; p<0.01 and 5.4±2.2%; p<0.05, respectively vs. baseline). After the 12 month treatment, the changes in BMD between group 4 and groups 1-2 were significant at both lumbar spine and femoral neck (p<0.00001). The changes between group 3 and groups 1-2 were also significant at both lumbar spine and femoral neck (p<0.00001). The difference in femoral BMD was significant also between groups 3 and 4 (P <0.0001). In groups 3 and 4 hydroxyproline excretion was significantly reduced (p<0.01), while osteocalcin mildly increased only in group 4.

In conclusion, supplementation with Calcium and Vitamin D and correction of hypogonadism are not sufficientl to prevent bone loss in young adult women after allo-SCT. Bisphosphonate administration is necessary to prevent and treat bone demineralization. Zoledronic acid was the only therapy improving femoral BMD. The choice of oral or intravenous bisphosphonate administration can be made on the bases of individual clinical conditions and prevalent site of bone loss

P306

INCREASED MIXED CHIMERISM AND POSITIVE MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENT AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A ROLE FOR PRE-EMPTIVE DONOR LYMPHOCYTE INFUSION

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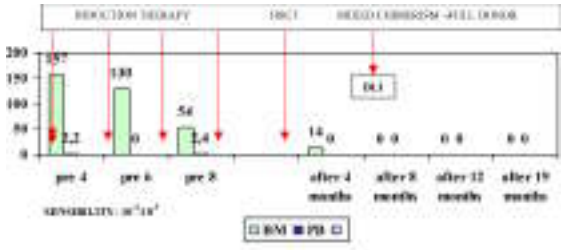
Several studies recently investigated the value of molecular minimal residual disease (MRD) monitoring in predicting outcome in adult B-lineage ALL using immunoglobulin antigen receptor genes as targets. On the other hand, it is found out that increased mixed chimerism is an important prognostic factor for unfavorable outcome in acute lymphoblastic leukemia patients after allogeneic stem cell transplantation. We report a case of acute lymphoblastic leukaemia patient who underwent allogeneic stem cell transplantation in morphological complete remission but with persistent positive MRD treated with preemptive donor lymphocyte infusion at the time of increasing mixed chimerism. On november 2003, a 26-years-old man presented with B-common ALL, FAB L1. A standard induction regimen was given according to the eight-cycles protocol for ALL applied in our institution, based on evaluation of MRD during induction treatment and subsequent different post-remission therapeutic approach on the obtained results. In this study evaluation of MRD is based on the principle that a monoclonal population of bearing leukemia cells undergoes a somatic VH-(DH)-JH immunoglobulin recombination event, unique in size and frequence for each B cell and its clonal progeny, so it is a a valuable leukemia-specific target for tracking MRD.

According to the MRD decreasing but persistent positivity after induction therapy and the presence of an available HLA pheno-identical mother donor, patient underwent allogeneic peripheral stem cell transplantation (PBSCT). Conditioning regimen applied was busulfan 16 milligrams/kg body weight po, from day -9 to day -6, cyclophosphamide 200 milligrams/Kg body weight intra-

venous, from day -5 to day -2. Patient received 7.64x10^6 CD34$^+$ cells. GVHD prophylaxis was cyclosporine plus "short" methotrexate. Supportive treatment (infection prophylaxis, VOD prophylaxis, transfusional support) were as standard practice for allogeneic PSCT. Toxicity was mild. Complete donor hematopoietic chimerism, assayed in the peripheral blood, was achieved and maintained until 7 months after PBSCT, when it became mixed; in the meanwhile no GVHD was observed. At the same time, MRD was decreasing but still positive so we decided to taper cyclosporine and to start a program of DLI infusion (4 courses) as a preemptive immunotherapy (table 1). CD3+ reinfused were 1x10^6/kg body weight at 1st cycle, 1 x 10^7/kilogram at 2nd ad 3rd cycles, 5 x 10^7/Kilogram at 4th cycle. Complete donor chimerism was achieved and MRD became negative after 2 cycles, so we decided to stop DLI after only 4 cycles, thinking about a possible severe acute GVHD and consequent increased mortality. GVHD I-II (skin, mouth) was observed without other toxicity. Patient is actually at > 570 day from PBSCT, in continuous complete remission and good clinical conditions.

Overt hematologic relapse of ALL can principally be prevented by withdrawal of cyclosporine and/or administration of low-dose DLI on the basis of chimerism. Serial analysis of chimerism reliably identifies patients with highest risk of relapse combined with monitoring of minimal residual disease. We experienced that pre-emptive immunotherapy can be effective in preventing relapse without serious adverse effects and DLI could be effective even in ALL when it is administered before hematologic relapse occurs and the tumor burden is low, as showed in some series.



P307

NODAL MARGINAL ZONE B CELL LYMPHOMA IN DONOR CELLS 20 YEARS AFTER ALLOGENIC BONE MARROW TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA

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In 1981 a 13-year-old girl was submitted to allogenic bone marrow transplantation (BMT) for severe aplastic anemia. At the time of the diagnosis she had been splenectomyzed for unknown reason. As conditioning regimen she received Cyclophosphamide 250 mg/Kg and "long" Methotrexate for GVHD prophylaxis. Transplantation was successful and the patient was well with full donor engraftment. No acute or chronic GVHD occurred.

In 1998, 17 years after BMT, she was diagnosed to be affected by HCV viral infection (genotype 2a/2c) with normal alanine aminotransferase.

In 2001 she had a spontaneous pregnancy with normal delivery.

In January 2003, 21 years after BMT, because of the appearance of a serum monoclonal component (IgM 1.7g/dL) a bone marrow aspirated and biopsy was performed which revealed 70% small to medium sized lymphocytes CD20+. Computed tomography scan of the chest and abdomen demonstrated a rounded hepatic hilar lymph node, 5.0 cm in diameter.

In June 2003 a diagnostic laparotomy was performed with multiple hepatic and nodal biopsies. The final diagnosis was nodal marginal zone B cell lymphoma, stage IVA with bone marrow and liver involvement. HCV RNA viremia by RT-PCR assay was 3.8x106U/mL. The analysis of polymorphism on lymph node, by microsatellites (apo B, MCT 118) documented the donor pattern polymorphism. Thus donor origin of lymphoma was documented. Bone marrow was persistently 100% full donor. At that time, donor, a 40-year-old woman, was healthy without any symptom of lymphoprolipherative disease and HCV negative. The patient was treated with recombinant interferon alfa-2b, 3 million units thrice weekly for 12 months. At the end of antiviral therapy the patient obtained a partial response with a 65% decrease in the size of lymph node and a decrease of bone marrow lymphocytes infiltration. Concomitantly the HCV viremia disappeared in the serum as well as the M component on immunofixation electrophoresis. This case describes a lymphoma as a late complication of BMT and underlines the role of host factors, mainly HCV infection, in the lymphomagenesis. Moreover we confirmed that in HCV-positive patient antiviral therapy may be an alternative to chemotherapy for the treatment of low grade B-cell non Hodgkin's lymphomas.

Posters II

Myelodysplastic Syndromes

P308

INTERNATIONAL PROGNOSTIC SCORING SYSTEM IDENTIFIES MYELODYSPLASTIC SYNDROMES WITH DIFFERENT PERIPHERAL BLOOD CD34+ CELL PATTERNS

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Introduction. The International Prognostic Scoring System (IPSS) defines the outcome and transformation risk of myelodysplastic syndromes (MDS). Peripheral blood (PB) CD34+ cells are known to stratify according to FAB subgroups. Whether a correlation exists between PB CD34+ cells and IPSS classification of MDS is undetermined.

Patients and Methods. PB CD34+ cells detected at the diagnosis of 29 MDS [according to WHO, 8 refractory anemias (RA), 7 refractory cytopenias with bi-/tri-lineage dysplasia, 5 RA with excess of blasts (RAEB)-I, 5 RAEB-II, 1 chronic myelomonocytic leukemia, 3 mixed MDS/myeloproliferative disorders] were compared with IPSS and its determinants [karyotype class, bone marrow (BM) blasts percentage, number of cytopenias]. Karyotype classes were as follows: good (GK): normal, isolated 5q-, 20q-, -Y; poor (PK): more than 2 anomalies, chromosome 7 abnormalities; intermediate (IK): other. Multiple group comparisons were made using Kruskal-Wallis one way analysis of variance. Continuous and categorical variables were compared by means of the Mann-Whitney U test and the Fisher exact test, respectively.

Results. According to IPSS, 10 (34.5%) low risk (LR), 10 (34.5%) intermediate risk (IR)-1, 8 (28%) IR-2 and 1 (3%) high risk (HR) patients (pts) were identified, respectively. GK, IK and PK were detected in 19 (66%), 6 (21%), and 4 (19%) pts, respectively. Chromosome 7 and chromosome 17 anomalies were the most frequently reported (14% and 10% of pts, respectively). Each 5q- and -Y occurred in 7% of pts. Neutrophil counts less than 1.8×10^9/L, hemoglobin less than 10 g/dL, and platelet counts less than 100×10^9/L were detected in 9 (31%), 19 (66%), and 14 (48%) pts, respectively. Less than 5%, 5-10%, and 11-20% BM blasts were observed in 18 (62%), 6 (21%) and 5 (17%) pts, respectively. Median PB CD34+ cells were 10.4/mcL (range, 2.7-66.4). Proportion of LR/IR-1 pts with 10 or more CD34+ cells/mcL was significantly lower than that of IR-2/HR pts (35% vs 100%, p=0.001). In the latter, median PB CD34+ cells (25/mcL, range 10-66.4) were significantly higher than those detected in LR (5.6/mcL, range 2.7-10.4) and IR-1 (14/mcL, range 3.1-37.3) pts, respectively (p=0.0003 and =0.045, respectively). Median PB CD34+ cells in the GK group (4.7/mcL, range 2.7-15) were significantly lower than those in the IK (30/mcL, range 4.6-56) and PK (36/mcL, range 13-66.4) groups, respectively (p=0.03 and =0.01, respectively). Similarly, median PB CD34+ cells were significantly lower in pts with less than 5% BM blasts than in pts with 11-20% BM blasts (4.9/mcL vs 25.2/mcL, p=0.036), and in pts with 0-1 cytopenias than in pts with 2-3 cytopenias (6.2/mcL vs 16.8/mcL, p=0.023).

Conclusions. By dividing MDS according to IPSS, definite patterns of PB CD34+ cells can be identified. Pts with intermediate-unfavourable cytogenetics, previously demonstrated to have reduced BM CD34+ cell apoptosis, have also high PB CD34+ cells. These findings support the prognostic impact of PB CD34+ cell assessment in newly diagnosed MDS.

P309

CLINICAL, MORPHOLOGICAL AND CYTOGENETIC FINDINGS IN MIXED MYELODYSPLASTIC/MYELOPROLIFERATIVE DISORDERS

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Introduction. According to WHO classification, mixed myelodysplastic/myeloproliferative disorders (MDS/MPD) are defined as conditions characterized by features of myelodisplastic syndrome (MDS) and myeloproliferation. Cytogenetic abnormalities, such as iso(17q), inv and t(3;3), have been frequently reported. Here we describe clinical-morphological characteristics and karyotype findings in 3 MDS developing proliferative features during follow-up.

Case Report 1. A 78-year-old male was diagnosed as RAEB-II in October 2003. Bone marrow (BM) cells karyotype was 47,XY,del(1)(p?34),+8[20]. Six months later, anemization, thrombocytosis, peripheral leukoerythroblastosis with normal peripheral blood (PB) CD34+ cells, lactate dehydrogenase (LDH) rise and spleen enlargement were observed. BM morfology showed hypercellularity with erythroblastosis and megakaryocytosis, and 8% myeloid blasts. Cytogenetics revealed a 47,XY,+8[2] /47,XY,del(1)(p?34),+8[5]/47,XY,del(1)(p?34),+8,add(17)(p1?2)[17]/48,XY,del(1)(p?34),+8,+13,add(17)(p1?2)[3] karyotype, demonstrating clonal evolution. In December 2004, the patient died for neurosarcoidosis.

Case Report 2. An 87 year-old female was diagnosed as RA in Jenuary 2004. At diagnosis, BM morphology showed megakaryocytosis and erythroblastosis with dysplastic features, myeloid hypoplasia without blast excess, and no reticulin fibrosis. BM cells karyotype was 46,XX, del(5)(q13q33), del (9)(q22) [5]/46,XX[14]. On December 2004, progressive thrombocytosis without leukoerythroblasto-

sis developed. Spleen was not enlarged. PB CD34+ cells were 37.3/mcL, and PB cell cultures demonstrated increased CFU-GM colony numbers [209/10^5 mononuclear cells plated, normal range: 1-18], confirming proliferative phase development. The patient is alive 13 months after diagnosis.

Case Report 3. A 76-year-old male was diagnosed as therapy-related MDS in August 2004. There was a history of multiple myeloma, in partial remission after melphalan-including schemes. In October 2004 leucocytosis, leuko-erythroblastosis with increased PB CD34+ cells (66/mcL), thrombocytopenia and LDH rise without spleen enlargement were registered. BM morphology showed trilineage dysplasia, myeloid hyperplasia without blast excess, deep megakaryocytic hypoplasia, focal reticulin fibrosis, and 10% plasma cell infiltration. The karyotype was 43,X,-Y,del(7)(q22),-15,-17,-18,+mar[13]. PB picture persisted until death, that occurred at the end of November 2004 for cardiac insufficiency.

Conclusions. These cases suggest the correlation between complex karyotype and poor prognosis to be mainteined during the proliferative phase of a MDS.

P310

EARLY MYELODYSPLASTIC SYNDROME: PRODUCTION AND POLYMORPHISM OF IMMUNOREGULATORY CYTOKINES

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Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders characterised by peripheral blood cytopenias, a bone marrow (BM) with dysplastic changes of hematopoieic cells, and risk of transformation in acute leukemia. Autoimmune phenomena and alteration of apoptosis have been recently reported in MDS. It's known that immunoregulatory cytokines, both up-regulating (IL-2, IFN-γ, IL-12, and TNF-α) and down-regulating (TGF-β, IL-4, IL-6, IL-10 e IL-13), control the autoimmune cytotoxic response. Furthermore, in several autoimmune and inflammatory conditions, an association with functional polymorphisms, known to modulate the production of immunoregulatory cytokines, is described. The aim of this study was to investigate IL-2, IL-4, IL-10, TNF-α, TGF-beta and IFN-gamma production in bone marrow and peripheral blood cultures from 20 patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS), compared with 28 controls (miscellaneous haematological conditions). Moreover, we investigated regulatory variants of 4 cytokine genes (TNF-α, IFN-γ, TGF-β, and IL-10) in the same cohort of patients compared with normal controls. We cultured PBMC from whole blood from MDS patients and controls. Cells were separated by density gradient and plated with PHA (2 micrograms/mL) for 48 hrs. All cytokines were evaluated in supernatants from bone marrow and peripheral blood cultures by ELISA assay. Cytokine producer genotypes were detected by PCR-SSP. Genotypic frequencies of the patients were compared with a series of 363 healthy Italian blood donors and differences among frequencies were estimated using chi squared test. We found that IL-2, IL-4, IL-10, TNF-α, and TGF-β production was significantly higher, whereas IFN-gamma was significantly lower in early MDS patients than controls (Table).

Concerning bone marrow cultures, IL-2, TNF-α, IL-10 and TGF-beta were reduced, although not significantly, in MDS compared with controls; IFN-γ and IL-4 were comparable (data not shown). Statistical analysis performed with chi squared test showed no significant differences in the frequencies of cytokine producer genotypes between patients and controls (data not shown). Our results showed that both Th-1 cytotoxic cytokines IL-2 and TNF-alpha and Th-2 inhibitory cytokines IL-4, IL-10, and TGF-beta were overexpressed in PB and not in BM in early MDS patients, suggesting that Th-1/Th-2 balance has no major role in BM failure and PB cytopenias. Consistently, neither cytokine producer genotypes demonstrated a shift towards a preferential Th profile.

Table.

	MDS patients	Controls
IL-2 (pg/ml)	1733±168*	504±52
IFN-gamma (pg/ml)	1200±9*	8969±795
TNF-alpha (pg/ml)	1076±151*	233±36
IL-4 (pg/ml)	202±9*	46±6
IL-10 (pg/ml)	1359±159§	973±133
TGF-beta (pg/ml)	7435±1576*	2430±184

*mean ± SE of 28 controls and 20 patients; * $p < 0.01$; § $p < 0.05$ patients vs controls*

P311

DEFERIPRONE IS USEFUL IN BLOOD TRANSFUSION-DEPENDENT PATIENTS AFFECTED BY MYELODISPLASTIC SYNDROME

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Blood transfusion is the only supportive therapeutic chance in MDS patients refratory to other treatments ("maturing" therapy, growth factors, low dose chemotherapy, biological response modifiers etc.). Repeated transfusions always cause an iron overload with an elevated associated comorbidity and mortality risk independently from their primitive hematological disease. Several studies (Rose C. *et al.,* Transfus. Clin. Biol., 2001, 8(5), 422-432) have demonstrated that patients with "good prognosis" (Refractory Anemia, Acquired Idiopathic Sideroblastic Anemia, and 5q- Syndrome) have an elevated morbidity and mortality risk after the transfusion of more than 100 units of blood red cells. On the basis of these results the use of iron chelators could reduce or prevent the iron overload damage. Until few years ago desferoxamine has been the only drug registered for clinical use. However, the chronic use of desferoxamine (s.c. administered) causes, sometimes irreversibile, ototoxicity and nephrotoxicity in polytransfused thalassemic patients. Deferiprone is a new iron chelator administered per os approved for clinical use. We have

treated 6 patients affected by MDS with deferiprone (4 AISA, 2 RA) refractory to any treatment modality and blood transfusion dependent form at least 1 year. All the patients showed before the beginning of the iron chelator treatment more than 2000 ng/mL of ferritinemia and a mean blood transfusion request of 1 unit of red blood cells every week in order to maintain Hgb levels higher than 8g/dL. After one month from the beginning of the therapy with deferiprone all the patients showed a reduction of ferritinemia (an about 15% decrease, r: 8-25%). Five out of six patients have shown a transitory increase of the transfusion request, however, after 3 months from the beginning of deferiprone therapy, a reduction of the transfusion request was recorded in all the patients (at the present, one unit every 14 days). Until to-day (8 months after the beginning of the therapy) we have not recorded either toxicity or adverse events. Further studies are warranted to define the role of deferiprone in reducing the blood transfusion request in patients affected by MDS refractory to any other kind of conventional therapeutic strategy.

P312

ABERRANT EXPRESSION OF THE PRION-LIKE PROTEIN DOPPEL IN BLAST CELLS OF ACUTE MYELOID LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES

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The PRND gene, located on human chromosome 20pter-p12, 27 Kb downstream from the prion protein gene, encodes for the Doppel protein (Dpl), that has many biochemical and structural properties in common with the prion protein (PrP). Both PrP and Dpl are associated with neurodegenerative disease by elusive mechanisms. Dpl is about 179 amino acid residues long, it is characterized by an alpha-helical conformation, and it is presented on the cell surface by a glycosylphosphatidylinositol anchor. Whereas PrP has a widespread tissue distribution, Dpl is expressed during embryogenesis and in adult testis and does not seem to be required for prion disease progression. No data are available on Dpl expression in hematopoietic cells. Since preliminary studies have demonstrated significant levels of Dpl expression in several types of cancers, we investigated the Dpl distribution in normal bone marrow cells, in leukemic cell lines and in bone marrow cells from patients with acute leukemia and myelodysplastic syndrome (MDS), in order to evaluate its possible ectopic expression in such disorders. Molecular and immunocytological studies were carried out on the human leukemic lines HL-60 and K562 and on bone marrow samples from 16 normal controls, 44 patients with acute myeloid leukemia (AML) and 63 patients with MDS (21 RA, 9 RARS, 14 RAEB, 7 RAEB-t and 12 CMML). A goat polyclonal antibody raised against a peptide mapping near the aminoterminus of Dpl of human origin (Santa Cruz Biotechnology, Inc.) was used for immunocytochemistry, flow cytometry and Western blotting. Normal samples were negative or showed very weak surface expression in rare immature cells (median 1%). Dpl expression was restricted to $CD34^+$ cells (71-82% positive) and down-regulated in stem cell differentiation. Dpl was detected in both cell lines and in most AML and MDS cases, with median percentages of positive blasts respectively of 13.5% (IQR 6.5-20%) and 16% (IQR 10-26%). Interestingly, in some pathological samples Dpl was ectopically localized in the cell cytoplasm and showed abnormal electrophoretic patterns. Quantitative RT-PCR revealed variable mRNA levels in almost all AML and MDS cases, but barely detectable levels in normal bone marrow (p=0.001). These differences were confirmed by in situ hybridization. On the contrary, prion gene expression did not differ between normal and pathological samples. PRND expression was higher in advanced compared with early MDS (p=0.01), but Dpl levels did not predict disease progression. In AML there was no correlation between Dpl levels and clinical or laboratory findings. Induction of HL-60 cells into granulocyte differentiation by ATRA was associated with down-regulation of Dpl expression. In conclusion, for the first time the expression of PRND has been demonstrated in human bone marrow cells. Its overexpression in leukemic and dysplastic cells could be explained by the immaturity or deranged differentiation of the transformed cells; anyway, the differential Dpl distribution in AML and MDS versus healthy subjects makes it a possible leukemia-associated antigen with important diagnostic and therapeutic applications. On the other hand, the Dpl expressing HL-60 and K562 cell lines may provide a useful model to study protein function and gene regulation.

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EXPRESSION OF MATRIX METALLOPROTEINASES 2 AND 9 IN MYELODYSPLASTIC SYNDROMES

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Matrix metalloproteinases (MMP) are a family of zinc-dependent endopeptidases which are able to degrade all the protein components of the extracellular matrix. MMP-2 (gelatinase) and MMP-9 (type 4 collagenase) have been implicated in tumor progression and metastasis and, recently, it was suggested that these enzymes may also contribute to leukemic dissemination. We analyzed the expression of MMP-2 and MMP-9 in bone marrow cells from 70 patients with myelodysplastic syndrome (MDS) (24 RA, 10 RARS, 21 RAEB, 5 RAEB-t, 10 CMML), not previously treated, and from 25 non hemopathic subjects, in order to evaluate whether abnormalities in their expression were associated with relevant laboratory and clinical findings. Moreover, a possible correlation was investigated between MMP positivity and altered apoptosis level, as measured by TUNEL technique, or altered proliferative activity, as evaluated by MIB-1 immunostaining. MMP-2 and MMP-9 were detected on bone marrow smears by an immunoalkaline phosphatase method (streptavidin-biotin complex) using primary murine monoclonal antibodies raised against human MMP-2 (clone A-Gel VC2, NeoMarkers) and human MMP-9 (clone IIA5, NeoMarkers). In normal samples MMP-2 was detected only in occasional

myeloid cells, whereas MMP-9 was expressed in some 30% of maturing myeloid cells. In MDS the percentages of cells positive for both MMP-2 (median 27%, IQR 12-43%) and MMP-9 (median 47%, IQR 35-58%) were significantly higher than those observed in normal controls (p=0.006 and 0.03 respectively); moreover, many erythroblasts expressed both enzymes. In early MDS (RA and RARS) percentages of MMP positive cells higher than in advanced forms were observed. In MDS a positive correlation between MMP-9 and TUNEL positivity was identified by the Spearman correlation test (p=0.04), whereas MMP-9 as well as MMP-2 expression was independent of the proliferative rate. A significant inverse correlation between either MMP-2 or MMP-9 and bone marrow blast cell percentage was observed (p=0.01 and 0.02 respectively), but no significant relationship was found between MMP levels and clinical and laboratory features such as age, leukocyte count or karyotype. A low MMP-9 expression was predictive of disease progression and shorter survival. Seven patients with early MDS were treated with thalidomide.Very interestingly, thalidomide treatment decreased the cellular expression of MMP-2 and MMP-9 as well as apoptosis in bone marrow erythroblasts of responsive cases. In conclusion, for the first time the relation between MMP abnormal expression profile and other biological and clinical features has been evaluated systematically in MDS. Our findings suggest that the production and release of these enzymes may influence hematopoietic cell death and behaviour, possibly by the processing of regulatory proteins in marrow, with a potential prognostic significance for disease progression. On the other hand, MMPs may represent specific targets for therapeutic intervention.

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DOES RECOMBINANT ERYTHROPOIETIN INFLUENCE SURVIVAL OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES? A SINGLE INSTITUTION RETROSPECTIVE ANALYSIS OF 169 CASES

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Evidence-based guidelines recommend the use of recombinant erythropoietin (r-EPO) with the aim of reducing transfusions and improving quality of life in anemic patients with cancer. Some reports, however, have recently raised the question of whether the use of r-EPO may also influence survival and clinical outcome of neoplastic patients who receive this drug along with the therapy of their disease. r-EPO is frequently used for the treatment of anemia in myelodysplastic syndromes (MDS). The efficacy of r-EPO in these patients have been extensively investigated in terms of improvement of Hb levels and quality of life parameters and its use is now recommended in selected settings of MDS. However, there is very small evidence in the literature about the long term effects of r-EPO in patients with MDS and, above all, about its possible relationship with survival. In particular, low-risk MDS, which generally do not receive cytostatic treatments, represent an interesting model to evaluate the impact of r-EPO on the natural history of these disorders.

We performed a retrospective analysis on our patients with MDS which was focused on determining overall survival and causes of death in relationship to the use or not of r-EPO. Between 1986 and 2001 346 patients affected by MDS were identified in our database. Among them, we selected 169 anemic (Hb < 10 g/dL) patients with well defined diagnosis of MDS according to FAB criteria (excluding CMMoL and RAEB-T), low-intermediate-1 IPSS score (retrospectively assessed) and regular follow-up. Ninety-nine patients belonged to the pre-r-EPO era or had never received r-EPO for other reasons. Seventy patients had instead received r-EPO (alpha or beta) for at least 12 weeks during the course of their disease. The two groups (r-EPO no/r-EPO yes) resulted comparable for age, type of diagnosis, additional treatments, Hb levels and transfusion requirement. Among patients who received r-EPO, 22 (31.4%) achieved an hematological erythroid improvement (HI-E), according to IWG criteria. In these patients the drug was given for 8-49 months (median 19).

There was no difference between the two groups in terms of leukemic evolution (19% vs 21% at 5 years) and causes of death (infections, hemorrhages, second tumors, cardiovascular events). No death could be directly attributable to r-EPO. No case of PRCA was documented. Median overall survival was also not statistically different between patients who had received (45 months) or not (41 months) r-EPO. However, when survival was analysed according to response to r-EPO, it was found significantly longer (p<0.03) in responders (median 54 months) than in non-responders (median 39 months), as well as than in subjects who had never received the drug. Such a difference was not modified by the time (early, during the first year from diagnosis, or later) of r-EPO administration. Response to r-EPO was associated with lower levels of endogenous EPO, lower percentage of marrow blasts and higher Hb values. However, at multivariate analysis of major prognostic parameters (including IPSS score), response to r-EPO maintained an independent prognostic value on survival. This is the first study focused on survival of MDS analysed according to the role of r-EPO. Although, overall, administration of this drug did not modify substantially the natural history of the disease, response to r-EPO was significantly associated with better survival, thus representing a possible new prognostic factor in MDS.

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DYSPLASIA DEGREE RELEVANCE AND REPRODUCIBILITY IN LOW-RISK MYELODYSPLASTIC SYNDROME: STUDY FROM PIEDMONT MDS REGISTRY

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Aims. Evaluation of the reproducibility of cytological criteria for low-risk MDS according to WHO classification and the degree of interobserver concordance in the diagnostic work-up.

Patients and methods. We reviewed 100 cases of MDS

with less than 5% marrow blasts, consecutively admitted in five hospital of Piedmont between 1998 and 2004, whose charts were recorded in the archives of Piedmont MDS Registry. Minimal requirements for patient selection were availability of at least two May Grunwald-Giemsa well stained and good-quality smears from bone marrow aspirate, and a complete set of clinical data such as hemogram, routine biochemistry, date of diagnosis, date and patient status of the last follow-up.

Smears were assessed for three-lineage presence and degree of dysplasia, according to WHO criteria: dyserythropoiesis (DE), dysgranulopoiesis (DG) and dysmegakaryopoiesis (DM) and percentage of blasts (at least 400 nucleated BM cells were counted). Presence of sideroblasts were evaluated on smears stained with Prussian blue. Minimal requirement for presence of dysplasia was the evidence of dysplastic characteristic in at least 10% of the nucleated cells of the same lineage. Each case was independently reviewed by two observers blinded to the clinical and laboratory data. The cases with an interobserver discrepancy were reviewed and re-discussed in order to reach a diagnostic consensus after 500 differential counts on BM. The interobserver concordance of each lineage was evaluated using the Cohen k test. Median survival were estimated by Kaplan-Meyer analysis: we considered patients with anemia (group A)versus pancitopenia (group B) and cases with unilineage versus multilineage dysplasia. IPSS score was evalueted for these groups of patients.

Results. The interobserver agreement for the three lineage dysplasia was very low (dyserythropoiesis: kappa=0.02, dysgranulopoiesis: kappa=0.5, dysmegakariopoiesis: kappa= 0.07). Forty cases with interobserver discrepancy on one or more lineage were revaluated and a consensus was reached by reviewing BM aspirate jointly.

We tried to classify patients according WHO classification: patients with definite clinical entities such as 5q- syndrome(7 cases), CMML(9 cases), RAEB(5 cases) were excluded from the analysis. Seventy-two patients were valuable: 20 with anemia only (Group A) and 52 with pancytopenia (Group B). Five out of 20 group A patients showed dyserytropoiesis only and therefore were classified as RA/RARS; the others didn't fulfill any of the known WHO category. Conversely 31/52 group B patients showed multiple dysplasia and therefore were classified as RCMD/RCMD-RS; the others, with a single marrow dysplasia didn't fulfill any of the known WHO category.

Group A cases showed a median survival longer than group B patients (median survival not reached versus 91 months)(p<0.01). By contrast all patients with unilineage dysplasia(group A+B) presented a median survival (not reached) not significantly different from patients with multilineage dysplasia (Group A+B) (91 months)(p= 0.1). Most cases with Group A had low IPSS score (12 low, 4 Int-1); by contrast Group B patients were in the intermediate IPSS range (2 low, 33 Int-1, 1 Int-2, 0 high).

Conclusions. 1) there was a low concordance rate between reviewers on the presence and grading of bone marrow dysplasias. This was in part due to the low quality of smears. 2)There are cases of anemia and dysplasia of 2 or 3 lineage and on the contrary cases with pancitopenia with only one dysplasia: these cases are not strictly classifiable according to WHO criteria. However, since the only prognostic relevant factor seems the presence of bi- or pancytopenia as opposed to single cytopenia (anemia), the impact of unilineage versus multilineage dysplasia on survival is uncertain.

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PROLONGED, HIGH DOSE RECOMBINANT ERYTHROPOIETIN IN MYELODYSPLASTIC SYNDROME: A CASE REPORT OF FURTHER ANEMIA IMPROVEMENT WITH INCREASING EPO DOSES.

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Human recombinant EPO improves anemia in approximately 20 % of patients with myelodysplastic syndromes (MDS) and 35% of those with low blast counts (<10%) for a median time of 12 months(Tergos et al Br J Haem, 2002; Stein R.S.Clin Lymph 2003) The percentage of responding patients further increases to about 50% with prolonged therapy (> 6 months) and/or high EPO doses (40.000 U x 2/week), with evidence of an improvement in quality life and some long-lasting erythroid responses (Clavio et al. Eur J Haematol 2004). Here we describe a 72 year old patient, with intermediate-risk MDS (RAEB 1; IPSS Intermediate I) who obtained a benefit from a further increase in EPO dosage to 40.000 U x 3/week. The patient had a MDS diagnosis in 1/8/02. He was transfusion dependent(2U/month), with EPO serum level 200 UI/l.The patient received rHuEpo at doses of 40.000Ux2/week + a "differentiative" therapy with 13-cis retinoic acid and dihydroxylated vitamin D3 (Ferrero D *et al.,* Leuk Res, 1996), this resulting in minor response, with a 50% reduction in transfusion need for one year. EPO dosage was then increased to 120.000 U week to improve precedent response and a major response was achieved, with no longer transfusion requirement and Hb level of 11 g/dL. The therapy was well tolerated and previous fatigue and dispnea improved; the patient achieved a good performance staus (1) and was self sufficient in daily endash life for three months: the patient then died for acutization of co-existing chronic obstructive broncopneumopathy, with an Hb level of 10,7 gr/dL.

Our patient presented with unfauvorable prognostic features for response to rHu EPO, such as: bone marrow blasts > 5%, transfusion requirement of 2U/month and high serum EPO level. Indeed, only a minor erythtroid response was initially achieved in spite of high dose EPO + "differentiative" therapy. Nevertheless, the response improved with a further increment in weekly EPO dosage. This suggests that very high rHuEPO dosage (120.000 U/week) can be well tolerated and may be effective in some patients partially refractory to 80.000 U/week and/or with low chances of erythroid response.

P317

MYELODYSPLASTIC SYNDROMES IN PATIENTS WITH LESS THAN 50 YEAR OLD: IDENTIFICATION OF HIGH RISK FEATURES

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Myelodysplastic syndromes are rarely described in patients under 50 years and the few reports mostly referred to patients from different institutions and/or included in bone marrow registries.

We report here on 62 patients under 50 years (9%) observed at our Institution among a consecutive series of 689 patients diagnosed as having MDS between July 1983 and December 2000. Diagnosis was made according to FAB criteria; parameters included in the analysis were age at presentation, sex, Hb, WBC, platelet and neutrophil count, percentage of peripheral blood (Pb) and bone marrow (BM) blasts, cell-lineage involvement, transfusional requirement, infectious episodes and cause of death. There were 26 males and 36 females (ratio 0.7), median age 43 years (30 patients <40 and 32 > 40 years); no history of familiar myelodysplasia was revealed in any patients. A prior occupational exposure to potential carcinogens was recorded in 13/62 (21%) patients. According to FAB classification 30 patients had refractory anaemia (RA) 3 patients refractory anaemia with sideroblasts (RARS), 18 patients refractory anaemia with excess of blasts (RAEB), 6 patients refractory anaemia with excess of blasts in transformation (RAEB-t) and 5 patients with chronic myelomonocytic leukaemia (CMML). Reclassification according to WHO distinguished among the 30 RA patients, 15 patients with pure RA and 15 with refractory cytopenia with multi-lineage dysplasia (RCMD) and in the RAEB category, 12 patients with RAEB-I and 6 with RAEB-II. In the whole series of patients, median Hb level was 9.8 gr/dL, median WBC count 4.3 x 10^9/l and median platelet count 109 x 10^9/l. Cytogenetic analysis showed a normal karyotype in 34/50 analysed patients, a trisomy 8 in 5 patients, a monosomy 7 in 3 patients and other aberrations in 8 patients. Exposure to carcinogens was associated with male sex, age > 40 years, presence of monosomy 7 (3 patients) and del(5q) (2 patients), prevalence of high risk disease (5 RCMD, 2 RAEB, 1 RAEB-t, 1 CMML) and high frequency of AML transformation. Univariate analysis of prognostic factors for survival showed that age >40<50 (p=0.02), presence of PB blasts (p=0.04), IPSS intermediate-2 and high risk (p=0.002) were statistically significant; prognostic factors significant at univariate analysis for risk of transformation to AML were FAB classification (p=0.0001), presence of PB blasts (p=0.0001), >5% BM blasts (p=0.001), stratification by Spanish score (p=0.0001), by Bournemouth score (p=0.02) and by IPSS (p=0.0003).

At a median follow-up of 20 months, 19 patients (31%) evolved to AML. As compared to the series of patients >50 years observed at our Institution in the same period, differences were noted as to sex ratio (0.7 vs 1.56 in patients >50 years, p=0.002), frequency of RAEB/RAEB-t in patients >50 years (p=0.02) and median survival (49 months vs 24.6 in older patients, p=0.001). In conclusion, some clinical and prognostic features appear to be characteristic of young MDS patients; our analysis showed the usefulness of IPSS which should be tested in larger series of consecutive young patients to confirm its predictive value.

P318

TREATMENT WITH IMATINIB MESYLATE IN PATIENTS WITH REFRACTORY ANEMIA WITH EXCESS OF BLASTS AND SECONDARY AML.

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Imatinib is an inhibitor of specific protein tyrosine kinases activity that was targeted to the platelet derived growth factor (PDGF) receptor, bcr-abl and c-kit. Several protein kinases are deregulated and overexpressed in human cancers. The product arising from the Philadelphia (Ph) chromosome of chronic myelogenous leukemia, the BCR-ABL tyrosine kinase is inhibited by Imatinib. The efficacy of Imatinib was also demostrated in gastrointestinal stromal tumors (GIST). There are mutations in c-kit in approximately 60% of cases. The proto-oncogene c-kit encodes a transmembrane tyrosine kinase receptor located on the long arm of chromosome 4 (4 q11-q12). Its ligand is the stem cell factor (SCF). This proto-oncogene has a role in the development of normal hematopoiesis and is also expressed in immature myeloid cells. In acute myeloid leukemia (AML), c-kit expression can be detected in 65-90% of the novo cases. SCF stimulation of leukemic blasts results in increased c-kit tyrosine phosphorilation and induction of proliferation, indicating a functional role of c-kit in AML. Recently a complete hematologic remission is described in a patient with c-kit + AML after administration of Imatinib. We have also, treated with Glivec two patients suffering from acute leukemia and AREB, whose blasts were expressing c-kit (CD 117 +). In the following, patients ' features and results are reported.

Case1. A 71 years old man, was diagnosed to suffer from a refractory anemia with excess of blasts (RAEB) in june 2000, IPI score 0.5. The bone marrow aspirate, performed in september 2001, after 14 months from diagnosis, revealed an increment of leukemic blast infiltration (25%) and fluorescence activated cell sorter (FACS) analysis showed a strong expression of c-kit receptor (CD117 and CD34 +) on 80% of the leukemic blasts. The patient was also anemic. The patient, given his written informed consent, received imatinib in a daily oral dose of 400 mg. A control of the bone marrow aspirate, after 4 and 8 weeks of treatment, showed a reduction of blast cell infiltration (25->7%) and immunological flow cytometric study confirmed the reduction of expression of c-kit receptor as well. We did not observe improvement in hemoglobin level, whereas a mild increment in neutrophil count occurred. The treatment was stopped after 12 weeks because the patient refused to go on. No adverse events were observed.

Case 2. A 60 years old patient, suffering from AREB diagnosed in1998, performed auto ed allo-graft in August 1999. In Novembre 2001 diagnosis of LMA French-American-British (FAB) subtype M2, was performed, with the evidence of blasts positive to CD13, CD33, CD34 and CD117 by FACS analysis. Normal karyotype. The hematological exams showed WB 30000 Bl 50% Hb 9.6 Plt 10000. In later on previously therapy and bad conditions of patient, no chemotherapy was give, and the treatment with imatinib 400 mg die for 2 weeks, followed by 600 mg/die for 2

months, was enterprised. The bone marrow aspirate performed after 30 days, showed a reduction of blast infiltration of the bone marrow itself (90%->50%) and a reduction of expression of c-kit (CD117) which persisted only on 50% of blasts. After 60 days we observed a further reduction of blasts expressing CD 117 (20%). No improvement in hemoglobin level and platelets count , but reduction of white blood cells we obteined. After 80 days the patient died from cerebral hemorrage due to heavy piastrinopenia.

Discussion. In our experience we have observed the reduction of blasts that were expressing c-kit documented by FACS analysis. In the first case we could think that a longer treatment could have given better results as far as reduction of bone marrow blasts concurred. In the case n.2, we have observed a reduction of number of blasts expressing c-kit, but therefore this has not changed in any way the ongoing of the disease. Activation of c-kit may be due to autocrine/paracrine signaling or to structural alterations, wich confer factor-independent proliferation. In AML, mutations have been described in exon 8,10,2,11,7 of the c-kit. In recent report, sequence analysis by standard PCR failed to detech such alterations. The response observed in our patients (reduction of number of blast cells) is most likely due to inhibition of c-kit signaling. However, whether c-kit is activated because of a mutation, autocrine production of kit-ligand, or exposure to kit-ligand on stromal cells cannot be determined. Altough the clinical outcome of the two patients has not been modified by the means of Imatinib, we think that the activity of this drug on leukemic cells expressing c-kit should be considered a challengig result. Anyway we think, as well, that further studies should be performed on a wider number of patients.

P319

PROGNOSTIC IMPACT ON SURVIVAL OF WHO RE-CLASSIFICATION IN FAB LOW-RISK MYELODYSPLASTIC SYNDROMES

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According to the WHO classification, low risk myelodysplastic syndromes (MDS) have been defined only when dysplasia is restricted to the erythroid lineage. We have reclassified according to this system, our series of consecutive FAB low-risk MDS patients, with the aim of verifying the clinical and prognostical impact of the new classification. From January 1990 to December 1998 we observed 240 patients who were classified according to FAB criteria in refractory anemia (RA, 214 patients) and refractory anemia with ringed sideroblasts (RARS, 26 patients). According to the WHO system, 35 of the 214 RA patients had two or more lineage dysplasia and were reclassified as refractory cytopenia with multilineage dysplasia (RCMD); of the 36 RARS cases, 9 were reclassified as RCMD with ringed sideroblasts (RCMD-RS). When analysing presenting features of patients, no differences were observed between WHO subgroups as regards age, haemoglobin level, neutrophil counts. Transfusional requirement was higher in RCMD category as compared to RA patients (p=0.05) and also infectious and haemorrhagic events were more frequent in multi-lineage dysplasia patients (p=0.04 and 0.05, respectively). Median survival of RCMD patients was significantly inferior as compared to RA patients (30 vs 48 months respectively, p=0.001); more evident was also the difference of median survival between the RCMD-RS patients and pure RARS patients (16 vs 31 months respectively, p=0.002).

WHO classification also had a strong impact on distinction of low-risk patients with higher probability of acute transformation: we detected a 34% of AML in the group of RCMD vs 14% of transformation in pure RA patients (p=0.001), and a 56% in RCMD-RS group vs 29% in RARS patients (p=0.0001). In the WHO redefinition, also the application of prognostic scoring systems were able to distinguish risk stratification, especially for Bournemouth score that clearly identified low and intermediate risk in terms of survival and risk of acute transformation.

In conclusion, our analysis shows that WHO application for low-risk MDS is useful and may be of help in the identification of patients who might benefit of more intensive therapies and/or stem cells procedures.

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ERYTHROPOIETIN ALPHA TREATMENT OF ANEMIC PATIENTS WITH LOW OR INTERMEDIATE-LOW-RISK MYELODYSPLASTIC SYNDROMES, SELECTED ON THE BASIS OF LOW PRE-TREATMENT ENDOGENOUS ERYTHROPOIETIN LEVELS. RETROSPECTIVE STUDY OF 30 PATIENTS

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Background. Recombinant human erythropoietin (rHuEPO), given daily or 3 times a week, subcutaneously, at a dosage of 30-70.000 U/week, has proven effective in relieving symptomatic anemia in 20-30 % of patients (pts) with myelodysplastic syndromes (MDS). A low or intermediate-low risk score, according to International Prognostic Score System (IPSS) (Greenberg et al, 1997) and low pre-treatment serum EPO levels are both associated with a higher probability of response.

Aims. as rHuEPO is expensive, and the percentage of responder MDS pts is low, an attempt to select MDS pts on the basis of basal serum EPO levels and IPSS score, is justified.

Methods. from September 2001, 30 pts (17 males, median age: 76, range 34-92 yrs) with low-or-intermediate risk MDS were treated with rHuEPO alpha because of symptomatic anemia (Hb < 10 g/dL). The pts received a high doses rHuEPO regimen: 40.000 U twice weekly, for the first month, followed by a single weekly dose of 40.000 as maintenance treatment, for at least 8 weeks. Only pts with a pre-treatment serum EPO < 200 U/l were selected for rHuEPO treatment.

Results. 28/30 pts showed a favourable response. 8 pts were transfusion-dependent. Among them, 6 showed a significant (at least 50%) decrease of their transfusion need. All the remaining 20 pts, with less severe anemia, not requiring transfusions, showed a clinical significant

response (i.e. a > 1g/dL increase of Hb). The median duration of response was of 13 (2-41) months, and 24 pts are still maintaining response under maintenance treatment.

Summary/*Conclusions.* although only 20-30% of MDS pts show a clinical significant response to rHuEPO, if pts are carefully selected on the basis of a low or intermediate-low risk score (following IPSS) and a low (< 200 U/l) pre-treatment endogenous EPO level, the percentage of responses is high.

P321

BONE MARROW BLAST EVALUATION IN MYELODYSPLASTIC SYNDROME: PROBLEMS EMERGING FROM THE EXPERIENCE OF THE PIEMONTE MDS REGISTER

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Background. Bone marrow blast count is crucial for both the WHO classification and the IPSS prognostic score of myelodysplastic syndromes (MDS). Non-RAEB, RAEB-I and RAEB-II are apparently easy to differentiate, based on bone marrow blast percentage, however some concerns arise about the reproducibility of this simple tool. In particular there are no so far data about the differences among cytology, histology and flow cytometry in blast counting in order to define non-RAEB, BAEB-I and RAEB-II categories.

Aim of work. To analyse the prognostic difference among cytology, histology and flow cytometry in defining different MDS categories based on bone marrow blast count.

Patients and Methods. Since 1999, clinical and laboratory data from 756 new cases of MDS were prospectively recorded into the Piemonte MDS register through our web site. Two hundred and eighty eight patients were excluded because bone marrow blast quantification according to conventional cytology was not reported in file, and 32 patients were excluded because RAEB-t. In the remaining 436 patients, who are the object of the present analysis, bone marrow blast count according to conventional cytology (BMC) was retrospectively compared to both histochemical evaluation of $CD34^+$ on bone marrow threphine biopsy (BMH), and flow cytometry count of $CD34^+$ cells (BMFC).

Results. Patients distribution according to BMC was: non-RAEB 255, RAEB-I 108, RAEB-II 73. BMH and BMFC evaluation was available in 282 and 124 cases respectively. A disagreement between BMC and BMH was evident in 93/282 cases (33%): BMH over-evaluated and under-evaluated the WHO class, as originally defined by BMC, in 75/282 (27%) and 18/282 (6%) cases respectively. When comparing BMC and BMFC the disagreement was shown in 41/124 cases (33%), with BMFC over-evaluating and under-evaluating blast percentage in comparison to BMC in 13/124 (10%) and in 28/124 (23%) respectively. The disagreement between BMH and BMFC was globally evident in 40%. As expected BMC predicted a significant prognostic difference ($p<0.05$) among non-RAEB, RAEB-I and RAEB-II patients. However when comparing the three different methods of computing bone marrow blasts, BMFC was better than the others two methods in order to predict the favourable prognosis of non-RAEB patients.

Conclusions. The distinction among non-REAB, RAEB-I and RAEB-II is far from accurate and reproducible. In comparison to the conventional BMC system, BMFC should probably offer a better tool in order to select a group of patients at favourable prognosis. Large multicenter prospective studies should be useful.

P322

ANALYSIS OF CAUSES OF DEATH IN MYELODYSPLASTIC SYNDROME: RESULTS EMERGING FROM THE EXPERIENCE OF THE PIEMONTE MDS REGISTER

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Backgroynd. The best treatment for myelodisplastic syndromes is difficult to define. Aggressive chemotherapy strategies are at high risk in a population of elderly patients, while results obtained with differentiation drugs are moderate. Therefore for the majority of patients the only feasible treatment is supportive care. There are no so far population studies that consider the causes of death in order to evidence how often they die of complications due to cytopenia and leukaemic transformation in comparison to other unrelated causes.

Aim of work. To analyse the causes of death of a large group of myelodysplastic syndromes and to evaluate the percentage of unrelated causes.

Patients and Methods. Since 1999, data from 756 unselected new cases of MDS were prospectively recorded into the Piemonte MDS register through our web site. As expected mean age was very high (72 years; range 27-95), with 22% of them older than 80 years. One or more comorbidities were present at diagnosis in a percentage as high as 91%. Thirty two patients were excluded from the present analysis because RAEB-t. Up to December 2004, one hundered and seventy patients have died, and the causes of death were recorded for 167 of them who are the object of the present study.

Results. The causes of death were as follows: complications due to cytopenia or leukaemic transformation in 57 patients (34%), infections in 21 patients (23%) and other causes age or comorbidity related in the remaining 89 patients (53%). No significant differences of causes of death were seen according to sex. As expected, deaths secondary to unrelated causes increased with increasing age: from 29% for patients under 60 years up to of 67% for patients over 80 years. Deaths due to cytopenia complications or leukaemic transformations were more frequent in patients without any comorbidity (64%), while no differences were

seen according to the number of concomitant comorbidities: 29% for patients with one associated disease; 28% for patients with two and to 30% for patients with 3 or more. A significant relationship was also evident between causes of death and diagnostic subgroups: deaths due to unrelated causes were maximum for RA (81%) and minimum for REAB (41%) ($p<0.05$). A similar relationship was evident between IPSS and causes of death: deaths due to unrelated causes were maximum for IPSS score low/intermadiate-1 (67%) and minimum for IPSS score intermediate-2/high (28%) ($p<0.05$).

Conclusions. The analysis of causes of death of MDS patients suggest that a high proportion of patients die of unrelated causes. Age and comorbidities play a major role in defining the treatment strategy of this group of patients. Aggressive and potential toxic regimen should be limited to a small group of patients with diagnosis of RAEB and high IPSS score, while the majority of patients should benefit from an improvement in supportive treatment.

P323

FLT3 EXPRESSION IN MYELODYSPLASTIC SYNDROMES: POSSIBLE CORRELATIONS WITH DISEASE PROGRESSION AND RESPONSE TO TREATMENT

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About one-third of MDS patients acquire an internal tandem duplication (ITD) or a point mutation of the fms-like tyrosine kinase 3 gene (FLT3) at the time of progression to AML. ITDs and point mutations of the FLT3 gene are the mechanisms through which a high expression of the gene is achieved. Therefore, we planned to evaluate FLT3 expression in relation to clinico-hematological features and disease outcome in 23 MDS patients. Our study was especially aimed at establishing whether FLT3 expression was increased in 7 MDS patients who progressed to AML and whether it correlated with response to treatment. Our MDS patients were evaluated at diagnosis and during disease outcome. Thirteen patients were males and 10 females, their median age was 57 years (range 36-74). According to FAB classification 5 were diagnosed as refractory anemia with ringed sideroblasts (RARS), 13 as refractory anemia (RA) and 5 as refractory anemia with excess of blasts (RAEB). Conventional cytogenetics identified a normal karyotype in 17 patients, a del(20q) in 3, a del(5)(q13q33) in one, a del(5)(q23q33) in one and a del(12p) in one. In eighteen patients blast cell percentage was 0-5%, in three it was 6-10% and in two it was 11-20%. According to IPSS, thirteen patients were classified as low-risk, seven as intermediate-1 risk and three as intermediate-2 risk. FLT3 relative quantification was obtained through a real-time polymerase chain reaction (PCR) which employed SybrGreen I as DNA-binding fluorescent dye. Standard curve for real-time quantification was obtained by serial dilution of total RNA isolated from mononuclear cells collected from a patient affected by AML, exhibiting FLT3 ITD and elevated expression of FLT3 mRNA. Gene expression was calculated by DDCt method. FLT3 levels were normalized to ABL and calibrated on a normal sample. At the onset of MDS FLT3 expression was similar to that of the normal sample in 20 patients, while it was two-four times increased in the other 3 patients. No correlation was found between a high FLT3 expression and any clinico-hematological feature. Seven patients (3 RA and 4 RAEB) evolved to AML after a median time of nineteen months (range 8-42). These three patients had showed an elevated FLT3 expression already on clinical diagnosis, while the remaining 4 had presented an abrupt increase of the expression of the gene just on progression to AML. In these 7 patients FLT3 was expressed two-seventeen times more than in the normal sample. Four patients received different courses of intensive chemotherapy. Two of them, who failed to respond to such treatments, showed persistently elevated FLT3 expression levels. The other 2, who achieved a complete remission (CR) of twenty and eight months duration, presented a normal FLT3 expression. Both the patients developed a clinical relapse and showed a quick rise of FLT3 expression, which was six and eight times that of the normal control. In conclusion in our series FLT3 expression i) is found elevated in 13% of MDS patients analysed on clinical diagnosis, ii) is not associated with any peculiar clinico-hematological features, iii) is significantly increased in the 7 patients who progressed to AML. Four of these last patients received different courses of intensive chemotherapy and in these patients FLT3 expression seems to be correlated with response to treatment and relapse.

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PROGNOSTIC RELEVANCE OF CHROMOSOME 3 ABNORMALTIES IN MYELODYSPLASTIC SYNDROMES

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Abnormalities involving the long arm of chromosome 3 (3q abnormalities) are detected in all AML cytotypes, in MDS and in megakaryoblastic crisis of chronic myeloid leukaemia. Patients carrying 3q21q26 abnormalities seem to have similar clinico-pathological characteristics and an identical molecular lesion. However, recent data point to the fact that a unique molecular mechanism is unlikely to be involved in all the 3q rearrangements. From a prognostic viewpoint these abnormalities are associated with a poor disease outcome in AML, while in MDS they are included within the IPSS intermediate risk cytogenetic category. The present study was aimed at detecting the incidence, clinical features and prognostic significance of 3q rearrangements in a series of 352 consecutive MDS patients. 3q abnormalities were discovered as a single defect in ten patients (2.8%). Three of them showed an inv(3)(q21q26), three a t(1;3)(p36;q21), one a t(3;3)(q21;q26), one a t(3;5)(q21;q33) and two a del(3)(q21). They were five males and five females and their median age was 64 years (range 46-87). According to FAB classification one patient was classified as Refractory Anemia (RA) without

ringed sideroblasts, five as RA with excess of blasts (RAEB) and four as RAEB in transformation (RAEB-t). Six patients presented at least one cytopenia, three showed two cytopenias and one showed three cytopenias. From a morphologic point of view abnormalities of megakaryocytpoiesis were observed in a total of four patients: one presenting the t(3;3) translocation and the remaining three patients presenting an inv(3)(q21q26). In these four patients the average number of platelets was 487.x109/l. Considering all the ten patients median blast cell percentage was 20% (range 5-28). According to IPSS four patients were classified as intermediate-1 risk, one as intermediate-2 risk and five as high-risk MDS. The 2-year survival probability for patients with 3q abnormalities was 0.22% (95% confidence intervals, 95% CI, =0.03-051) and their mortality rate was 5.8 times (95% CI= 2.6-12.9; p-value=0.001) higher than that of chromosomally normal patients. An evolution into AML occurred in four patients, who were submitted to various courses of intensive chemotherapy without achieving any response. The 2-year progression-free interval (PFI) for patients with 3q abnormalities was 0.4% (95% CI= 0.01-0.82) and their probability of disease progression was 3.4 times (95% CI= 1.1-10.0; *p*-value= 0.025) higher than that of chromosomally normal patients. When compared to patients included in the IPSS intermediate risk cytogenetic group, patients with 3q abnormalities showed a significantly higher mortality rate (hazard ratio = 0.37 with 95% CI = 0.17-0.80 and p-value=0.013), which was similar to that of patients included in the IPSS high risk cytogenetic category (hazard ratio = 1.1 with 95% CI = 0.52-2.40 and p-value=0.7). In addition, their PFI was similar to those of patients included either in the IPSS intermediate or in the high risk cytogenetic group (*p*-values = 0.8 and 0.2 respectively). Our data show that: i) 3q rearrangements have a low incidence in MDS, ii) a high platelet number and abnormalities of megakaryocytopoiesis are clinical and morphologic features only present in patients with either inv(3) or t(3;3), iii) patients with 3q abnormalities could be segregated from the IPSS intermediate risk cytogenetic category when their mortality rate is analysed, however they are correctly included within this IPSS cytogenetic category when the probability of disease progression is considered.

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ANALYSIS OF THE USE OF RECOMBINANT HUMAN ERYTROPOIETIN IN MYELODYSPLASTIC SYNDROMES ACCORDING TO THE INDICATIONS OF THE ITALIAN MINISTRY OF HEALTH

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Treatment with rHu-EPO in MDS is limited by problems of cost and efficacy. Nevertheless the Italian Ministry of Health (IMH) allows the use of rHu-EPO in specific FAB subtypes and by setting financial limitations, so that is possible to give a weekly dose of 30.000 UI to patients with refractory anaemia (RA) and refractory anaemia with ringed sideroblasts (RARS). The indication for refractory anaemia with excess blasts (RAEB) is unclear, while RAEB in transformation is excluded. We have evaluated the erythroid response to rHu-EPO given according to IMH indications in MDS patients diagnosed at our centre from January 2001 to November 2004. Of 148 cases, 72 were eligible to rHu-EPO (symptomatic or transfusion-dependent anaemia, diagnosis of RA, RARS or RAEB) and 49 were actually treated (33% of all MDS), including 27/35 RA (77%), 6/7 RARS (86%), 12/30 RAEB (40%), as well as 1 LMMC and 3 MDS, undefined. Seventeen patients received more than one therapeutic cycle. Median age was 72 ys (range 60-92). The International Prognostic Score System (IPSS), evaluable in 57/66 cycles, was low (L) in 32 (56%), intermediate 1 (Int1) in 17 (30%), intermediate 2 (Int2) in 5 (9%) and high (H) in 3 (5%) cases. Rhu-EPO treatment, given at 10.000 UI x 3/w s.c., was stopped after 4-6 wks in 14 non-responders (NR). In 7 of them, as well as in 3 PR and in 4 patients relapsing while on effective treatment, rHu-EPO was increased to 10.000 UI x 6/w s.c for 3-4 wks according to SIE guidelines (Haematologica, 2002). Response was evaluated according to Cheson (Blood, 2000). Results are shown in the Table.

Table.

	CR (%)	PR (%)	NR (%)	TOT
RHuEPOx3	21 (40)	17 (32)	14 (27)	52
RHuEPOx6	3 (21)	2 (14)	9 (64)	14
PAZIENTI	21 (43)	15 (31)	13 (26)	49

Twenty of 43 cases (43%) lost transfusion dependence. Median hemoglobin value rose from 8.6 g% to 10.4 g% ($p<0.0001$). Complete response (CR) correlated with IPSS (L/Int1: 44% vs. Int2/H: 0%; $p<0.04$). No SAE were noted. Treatment was stopped in 2 cases, because of fever (x6) and itching erythema (x3). In 10/38 responsive cases, response was lost after 4.5 months (range 1-29). It was not reachieved by doubling rHu-EPO dose in 4/4 cases. Treatment duration in responsive cases was 7 months (range 1-45), with 19 patients still on rHu-EPO after 12 months (3-45+). In conclusion: 1) Rhu-EPO given to 79% of eligible MDS patients according to IMH indications, had higher than expected (40% CR) and sustained efficacy, even at doses lower than those recommended by scientific guidelines 2) Its use in RAEB patients is questionable, considering the low response rate (0% CR; 67% PR) 3) Complete response significantly correlated with a L/Int1 IPSS 4) Doubling Rhu-EPO dose rescued about 30% of non-responsive cases, but no relapsing patient. 5) This study supports the IMH indications to Rhu-EPO use in MDS, particularly when considering the issue of a rational use of economic resources.

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TUSCAN MYELODYSPLASTIC SYNDROME REGISTRY: A TOOL FOR REGIONAL COORDINATION AND IMPROVEMENT OF THERAPY IN THE ELDERLY

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Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal diseases characterized by ineffective hemopoiesis and high probability of transformation into acute myeloid leukemia. Despite the fact that multiple therapeutic approaches have been tempted for these pathologies, most of them have been successful only for scanty percentages of patients. This has been ascribed to the poor understanding of molecular causes of MDS and thus to an insufficient characterization of subgroups of MDS. Hematologists face the problem of classifying and treat different types of MDS daily, and although IPSS and WHO classification have been helpful in this sense, most of the times it is extremely hard to follow up the patients. One of the major problems is that elderly patients, thus the majority of patients with MDS, are referred rarely, or very late during the course of the disease, to specialized centers, and precise characterization of their pathology is often estimated not relevant.

Therefore real epidemiologic data are sporadic, and true incidence of MDS is underestimated, as this group of diseases is not included into cancer registries. Based on limited number of regional studies in Europe, we make a crude estimate of 2.1-12.6 cases/100.000 /year, increasing to 15-50cases/100000/year over 70 years. Constant aging of population of course contributes to an increase in incidence of MDS. Due to the potential increase in life expectancy and awareness in MDS in Europe, the incidence in MDS is expected to rise further in the next decade. For these reasons, although our center participate to the Italian MDS registry and to MDS Foundation registry, we very recently decided to establish a regional screening and clarify incidence as well as unify diagnostic criteria in Tuscany. Our region has a population of 3.530.000 inhabitants, mean age of 45 years, people over 65 are 679.400. The expected rate of MDS limited to elderly people should than be 90-300 new cases yearly. The Dept of Hematology, University of Florence and CISPO, Centro per lo Studio e la Prevenzione Oncologica have distributed to all hospitals in Tuscany an easy form to be filled at diagnosis of any MDS case, and updated every 3 months. The form includes anamnestic and work exposure data, chromosomal and molecular abnormalities, bone marrow morphology data, FAB, WHO classifications and IPSS score. Since January 2005, 30 new MDS cases have been registered, mainly IPSS score 1.5-2. Forms can be submitted by mail or fax and this has prompted many clinicians to referral of patients for experimental therapeutical trials or evaluation. Even if initial recruitment is slow and awkward, the reactions of small hospital clinicians has been positive, thus we believe that the regional registry can be an incentive to improve diagnosis and standard of treatment, expecialy for elderly MDS patients

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HEMATOLOGICAL IMPROVEMENT OF REFRACTORY ANEMIA OCCURRING DURING MYCOPHENOLATE MOFETIL THERAPY

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We describe the case of a man with systemic lupus erythematosus (SLE) and refractory anemia (RA) who reported a hematological improvement during treatment with mycophenolate mofetil (MMF).

G.T., a 57-year-old man, was admitted to our Department in November 2003 with complaints of tiredness and tachycardia; he had a 1-year history of SLE treated with corticosteroids, hydroxychloroquine and azathioprine.

At the admission peripheral blood evaluation showed severe anemia with hemoglobin (Hbg) 6.2 g/dL, and mean corpuscolar volume 105 fl; vitamin B12, folic acid, iron, transferrin and ferritin levels were in the normal range. HIV-antibodies, HCV-antibodies, Hbs antigene and antinuclear antibodies were negative. Bone marrow evaluation showed a three-lineage dysplasia with normal cytogenetic profile (22/22 metaphases 46XY). The diagnosis of refractory anemia, according to French-American-British (FAB) classification, was done IPSS score 0.5. We stopped treatment with hydroxychloroquine and azatioprine, and went on with steroid therapy only. However, the patient's red blood cells transfusion needs remained elevated (about once a week) and every attempt to reduce steroids resulted in the development of diffuse myalgias, arthralgias and diffuse skin erythematous swellings. In April 2004, due to the persistence of severe anemia, the patient started therapy with subcutaneous erythropoietin, 40000 UI once a week, without benefits. In September 2004, in presence of increased transfusional needs associated with worsening autoimmune syndrome, we started treatment with chemotherapy according to the FLAG-Ida schedule. The patient received fludarabine 30 mg/mq (total 50mg/day) on days 1-5, ara-C 2 g/mq (total 3.4 g/day) on days 1-5, idarubicine 10 mg/mq (total 17 mg/day) on days 1-3, and filgrastim 300 mcg/mq on days 0-5 and from day 12 to neutrophils over 1500/mmc. Meanwhile, we tapered steroid therapy until interruption. During the next two months we didn't observe major improvement: the patient still suffered from anemia, myalgias, arthralgias, pleural effusion, and skin lesions; these conditions led us to go back to steroid treatment (prednisone 75 mg/day).

In December 2004, as a treatment for SLE we started therapy with MMF 1 g twice a day. A progressive recovery of patient's conditions was observed: hemoglobin levels gradually reached a stable Hbg of 11,5 g/dL. The erythematous lesions ameliorated until almost complete disappearance, and steroid treatment was stopped. The patient is currently off transfusion, with a marked improvement in quality of life. To our knowledge, no other reports in the literature describe the use of MMF as a therapy for myelodysplastic syndromes. This observation suggests that an immune-mediated mechanism underlies this case of refractory anemia, and that myelodysplastic syndromes associated with autoimmune disease may benefit from MMF treatment.

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ANALYSIS OF EVI-1 GENE EXPRESSION BY QUANTITATIVE REAL TIME PCR IN MYELODYDPLASTIC SYNDROMES

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The EVI-1 gene is located in chromosome 3q26 and it codes for a zinc finger protein that may activate transcription under certain circumstances, but more generally is presumed to act as a transcriptional repressor.EVI-1 and its variant form, MDS1/EVI1, have been reported to act in an antagonistic manner and be differentially regulated in samples from patients with acute myeloid leukaemia and rearrangements of the long arm of chromosome 3. MDS1/EVI1 differs from EVI-1 only by the presence of an extended N-terminus. Rearrangements of the EVI-1 locus in chromosome band 3q26 are associated with a poor prognosis in myeloid malignancies. Recently, it has been demonstrated that the overexpression of EVI-1 gene confers a high degree of sensitivity to Arsenic trioxide therapy. The aim of the present study was to investigate the expression level of EVI1 and its variant form MDS1/EVI1 in a series of MDS patients and secondary acute myeloid leukaemia (s-AML).We analyzed the expression levels of EVI-1 and MDS1/EVI1 in 120 BM samples and 62 PB collected from 143 MDS patients. 65 were refractory anaemias (RA), 40 refractory anaemias with excess of blasts (RAEB) and 38 secondary AML (s-AML). Moreover we tested 10 de novo AML patients. 4 AML and 1 MDS patients showed the presence of 3q26 rearrangement detected by cytogenetic analysis. In addition, we analyzed the transcript amount in 12 normal BM and 15 PB samples obtained from healthy volunteers as control. The expression level of EVI-1 and MDS1/EVI1 was established using quantitative Real-Time PCR based on two specific sets of primers and probe (Assays-on-Demand, gene expression products, Applied Byosystems). The values obtained were normalized using ABL as housekeeping gene and the final results were expressed using the DeltaDelta Ct method. We found that normal samples do not expressed Evi-1 and they express very low levels of MDS1/EVI-1 (range of 2(e)-DeltaDelta Ct= 0,37-4,4 in PB and 6-13 in BM) . By contrast, samples from patients with 3q26 rearrangements expressed very high levels of EVI-1 (range 294-35120). In 36 MDS patients out of 143 we detected abnormal levels of EVI-1. Particularly, the patients who overexpressed EVI-1 were: 6 out 65 RA with a mean value of 2(e)-Delta Delta Ct of 42 (range 11- 64), 14 out of 40 RAEB with a mean value of 123 (range 60-264) and 16 out of 38 s-AML with a mean value of 2196 (range 162-6653). 2 out of 10 de novo AML showed abnormal expression of EVI-1 with values of 530 and 56. In all the cases we found a good correlation between the expression level in PB and BM. Finally, EVI-1 expression was evaluated during follow-up of three patients who converted into overt leukaemia and in all the cases EVI-1 increased during progression. These data allow to conclude that the overexpression of EVI-1 is present in about 25% of MDS patients regardless of the presence of the 3q26 rearrangement. The overexpression seems to be more frequent in RAEB and s-AML respect to RA and it increases during disease progression.

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HLA-DPB1*0302:A NEW ALLELE HLA DPB1* IN A SICILIAN NHL PATIENT CANDIDATE A TO BONE MARROW TRANSPLANTATION

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We report here the identification and characterisation of a novel HLA-DPB1 allele that was subsequently named HLA-DPB1*0302† by the WHO Nomenclature Committee. HLA-DPB1*0302 was identified in a single Sicilian individual by a combination of sequence specific primers (SSP), reverse line sequence specific oligonucleotide probing (SSO) and sequence based typing (SBT).

The DPB1*0302 allele is most similar to the DPB1*3101 allele, differing by a single mismatch at nucleotide position 301 (T to G). We report here in the identification of a novel HLA-DPB1 sequence, DPB1*0302. The DPB1*0302 allele was initially revealed during the course of routine HLA genotyping of a bone marrow transplant patient in Sicily. Genotyping technologies employed were PCR-SSP (Dynal HLA-DPB1 Allset+ SSP kit, Dynal Biotech Ltd. UK and GenoVision HLA-DPB1 SSP kit, GenoVision Vertriebsgesmb H, Austria) and reverse line PCR-SSO (InnoLiPa, Immunogenetics, Belgium). Confirmation of the unusual typing results was achieved when DNA from this individual was tested using the Dynal SSP technology in the R&D department at Dynal Biotech Ltd. The specific amplification pattern obtained did not correspond to any known DPB1 allele. Further HLA genotyping revealed this individual's HLA genotype to be: HLA-A*0301,*6801; B*1402,*3801; Cw*0802,*1203; DRB1*1104,*1301; DRB3*0101,*0202; DQB1*0301,*0603; DQA1*0103,*0505

The new HLA-DPB1 allele was characterized by direct sequencing of exon 2 in both directions, following separation of the DPB1*0302 allele from the DPB1*0401 allele using the allele-specific and sequencing primers described below. The complete exon 2 sequences of both DPB1 alleles were amplified with the following primers: 'DPB1F' 5'TGTAAAACGACGGCCAGTGAGAGTGGCGCCTCCGCTCA and 'DPB1R' 5'CAGGAAACAGCTATGACCCCGGCCCAAAGCCCTCACTC

The bold sequence in the forward primer matches HLA-DPB1 intron 1 (41-20 bases 5' from exon 2 i.e. 4491-4511 in intron 1), whereas the bold sequence in the reverse

primer matches HLA-DPB1 intron 2: 3-23. The 5' sequences (the first 17 bases of each primer) were designed as targets for the sequencing primers. Confirmation of the novel allele DPB1*0302 was performed by allele-specific amplification that separated DPB1*0401 from DPB1*0302. Amplification for DPB1*0401 utilized the forward 'DPB1F' as described above, in conjunction with the following reverse sequencing primer: 'DPB0401R' 5'CTGCAGGGTCATGGGCC DPB1*0302 was amplified utilizing the same forward primer 'DPB1F' in conjunction with the following primer: 'DPB3101R' 5'CTGCAGGGTCACGGCCT. Both reverse primers were located at nucleotide position 341. Sequencing was performed in two separate reactions using the reverse primers in conjunction with a forward primer matching the 'DPB1F' target sequence. The amplification and sequencing were based on methods previously described from DRB1 sequencing by Sayer et al using an ABI 3100 capillary sequencer (Perkin Elmer, Applied Biosystems, Warrington, Cheshire, UK)

Sequencing confirmed the presence of HLA-DPB1*040101, and identified the subsequently named DPB1*0302 allele. As shown in Figure 1, the DPB1*0302 allele most similar to DPB1*3101 with a 'T' to 'G' substitution at nucleotide 301. This single base change results in a coding change in codon 72 from leucine to valine as shown in Figure 2. This change is located on the exposed alpha-helix and thus may have some functional relevance.

DPB1*0302 is identical to DPB1*040101 up to nucleotide 280 and thereafter identical to DPB1*030101 and so DPB1*0302 may have arisen from a gene conversion event involving commonly found alleles such as DPB1*040101 and DPB1*030101. Alternatively, DPB1*0302 allele may have arisen by point mutation from the similar DPB1*3101 allele. The frequency of DPB1*0302 in the general population is unknown, but we can speculate that as DPB1*0302 was readily detected with popular, commercially available DPB1 typing systems, so it follows that this new allele is likely to be uncommon for it not to have been previously discovered.

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UNCOMMON CYTOGENETIC FINDING IN A PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENT

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Chronic lymphoid leukaemia is a B cell chronic lymphoproliferative disorder characterized in 98% of patients by proliferation and accumulation in peripheral blood, bone marrow and lymphoid tissues, of small size B lymphoid cells. The immunological pattern is the following: CD5+; CD19+; CD20+; CD23+; CD24+; CD43+; SIg+ at low intensity and CD38 with variable positivity corresponding to positive or not molecular findings for the IgVH rearrangement. The caryotype shows frequently: deletion 13q, deletion 11q, trisomy 12q, and deletion 17p.

A 78-year old man was referred to our Haematology Division in August 2004 because of leukocytosis (WBC: $60x10^9$/L) with lymphocytosis ($50x10^9$/L), absence of anaemia and thrombocytopenia. At the physical examination small nodes (less than 2 cm in size) were present on all superficial sites. Total body CT scan showed increase of spleen size (185 mm) and lymphoadenomegalies in many deep sites. The morphological examination of peripheral circulating cells showed small size lymphoid cells with immunological pattern CD5+; CD19+; CD20+; CD23+; CD24+; CD38+ (64.9%); SIgM+ at low density . The cytological examination of the bone marrow showed a lymphoid infiltration up to 70% of total nucleate cells. Bone marrow biopsy confirmed the lymphoid infiltration. Molecular finding was positive for IgVH rearrangement and negative for TCRβ rearrangement. The caryotype was: 46,XY with clonal expression of t(2;18)(q32;q12) and del(13)(q21). Both anomalies were confirmed by FISH painting for the chromosomes 2, 13 and 18. Hepatitis C antibodies were negative. Therefore, the patient was subjected to the following treatment schedule: 25 mg/sqm/day Fludarabine i.v. for 5 days every 4 weeks. Six total treatment cycles were performed and the treatment was well tolerated. Disease restaging (CT scan total body and bone marrow biopsy) has shown a partial remission. The translocation 2;18 has been described in rare cases of CLL, involving Ig kappa and bcl-2 genes (p11;q21). At our knowledge, the t(2;18)(q32;q12) has not been described in CLL until now . Its clinical significance and, especially, the association with the del(13) and the CD38 overexpression are unclear but may identify a new subset of high risk patients.

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GRANULOCYTIC SARCOMA ASSOCIATED WITH I(8)(Q10) IN A THERAPY-RELATED ACUTE MYELOID LEUKEMIA

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Granulocytic sarcoma (GS) is a rare extramedullary tumor of immature myeloid cells, usually developing during the course of acute myeloid leukemia (AML) and in myeloproliferative or myelodysplastic disorders. Several features, including chromosomal abnormalities [t(8;21), inv(16)], cell-surface markers (CD56, CD2, CD4, CD7) and Franch-American-British (FAB) subtype (M2, M4, M5) have been implicated as predisposing risk factors to GS. We report a patient with a therapy-related AML which developed a GS involving the cauda equina and the conus medullaris nine months after the complete hematological remission. At the diagnoses of leukemia immunophenotypic analysis of bone marrow aspiration demonstrated a population of blast cells exhibiting a typical phenotype of a M5b AML, characterized by the expression of CD4dim, HLA-DR, CD15, CD56, CD33, CD38, and negativity for CD34, CD117 (c-kit) and CD14 antigenes, Cytogenetic analysis of bone marrow revealed two abnormal clone with the following Karyotype: 46,XX, del(20)(q10), 1-2dmin [9]/46,XX,-20,+mar, 2-3 dmin [6]. The patient was

treated with three course of intensive chemiotherapy and complete remission was achieved. When GS developed, bone marrow aspirate showed a population of immature cells, corresponding to 10-12%, which lost the morphology showed at diagnoses of leukemia, appearing poorely differentiated with vacuolated cytoplasm. Flow cytometric analysis didn't show blast population of diagnoses too. Chromosome analysis revealed a new clone in 12 of 42 banded cells, expressed as follows: 47,X,-X, +i(8)(q10), +mar, 2-4 dmin. None methaphases showed the karyotype of diagnoses. The patient died two months after development of GS due to brain involvement. FISH analysis with c-myc (8q24) probe and chromosome(#) 8 centromeric probe (Vysis, Downers Grove, IL) was performed to confirm the presence of i(8q) and the possible amplification of c-myc on double minutes chromosomes (dmin). FISH showed one copy of c-myc in both normal #8 and two copies on the isochromosome, demonstrating tetrasomy 8q, without evidence of c-myc amplification on dmin. FISH analysis with MLL dual color/break apart probe (Vysis, Downers Grove, IL) was also performed to detect the possible amplification of MLL gene on dmin. FISH failed to show MLL amplification, but it revealed MLL split signal in keeping with gene rearrangement. On methaphase cells FISH gave evidence of MLL translocation on p arm of a C #. To our knowledge this is the first report of i(8)(q10), MLL rearrangement and dmin in a patient with GS. The i(8q) is a rare finding in myeloid malignancies, being more frequent in chronic lymphoprolyferative disorders. Also dmin, products of gene amplification associated with rapid disease progression and short survival , are rare events in myeloid malignancies and in hematologic disorders in generally. c-Myc or MLL appears to be the most frequently amplified gene in AML; cases showing amplification of other genes are rarely reported.

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FREQUENCY OF H63D MUTATION IN THE HFE GENE IN ADULT ACUTE LEUKEMIA

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Hemochromatosis is a common autosomal recessive genetic disorder leading to progressive iron accumulation and parenchymal organ damage due to excessive absorption of dietary iron. Among general population, the allelic frequencies of C282Y and H63D, the more frequent mutations of HFE gene in Italy, are 0.037 and 0.16 in the Northern regions and 0.015 and 0.16 in the Southern regions. Different authors have reported an increased incidence of HFE gene mutations in neoplastic diseases including malignant blood disorders. In this study, we investigated the allelic frequency of HFE gene mutations in a series of 154 patients with acute leukemia, including 107 cases of acute myeloid leukemia (AML), 20 cases of acute promyelocytic leukemia (APL), and 27 of acute lymphoblastic leukemia (ALL). As controls, a group of 230 healthy blood donors of the same hospital district was adopted. HFE genotyping was performed using a polymerase chain reaction (PCR) method using sequence-specific-primers, and the products were analyzed on agarose gel. Statistical differences between the prevalence of HFE genotypes in the patients and control as well as between the different subtype of leukemia were assessed using the chi square test; p values <0.05 were considered as statistically significant. We did specifically focus on H63D mutation, which either in the study group or in the controls was the most frequent. Overall, the allelic frequency of H63D mutation was 0.16 in the group of patients with acute leukemia and 0.11 in the controls (p:0.09). There was neither statistically significant difference between patients with AML and controls (0.15 vs. 0.11, p:0.10), nor between APL and controls (0.05 vs. 0.11, p:0.31); on the contrary, the H63D mutation was significantly more frequent in ALL patients as compared to controls (0.22 vs. 0.11, p:0.04). The comparison of allelic frequency of the mutation among the three different subtypes of acute leukemia demonstrated a border line value (p:0.07), mainly due to difference between ALL and APL. Finally, we dissected the AML patient population according to age more or less than sixty years, a previously diagnosed myelodysplastic syndrome and the presence of trilinear dysplastic changes at diagnosis. No differences were found in allelic frequency of H63D as all the above parameters were concerned.

We conclude that as compared to general population the H63D mutation of HFE gene is more frequent in patients with ALL. The lowest allelic frequency was found in patients with APL, while AML patients had intermediate value. The clinical relevance of such findings clearly needs to be investigated in larger cohorts of patients.

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INTERPHASE-FISH IS COMPARABLE TO VNTR /STR POLYMORPHISMS IN THE ASSESSMENT OF DONOR CHIMERISM AFTER SEX-MISMATCHED BONE MARROW TRANSPLANTS

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Monitoring bone marrow chimerism after allogeneic bone marrow transplantation (BMT) is helpful in documenting the success of the engraftment. Several techniques are now available to detect minimal residual disease (MRD) and mixed chimerism after BMT. Standard cytogenetics of bone marrow cells from sex-mismacthed transplant recipients is limited by the low number of metaphases that can be analyzed and is time consuming. Moreover a mosaicism of 1% may be excluded only if more than 200 metaphases are analyzed. Interphase FISH easily overcome these problems.

We used i-FISH to monitor donor chimerism after sex mismatched BMT in 47 patients, and compared the results with DNA polymorphism obtained with a set of VNTR microsatellite loci or STR. Donor chimerism was assessed at month 1,3,6,12 and every 6 months thereafter. FISH was performed with a CEP XY probe (Vysis, Downers

Grove,IL, USA) following manufacturer's instructions. A total of 200 cells were analyzed by two different observers. DNA polymorphism analysis was performed by PCR amplification followed by electrophoresis of 1/10 of PCR product through a standard 8% polyacrylamid gel as previously described with a set of height variable number tandem repeats. Forty- seven patients (M/F: 21/26) undergoing a sex-mismacthed transplant from January 2003 through February 2005 in two different Hematology divisions were included in this study. Median age was 55 (range 38-64). Diagnosis was acute myelogenous leukemia (AML) in 20, acute lymphoblastic leukemia (ALL) in 7, chronic myelogenous leukemia (CML) in 6, Hodgkin lymphoma (HD) in 3, non-Hodgkin lymphoma (NHL) in 2, multiple myeloma (MM) 2, chronic lymphocytic leukemia (CLL) in 2, others 5. Conditioning regimens included total body irradiation,(TBI), thiotepa, antithymocyte globulin, fludarabine, cyclophosphamide. Donor bone marrow was harvested by standard techniques and donor PBSC were enriched after subcutaneous G-CSF. No discrepancies were observed between i-FISH and VNTR/STR. FISH confirms as a reliable method for the detection of donor chimerism at the same level as VNTR polymorphisms. These two analyses may represent alternative approaches for transplant monitoring. Data will be shown on all 47 patients.

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HEREDITARY HYPERFERRITINEMIA-CATARACT SYNDROME AND COEXISTENCE OF HETEROZYGOTIC MUTATION OF THE GENE HFE OF THE HEMOCROMATOSYS: REPORT A CASE OF A FAMILY GROUP

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The author brings the case of a woman of 38 years that arrive at his observation because during the pregnancy a hyperferritin was discovery (value 1024 ng / ml) with normal transferrin and serum iron concentration . In the history of the patient most recent had been submitted to surgical intervention of cataract when she still wasn't known the elevated value of the ferritin. Her daughter at the birth was examined and presented hyperferritinemia. After the evaluation of the family nucleus he discovered that her mother and one of the two brothers had hyperferrytinemia. Also her brother had been operated of cataract without knowing to have elevated values of the ferritin . The analysis of the DNA of the whole family nucleus has underlined that the patient and her daughter introduced the genetic alterations of the syndrome hyperferritinemia cataract and the heterozygote for hemochromatosis while one brother and her mother only the hereditary line of the first syndrome. Molecular analysis underlined the presence of the mutation C33T to the state heterozygosis in the esone 1 not translated of the gene FLT (compatible with the state of illness) in the patient, in her doutgher, in her mother and his brother as well as the mutation of Cys 282Tyr to the heterozygote state (and the absence of the mutation alone Hys63Asp) in the patient and her daughter. From the evaluation of the values of the ferritin and the serum iron concentration , as from the familiarity for hyperferritinemia – cataract, it was concluded that for the patient there was no relationship among the state of healthy carrier of hemocromatosys and the condition of hyperferritinemia for which as healthy bearer of hemocromatosys was identified and she cuts from syndrome hyperferritinemia / cataract transmit as autosomic dominant character. The case results unusual for the double mutation and for the casualness with which she has reached the diagnosis both clinic and genetics. In literature the case of another Danish family group is brought only. The HHCS is an autonomic dominant disease, characterized by bilateral cataracts and increased serum L-ferritin, in the absence of iron overload (with normal serum iron concentration and transferrin) . Under physiological conditions, ferritin synthesis is finely regulated at the translational level by iron availability. This regulation is achieved by the high-affinity interaction between cytoplasmyc mRNA – binding proteins (iron regulatory proteins, IRPs), and mRNA stem-loop structures, know as iron responsive elements (IREs), located in the untranslated regions (UTRs) of the mRNAs. A single IRE is located on the 5'UTR of a series of genes involved in iron metabolism, like L-ferritin, and the binding IRE – IRPs represses these genes translation. The deregulation of ferritin production responsible of HHCS is caused by heterogeneous mutations in the iron regulatory element (IRE) of L-ferritin that interfere with the binding of the iron regulatory proteins, disrupting the negative control of L-ferritin synthesis and causing the constitutive up-regulation of ferritin L-chains. In these patients therefore the production of ferritin is totally released by the presence of the iron and it is produced in excess. They forms so in the fabrics you accumulate of "hulls" of empty ferritin (without iron) that cannot be removed in some way. The blood-letting therapy commonly employee in the treatment of the iron overload, doesn't have any effectiveness in these patients and is, rather, self-defeating, determining the express development of an anaemia without any reduction of the value of the ferritin in the blood. This to further confirmation that the hyperferritinemia is not accompanied to the accumulation of ferritin in the different tissue and it doesn't determine some particular problem except that in the crystalline causing the cataract. The surgical intervention resolves the problem. Patients with HHCS may be recognized by a family history of cataracts and hyperferritinemia without increased serum iron. Hereditary hemochromatosis (HH) is instead an autosomal recessive disorder (HFE gene mutations C282Y and H 63 D are responsible for the majority of HH cases) of iron metabolism characterized by increased iron adsorption and progressive storage resulting in organ damage.

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EIGHT YEAR EXPERIENCE IN PRENATAL DIAGNOSIS FOR HAEMOPHILIA AT CASTELFRANCO VENETO

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Genetic counselling in families with haemophilia A (HA) and B (HB) requires determination of carrier state before or during pregnancy in females at risk of transmitting the disease. In our centre 344 and 155 females respectively for HA and HB have been investigated starting from 1997. After molecular F8 and F9 gene analysis, 192 (56%) and 110 females (71%) were found to be carrier respectively of HA and HB. To date, we have performed 34 prenatal diagnosis (PND), 22 for HA and 12 for HB, 32 from chorionic villus samples (CVS) and 2 from amniocentesis, on 24 severe HA and HB carrier women, as 10 women performed PND twice. Moreover, in 12 further cases the carrier women required PND but the DNA analysis was not performed because of miscarriage (6 cases) or female foetal gender determination obtained from caryotypic or maternal peripheral blood analysis (4 and 2 cases respectively). In total, 33 carrier women asked for 46 PND. In 34 out of 46 counselling requests (74%) DNA was analyzed. Genomic DNA was obtained and used for foetal gender determination, causative mutation identification and linkage analysis. For sex determination we used specific primers for single copy amelogenin-encoding gene (AMD) mapping both on the X and the Y chromosomes. In male foetus the mutation detection was performed by Long Distance PCR for F8 gene inversion involving intron 22 or PCR followed by CSGE screening or direct sequencing of the previously identified mutated F8 or F9 gene fragment. F8 gene inversion represents the most important causative mutation responsible for severe cases of haemophilia A, accounting for 9 out of 22 causative in our cohort. In our experience, DNA extraction and PCR conditions have a crucial role for the efficacy of Long Distance PCR in foetal tissues (Belvini et al, Haemophilia, 2001). Diverse point mutations account for 13 HA and all the 12 HB cases. PNDs were generally complete in two to four days starting from the CVS sampling, in order to give the pregnant women the genetic analysis results as soon as possible. In this cohort, the gender sex determination was done for 40 foetal samples and we found 15 female foetus (52.5%) and 19 male foetus (47.5%). Among the male foetus for which we carried on with molecular analysis, 12 were affected and 7 were not affected (30% and 17.5% respectively). The vast majority of the affected foetus' mothers decided for voluntary interruption of pregnancy within the first trimester.

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FISH STUDIES ON 5Q35/HOX11L2 ABNORMALITIES IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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The cryptic t(5;14)(q35;q32), is one of the most frequent abnormalities in childhood T ALL, with variant 5q35 translocations involving different chromosome/gene partners also being reported. Common to all is activation at 5q35 of the orphan homeobox HOX11L2, a transcriptional regulator that is closely related to HOX11. HOX11 is another orphan homeobox that is deregulated in T ALL with t(10;14)(q24;q11). Recently, extra-chromosomal amplification of ABL1 associated with a NUP214/ABL1 transcript was found in a subgroup of T ALL with deletion of CDKN2A and abnormal expression of HOX11L2 or of HOX11. Since the 5q35/HOX11L2 are cryptic rearrangements at conventional cytogenetics, we applied FISH to study these changes in a series of T cell ALL belonging to the GIMEMA Study Group. To date, we have identified three cases. Patient 1 was a 17 year old female. The immunophenotype indicated a mature T ALL with cCD3 and sCD3 positivity. Cytogenetics showed a complex karyotype: 47,XX,der(2), add(5)(q35),del(7)(q21q35),+ marker. Metaphase FISH with whole chromosome paints 2, 5, and 7, and subtelomeric clones for 2p (RP5-892G20) and 2q (RP5-1011O17) showed the karyotype was as follows: 47,XX,t(2;2)(p25;q35),t(5;7)(q35;q21),+marker. Patient 2 was a 24 year old male, with CD7 and cCD3 positive T ALL and the following karyotype: 46,XY[18] /46,XY,add(2)(q)[5]. Patient 3 was a 20 year old male, with CD7 and cCD3 positive T ALL and normal karyotype.

Although the three cases showed different chromosomal changes, HOX11L2/5q35 involvement was demonstrated by applying the TLX3 FISH DNA probe, split signal (DakoCytomation, Denmark A/S). In patient 1, the TLX3 probe identified the 5q35 breakpoint of t(5;7) showing one fusion signal (1F) on normal chromosome 5, 1 red signal (1R) on der(5), and 1 green signal (1G) on der(7). The 5q35 breakpoint was narrowed to within clone RP11-546B8 which gave three hybridization signals on normal 5, on der(5), and on der(7). In patient 2, the TLX3 probe gave 1F on normal chromosome 5, and 1G signal on the other normal 5. The 1R signal was missed, indicating a rearrangement at 5q35 with loss of material telomeric to HOX11L2. Double color FISH with clones RP11-546B8 and CTB-45L16 for 5q35 and clones RP11-431B1 and RP11-1070N10 for BCL11B1/14q32 and TCL/14q32, respectively, demonstrated an unbalanced t(5;14) and identified the 14q32 breakpoint within BCL11B1. In patient 3, interphase FISH showed 1F, 1R, and 1G in 85% of nuclei. Interphase FISH with the BCR/ABL1 ES dual color probe (Vysis) did not detect amplification of ABL1 in any case. The FISH technique fully characterizes chromosomal changes underlying HOX11L2 transcriptional activation either in

metaphase or interphase cells. Common clinical and hematological features observed in our patients, i.e. young age, positivity of cCD3 antigen, and early relapse, overlap with previously published HOX11L2 positive T ALL.

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4-WAY TRANSLOCATION T(5;9;22;17) IN A CASE OF CHRONIC MYELOID LEUKEMIA VARIANT PRESENTING WITH BONE MARROW FIBROSIS AND SEVERE THROMBOCYTOSIS

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In variant chronic myeloid leukemias (5-10% of all cases) Philadelphia (Ph) translocation involves one or more chromosomes in addition to 9 and 22. The novel variant here described t(5;9;22;17) generates a rearrangement between chromosomes 17 and 22 thus creating a translocation - t(17;22) - which is typically associated with the cutaneous malignant tumor dermatofibrosarcoma protuberans (DFSP). It has been recently demonstrated that t(17;22), which activates platelet-derived growth factor-B (PDGFB) gene by placing its transcription under the control of the collagen 1A1 promoter (COL1A1), renders DFSP responsive to targeted therapy with the PDGFB-receptor tyrosine kinase inhibitor imatinib mesylate.

A 69-year old man was referred to our institution following the detection, during routine blood tests, of moderate anemia (10.8 g/dL), leucocytosis (36.000/mmc) and severe thrombocytosis (1.400.000/mmc). Patient had no splenomegaly or hepatomegaly. Skin examination was negative. Bone marrow aspirate was characterized by a significant prevalence of megakaryocytes; bone marrow biopsy showed a marked hyperplasia of hypolobulated megakaryocytes and fibrosis. The cytogenetic diagnosis from bone marrow aspirate was 46,XY,t(5;9;22;17)(q12;q34;q11.2;q11). The BCR/ABL ES FISH probe demonstrated the BCR/ABL fusion gene in all metaphases and in 300 interphase cells. In addition, FISH analysis with WCP probes confirmed the involvement of the chromosomes 5, 9, 22 and 17 in the 4-way translocation. The fusion transcript BCR/ABL (b2a2) was confirmed by RT-PCR. ABL gene point mutation was excluded by D-HPLC on BCR-ABL rearrangement.

Following the diagnosis of CML (Sokal risk 1.602) the patient was enrolled in protocol ICSG/CML 022 and randomized for the imatinib mesylate 800 mg/day arm. After few weeks (the first and second with imatinib mesylate at 400 mg/day) characterized by the persistence of an elevated thrombocytosis, a rapid normalization of the leukocytes and platelets count was documented. After 3 months the patient underwent a complete evaluation including a cytogenetic analysis showing a major response (95% Ph-). Presently, after 6 months from diagnosis, the cytogenetic and molecular response is complete.

To our knowledge the variant 4-way translocation t(5;9;22;17)(q12;q34;q11.2;q11) is here described for the first time. Interestingly, the rearrangement between chromosomes 17 and 22 could explain some of the morphologic and clinical features of this case at diagnosis. PDGFB is in fact a potent growth-factor locally promoting an autocrine and paracrine tumor growth other than acting as a mitogen and chemo-attractant for a variety of cells of mesenchimal origin. It is conceivable to hypothesise that its overexpression, by stimulating bone marrow fibroblasts expansion and collagen synthesis, might have sustained the fibrosis and indirectly the megakaryocytes proliferation. Finally, the inhibitory action of imatinib mesylate on both tyrosine kinases (ABL and PDGFB-receptor) might explain the pattern of response to STI-571 observed in this case.

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CHROMOSOME ABNORMALITIES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA DETECTED BY INTERPHASE FLUORESCENCE *IN SITU* HYBRIDIZATION: CORRELATION WITH BIOLOGICAL FEATURES

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Background. B-CLL has a variable history with respect to time to progression and response to standard cytotoxic therapies. FISH interphase cytogenetic analysis is superior to standard karyotype analysis in identifying known abnormalities and is able to reveal chromosome changes in the majority of B-CLL samples (1,2). The most common abnormalities are del(13)(q14.3), del (11)(q22-23), trisomy 12 and del(17)(p13.1). Del(11q) and del(17p) are definitely associated with a bad prognosis(1). Some Authors have correlated FISH features with other biological parameters, including sex, age, Rai stage, IgVH mutational status and CD38 expression (3). We tried to establish such correlations in a series of patients coming from several Centers in Romagna.

Materials and methods. FISH was carried out using VYSIS probes for each of the above mentioned abnormalities.

Results. Up to now, 25 B-CLL patients have been studied. 15 were males and 10 were females, their age ranged from 36 to 86 years. FISH results are shown in Table 1.

The 2 pts with del(17p) seem to present with a higher lymphocyte count and a higher percentage of CD38+ cells, with respect to the other cathegories. Up to now, no del(11q) has been observed.

Conclusions. Due to the still low number of pts analysed, no clear-cut correlation can be drawn. Preliminary data seem to confirm the results of the literature. The work is still in progress. Cytofluorimetric detection of ZAP70 will be added soon.

Table 1.

	NO Abn.	Del(17p)	Del(13q)	Tri(12)
N° Pts	8	2	12	3
M/F	5/3	1/1	8/4	1 / 2
Age				
(mean)	48	70	51.2	52.6
(extr.)	[36-61]	[54-86]	[34-62]	[35-67]
(median)	48	70	54	56
Ly/mm^3				
(meanx10^3)	23.9	42.5	24.1	11.2
(extr.)	[6.1-49.2]	[37.5-47.6]	[8.7-59.8]	[6.2-20.7]
(medianx10^3)	20.7	42.6	15.4	6.5
CD38+ (%)				
(mean)	19.8	47.5	2.8	32
(extr.)	[0-46]	[1-94]	[1-4]	[3-53]
(median)	9.0	47.5	3.5	40
RAI stage				
0	5		6	2
I	3		5	1
II		1	1	
III				
IV		1		

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NOVEL FINDINGS FROM THE MOLECULAR CYTOGENETIC CHARACTERIZATION OF DELETION ON DER(9) IN CHRONIC MYELOID LEUKEMIA

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Background. The t(9;22)(q34;q11), generating the Philadelphia (Ph) chromosome, is found in more than 90% of patients with chronic myeloid leukemia (CML). Deletions adjacent to the translocation junction on the derivative chromosome 9 were described by several groups. These studies revealed two primary points:1) genomic microdeletions were concomitant to the t(9;22) rearrangement; 2) deleted sequences location was upstream to ABL1 and downstream to BCR genes. We report two CML cases bearing deletions on der(9) without the characteristics reported above.

Patients and Methods. A screening of 291 CML cases with the BCR/ABL rearrangement detected by RT-PCR was performed by Fluorescence In Situ Hybridization (FISH) with BAC and PAC clones specific for ABL1 and BCR genes, as previously reported. A set of BAC/PAC probes (clones proximal and distal to ABL1 and BCR, respectively) belonging to 9 and 22 chromosomes allowed us to define precisely the deletion size.

Results. Case #1. FISH studies revealed the presence of a variant complex t(6;9;12;22)(p22;q34;q13;q11). Surprisingly, the detailed molecular cytogenetic characterization of chromosome 9 breakpoint showed genomic loss of about 400 Kb downstream to ABL1 gene. NUP214 is the alone gene with known function mapping in the deleted region. Case #2. Conventional cytogenetic analysis revealed a normal karyotype. FISH analysis with clones specific for ABL1 and BCR genes showed a single fusion signal on der(9), suggesting the occurence of an insertion event. Further FISH experiments confirmed that a chromosome 22 segment proximal to BCR was inserted on der(9) and revealed the loss of chromosome 9 and 22 sequences, proximally to ABL1 and distally to BCR genes. The size of chromosome 9 and 22 deletions was about 0.8 and 1.1 Mb, respectively.

Conclusions. Our data indicate that (1) deletions on der(9) in CML cases could involve chromosome 9 sequences located telomeric to ABL1 gene other than those centromeric previously described; (2) genomic microdeletion can be associated to rearrangements involving 9 and 22 chromosomes, such as insertion event, different from the reciprocal translocation. Further studies are needed to establish whether CML cases with these features could have a different prognosis.

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A HIGHLY SENSITIVE AND SPECIFIC FLUORESCENCE IN SITU (FISH)APPROACH FOR THE DETECTION OF T(9;11)(P22-23;Q23)IN ACUTE MYELOID LEUKAEMIA

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The most frequent partner in translocations involving the MLL gene at 11q23 (3-5% of adult de novo acute myeloid leukaemias, dnAMLs) is the AF9 gene at 9p22-23. The detection of these rearrangements is important because a) MLL rearrangements represent a cytogenetic group characterized by low survival rate and poor response to treatment and b) it was reported that MLL/AF9 fusion may carry a less severe prognostic significance as compared with other 11q23/mLL rearrangements. We set up a highly sensitive and specific DNA probe for the detection of t(9;11)(p22-23;q23)-MLL/AF9 fusion by interphase (FISH). A dual colour dual fusion probe was designed encompassing the MLL and the AF9 gene, resulting in the correct identification of the two derivative chromosomes (visible as two fusion signal) as well as of the two normal alleles (single red and green signals, respectively). The aims of the study were: a) to establish the incidence of MLL rearrangements in a series of unselected dnAML, b) to study the sensitivity of FISH in a series of AML cases carrying 11q23 rearrangements by conventional cytogenetics and/or molecular methods and, c) to ascertain the frequency of deletions possibly surrounding the translocation breakpoints.

Interphase FISH was used to screen a variable number of nuclei (100-252) in a total of 73 unselected dnAML cases and in 22 additional cases bearing 11q23 rearrangements at karyotype. Cytogenetic analysis was carried on in all cas-

es. RT-PCR for the detection of MLL-AF9 fusion transcript was used to confirm the molecular cytogenetic data.

Results. We analyzed 73 unselected cases of adult dnAML with a set of two PACs, 217a21 and 167k13, mapping respectively 5' and 3' to the breakpoint cluster region: 66 cases (90,4%) resulted in a germline FISH pattern (96,9-100% interphase with two fusions), confirming the results of conventional cytogenetic analysis. In 7 cases (6,8%) segregation of green and red signals suggestive of MLL gene rearrangement was observed in 30% to 96,5% of the cells (FISH-positive cases): in 5 patients an 11q23 break was detected at karyotype, whereas in 2 cases an 11q- chromosome was reinterpreted as an 11q23/mLL translocation after FISH and molecular genetic studies.

We then applied the dual colour dual fusion probe to an enlarged series of 27 AML cases with 11q23 rearrangements and 7 cases without involvement of band 11q23 at G-banding. In all cases studied FISH correctly detected MLL-AF9 fusion when compared with G-banding analysis and RT-PCR studies, except in one case where an atypical t(9;11)(p23;q23) involving a 9p region distal to AF9 was detected. 3' MLL deletion was found in 1/27 cases, whereas no cases with t(9;11) and deletion flanking the 9p breakpoint was found in this series. We arrived at the following conclusions: a) This dual-colour dual-fusion system is sensitive and specific for the detection of MLL/AF9 fusion in AML, b) It may be of value for the identification of MLL involvement in cases with an 11q- chromosome at karyotypic analysis, c) The incidence of deletions surrounding MLL and AF9 breakpoints may be lower than previously reported in the literature.

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THE 5'RUNX1/3'CBFA2T1 FUSION GENE MAY BE GENERATED BY DIFFERENT CHROMOSOMAL MECHANISMS IN ACUTE MYELOID LEUKEMIA

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Background. Translocation t(8;21)(q22;q22) is a common karyotypic abnormality detected in about 15% of Acute Myeloid Leukemia (AML) cases. The rearrangement results in fusion of the RUNX1 (also known as AML1) and CBFA2T1 (also known as ETO) genes generating a 5'RUNX1/3'CBFA2T1 transcriptionally active fusion gene on derivative chromosome 8. In 1 to 8.5% of AML cases insertions events generating a 5'RUNX1/3'CBFA2T1 fusion gene have been reported, whereas the occurrence of inversions accompanying the t(8;21) has never been observed.

Aims. We performed a molecular cytogenetic analysis by FISH to verify the frequency of chromosomal insertion and inversion events in AML cases bearing the 5'RUNX1/3'CBFA2T1 rearrangement. A detailed breakpoints characterization has been performed in all cases with insertions and inversions.

Methods. We report a screening of 82 AML cases bearing the RUNX1/CBFA2T1 rearrangement detected by RT-PCR; all cases were tested by Fluorescence In Situ Hybridization (FISH) with BAC and PAC clones specific for CBFA2T1 and RUNX1 genes.

Results. Our results revealed that 8 (9.8%) AML cases showed cryptic chromosomal insertions or inversions. FISH co-hybridization experiments with CBFA2T1 and RUNX1 probes revealed the presence of a functional fusion gene on the der(21) instead of the der(8) chromosome in five cases with ins(21;8); a single fusion signal on the der(8) chromosome was detected in the case with ins(8;21). The use of the same clones in FISH studies showed the presence of a single unexpected fusion signal on the 8p derivative chromosome in addition to faint CBFA2T1 and RUNX1 signals on the long arm of der(8) and der(21) chromosomes, respectively. These results suggested that in two cases a pericentric chromosome 8 inversion involving CBFA2T1 gene occurred and that the chromosome 21 was rearranged with the 8p derivative chromosome.

Appropriate chromosome 21 and 8 BAC clones were employed to precisely define the size of inserted regions in cases with insertions and the breakpoint on the 8p derivative chromosome in cases showing pericentric chromosome 8 inversion. The insertion size turned out to be very heterogeneous, ranging from a minimum of 2.4 Mb to a maximum of 44 Mb. In both cases with chromosome 8 inversion, the CBFA2T1 gene represents the breakpoint at the chromosome 8 long arm whereas the 8p breakpoint showed different mapping positions in 8p21.3 and 8p21.1, respectively.

Conclusions. Our study allowed us to reveal five cases with ins(21;8), one with ins(8;21), and two with a pericentric chromosome 8 inversion followed by a t(8;21) translocation. Our results illustrate that (1) heterogeneous mechanisms can lead to the generation of the 5'RUNX1/3'CBFA2T1 chimeric gene; (2) molecular cytogenetic techniques may identify cryptic chromosomal changes, not detected by conventional cytogenetic analysis (3) the crucial role of the 5'RUNX1/3'CBFA2T1 fusion gene in leukemogenesis does not depend on its location.

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INTERPHASE MOLECULAR CYTOGENETICS FOR DEL(13)(Q14) SCREENING IN MULTIPLE MYELOMA PATIENTS: UNEXPECTED RESULTS

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The presence of del (13)(q14) interstitial chromosomal deletion in Multiple Myeloma (MM) is a well known adverse indipendent prognostic factor. Screening our MM patients for this rearrangement has become a routine practice. A fast, affordable and reliable screening method is represented by interphase molecular cytogenetics, (FISH). In a previous study (2000, unpublished data) by our Laboratory. we have selected two probes for 13q14 region, among the four commecially available at the moment: LSI 13 q14 Spectrum Green and LSI 13q14 Rb-1 Spectrum Orange for

their capability, compared with LSI13q14 D13S319 Spectrum Orange and LSI 13q14 D13S25 Spectrum Orange to identify respectively: the chromosome 13 copy number in the individual's karyotype both in metaphase cells and in interphase nuclei, and the deletion of clinical interest. The two probes differ in lenght : LSI 13q14 Spectrum Green spanning over the Rb-1 region(180 kb) and covering the regions adjacent to the locus both centromerically and telomerically with a total lenght of 440 kb, and LSI 13q14 Rb-1 just covering the entire gene (220kb). In the aim of achieving the triple issue of screening the presence of deletion, of evidentiating a possible chromosome 13 aneuploidy and of providing an internal control into the experiment, we co-hybridized the two probes on the same slide. Due to their construction that makes them behave as *regional painting probes* the overlapping regions (Rb-1 locus) at epifluorescence observation, show a Yellowish (Green + Orange) colour, giving a typical pattern that can be interpreted as follows:

TWO GREEN SIGNALS BOTH WITH A YELLOW SPOT INSIDE THEM = NORMAL

TWO GREEN SIGNALS ONE WITH A YELLOW SPOT INSIDE IT = 13q14 Deletion

ONE GREEN SIGNAL WITH A YELLOW SPOT INSIDE IT = Monosomy 13

We report here a case with *unexpected results* at FISH analisys. The patient , a forthy-seven years-old male was diagnosed MM with 30% plasma-cellular bone marrow infiltration, Conventional cytogenetics could not evidentiate any subtle rearrangement yelding low resolution metaphases. FISH analisys showed an unexpected pattern with two green signals and one red signal rather distant from any of the two green ones. Altough the presence of a single red signal alone, indicates del (13)(q14), we performed a further experiment with co-hybridization of a chromosome 13 painting library (FITC labelled) and the Rb-1 probe (Spectrum Orange) that confirmed our data, also excluding any translocation involving chromosome 13 . Trying to investigate among the possible chromosomal rearrangements that could produce such a result, the occurrence of a paracentric inversion with one break-point at the centromeric end of the Rb-1 region and the other at a distance of at least one chromosomal band, could explain a change of loci order along the chromosome, creating a distance between them. The lack of cohybridization at Rb-1 where we could only observe the red signal could be the consequence of a mismatch of the LSI 13q14 probe segment at the breakpoint.

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PROGNOSTICALLY UNFAVOURABLE CHROMOSOMAL ABNORMALITIES STUDIED BY FISH IN CLL PATIENTS AT DIAGNOSIS

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Routine cytogenetic analysis of B-cell chronic lymphocytic leukemia (B-CLL) frequently fails to identify an abnormal clone due to the low rate of spontaneous mitoses and poor response to mitogen stimulation. Recent studies utilising interphase fluorescence in situ hybridization (FISH) suggest that prognostically significant chromosomal abnormalities occur more frequently in B-CLL than has been previously recognised. The aim of this study was to detect the most frequent unfavourable chromosomal abnormalities by FISH in cases of B-CLL at diagnosis, to correlate those with clinical features and give useful information to decide the beginning of treatment. 46 consecutive cases of CLL referred to our Department between October 2003 and July 2004 were studied at diagnosis for trisomy 12 and for rearrangements involving 11q22 and 17p13. The median age of the patients was 69 years (range, 39-90 years); 29 male and 17 female. Median WBC count, Hb and platelets count were respectively 16,46x10^9/L (range: 6,33-58,79), 13,9 g/dL (range: 9,8-16,9) and 188x10^9/L (range: 100-519). Clinical staging of B-CLL patients showed that 29 pts were in stage 0 Rai, 12 in stage I and 5 in stage II. CD 38 was positive in 7/41 pts (17%). FISH studies were abnormal in 24% of cases. The most common abnormality was trisomy 12 (54%) with an incidence of 13%; rearrangements involving 11q22 and 17p13 had an incidence of 6% and 4% respectively. CD38 was positive in 50% of patients with trisomy 12. At the contrary of data reported in literature, we had a lower incidence of unfavourable chromosomal abnormalities and no correlation was observed between aberrations involving 11q22 and lymphoadenopathies. Our results suggest that a reduced incidence of chromosomal abnormalities may be due to an early analysis performed on CLL cases at diagnosis.

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A NEW CHROMOSOME 3 REARRANGEMENT IN B-NHL

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The BCL-6 gene, which functions as a transcription repressor, is involved in the breakpoints of various chromosomal translocations in NHL. These rearrangements occur in the nontranslated region of the BCL-6 gene, juxtaposing regulatory sequences of the diverse partner genes to the open reading frame of the BCL-6 gene and thus are thought to deregulate the BCL-6 expression. The levels of expression of the BCL-6 gene and protein have a prognostic value in predicting the clinical outcome of diffuse large B-cell lymphomas. Chromosomal rearrangements affecting the 3q27 band, where the BCL-6 gene is localized, is one of the most common genetic abnormalities in B-NHL.The rearrangements occur within the major translocation cluster (MTC) of BCL-6, and often the traslocation results in juxtaposing an immunoglobulin gene to a coding region of BCL-6. On November 2004 a 74-y man was diagnosed at first as LLC because of his leucocytosis (15.6x10^3/microliters WBC) with lymphocytosis (9.0 x10^3/microliters). He presented splenomegaly due to his microcythaemic condition. The diagnosis changed to B-NHL after immunophenotypic characterization (B-cell 74%; CD20+, CD79b+, CD95+, CD5-,CD23-,CD19-; sIgD-M/k+). The cytogenetic study showed the following complex caryotype: 45,XY,der(3)(3pter-3q29::3q29-3q25::4q28-4qter),i(17)

(q10),der(13;22)(q10;q10) [13]/45,idem, der(13;13)(q10;q10) [3]/46,XY[12].

The rearranged chromosomes 3 and 4 were characterized by FISH (paint probes). The chromosome 3 showed a duplication (q25-q29) including the region 3q27, usually involved in traslocations. Then we suppose an increase of BCL-6 gene expression in this patient as the result of the gene duplication.

P345

FLUORESCENCE IN SITU HYBRIDATION FOR DEL 13Q AND IGH REARRANGMENTS IN 20 MULTIPLE MYELOMA PATIENTS AT DIAGNOSIS

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Conventional cytogenetics studies find abnormal karyotypes in 20-50% of patients affected by multiple myeloma (MM). Studies using fluorescence hybridation in situ (FISH) techniques have demonstrated that the incidence of numerical and structural abnormalities in these patients is higher than previously reported. The most frequent structural abnormalities are chromosome 13q deletions and translocations involving the immunoglobulin heavy-chain (IgH) locus on 14q32. With the exception of t(11;14), these abnormalities have been associated with an adverse outcome. We report the results of the FISH studies of 20 consecutive patients affected by MM at diagnosis referred to our Unit from January to December 2004. Patients characteristics were: M/F 14/6, mean age was 55 years (range 31-76 years), 13 pts were IgG, 5 IgA and 2 BJ. The majority of patients were in Durie and Salmon stage III (15 pts), 3 pts in stage II and 2 in stage I; 7 pts had a previous MGUS and 2 pts had AL amyloidosis. For all patients CD138+ magnetic separation of bone marrow plasma cell was performed and the samples were tested for 13q deletion (spectrum orange LSI RB1, Vysis) and translocations of chromosome 14q32 (dual colour orange-green LSI, Vysis): t(4;14), t(11;14) and t(14;16) involving IgH gene with the following partner genes FGFR3, CCND1 e MAF. In our experience, half of the patients showed at least one of the tested chromosomal abnormality in FISH. The most common abnormality was t(4;14) observed in 25% of patients, followed by 13q deletion (20%) and t(14;16) in 15%. Only one patient was positive for t(11;14). Moreover in 3/4 patients positive for del 13q a translocation involving chromosome 14q32 was observed: 2 pts were positive for t(4;14) and 1 had t(14;16).

Our results are consistent with those reported in literature, but the lower incidence of t(11;14) may be due to the absence in our case of IgM or nonsecretory MM, more frequently associated to this translocation. The 3 pts carrying del 13q and 14q32 translocations were all in stage III with aggressive disease. Our FISH study performed on CD138+ cells seems to be feasible and effective in detecting recurrent chromosomal structural abnormalities in MM.

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MUTATIONAL ANALYSIS OF THE CODING REGION OF PDGFR-BETA IN PATIENTS WITH CMML AND ATYPICAL CML

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Background. A subset of patients diagnosed with chronic myelomonocytic leukemia (CMML) and atypical chronic myelogenous leukemia (aCML) have been shown to have balanced translocations involving the platelet derived growth factor receptor beta (PDGFRbeta) gene. To date, nine PDGFRbeta fusion partners (ETV6, CEV14, HIP1, H4/d10S170, RABEP1, Myomegalin/PDE4DIP, NIN, HCMOGT-1, and KIAA1509) have been identified, and there are additional reports of translocation involving PDGFRbeta where the fusion partner is not yet known. The resulting fusion proteins have constitutive enzymatic activity and deregulate the growth in a manner analogous to that induced by BCR-ABL. Imatinib Mesylate have been shown to inhibit the growth of cells which carry these translocations. In contrast to other class III Receptor Tyrosine Kinases, oncogenic versions of PDGFRbeta resulting from point mutations have not been reported. Nevertheless, it has been demonstrated that a single amino acid substitution in the cytoplasmic juxtamembrane region of the PDGFRbeta receptor is sufficient to constitutively activate this receptor.

Study aim. We aimed to search for mutations in the coding region of PDGFRbeta in patients diagnosed with CMML and acme, and to correlate the PDGFRbeta status with the in vitro sensitivity to Imatinib Mesylate.

Materials and Methods. Patients were studied by conventional cytogenetic analysis and screened for the presence of BCR/ABL rearrangement. Mutational analysis of PDGFRbeta was conducted by amplifying and direct sequencing the entire coding region of the gene.The ability of Imatinib Mesylate to inhibit primary patient derived cell growth was explored by clonogenic assays.

Results. At the time of analysis 8 patients have been studied. Five of them had been diagnosed with CMML, 2 with aCML, and 1 with AML secondary to CMML. Five patients had normal caryotype, 1 of them complex caryotype, 1 patient non clonal abnormalities. In one case cytogenetic analysis was not available. Seven patients tested for the presence of BCR/ABL rearrangement showed negative results. Imatinib Mesylate failed to inhibit the growth of primary patient derived cells when spontaneous growth was found. Nevertheless we pursued to search for mutations in the coding region of PDGFRbeta because, in analogy to other class III Receptor Tyrosine Kinases, the presence of a point mutation might had conferred resistance to Imatinib Mesylate. To date 22 out of 23 exons have been sequenced and sequence variations with pathogenetic meaning have not been found.

Conclusions. In contrast to other class III Receptor Tyrosine Kinases, oncogenic versions of PDGFRbeta resulting from point mutations have not been reported. At the time of analysis sequence variations with pathogenetic meaning have not been found. Understanding the reason of the absence or rarity of this phenomenon requires further investigations.

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HFE GENE MUTATIONS: THE EXPERIENCE OF THE CATHOLIC UNIVERSITY IN ROME AND MOLISE

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Hereditary Hemochromatosis (HH) is the most common inherited autosomal recessive disorder in the white population. Its prevalence in North Europe is estimated from 1:200 to 1:500 and shows a cline from north to south and from west to east of Europe. In the majority of cases, the HFE gene is involved in the aetiology of the disease. Three HFE mutations are the main responsible of the disease: C282Y, H63D, S65C. The homozygous state for the first variant is the most common genotype revealed in HH patients, less frequently the other two mutations (in homozygous state or compound heterozygous state with the C282Y) are detected.

The epidemiological data about the allele frequencies of these mutations in Central Italy are scanty, overall because unbiased studies on a large sample of people are still lacking. It could be useful to have studies reporting the relative prevalence of the HH genes principal variants in regional populations, concerning patients suspected for HH on the basis of clinical or laboratory iron overload and in their relatives. These studies could also contribute to better understand the clinical penetrance of each genetic alteration and to plan a specific policy concerning the diagnostic procedure, the genetic consulting and the life habitude also in relation with other hereditary or acquired diseases. We have studied two groups of subjects referred to our laboratory for suspected or diagnosed iron overload by the family physicians or by the specialist from Rome (n=93) and from Campobasso (Molise and the surrounding provinces of Avellino and Benevento: the ancient Sannium)(n=36) (Total n=129). While the Campobasso group, was more homogeneous regarding the ethnical composition, the Rome group was more heterogeneous both for the relevant historical and actual immigration both for the referral of subjects to the laboratory also from other regions. For genetic analysis DNA was prepared by standard protocols and samples were genotyped using a reverse hybridization assay (Haemochromatosis StripAssay, Nuclear Laser, Settala, Milan, Italy) for the simultaneous detection of HFE, TFR2 and ferroportin mutations.

The results we have obtained are in the Table 1:

Table 1.

	WT WT	C282Y WT	C282Y C282Y	H63D WT	H63D H63D	S65C WT	C282Y H63D	H63D S65C	Other	Total Mut	C282Y allele frequecy	H63D allele frequency
Rome N=93	65	2	4	16	3	1	0	0	2	28/93 30,1%	5,4%	11,8%
Molise N=36	14	1	1	15	1	1	2	1	0	22/36 61,1%	6,9%	27,8%
Total N=129	79	3	5	31	4	2	2	1	2	50/129 38,7%	5,8%	16,3%

Our data do not represent any reliable index of the absolute prevalence of the HH gene mutations in the studied populations, overall because the recruitment of the subjects is not random, but it has been determined by the physician suspect or indication. However some preliminary interesting considerations can be made. In both groups the H63D seems to be the most frequent mutation, but in Molise patients it seems to have an higher prevalence, as suggested by the allelic frequency. These data could be explained by the more homogeneous composition of the population from Molise, but they need to be confirmed by epidemiologic studies conducted on the general population. Furthermore we have observed that some patients heterozygote for the H63D mutation showed iron overload without evidence of any other pathological conditions (such as inflammatory, haematological, cancer or liver diseases) affecting the iron balance. Thus further studies on a larger number of subjects, could contribute to evaluate if the style of life and/or genetic polymorphisms can influence the development of iron overload.

P348

EVALUATION OF FIP1L1-PDGFRA REARRANGEMENT IN IDIOPATHIC HYPEREOSINOPHIC SYNDROME

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Introduction. Idiopatihic Hypereosinophilic Syndrome (HES) is a rare disorder characterized by persistent eosinophilia associated with multi organ involvement without evidences of other causes, particularly allergic or parasitic diseases. Many HES presented a chronic and indolent course, but sometimes they are characterized from elevated aggressiveness and multi-organ damage. HES could be called Eosinophilic Leukemia (EoL) when a clonal origin is demonstrable (by chromosome X inactivation assay or clonal karyotipic abnormalies). Aggressive HES have been treated with steroids and cytotoxic drugs without any relevant successes, recently low-dose of Imatinib mesylate (100 mg/day) led to partial or complete response in about 50% of these patients. Recently, Cools *et al.,* have identified a clonal abnormality consisting in an interstitial deletion on chromosome 4q12 which led to the creation of a chimaeric gene by juxtaposition of the genes FIP1L1 and PDGFR-alpha (PDGFRA). Due to the gene fusion the tyrosin-kinase PDGFRA becomes constitutively activated. Gene sequencing of this region has evidentiated different breakpoints on FIP1L1, ranging from exon 8 to exon 10, instead breakpoint on PDGFRA occurs always on exon 12. FIP1L1-PDGFRA rearrangement is not detectable with conventional cytogenetic analysis but with reverse transcriptase polymerase chain reaction (RT-PCR). The majority of these patients presented a clinical response to Imatinib treatment, maybe for the inhibitory effect of this drug on the tyrosine-kinase activity of PDGFRA. We evaluated RT-PCR on our HES patients in order to find the rearrangement FIP1L1-PDGFRA.

Patients and Methods. We considered HES patients who presented sustained eosinophilic count (>1500/mmc) with-

out any evidence of allergic (normal IgE value) or intestinal parasitic disease. HES was confirmed if marrow eosinophilic infiltration was > 15%, in these patients RT-PCR have been performed. Also cytogenetic analysis was performed to identify other abnormalities. Sequencing of c-DNA was done on positive samples in order to confirm the interstitial deletion and define the breakpoint region.

Results. We tested 10 patients affected by HES, 2 of them resulted positive for FIP1L1-PDGFRA rearrangement by RT-PCR analysis and were confirmed with c-DNA sequencing. Both the patients presented the same FIP1L1 breakpoint on intron 8. One of these patients was treated with low dose of Imatinib mesylate obtaining a clinical response, the other instead died before starting the treatment. No cytogenetic alteration have been found.

Discussion. We retain that FIP1L1-PDGFRA rearrangement have to be detected in all the patients with clinical suspect of HES. In fact the found of this chimaeric transcript can justify the treatment with Imatinib and get a rationale for its success. The first analysis remain RT-PCR, for its praticity, but sequencing needs to confirm the result and to analyse the breakpoint. Further study on more patients need to confirm the role of FIP1L1-PDGFRA rearrangement in this pathology.

P349

DIFFERENTIAL EXPRESSION OF PRAME GENE IN HODGKIN'S DISEASE AND NON-HODGKIN LYMPHOMAS

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PRAME is a member of the large family of cancer testis antigens (CTA) that are expressed during embryonic development and fetal life, being then silenced by promoter methylation in all normal adult tissues except testis. They encode for antigenic peptides able to elicit an autologous and specific CTL response in the context of appropriate HLA-I presentation. The physiological role of proteins encoded by CTAs remains largely unknown. No information is currently available as to the role of PRAME gene in the pathophysiology of human lymphomas. We therefore undertook an extensive study, to assess the expression patterns and the biologic significance of deregulated PRAME expression in both Hodgkin's disease (HD) and non-Hodgkin lymphomas (NHL). High levels of PRAME-specific transcripts were detected by quantitative PCR with Real Time technology (Q-RT-PCR) in all of HD-derived cell lines (HDLM2, KMH2, L540, L428, L1236 ed HDMYZ) analyzed, suggesting that PRAME deregulation represents a constant phenomenon in HD. The study of 48 primary HD lymph node (LN) samples (34 nodular sclerosis, NS; 9 mixed cellularity, MC; 5 nodular lymphocyte predominance, NLP) and 10 reactive LN tissues revealed that all samples of NS-HD show a significantly higher constitutive levels of PRAME mRNA than those present in non-neoplastic LN. In contrast low levels of PRAME were detected in MC-HD, while NLP cases were PRAME negative. Isolation and analysis of purified CD30+ Hodgkin and Reed Sternberg (H-RS) cells from primary LN evidenced that the overexpression of PRAME, in HD tissues, is confined to tumor cells. We also demonstrated that the 5'-end region of PRAME gene is constantly demethylated in H-RS cells. A parallel analysis of de novo methylases (DNMT3A, DNMT3B), maintenance methylase (DNMT1) and demethylase (MBD2) genes, did not evidence significant differences in their relative levels of expression, among normal and HD-derived LN, except for the detection of a lower amount of DNMT1-specific transcripts in tumor tissues. HD patients analyzed, however, did not show a prevalence of the MHC Class I alleles (HLA-A24, Cw 1, 3, 7) implicated in the activation of a PRAME-specific immune response. Analysis of primary LN tissues from NHL patients disclosed a statistically significant (p=0.002) difference in the relative levels of PRAME expression between diffuse large B cell lymphoma (DLBCL; n=35) and follicular lymphoma (FL; n=37) of different grading (G1, n=11; G2, n=15; G3, n=11). The majority (21/35, 60%) of DLBCL were positive for PRAME expression as opposed to only 25% (9/37) of FL samples. Accordingly, the absolute amounts of PRAME RNA in FL samples was always significantly lower than in DLBCL. To exclude that such differences were due to a lower amount of tumor cells in FL samples, as opposed to DLBCL, data were correlated to BCL-2/JH quantitative evaluation in the same samples. The analysis confirmed that PRAME overexpression in DLBCL is due to intrinsic tumor cells deregulation. PRAME gene maps to chromosome 22, being placed inside the immunoglobulin lambda variable chain gene locus. Based on our data, it can be speculated that mechanisms associated to a 'disturbed' somatic hypermutation process, may concurrently result in DNA methylation changes in turn responsible for PRAME re-expression in specific lymphoma cell types. The constant overexpression of PRAME in tumor cells of classical HD and DLBCL, both typically characterized by disturbances in the somatic hypermutation processes, seems to confirm this hypothesis. In contrast, tumor cell types (NLP-HD and FL), 'smoothly' undergoing productive hypermutation are mostly PRAME-negative. In addition, at least in NHL, PRAME expression appears to be somehow linked to the transformation of tumor B cells to a more aggressive phenotype, while the relevance of PRAME expression on clinical features and/or survival in HD is currently being assessed. Taken together our results demonstrate that PRAME deregulation is constant phenomenon in classical HD and DLBCL as opposed to NLP-HD and FL, respectively. While it is yet not known whether patients are able to mount an endogenous immunological response to PRAME, a specific immunotherapeutic approach targeting PRAME-encoded peptides is worth to be explored in specific subtypes of human lymphomas.

Myeloproliferative Syndromes II

P350

CYTOMETRIC CHARACTERIZATION OF ATYPICAL MONOCYTES AND MINIMAL RESIDUAL DISEASE MONITORING IN PATIENTS WITH CHRONIC MYELOMOCYTIC LEUKEMIA TREATED WITH AML-LIKE CHEMOTHERAPY

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Chronic myelomonocytic leukemia (CMML) is a malignant blood disorder characterized by increased monocytes in the bone marrow (BM) and peripheral blood, variable degree of dysplastic abnormalities and terminal transformation in acute myeloid leukemia (AML). CMML affects predominantly elderly people and in most cases standard therapy involves hydroxyurea and/or supportive care. In younger patients, AML like chemotherapy has been reported as able to induce complete remission (CR) in nearly half of the cases. However, standard AML criteria of response evaluation are hardly applicable to CMML, namely in cases with low blast percentage in the BM. In addition, the degree of BM monocytosis is highly variable and its morphologic evaluation can be poorly reliable for the assessment of therapeutic response. Till now, few studies have specifically addressed the immunophenotypic pattern of atypical monocytes in CMML. Notwithstanding, a precise cytometric characterization of such cells could be of major utility for the evaluation of response to any treatment aimed at CR achievement as well as at monitoring minimal residual disease (MRD). In this study, we report results from four patients with CMML treated with fludarabine, cytarabine and G-CSF (FLAG), in whom cytometric evaluation of bone marrow monocytosis was performed at diagnosis, at CR achievement and after consolidation therapy including, in three cases, allogeneic stem cell transplantation (allo-SCT). In order to detect, by flow cytometry, neoplastic cells in bone marrow aspirates from patients affected by CMML, we used three combined approaches. First of all, we tried to identify, if present, immature blast cells, by using CD45, CD34, CD14 and CD33 antigens, along with mature specificities as CD66b and CD15. The expression of atypical antigens, such as CD7 and CD19 was carefully searched for. Second, we focused on monocytic population in order to define the presence of immature (CD36+, CD14-) and atypical cells, either immature (CD56+/CD36+/CD14-) or mature (CD56+, CD36+, CD14+). Third, we analysed cytometric patterns of dysgranulopoiesis, by using the following antigen combinations: CD16/CD11b/CD45/CD14, CD16/CD13/ CD45/ CD14 and CD64/CD11b/CD45/ CD14). These three lines of cytometric analysis were carried out at diagnosis and during the follow up. At diagnosis, dysplastic hematopoiesis as well as a specific immunologic "fingerprint" of atypical monocytes consisting of asynchronous and or aberrant antigen expression, were found in all patients. In particular, abnormal membrane patterns included: CD36+/CD14+/CD56+ and CD36+/14-/CD56+. In all patients achieving CR after FLAG we observed the complete absence of blasts as well as of immature and/or atypical monocytes, and the restoration of normal patterns on granulopoietic differentiation as evaluated by morphology and flow cytometry. We conclude that cytometric evaluation of bone marrow enables a precise evaluation of therapeutic results in CMML patients treated with aggressive chemotherapy and/or transplantation procedures and can be extremely useful for the monitoring of minimal residual disease.

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ANALYSIS OF RISK FACTORS FOR THROMBOTIC EVENTS IN 306 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Introduction. Essential Thrombocitemia (ET) is a chronic myeloproliferative disorder of unknown etiology, involving the expansion of multipotent hematopoietic stem cells. Peripheral thrombocytosis and abnormal proliferation of megakariocytes in the bone marrow are the hallmark of the disease. Even though the clinical course of the disease is often complicated by thrombotic and, less frequently, hemorrhagic complications, life expectancy does not appear to be significantly different from the healthy general population of the same age. With this retrospective study, we aimed to investigate clinical and laboratory characteristics associated with the occurrence of thrombotic events, with the purpose of identifying subgroups of patients who could benefit from antiaggregant and/or cytoreductive treatment.

Patients and Methods. 306 patients who were diagnosed with ET in our Institution in the time period from January 1979 to December 2002 were included in this study. At the time of analysis, 196 patients were still alive with a median follow-up of 96 months. Variables that were investigated for association with the risk of thrombosis were age, platelet count, previous history of thrombotic events, time elapsed from the diagnosis of ET, ongoing treatment with antiaggregant and/or cytoreductive agents, and cardiovascular risk factors (arterial hypertension, obesity, hypercholesterolemia, diabetes, cigarette smoking).

Results. The study group comprised 109 men (35%) and 197 female (65%). Median age was 58 years. At the time of last follow up, 46 patients (15%) experienced at least one thrombotic event, whereas no thromboses were observed in the others 260 patients (85%). The occurrence of thrombotic events were observed in 26 patients (40.6%) out of 64 with previous history of thrombosis and in 20 patients (8.3%) out of 242 with no previous history of thrombosis ($p<0.0001$ Fisher's exact test, odd ratio 7.6). A significant difference between the two groups of patients was also confirmed when Kaplan Meier estimates of thrombosis-free survivals where compared by the log-rank test ($p<0.0001$). By logistic regression, platelet number at diagnosis did not associate with occurrence of thrombosis in the whole patient population. When patients with no

previous history of thrombosis were stratified according to the number of cardiovascular risk factors (none vs one vs more than one), we observed a significant correlation with occurrence of thrombotic events (Mantel-Haenszel Chi-square 5.47, $p<0.05$).

Conclusion. This study confirmed that history of thrombosis strongly associates with the risk of developing further thrombotic events in patients with ET, while platelet number does not seem to represent a prognostic factor. In patients with no previous history of thrombosis, the presence of other cardiovascular risk factors have to be taken into account when establishing the therapeutic approach.

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SERUM LEVELS OF ANGIOPOIETIN-2 AND ITS RECEPTOR TIE2 IN CHRONIC MYELOPROLIFERATIVE DISEASES

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Angiogenesis is important for progression of haematologic malignancies including chronic myeloproliferative diseases (CMD). Recently, the Angiopoietin family has been the subject of intense investigation. In general, high levels of Angiopoietin-2 (Ang-2) by tumor or vascular tissues have been documented in a wide variety of tumors such as malignant glioblastoma, hepatocellular carcinoma, gastric carcinoma. Further, the expression of Ang-2 in cooperation with Vascular Endothelial growth factor (VEGF) has been correlated with poor prognosis in gastric, hepatocellular and breast cancers.But no data are available on soluble Ang-2 and Tie2 in haematopoietic malignancies. Therefore, in this study we investigated the clinical significance of Ang-2 and its receptor Tie2 in sera of patients with Chronic Myeloproliferative Disorders (CMD). The study included 15 patients with Chronic Myeloid Leukemia (CML) and 25 patients with Essential Thrombocythaemia (ET).

Enzyme-linked immunosorbent assay (Quantikine human Angiopoietin-2, Tie2 immunoassay; R&D Systems) detected (p <0,01)higher levels of Ang-2 in CMD compared with healthy subjects (1684.84 ± 1205,16 ng/mL vs 563,75± 322,95 ng/mL, respectively); the highest levels of Ang-2 were detected in CML patients (2013,3 ± 867,9 ng/mL). Lower levels were present in ET patients (1494,66 ± 1348,10 ng/mL). Moreover, in CML patients, levels of Tie2 (17,04 ± 8,9 pg/mL) were higher ($p< 0,01$) compared with healthy controls (9 ± 3,5 pg/mL).The highest levels of Tie 2 were observed in ET patients with history of thrombotic episodes compared with those of ET patients without thrombotic complications, although no significant differences were found. No correlation was found between levels of Ang-2 and levels Tie2 or platelet count among ET patients. Interestingly, CML patients who achieved haematological remission after Interferon or Imatinib therapy showed circulating levels of Ang-2 significantly ($p<0,05$) decreased when compared with those at diagnosis. Our results suggest a role of Angiopoietin/Tie2 system in the pathogenesis of CMD.

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CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED GENES IN CD34+ CELLS FROM PERIPHERAL BLOOD OF IDIOPATHIC MYELOFIBROSIS PATIENTS USING MICROARRAY ANALYSIS

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Idiopathic myelofibrosis (IMF) is a clonal myeloproliferative disorder, characterized by bone marrow fibrosis, myeloid metaplasia, usually accompanied by leukoerythroblastic blood smear, variable degree of pancytopenia or leucocytosis, splenomegaly, and increased number of $CD34^+$ cells in the peripheral blood (PB). A part for the recently described mutation in JAK2 exon 12 in about half of patients, no recurrent chromosomal abnormality nor molecular defect has been described to date. We aimed to identify disease-specific gene abnormalities and their possible pathogenetic role in $CD34^+$ cells purified from the peripheral blood of patients with IM, as compared to $CD34^+$ cells obtained from the bone marrow of normal subjects. For this purpose, we prepared three pools of purified (purity >98%) $CD34^+$ cells from IM subjects, and two pools from normal BM donors, each comprising five subjects. The cDNA was hybridized an Affymetrix HG-U133A oligonucleotide microarray chip representing 22,283 genes. 1100 genes were found to be differentially expressed: of these, 632 sequences were increased and 468 were decreased. Among the genes that were increased in $CD34^+$ cells of IM patients, attention has been focused on some involved in the megakaryocyte commitment (HOXA7, CD9, ROD1) and in bone marrow fibrosis/angiogenesis (TIMP3, TIMP1, ANGPT-1). Of potential interest were also some transcription factors, like WT1, GAS2, (p45)N-FE2, RUNX1, or adhesion molecules such as DLK1, vWF, OB receptor. On the contrary, among the genes that were decreased, there were IL-8, AIM1, FOSB, TGFBI and CXCR4. Interestingly, the latter is significantly down-regulated in IM $CD34^+$ cells and we speculate that it might be related to the constitutive mobilization of $CD34^+$ cells in these patients. Among these, 50 genes, that we considered as biologically important in the pathophysiology of IM, was then validated by RT-PCR analysis, using $CD34^+$ cells purified from additional 20 IM patients and 7 BM controls.

The ongoing functional characterization of these genes and their gene products might help to identify aspects potentially important for the pathogenesis, and would facilitate the molecular diagnosis of IM.

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SPLEEN VOLUME SIZING IN PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE DISEASES

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Spleen volume (SV) enlargement is an important parameter for the clinical evaluation of patients with hypercytosis, particulary in those affected by Phyladelphia negative myeloproliferative diseases, in which spleen parenchyma may be active site of disease. The most common parameter utilised for spleen sizing is the cranio-caudal diameter (CCd) determined by ultrasound scan (US), as a surrogate for spleen volume (SV). The aim of our study was to verify whether US-determined SV might be more sensitive in detecting spleen size changes. In the past five years, 200 out-patients referred to our unit for hypercytosis were recruited in the study; they presented with the following diseases: 73 erithrocytosis, 24 thrombocytosis, 30 polycythemia vera (PV), 73 Essential Thrombocythemia (ET). PV and ET patients were diagnosed according the PVSG criteria. In agreement with the literature, a multislice CT scan was considered the gold standard for spleen volume sizing; in 30 individuals SV measured by CT scan and US scan were directly correlated and the correlation index was excellent (r = 0.945; p=0.0001). The US-determined SV showed a trend of about 20% in overestimating the SV. For this reason we considered 250 ml as the upper normal limit of SV instead of the commonly accepted 200 mL. US measured SV showed higher sensitivity compared to CCd measurement, and detected a splenomegaly in more than 25% of patients whose spleen CCd was within normal limits. Furthermore spleen volume showed a higher degree of correlation with other parameters influenced by the basal disease, such as red cell mass, bone marrow fibrosis, serum LDH. The results of this study in a large series of haematological patients suggests to consider the volume as the preferred parameter to be utilised for spleen sizing myeloproliferative disorders.

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SECOND MALIGNANCIES IN ESSENTIAL THROMBOCYTOSIS: A RETROSPECTIVE ANALYSIS OF 331 PATIENTS FROM A SINGLE INSTITUTION

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Background. Alkylating agents (ALK) has been demonstrated to increase risk of acute leukaemia and myelodysplastic syndromes in patients with myeloproliferative disorders. Hydroxyurea (HU) is the most used cytoreductive drug in essential thrombocytemia (ET), having a lower leukemogenic potential.

Aims. To retrospectively investigate long-term development of haematological and non haematological second malignancies in patients with ET, analyzing possible associations with cytoreductive treatments.

Methods. Data from 331 patients with ET referred to our Institution from January 1977 to November 2002 were retrospectively collected, particularly with respect to development of second malignancies. Diagnoses were confirmed according to the WHO criteria. Estimates of survival were based on the Kaplan-Meier method. Continuous independent variables between patient groups were compared by Wilcoxon two-sample test. Treatment with HU, Melphalan and Busulphan was categorized as 3="no drug used", 2="low dose used", 1="high dose used". High and low doses were selected based on median value. A logistic regression model was then applied including emergence of malignancy during follow-up as the dependent variable and HU, Melphalan, Busulphan and use of HU after ALK, as the independent variables.

Results. The patient population included 214 females and 117 males, with a M/F ratio of 0.55. Median age was 61 yrs (range 18-87). At the time of analysis (December 2004), 189 patients were still alive. Median follow-up of the alive patients was 124 months (range 23-304). Median survival of the whole population was not reached, with more than 60% of patients still alive 240 months after diagnosis. At diagnosis, median platelet count was $846x10^9$/L (range 443-3200), median hemoglobin value was 14 g/dL (range 9-17), and median WBC count was $8.7x10^9$/L (range 4-22.2). After referral, 137 patients did not receive any treatment, whereas 194 were treated with chemotherapy; 116 patients received only HU, 38 only ALK (Melphalan/Busulphan), and 40 ALK followed by HU. A second malignancy was detected in 43 patients, with a median time from diagnosis of 86 months (11 acute leukemias, 4 NHL, 28 solid tumors). Treatments administered for ET before development of second malignancy were as follows: 10 (23%) no treatment, 13 (30%) HU, 10 (23%) ALK only, and 10 (23%) ALK followed by HU. Of the 288 patients who did not develop secondary malignancies, 127 (44%) were never treated, 104 (36%) received HU only, 28 (10%) received ALK only, and 29 (10%) received ALK followed by HU. Cumulative doses of HU were significantly different in patients who received only HU compared to patients who received HU sequentially to ALK (p<0.05). Among patients who were treated with HU or ALK single agents, average drug cumulative doses were significantly higher in those who developed second malignancy compared to those who did not. In contrast, when patients were treated with ALK followed by HU, no significant differences between the two groups were observed in drug cumulative doses. By multivariate analysis, high dose of Melphalan was statistically different with respect to no use of Melphalan (Wald test=12.1 p value=0.0005, odds ratio 6.56 with 95% confidence interval from 2.27 to 18.94); high dose of Busulphan was statistically different with respect to no use of Busulphan (Wald test=4.4 p value=0.0370, odds ratio 3.67 with 95% confidence interval from 1.08 to 12.47). HU was not significant with both doses. Use of HU after ALK was not significant.

Conclusions. Our study, including a large population of unselected ET patients with prolonged observation times, suggest that treatment with single agent high cumulative doses of alkylating agents, but not of HU, is associated

with higher risk of developing second malignancies. However, such risk seems to be enhanced by relatively low cumulative doses of HU in patients who received previous alkylating agents.

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RELIABILITY AND SAFETY OF ANAGRELIDE THERAPY IN ESSENTIAL THROMBOCYTEMIA: A SINGLE CENTER EXPERIENCE

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Background. Differently from Secondary Thrombocytosis, Clonal Thrombocytosis needs a cytoreductive treatment to reduce thrombohembolic risk. The ideal drug should be effective, tolerable, should reduce the thromboembolic risk as well as negative effects (leukemias and secondary myelodysplastic syndromes). In this single center retrospective study, we evaluate the reliability and safety of the therapy with Anagrelie, a selective inhibitor of megakaryocytopoiesis and of platelets production in patients with Essential Trombocytemia. (ET).

Patient's Characteristics. We analysed 25 patients with diagnosis of ET (median age 42 years - range 20-83); 2 patients had a mild marrow fibrosis; 17 patients (68%) received pre-treatment with HU, Alkylants or IFN; 16 patients (64%) were asymptomatic; 5 (20%) had an high thromboembolic risk. Median platelets count at start was 1.110x109/L Initial dose of Anagrelide was 0,5 mg/day for a week,while the average maintenance dose of 2 mg/day(0.5-4 mg)divided into three administrations. All patients received also an anti-platelet drug. Median follow-up was 44 months (range 6-84 months).

Results. After one month therapy, 22 patients (88%) had a significant decrease of platelets. Of these, a complete (platelets less than $450x10^9$/L) and a partial response (platelets between 450 and $600x10^9$/L), were observed in 12 (48%) and 10 (40%) patients, respectively. Eight patients (32%) interrupted therapy for non response (3 patients), atrial fibrillation (1 patient 83 years old), evolution in Idiopatic Myelofibrosis (1 patient with slight bone marrow fibrosis at diagnosis), pregnancy (1 patient), anemia (1 patient), intolerance (1 patient). Mild side effects (tachycardia, headache, nausea, edema) were present in 5 patients (20%) and disappeared after two weeks of therapy. During treatment 52% of patients had a reduction of at least 1g Hb (1-4 g) compared to initial values (p=0.03). Only 1 patient had a thrombotic event during the follow-up (cerebellar ictus) after a three months voluntary interruption of therapy. 83% of symptomatic patients at onset, still in treatmente, did not show symptoms. No case of acute leukemia or myelodisplastic syndrome was observed.

Conclusions. In our hands Anagrelide has been a reliable, safe and well tolerated drug. The most frequent adverse effect was a modest anemia, always revertable at the interruption of therapy. We think that this is a reliable and safe therapeutic approach in ET even if it should be confirmed in lager studies and longer-term evaluations.

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ESSENTIAL THROMBOCYTHEMIA FOLLOWING SOLID NEOPLASIA

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Introduction. Hematological malignancies following neoplastic diseases are increasingly described as an important late complication in cancer patients attributable to the chemotherapy and/or radiotherapy regimens administered, as well as to immunosuppressive treatment following allogenic organ transplantation. Recently, we have reported the characteristics of Chronic Myeloid Leukemia cases presenting as secondary neoplasms, demonstrating that there were peculiar clinical features in these patients. The frequency and the clinical characteristics of essential thrombocythemia (ET) cases following solid neoplasias have never before been reported in literature. In this study we focused our attention on ET cases following prior neoplasia, analyzing the data of our Centre to assess whether this kind of ET has different characteristics from those in patients without previous cancer.

Patients and Methods. Five-hundred and eighty ET cases (diagnosed from January 1988 to March 2005) were considered in this study. Among them, 20 (3.4%) patients had previously been treated for cancer. A historical group of 39 ET patients having similar features in terms of age and treatment were compared to these cases with ET as second malignancy. The Kolgomorov and Smirnov test was used to assess whether the data sample belonged to a population with a Gaussian distribution. Student's t-test or the Mann-Whitney test were performed for comparisons of means. Two tailed Fisher's exact test was used to compare categories. The duration of overall survival (OS) was calculated according to the Kaplan-Meier method and the log-rank test was used to compare curves. Only P-values <0.05 were considered to be significant.

Results. The distribution sites of the first neoplasia in the cases with ET as second malignancy were : breast in 6 (30%) cases, thyroid in 3 (15%), bowel in 3 (15%), prostate in 2 (10%), skin in 2 (10%), bladder in 2 (10%), testis in 1 (5%), and adrenal cortex in 1 (5%); among these cases, 18 (90%) were treated with surgery and the remaining two with surgery plus chemotherapy. The median time latency between the first neoplasm and the onset of ET was 8.5 years. The comparison between ET following neoplasm and the historical group did not show significant differences in terms of sex distribution, platelets count at diagnosis, thrombotic and/or hemorrhagic events, and OS. Four (20%) cases with secondary ET had one or more first-degree relatives affected by cancer, whereas no case was detected in the historical group (p=0.02).

Conclusions. ET following solid neoplasm is a novel entity never previously described in literature. From the results of our study this kind of ET does not seem clinically different from those usually reported. However, three points are noteworthy: firstly, these cases show an evident cancer familiarity; secondly, ET arises in several cancers in which thrombocytosis is frequently overt as a paraneoplastic syndrome; thirdly, in these cases the genotoxic effects of previous chemotherapy probably have not a role.

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FUNDAMENTAL ROLE OF T LYMPHOCYTES IN THE PATHOGENESIS OF IDIOPATHIC HYPEREOSINOPHILIC SYNDROME

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The Hypereosinophilic Syndrome (HES) is a rare haematologic disorder characterized by unexplained eosinophilia persisting for more than 6 months, in absence of reactive causes, with signs and symptoms of organ involvement. The pathogenesis of HES is still not completely understood. Nevertheless, one interesting pathogenetic hypothesis is that an occult T-lymphocyte clone would be responsible for proliferation of eosinophils by cytokine secretion (IL4, IL5). The molecular evidence of a clonal rearrangement of T cell receptor (TCR) γ/δ chains in some patients could sustain this mechanism.

These criteria are now under revision after the recent identification of the FIP1L1-PDGFRα fusion gene in about half cases of HES.

Imatinib mesylate (STI571) is a potent selective inhibitor of several tyrosine-kinases, such as BCR-ABL, c-KIT, and platelet-derived growth factor receptors (PDGFRα and PDGFRβ). Consequently, FIP1L1-PDGFRα fusion protein seems to be another ideal target of this new effective drug.

We would like to present experience of our Institution on 13 patients affected by HES, presented to our institution between 2000 and 2004. There were 2 female and 11 male, with a median age of 59 years (range 19-76). The BCR/ABL rearrangement was tested by RT-PCR: all cases resulted negative, thus excluding the diagnosis of chronic myeloproliferative disorders. The FIP1L1- PDGFRα fusion protein was detected in 4/13 (30.7%) of retrospectively analyzed cases. A clonal TCR rearrangement was detected by fluorescent PCR in 11/13 (84.6%) of tested cases: in 9/13 (69.2%) a clonal b rearrangement was found. Moreover, a clonal d rearrangement was detected in 6/13 (46.1%) cases; 4 of them showed either b or d clonal rearrangement. All 4 FIP1L1- PDGFRα-positive cases presented a clonal b TCR rearrangement also. Only one patient did not show anyone of tested molecular rearrangements. Nine patients received α-IFN as first or second-line therapy: 2 cases achieved a CR, 6 a PR and one was resistant (this one in the subgroup of TCR-negative cases). Six cases received Imatinib, 3 in the subgroup of FIP1L1- PDGFRα-positive and 3 in the subgroup of negative cases (one patient refused this treatment, notwithstanding the FIP1L1-PDGFRα positivity, retaining the treatment with α-IFN). All cases FIP1L1-PDGFRα-positive achieved a CR, and the remaining ones a PR. Interestingly, a FIP1L1-PDGFRα-positive case achieved a stable molecular remission after Imatinib administration; on the other hand, the other FIP1L1-PDGFRα positive case that refused imatinib did not achieve PCR negativity.

This experience suggests: 1) that the majority of HES cases present a clonal TCR rearrangement, thus sustaining the fundamental pathogenetic role of an occult lymphoproliferative disorder; 2) the role of imatinib in HES patients, even in the eradicating molecular abnormal rearrangements.

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IRON OVERLOAD CHELATION THERAPY WITH ICL670: A CASE REPORT OF UNEXPECTED CORRECTION OF ANEMIA IN A PATIENT WITH IDIOPATHIC MIELOFIBROSIS

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Tranfusional iron overload in patients with chronic acquired anemias can result in multiple organ failure, if not treated. ICL670 (deferasirox, Exjadeâ) is a once-daily, oral iron chelator in clinical development that has demonstrated efficacy and acceptable tolerability in patients with transfusional iron overload.

We report observations in a 63-year old woman, with Idiopathic myelofibrosis with myeloid metaplasia (MMM) and related transfusional-dependent anemia who received chelation therapy with ICL670. The patient experienced a reduction in iron overload and an "unexpected" complete reduction in blood transfusion requirements.

The patient was diagnosed with MMM intermediate risk (Dupriez score) in March 2002, according to the Italian Consensus Conference Criteria for the Diagnosis of MMM. Clinical characteristics at the time of diagnosis were: haemoglobin levels at 5-7 grams/deciliter, CD34 positive cell counts in peripheral blood of 16.2/microliter, positive direct Coombs test without hemolysis and anisopoikilocytosis with teardrop cells. A bone marrow biopsy showed 20% cellularity with diffuse fibrosis (grade 2). During the period May 2002 to June 2003, the patient required about 4 units of packed red blood cells per month.

In June 2003, the patient entered a Novartis phase II trial, CICL670A0108, which was designed to investigate the safety and efficacy of ICL670 (Exjade®) in rare anemias. At entry, the patient's baseline liver iron content (LIC) was 10.1 milligrams Fe /grams dry weight and serum ferritin was 3000 nanograms/microliter. The patient also had a further 10% decrease in marrow cellularity, but the clinical condition was unmodified. Based on the LIC evaluation at baseline, the patient was given ICL670 at a dose varying between 15-20 milligrams/kilogram body weight/day for 1 year. After 2 months of receiving ICL670, the patient's transfusion requirements progressively decreased and by early November 2003 (5 months of receiving ICL670), the patient was no longer being transfused. The patient's hemoglobin was stable between 11.4-12.4 grams/deciliter. At the end of study, in June 2004, the patient's LIC and serum ferritin were largely reduced to 2.8 milligrams Fe /grams dry weight and 1100 nanograms/microliter, respectively. Myelofibrosis re-evaluation revealed that haemoglobin had increased to 12.5 grams/deciliter transfusion free, CD34 positive cell counts in the peripheral blood had decreased to 11.1/microliter, direct Coombs test was negative and there was a reduction in teardrop cells. Bone marrow biopsy showed 15% cellularity with no modification in fibrosis score. At the time of writing this abstract case report, the patient's clinical condition is as stable as was in June 2004 and the patient is still transfusion free.

This is a case of unexpected improvement in the clinical condition of a case of MMM while undergoing chelation

therapy with ICL670. The relevance of this case strongly supports the need to fully investigate, in a clinical study setting, the correlation between iron overload and proliferative hematological diseases with severe anemia and the potential beneficial effect of iron chelation therapy.

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BCR/ABL NEGATIVE CHRONIC MYELOGENOUS LEUKAEMIA. DESCRIPTION OF 7 PATIENTS

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Patients with clinical characteristics of chronic myeloid leukemia (CML) in whom neither the Philadelphia chromosome nor the bcr/abl rearrangement are present constitute a long-disputed clinical entity defined as true Ph-negative, bcr/abl negative CML and, more recently, atypical CML (a-CML). We describe clinical characteristics of seven patients which were referred to our department from December, 2002 through January, 2005. All the patients fulfilled the recommended criteria (Kantarjan et al: Cancer 1986;58:2023-2030) for establishing the diagnosis of Ph-negative CML: 1) absence of Ph on analysis of at least 20 bone marrow mitoses; 2) hypercellular bone marrow with granulocytic hyperplasia; 3) bone marrow blasts < 30%; 4) absence of significant bone marrow dysplasia and fibrosis; 5) persistent, unexplained peripheral granulocytic leukocytosis (WBC count > 10x 10^9 per liter); 6) peripheral blast cells < 30%; 7) absolute monocyte count <1x10^9 per liter.

Table 1. Characteristics of the patients at diagnosis.

Pts	age	gender	WBC (x10^9/L)	Plt (x10^9/L)	Hb (gr/dL)	spleen (cm)	Baso. %	blasts %	caryotipe
1	38	F	57	65	9,2	0	3	12	46,XX
2	61	M	101	130	8,8	7	0	2	46,XY
3	65	M	102	430	14,7	4	0	0	46,XY
4	68	M	37	59	9,9	0	8	7	47,XY,+8
5	75	F	39	65	5,4	4	2	0	46,XX
6	48	M	31	490	5,0	3	2	0	46,XY
7	82	M	240	78	8,0	7	1	0	46,XY

The characteristics of our patients are shown in Table 1: median age 68 years (range 38 – 82), M/F ratio 5/2, all but one had anemia, five out of seven had thrombocytopenia and splenomegaly. All the patients had cytogenetic and molecular analysis performed routinely. Trisomy 8, which has been reported to be the most common abnormal karyotype in a-CML, was found in one patient. Blastic transformation occurred in the three patients who had blasts in the peripheral blood at the presentation (pts n°1,2,4). In patient 2 cytogenetic analysis at the time of leukemic transformation showed iso (q17). In this patient the disease was resistant to different therapeutic options included imatinib mesylate and two different intensive chemotherapeutic regimen. Patient 4 died of pulmonary infection; patient 1 was successfully treated with chemotherapy (daunorubicin, etoposide and high dose cytarabine) and obtained complete remission lasting since May, 2003. Bone marrow failure was the cause of death in patients 5 and 7. Patients 3 and 6 are treated with hydroxyurea; patient 6 needs frequent blood transfusions.

Onida et al (Cancer 2002; 95: 1673-1684) investigated patient-associated and disease-associated variables in order to better define this rare clinical entity and to design a prognostic scoring system (PSS). On this basis, bcr/abl negative CML seems to be a well-defined category distinct from bcr/abl positive CML for older age, male predominance, higher incidence of anemia and thrombocytopenia, worse outcome, and distinct from myelodysplastic syndromes for different pattern of cytogenetic abnormalities. The proposed PSS stratified the patients into a low-risk group and a high risk group with different outcome. According to this PSS, five out of seven patients were high risk and two low risk. Our data are in agreement with previous reports which supported the hypothesis that bcr/abl negative CML is a distinct clinical entity whose natural history is not well known because of its rarity. A prognosis improvement is likely to reside in the identification of gene alterations and of signalling pathways involved in the disease process

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ABNORMAL EXPRESSION OF GATA-1 IN MEGAKARYOCYTES FROM PATIENTS WITH IDIOPATHIC MYELOFIBROSIS

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The abnormal megakaryocytopoiesis associated with idiopathic myelofibrosis (IM) is believe to play a central role in its pathogenesis through the release of an array of cytokines that on turn activates stromal cells. We have previously shown that genetically-modified mice presenting defective expression of transcription factor GATA-1 (GATA-1low mutants) show a spectrum of megakaryocyte alterations resembling those observed in IM patients, and eventually develop myelofibrosis. Therefore, we investigated the possible occurrence of GATA-1 abnormalities in IM patients. To this end, $CD34^+$ cells were purified from the peripheral blood of 12 IM patients or the bone marrow of 8 healthy donors; GPA+ erythroblasts and CD61+ megakaryocytes were then immunomagnetically purified starting from unilineage cultures of $CD34^+$ cells. These cells were then analyzed for the expression of mRNAs for GATA-1, and two related transcription factors GATA-2 and FOG-1, by a sensitive RealTime RT-PCR. We found that the levels of these mRNAs in purified CD61+, GPA+ and $CD34^+$ cells from IM patients were comparable to controls. The protein content of both GATA-1 and GATA-2 was analyzed in cell extracts; CD61+ cells from IM patients were found to contain significantly reduced GATA-1 protein (44±12% of controls) unlike both $CD34^+$ and GPA+ cells. Furthermore, 45% of megakaryocytes (range, 18-67%) in bone marrow biopsies from IM patients did not stain with anti-GATA-1 antibody (GATA-1neg megakaryocytes), while in controls and patients with

Essential Thrombocythemia GATA-1neg megakaryocytes were <10%. Interestingly enough, the number of GATA-1neg megakaryocytes was significantly higher than controls and ET (but still significantly lower than IM) in BM biopsies form 10 patients with PV (11%, range 1-25%). These abnormalities were specific for GATA-1 since immunoreactivity for FOG-1 was comparable in all subjects evaluated. No mutation in GATA-1 coding sequences was found in IM cells. Finally, we also determined the JAK2 mutational status in 14/17 IM subjects whom BM biopsies had been evaluated; 10/14 patients beared the mutation. There was no clear correlation between the presence of mutation and the percentages of GATA-1neg megakaryocytes. These results indicate that megakaryocytes from IM patients have reduced GATA-1 content, that might contribute to the disease pathogenesis, as in the GATA-1low mice, and also represent a novel IM-associated marker. Furthermore, abnormalities of GATA-1 are unlike to be directly dependent on JAK2 mutation.

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OVEREXPRESSION OF CD9 IN PERIPHERAL BLOOD CD34+ CELLS OF IDIOPATHIC MYELOFIBROSIS PATIENTS

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Idiopathic myelofibrosis (IM) is a clonal myeloproliferative disorder (MPD) characterized by abnormal proliferation of multipotent hematopoietic progenitors, reactive fibrosis and extramedullary hematopoiesis. The number of CD34+ cells circulating in the peripheral blood is typically increased. The pathogenetic mechanisms that lead to the abnormal proliferation of hematopoietic cells, and especially to the defective megakaryocyte proliferation and maturation, remain unknown. The identification of gene expression abnormalities resulting from comprehensive molecular analyses, as through microarrays, might potentially help in elucidating mechanisms and eventually represent novel biomarkers IM. From the analysis of data resulting from a microarray analysis performed on purified PB CD34+ cells from 15 IM patients, that were compared to bone marrow (BM) CD34+ cells of normal subjects, we identified an abnormally high expression of the CD9 mRNA in IM CD34+ cells. The CD9 molecule, a member of the tetraspanin superfamily, is expressed by a various types of hematopoietic cells including B-lymphoid and megakaryocytic lineages, and it is involved in the regulation of megakaryocytopoiesis. The increased expression of CD9 mRNA was further confirmed by validation experiments using TaqMan Assay on additional 15 IM patients and 10 normal controls. We have also observed by FACS analysis that the number of CD9 molecules, expressed as Mean Fluorescence Intensity (MFI), and the percentage of double-labelled CD34/CD9 cells, are both significantly increased (p<0.01)in IM patients (5.43 and 42.6% respectively) as compared to: i) normal controls (1.6 and 15.8%), ii) Polycythemia Vera (3.4 and 34.8%) and iii) Essential Thrombocythemia (2.3 and 23.44%) patients. These observations support the utility of CD9 evaluation by FACS analysis for the diagnosis of IM, as recently exploited also for acute megakaryoblastic leukemia. We have investigated the possible involvement of CD9 in the abnormal regulation of IM megakaryocytopoiesis by using in vitro culture assays. As reported (Blood, 2001,97:1982), the engagement of the CD9 molecule with a anti-CD9 monoclonal antibody (mAb) resulted in an increased number of CFU-Mk from normal CD34+ cells. Therefore, we investigated the effects of anti-CD9 mAb in both semisolid and liquid cultures of IM and normal CD34+ cells. We have found that the number of both BFU-E and CFU-Mk were significantly increased in cultures supplemented with anti-CD9 antibody in IM patients unlike in controls; such an increase was particularly evident for CFU-Mk, that were increased 1.8 fold as compared to 1.3 in controls (p<0.05). Our data suggest that the abnormal expression of CD9 in CD34+ cells might allow to selectively identify IM patients versus other MPD, eventually representing a novel biomarker of this disease.

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CYTOGENETICS IN THE ESSENTIAL THROMBOCYTHAEMIA: PRELIMINARY REPORT OF THE GENETICS COMMITTEE OF THE REGISTRO ITALIANO TROMBOCITEMIA

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The Essential Thrombocythaemia (ET) diagnosis is fundamentally done by excluding the secondary thrombocytosis, the other chronic Myeloproliferative Disorders (MPD) and the Myelodysplastic Syndromes (MDS). According to the PVSG diagnostic criteria, the cytogenetic study is primarily required to document the absence of the Ph chromosome, although today it is more convenient and cheaper to exclude the CML diagnosis by the documentation of the bcr-abl transcript absence. Moreover, the cytogenetic study, particularly when integrated with molecular techniques, is very useful to discriminate the ET from the MDS with thrombocytosis and, in general, to identify genetic abnormalities with potential prognostic value. In the old series of patients of the Registro Italiano Trombocitemia (RIT) the cytogenetic study at diagnosis was available and valuable in 958 (44.8%) of the 2139 cases. In 47 patients (4.9%) were reported aspecific abnormalities that now are object of a centralised evaluation by the Genetics Committee of the RIT.

Concomitantly, the cytogenetic data of the ET patients consecutively observed in the Haematological Centres of Ravenna, Reggio Emilia and Perugia are object of the centralised evaluation. In the series of Ravenna (65 patients, 25 males, 40 females, median age 60 yrs) aspecific cytogenetic abnormalities were documented in height cases (12,7%): 46,XO,-Y; 46,XY,der(20)t(1;20)(q21;q13); 46,XY,t(5;11)

(q31;p15),del(10)(q24); 46,XY,del(3)(p13p21.2); 46,XO;-Y; 45,XX,-17;47,XX,inv(3) (q21q26),+12; 46,XX,del (20) (q11q13). In the series of Reggio Emilia (49 patients, 19 males, 30 females, median age 58 yrs) four patients (8.2%) showed aspecific abnormalities: 45,X,-Y/46,XY; 47XY,+ 9/46,XY; 47,XY,+del(1) (p13p36)/46,XY; 45,XY,rob(14;21) (q10;q10). In the series of Perugia nine patients showed the following abnormalities: 47,XY,+21; 46,XY,t(13;14) (q32;q32); 46XX,t(1;7)/ 47,XX,t(1;7),+8; 48,XX,+ mar1, +mar2; 46,XX,del(3) (p11p21)/46,XX; 46,XX/46,X, t(X;5) (q13;q33); 46,XY/46, XY,t(11;21)(q25;q22); 46,XX, t(5;13); 46,XX,t(4;9). Complementary molecular studies will be presented together with the cytogenetic data.

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CARDIOVASCULAR EVALUATION IN 130 ESSENTIAL THROMBOCYTHAEMIA PATIENTS TREATED WITH ANAGRELIDE: PRELIMINARY REPORT OF THE ANAGRELIDE COMMITTE OF THE REGISTRO ITALIANO TROMBOCITEMIA

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Anagrelide treatment is considered potentially responsible of cardiovascular toxicity. For this reason, we have retrospectively evaluated 130 patients (pts) with Essential Thrombocythaemia (ET) treated with Anagrelide in 10 Italian Haematological Centres. The pts, 55 males and 75 females,had at diagnosis a mean age of 43 years (range 13-82) and a mean platelet (PLT) count of 1086x10^9/L (range 572-2540; over 1000 49%). The Anagrelide treatment was started after a mean of 47 months from diagnosis(range 0-196). A previous treatment with cytoreductive drugs (mainly HU and IFN) and antiplatelet agents (mainly ASA), was performed in 65% and 98% of cases, respectively. The mean Anagrelide dose was 1 mg/day at start and 1.7 mg/day in the maintenance phase, when the mean PLT count was 473x10^9/L. Twentyfive patients (19%) withdrew the treatment after a mean of 27 months (range 1-85) for the following reasons: compliance loss (n8), inefficacy (n7), cardiotoxicity (n6), intolerance (n4), anemia (n1), renal toxicity (n1), thromboembolism (n1). During the follow-up (median 28 months) 4 thrombotic complications (1.15/100 pt-yrs) and 2 minor haemorrhages (0.57/100 pt-yrs) were registered. Moreover, 15 pts showed non lethal cardiovascular events: angina (n6), cardiomiopathy (n4), arrhythmia (n3), AMI (n2). An instrumental cardiovascular study with Echocardiography (ECHO) and ECG was performed at Anagrelide start in 58 pts: the Ejection Fraction (EF)was abnormal in one pt only (EF 47%). The instrumental monitoring was repeated in 28 pts every 6-12 months. Four out of these 28 pts showed a decrease of the EF below the level of 50%(43%,45%,47%, and 49%), but only 2 pts withdrew Anagrelide. The clinical and instrumental cardiovascular evaluation in ET patients receiving Anagrelide is becaming of routinary application and the data obtained by the partecipating Centres are now object of study by the Registro Italiano Trombocitemia (RIT).

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GENE EXPRESSION ANALYSIS BASED ON REAL TIME PCR OF 95 GENES CODING FOR TYROSYNE KINASES IN PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Ph negative Chronic Myeloproliferative Disorders (CMPD) are likely characterized by a deregulated tyrosine kinase (TK) activity which is well identified and characterized only in a small subset of patients. The possibility to identify additional TK involved in the pathogenesis of CMPD possesses a relevant clinical significance since it offers the possibility to design new molecules able to inhibit in a selective manner the specific target, giving the patient the possibility to be treated with a new molecular approach. The aim of the present study was to identify the presence of activated TKs in CMPD through a gene expression analysis based on Real Time PCR of 95 genes coding for Tyrosyne Kinases. We analysed the expression level of 95 genes in 16 patients affected by CMPD and 10 BM samples obtained from healthy volunteers. The quantitative analysis was performed using the ABI Prism 7900 (Applied Byosystems) using the micro fluidic card and the assays-on-demand system. The micro fluid card configuration was designed in order to allow the analysis in a single card of the expression level of the selected 95 TKs and three housekeeping genes in duplicate. The series of the patients studied included 3 patients affected by primary eosiniphilic syndrome (HES), one of them was characterized by the presence of the hybrid transcript FIP1L1-PDGFRα and one by the presence of a cytogenetic marker, 4 patients were affected by chronic myelomonocitic leukaemia (CMML) and 9 patients were affected by Ph negative CML like diseases, four of them characterized by a cytogenetic marker The final value of expression has been calculated using the software SDS 2.1. The first result obtained is represented by an impressive homogeneous expression of the TK within the normal samples. By contrast, in our series of patients we were able to identify 30 genes which resulted to be expressed in a significant different way respect to the normal samples. The majority of them were upregulated and few of them significantly downmodulated. The selected genes have been then clustered based on their biological significance. A further analysis allowed us to select 6 genes which appeared to be of particular interest in different subgroups of patients. These genes have been further studied in an enlarged series of patients. This study allowed us to identify a pattern of TK expression in different subgroups of CMPD and particularly to identify 6 genes which probably play a key role in the pathogenesis CMPD.

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BONE MARROW ANGIOGENESIS IN AUTOIMMUNE MYELOFIBROSIS

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Autoimmune myelofibrosis (AM) is an emerging clinicopathological entity, recognizing immune pathogenetic mechanisms and occurring isolately or in association with systemic and/or organ specific autoimmune diseases. It results in chronic cytopenias, and is defined by a pattern including bone marrow, peripheral blood, serological and clinical features. It has to be distinguished from other disorders having myelofibrosis. Among these, the most relevant differential diagnosis is with myelofibrosis with myeloid metaplasia (MMM). This is characterized by bone marrow histological findings, essentially consisting of increased reticulin fibrosis and megakaryocyte clusters; further, it is significantly associated with increased bone marrow angiogenesis. Otherwise, AM can be identified by increased reticulin fibrosis, not clustered megakariocytes, reactive lymphoid infiltration in bone marrow biopsy. The purpose of our study was to assess the angiogenetic aspects in bone marrow biopsies of patients with AM. Eight patients (median age 65,8; 2 males) were included in the study. They had been diagnosed as having AM on bone marrow, peripheral blood, serological and clinical features, The controls were represented by 10 bone marrow biopsies obtained from 10 patients affected by MMM (4 of them had cellular phase agnogenic myeloid metaplasia, 4 had post-polycythemic myeloid metaplasia, and 1 had post-thrombocytemic myeloid metaplasia). In all the specimens, bone marrow angiogenesis was evaluated by assessing microvessel density through immunohistochemical staining for the CD34 antigen, and by counting the number of vessels seen in 10 microscope fields (400 X). The results were expressed as median value; in particular, the bone marrow biopsies of the patients with AM gave the result of 46.4 (range 9-112), whereas those of the patients with MMM gave 180 (range 147-198). The microvessels of the patients with AM appeared narrowed and smaller than those observed in the control specimens. Our results showed that in AM bone marrow angiogenesis is decreased as compared to that of MMM. This finding may be considered as a further feature contributing to the differential diagnosis between the bone marrow pattern defining AM and that of MMM.

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LEUKEMIC POTENTIAL OF PIPOBROMAN : REPORT OF TWO CASES OF ACUTE MEGAKARYOBLASTIC LEUKEMIA EVOLUTED FROM ESSENTIAL THROMBOCYTEMIA

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Essential thrombocytemia (ET) is a chronic myeloprolipherative disorder with a benign course and morbidity and mortality caused by bleeding and thrombotic complications. Differently from others myeloprolipherative disorders that have a potential, to a greater or lesser degree, to spontaneously convert to acute leukemia, this association is less clear in ET. Treatment with radiophoshosphorus, alkylating agents and probably also hydroxyurea enhances the leukemic potential of disease. Patients who develop acute myeloid leukemia and myelodysplastic syndromes show often chromosome 17p deletions and other characteristics of the 17p- syndrome. We describe two cases of a essential thrombocytemia with normal caryotype transformed in acute megakaryoblastic leukemia.

Case 1. A 70 years old man was admitted at our institution in December 1998 with a stable increased platelet count documented from 1993 always upper 800.000 mm^3. FAL score , cytogenetic ,RT- PCR for BCR-ABL transcript, trephine biopsy was consistent with a diagnosis of ET. Treatment with hydroxyurea was started at 500 mg or 1000 mg t.d. daily with normalization of platelet count. In september 2001 hemoglobin level and hematocrit progressively arised and disease was clinically consistent with polycytemia vera. Therapy with pipobroman was perfomed with a total dose of 25 mg daily with hematological response. Patient was stable until march 2003 when was documented a progressive anemia and diagnosis of myelodysplastic syndrome (refractory anemia) was made . Therapy with folic acid and rh-EPO was given without sustained benefit. In october 2003 a new trephine biopsy was performed. Histologic findings were consistent with an increased number of megakaryocytes showing a pattern of dysplastic and pleomorphic abnormalities and mitotic activity. Bone marrow smears displayed micromegakarioblastic cells in amount of 51% with erithroblasts 12% granuloblasts 30% and lymphocytes 7%. The panel of monoclonal antibody on marrow blood showed : CD 41a :26% , CD 61 :26% , CD 41a/CD 61: 26% , CD 13 : 77%, CD 33 :77%, CD 13/CD33 :33%, MPO :37% ,cCD 41a :64% , CD 61:59% . Diagnosis of Acute Megakaryoblastic Leukemia (FAB M7) was performed and antiblastic and supportive therapy was started.

Case 2. A 74 years old man was admitted at our institution in october 1998 coming from another institution with acclared diagnosis of essential thombocytemia and therapy with pipobroman in course from June 1998. Treatment was switched with hydroxyurea and stable disease as far as September 2002. Then the patient was lost to follow-up and after a year displayed a low WBC count with 7 % rate of marrow blasts. On May 2004 the patient was admitted with severe anemia and mild thrombocytopenia. Bone marrow smears displayed megakaryoblastic-like cells in amount of 50% , erithroblasts 30%, granuloblasts 12%, lymphocytes 5% and plasma cells 3%.

Discussion. The reports of acute leukemias evoluted from essential thrombocytemia are aneddotical in the literature, mainly in patients with normal caryotype.Nevertheless leukemic potential of alkylating agents is universally acclared. Both cases were treated with hydroxiurea and pipobroman and if two drugs worked sinergically it is hard to estabilish. However, in agreement of very low leukemic potential of hydroxiurea we suggest to loose pipobroman from therapy of chronic myeloproliferative disorders.

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GM-CSF RECEPTOR AS A POSSIBLE MARKER TO DISTINGUISH PROLIFERATIVE AND DYSPLASTIC VARIANTS OF CHRONIC MYELOMONOCYTIC LEUKEMIA

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Chronic myelomonocytic leukemia (CMML) is a heterogeneous hematological malignancy, which shows features of both myelodysplastic and myeloproliferative disorders. For this reason, CMML has been included in a new category of MDS/MPD disorders in the last WHO classification of myeloid malignancies. An arbitrarily chosen leukocyte count has been proposed by the FAB group to differentiate between a "dysplastic" type (MD-CMML, with $<12x10^9$ WBC/L) and a "proliferative" type (MP-CMML, with $>12x10^9$ WBC/L) of CMML. However, apart from the WBC count, no biological difference has been identified to support distinction between these two disease-entities. Among factors that have been implicated in pathogenesis of CMML, GM-CSF produced by either autocrine or paracrine mechanisms has been shown to be a major growth determinant. In this study, peripheral blood samples from normal controls and from patients affected by proliferative and dysplastic variants of CMML were used to investigate expression of intracytoplasmic GM-CSF and expression of GM-CSF membrane receptor. Briefly, mononuclear cells (MNC) were isolated on Ficoll-Paque density gradient and cryopreserved in FCS 10% DMSO. In a first set of experiments samples from 5 healthy controls, 5 MP-CMML and 5 MD-CMML were thawed, permeabilized and stained with GM-CSF PE (Caltag) to evaluate expression of the intracytoplasmic cytokine by FACSCalibur flow cytometer (BD). Median percentage of GM-CSF expression was 0.3 (range 0-2.5) in normal controls, 39.3 (range 14.5-90.7) in MP-CMML and 0.9 (range 0-9.3) in MD-CMML. The difference between MP and MD disease was statistically significant. To further investigate the possible role of GM-CSF cytokine in the pathogenesis of CMML, in a second set of experiments, thawed MNC from 5 normal controls, 12 MP-CMML and 10 MD-CMML samples were stained with GM-CSFR (CD116 Pharmingen) and then with Goat Anti-Mouse FITC (BD) to evaluate the expression of the cytokine receptor. Median percentage of expression of GM-CSFR was significantly higher in CMML samples (53.9, range 15.7-69) than in normal controls (18.9, range 16.4-27.3). No difference was detected between subtypes of MP-CMML and MD-CMML. When we considered median intensity of GM-CSFR expression, we observed a significantly higher values in MP-CMML than in MD-CMML (120 and 60.8, respectively), whereas no significant difference was detected between normal samples and MD-CMML. The higher levels of intracytoplasmic GM-CSF and the increased density of the cytokine receptor in MP-CMML suggest a possible role of GM-CSF in malignant cell proliferation of CMML patients. If our results will be confirmed, these findings could be utilized as a possible biological marker to distinguish proliferative and dysplastic variants of the disease. Further studies are warranted to investigate possible therapeutic applications.

Autologous Transplantation

P369

RITUXIMAB IN ASSOCIATION TO MACHOP FULL INDUCTION THERAPY PLUS AUTOLOGOUS STEM CELL TRANSPLANTATION IMPROVES THE OUTCOME OF PATIENTS WITH POOR RISK DIFFUSE LARGE B-CELL LYMPHOMA

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Background. and objectives. The prognosis of patients with poor risk, aggressive non-Hodgkin's lymphoma (NHL) is dismal. The role of front line consolidation therapy with high-dose treatment (HDT) and autologous hemopoietic stem cell transplantation (ASCT) remains unclear. Aim of this study was to evaluate the impact of a front-line intensive program based on the administration of a full intensified CHOP-based induction therapy followed by HDT and ASCT in an unselected cohort of patients with poor-risk peripheral diffuse large B-cell lymphoma (B-DLCL).

Design and methods. One hundred-twenty consecutive adult patients, 60 years old or younger, with previously untreated B-DLCL were evaluated for the study. Patients were considered at poor risk if at least one among Ann Arbor stage III-IV, bulky disease (tumor mass greater than 7 cm) or B-symptoms were present at the time of diagnosis. The therapeutic program consisted in the administration of 6 courses of a MACHOP regimen (prednisone, vincristine, doxorubicin, cyclophosphamide, methotrexate, cytosine-arabinoside) with or without rituximab (depending of the study period), radiotherapy to initial bulky or localised residual disease, HDT (using BAVC as conditioning regimen) and ASCT.

Results. Eighty-eight out 120 patients completed the therapeutic program with HDT and ASCT, as planned, while the remaining did not principally because of toxicity, no response/progression during induction treatment, inadequate stem cell collection. Median follow-up was 46 months (range 1-190). Overall, projected OS and EFS are 74% and 71%, respectively. The addition of rituximab to initial induction therapy was associated with an improve of outcome in patients with IPI high-intermediate and high. Treatment related mortality was 2.5 % (3 patients), while long-term grade III-IV toxicity was 8.3 % (10 patients); no case of myelodisplasia or acute leukaemia were registered.

Interpretation and Conclusion. Our results compare well with historical data from the literature and support the application of an intensive therapeutic program inclusive of a full intensified rituximab containing CHOP-based induction chemotherapy and HDT for the treatment of patients with poor risk B-DLCL.

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AUTOLOGUS STEM CELL TRANSPLANTATION AS POST-REMISSION THERAPY IN 26 ALL ADULT PATIENTS, WITHOUT HLA SIBLING DONORS, IN FIRST OR LATER COMPLETE REMISSION.

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Introduction. The current therapies for Acute Lymphoid Leukemia (ALL) result in a high complete remission (CR) rate (80-90%) but only 25-30% of patients (pts) achieve a long-term Disease Free Survival (DFS) that is the most important challenge in ALL. The role of Autologus Bone Marrow Transplantation (ABMT) and its optimal timing in adult ALL pts is still a matter of debate and conclusive statements are limited by the short follow-up periods. Herein we report our single centre experience about this procedure.

Patients and Methods. Over a 12 years period, 26 consecutive ALL pts, without an HLA-identical sibling donor, received ABMT as consolidation therapy after remission. Pts median age was 27 years (range 16-58), 17 were males and 9 females. ALL B 19/26 and T 7/26, Standard-Risk (SR) 11/26 (42%) and High-Risk (HR) 15/26 (58%). There were 5 Ph+ ALL. The median time from diagnosis to ABMT was 9,5 months (range 2-15). The disease status at ABMT was: 1°CR in 20/26 (77%) of cases, 2° CR in 6/26 (23%). Hematopoietic stem cells source was peripheral blood in 16 pts (62%) and bone marrow in 10 (38%). In 21/26 (81%) pts the harvest was done after priming with granulocyte colony-stimulating factor (G-CSF). Conditioning regimens were: busulfan + cyclophosphamide in 25/26 (96%) of cases and melphalan + TBI in 1/26 (4%) of cases. The mean of CD34 positive unpurged cells reinfused was $2,9\pm2,7x10^6$/Kg.

Results. The median follow-up from diagnosis was 152 months (range 28-398). With a median follow-up from transplant of 66 months (range 4-144), 17 (65%) pts are alive and in complete remission while 9 (35 %) were death after a relapse that occurred at a median time of 9,6 months (range 2,2-59) from ABMT. The probability of OS and DFS of the whole population was 65% and 59%, respectively, at a median time from transplant of 60 months. There was a statistically significant difference in OS (p 0,004 by the log-rank test and p 0,002 by the Wilcoxon test) and DFS (p 0,001 by the log-rank test and p 0,002 by the Wilcoxon test) comparing ALL pts transplanted in first CR with those in II° CR. No transplant related mortality was recorded; transplant related complications were not important and all pts obtained a fast granulocyte recovery. No secondary MDS or malignances were observed during the post-transplant follow-up.

Conclusions. Our experience, with the caution of the small number of cases, would confirm that ABMT can be a well tolerate and effective therapeutical option in adult ALL pts without an HLA-sibling donor particularly those who received ABMT in first CR. In this setting (ALL pts in I° CR without a donor) randomized and multicentric studies are needed to compare the outcome of ABMT ± maintenance

therapy versus maintenance therapy alone and in order to define the best therapeutic approach after induction/consolidation treatments.

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MAFOSFAMIDE-PURGED AUTOLOGOUS BONE MARROW TRANSPLANTATION IN 1CR AML: EFFECTIVENESS OF PURGING IS MORE EVIDENT IN PATIENTS NOT HARBOURING GOOD CYTOGENETIC ABNORMALITIES

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In a single institution, 36 patients affected with Acute Myeloid Leukaemia (AML) in 1st Complete Remission (CR) received autologous bone marrow transplantation (ABMT). Mafosfamide was employed in a not randomised fashion to purge marrow, in 19 cases bone marrow cells were purged, while in 17 were left unpurged. All patients reached a Neutrophil count above $0.5x10^9$/L in a median of 25 days (range 8-49). Time to Neutrophil engraftment was not different in purged group compared to one, 24 days versus 25.5 days (log rank p=0.39), all patients reached a PLT count of $20.0x10^9$/L and median time for PLT engraftment was 60 days (range 17-270), it was significantly longer after purged transplants than unpurged, 85 days vs. 59 days (log rank p=0.041). Using Cox analysis, dose of infused Total Nucleated Cells (TNC) was an important factor for myeloid engraftment (p=0.02). No TRM was registered, Leukaemia Free Survival (LFS) for the entire group of patients was 53 %. LFS was 61 % in purged and 46 % in unpurged groups (p=0.31). Patients having a good prognosis cariotype had a LFS of 100% while the group of all others patients had a LFS of 42 %. Purging improved LFS in patients not harbouring good cytogenetic abnormalities (LFS 55 % vs 22 %, log rank p=0.06). Patients receiving a TNC > median had a LFS of 31 % and those receiving TNC < median had a LFS of 70 % (p=0.011). In conclusion, our results show a relationship between infused cell dose and engraftment and between cell dose and LFS. The use of purging did not worsened aplasia length and was clinically beneficial in a subgroup of patients identified by cytogenetic.

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BUSULFAN–MELPHALAN REGIMEN IN AUTOLOGOUS STEM CELL TRANSPLANTATION FOR ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Autologous stem cell transplantation (ASCT) improves the survival of patients with acute myeloid leukemia (AML). In the past, the high-dose regimens utilized in ASCT were derived from those used in allogeneic setting. Since these regimens (TBI-Cy, BU-Cy, BU-VP-Cy) provide both cytoreduction and immunosuppression to facilitate the allogenic engraftment, we evaluated the efficacy of a regimen that theorically gives the maximum therapy to eradicate the disease. Between January 1998 and October 2004, 24 AML patients (14 females and 10 males; median age 40 years, range 15-65; 5 patients had FAB M1 AML, 9 FAB M2 and 10 FAB M4; finally, 23 patients were in CR and 1 in PR) underwent to ASCT. The conditioning regimen consisted of 4d Busulphan (4 mg/ Kg from day -5 to -2) followed by Melphalan (140 mg/m^2) for 1d (day -1). Twenty-three patients (96%) achieved a full hematological recovery. The source of stem cell was bone marrow (BM) for 5 patients (20%) and peripheral blood stem cells (PBSC) for the others. Patients autografted with BM received a median of 1.55 x 108/Kg nucleated cells (range 0.4-2.4) while those autografted with PBSC received a median of $4.8x10^6$/Kg CD34$^+$ cells (range 2.5-11.1). The median number of days to neutrophil count of 0.5 x 10^9/L and platelet count of $20x10^9$/l was 19 (range 12-33) and 25 (range 14-145) respectively. As major extra-hematological treatment-related toxicity all the patients developed a mucositis episode which was severe (grade III-IV WHO) in 9 of them. There were 8 documented infection (all bacterial), while 11 patients had fever of unknown origin (FUO). After a median follow-up of 30 months (range 2 – 108), 17 patients (70%) are alive and in CR while 7 patients relapsed after 2, 3, 4, 6, 8, 11 and 14 months respectively and all of them died from refractory disease. In conclusion, despite the reduced number of patients and the short follow-up, our results demonstrated that feasibility of BU-MEL regimen, as conditioning treatment for AML patients who will undergo ASCT and secondarily the efficacy of this schedule as evidenced by the high number of continous complete responder patients.

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PHASE I-II STUDY OF SELECTION AND TRANSPLANTATION OF AUTOLOGOUS HIGHLY PURIFIED CD133$^+$ STEM CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH ADVANCED DISEASE

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CD133$^+$ antigen is expressed on hematopoietic stem cells (HSC) capable of long-term reconstitution of human hematopoiesis in immunocompromised mice. However, few studies have evaluated the infusion of allogeneic or autologous CD133$^+$ cells for the recovery of bone marrow function after myeloablative chemotherapy. In this study, 9 Chronic Lymphocytic Leukemia (CLL) patients with advanced disease (median age:52 years, range:36-58) received autologous transplantation of highly purified CD133$^+$ cells. A median number of $5x10^6$/Kg CD34$^+$ cells and of $3.9x10^6$/Kg CD133$^+$ cells were collected from the peripheral blood after mobilization with high dose cyclophosphamide and G-CSF. Immunomagnetic selection with an anti-CD133 monoclonal antibody allowed the purification of a median number of $3.9x10^6$ CD133 (median purity: 95.3±6.6%, median recovery 99.6±4.7%) and

$5x10^6$ $CD34^+$/Kg (purity: 93.1±9.4%, recovery 80.2±17.1%). Positive selection of $CD133^+$ cells produced a median T-cell depletion of 4±0.9 Log and resulted in the median purging of 2.9±1.4 Log of $CD19^+/CD5^+$ leukemic cells. Seven out of 9 patients have been reinfused so far after a conditioning regimen including Busulfan (16 mg/Kg) and Melphalan [140mg/m^2] with a median number of $2.6±1.7x10^6$ $CD34^+$ cells/Kg and $2.5±1.5x10^6$ $CD133^+$cells /Kg. Autologous graft of 7 patients contained a median number of $5.6±18.1x10^3$/Kg T cells and $2.04±1.5x10^4$/Kg $CD5^+/CD19^+$ cells. At the phenotypic level, 2/7 patients were reinfused with tumor-free autografts. Molecular analysis was also used to evaluate residual disease in vitro and *in vivo* after transplant. All patients showed a rapid and sustained engraftment with a median time of 13 and 16 days to ANC>$0.5x10^9$/L and to Plt>$20x10^9$/L, respectively. No unusual adverse events were observed following transplantation of autologous highly purified $CD133^+$ HSC. However, one patient developed sinusoidal obstructive syndrome (SOS) 30 days after the conditioning regimen. As expected, the immunological reconstitution after transplantation of $CD133^+$ HSC was delayed, with an absolute median value of $CD4^+$ cells below 0.5 $x10^9$/L up to 12 months from the reinfusion. However, no infectious complications were observed and none of the patients were readmitted into the Hospital for late infections. In conclusion, reinfusion of highly purified CD 133^+ HSC allows rapid and sustained recovery of hematopoiesis in CLL patients with advanced disease. Positive selection of $CD133^+$ cells induces significant T and B- cell purging. A longer follow-up and a larger cohort of patients are warranted to evaluate the impact of autologous stem cell transplantation of $CD133^+$ HSC on the clinical outcome of CLL patients.

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A PROSPECTIVE RANDOMIZED TRIAL OF TWO DOSE-INTENSIVE MELPHALAN REGIMENS (100 VS 200MG/MQ) IN MYELOMA NEWLY DIAGNOSED PATIENTS: AN INTERIM ANALYSIS

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Background. Several trials have shown the superior impact of high-dose melphalan (usually 200 mg/m^2, MEL200) versus standard therapy in myeloma patients.

Intermediate-dose melphalan (100 mg/m^2, MEL100) is also superior to the standard dose, but has not been clinically compared with MEL200 in a randomized study. In our last case-matched study, we demonstrated that MEL100 was less toxic than MEL200, MEL100 was inferior to MEL200 in terms of EFS but not in terms of OS. We now compare the efficacy and toxicity of MEL100 and MEL200 in a prospective randomized trial.

Aims. The end points of the study were: response, EFS, OS and toxicity.

Methods. All patients were untreated, with measurable disease and aged < 65. The Southwest Oncology Group (SWOG) diagnostic criteria and Durie and Salmon staging system were used. Exclusion criteria were prior treatment for myeloma, abnormal cardiac function (systolic ejection fraction <50%), respiratory disease (vital capacity or carbon monoxide diffusion < 50% of normal), abnormal liver function (serum> aminotransferase value > 2.5 of normal), abnormal renal function (serum creatinine > 3 mg/dL), HBV, HCV, or HIV positivity, concomitant cancer or > psychiatric disease. The institutional review board approved the protocol and written informed consent was obtained from all patients. The MEL100 regimen included: 2 DAV debulking courses (dexamethasone - doxorubicin [adriamycin]- vincristine; adriamycin 50 mg/m^2 day 1, vincristine 1 mg day 1, dexamethasone 40 mg days 1, 2, 3, 4, each course repeated every 28 days), cyclophosphamide 4 g/m^2 plus G-CSF and subsequent leukapheresis, double MEL100 with stem cell support. The MEL200 regimen was as MEL100, but the double autografts were conditioned with MEL200.

Results. An interim analysis has been planned after the first 200 newly diagnosed myeloma patients, median age 58, range 33-65, entered the study, between January 2002 and January 2005. At present, 83 patients have a follow-up superior than 1 year and have completed the entire program: 40 in MEL100 arm and 43 in MEL200 arm. Patient characteristics were similar in both groups (MEL100 and MEL200 respectively): median age 58 vs 59 yr, patients > 60 yr 48% vs 51%, Durie-Salmon stage II 31% vs 31%, Durie-Salmon stage III 64% vs 63%, Durie-Salmon stage B 5% vs 6%, median Beta 2 microglobulin 2.4 vs 2.6 mg/L, median Hb 10.4 vs 10.8 g/dL, median serum calcemia 2.39 vs 2.52 mmol/L, median albumin 3.56 vs 3.36 g/dL, median plasma creatinine 0.8 vs 0.8 mg/dL.

Conclusions. An interim analysis is ongoing and will be finished in April 2005, the results of this analysis will be presented.

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THE ROLE OF PEG-FILGRASTIM AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: A CASE-CONTROL STUDY

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Peg-filgrastim is a novel long-acting, self-regulating form of recombinant G-CSF created by attaching a poliethylenglycol molecule to filgrastim. The prolonged terminal half-life of peg-filgrastim allows single, once-per-cycle administration to stimulate neutrophil recovery after standard chemotherapy in both solid tumors and hematological malignancies. Limited data, however, are currently available about the use of peg-filgrastim in the setting of myelosuppressive, high dose chemotherapy followed by autologous stem cell tranplantation (AuSCT).

We investigated the effects of a single s.c. injection of peg-filgrastim (6 mg) on day 3 after AuSCT in 36 patients with multiple myeloma (MM). Mean age was 55.4 years (range 23-66). In 33 patients AuSCT was performed as consolidation therapy in first complete or partial remission, three patients received the procedure as salvage treatment for chemo-sensitive relapse. Conditioning regimens consisted of intermediate (100 mg/sqm in 12 patients) or high dose (140-200 mg/sqm in 24 patients) melphalan. For comparison, we selected a group of 36 historical controls matched for age, status of disease, treatment and number of $CD34^+$ stem cells infused, who had received standard daily s.c. administration of filgrastim (300 mcg/d) from day 5 to neutrophil recovery after AuSCT.

No serious adverse events occurred in patients treated with peg-filgrastim. There was no difference (p n.s.) between the two groups (peg-filgrastim vs filgrastim) in terms of median time to neutrophil recovery > 0.5 x 10^9/L (10 +/- 1.3 vs 11 +/- 2.4 d), hemoglobin recovery > 10 g/dL (12.5 +/- 6.6 vs 14 +/- 4.8 d), days of febrile neutropenia (1 +/- 2.5 vs 1 +/- 2 d) and number of red cell (0 +/- 1.6 vs 1 +/- 1.8 units) or platelet (1 +/- 1.6 vs 1 +/- 1.8 units) transfusions. Likewise, the median number of days with severe neutropenia < 0.5 x 10^9/L (5 +/- 1.8 vs 5 +/- 2.1) and < 200 x 10^9/L (3.4 +/- 1.7 vs 3 +/- 2.4), as well as the duration of hospitalization (16 +/- 5.1 vs 16 +/- 3.5 d) were also not statistically different. The median time to platelet recovery > 20x10^9/L (11 +/- 6.6 d, range 0-23 vs 14 +/- 5.1 d, range 3-28) was shorter, but not significantly different, in peg-filgrastim group. The mean of filgrastim injections in controls was 11.6 (range 7-20), so that the cost of this drug for single patient was 997 euro, as compared to 910 euro for patients receiving peg-filgrastim.

We conclude that peg-filgrastim and filgrastim have comparable safety and efficacy profiles in patients affected by MM who undergo AuSCT. In this specific setting of patients, peg-filgrastim could be slightly cost-effective. Whether peg-filgrastim may be superior in reducing the period of thrombocytopenia, requires to be confirmed by further investigations.

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OUTPATIENT MANAGEMENT OF PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Autologous stem cell transplantation (ASCT) represents the standard therapy for patients with multiple myeloma (MM). The inclusion into ASCT programs of an increasing number of patients aged over 60 years results in increasing pressure on hospital beds. Given that toxicity of high dose melphalan is mainly hematologic and rapidly corrected by peripheral blood stem cells (PBSC) infusion, we developed a program of outpatient management of aplastic phase after ASCT, based on immediate discharge following conditioning and PBSC infusion. Criteria for re-hospitalization were febrile neutropenia and any WHO grade 3-4 toxicity. In a preliminary study, we demonstrated the safety of this approach on a small series of 28 patients (Ferrara *et al,* THJ, 2003). Here we report our updated experience by adopting such a model on a series of 68 ASCTs performed for patients affected by MM. The program started on January 2001 and up to February 2005 has been offered to 63 patients for a total of 68 procedures (5 double autografts), out of 81 total consecutive ASCTs for MM (inclusion rate: 84%). Thirteen patients were excluded because of lack of caregiver (n=3), conditioning with BEAM regimen (n=9), poor performance status (n=1). Median age of patients was 58 years (35-73). Myeloma subtype was IgG or IgA in 40 cases, micromolecular in 14, non secretory in 3, and solitary plasmocytoma in the final 6 patients. Before ASCT 34 patients (50%) were in complete remission, 34 (50%) in partial remission, according to SWOG criteria. Forty-six patients were conditioned with melphalan at 200 mg/sqm, 22 at 140 mg/sqm (age >65 years), with a median $CD34^+$ cells infused of 6.6x10^6 (1.8-25.5). G-CSF was given at onset of severe neutropenia (in most cases at day +5 from PBSC infusion). All patients (100%) were discharged on the day after stem cell infusion. Readmission was necessary in 20 cases (29%), main reasons being febrile neutropenia (n=10), mucositis needing total parenteral nutrition (n=8) and patient's anxiety (n=1). Hematological recovery was not significantly different between patients readmitted and non-readmitted days to PMN>500/cmm and Plt>20000/cmm being 12 (9-15) vs 12 (9-20) and 11 (0-22) vs 11 (0-17), respectively, *p*:0.65 and 0.72]. Neither number of $CD34^+$ cells infused, nor patient and disease characteristics before ASCT were significantly different between the two groups. The median number of days of hospitalization for the whole patient population was 4 days (4-21); patients not readmitted required a median of 4 days as opposed to 13 days for those who were rehospitalized (*p*:0.001). Transplant related mortality was absent and no severe bacterial or fungal infection occurred. An high level of satisfaction was reported by all patients. Median reduction of costs for single procedure as compared to a series of 27 patients previously autografted with an inpatient approach was Euro 9.500. In conclusion, our study confirms the feasibility and safety of ASCT on an outpatient basis in patients with MM, including those aged >65 years. Patient level of satisfaction is high and costs are substantially reduced.

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INCIDENCE OF MYELODISPLASIA AND SECONDARY NEOPLASIA FOLLOWING HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION IN 141 PATIENTS WITH LYMPHOMA

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Background. Increased incidence of secondary myelodysplastic syndromes (MDS), acute leukemias (AL) and solid neoplasms (SN) after autologous HSCT has been reported.

Patients and Methods. Between March 1993 and March 2004 141 consecutive pts. (m 78; f 63) have been treated with high-dose chemotherapy (HDT) and autologous HSCT in our Institution. 41 pts. were affected by Hodgkin's lymphoma (HL) and 100 by NHL (follicular 31, diffuse

large B cell 26, centroblastic evolved from low-grade 12, anaplastic 12, mantle-cell 11, peripheral T cell 4, mediastinal B 3, MALT 1). Median age at ASCT was 44 yrs (16-66). Among HL, 33 had relapsed after ABVD or MOPP; 8 (20%) were refractory to first-line treatment. Among NHL, 20 had relapsed after CHOP or CHOP-like therapy, and 3 after alkilating agents; 4 were refractory to first line treatment. 27 pts. were intensified with HDT after partial response to conventional therapy. 46 pts, all among the NHL group, were treated up-front with intensified high-dose therapy because of adverse prognostic factors at diagnosis (19 high-grade and 26 low-grade). All the pts. have received one or more courses of chemotherapy; 34 pts. (NHL 11; HL 23) had also received limited (n=22) or extended field (n=12) radiotherapy before HSCT. Sequential high dose chemotherapy was administered to all pts. as previously reported (CTX 7g/m^2, MTX 8g/m^2+VCR 1mg/m^2, VP-16 2g/m^2, Mitoxantrone 60 mg/m^2, Melphalan 140 mg/m^2 - Gianni *et al.,* Ann Oncol 1991 Oct; 2:645-53). At present 88 pts. (62%) are alive; median follow-up is 64 months (range 12-143).

Results. 12 pts. (8%; m 8, f 4) developed secondary neoplasms: MDS 6 (refractory anemia 3, refractory anemia with excess of blasts 3), AL 2 (T- and B-ALL) and SN 4 (breast, gastric, lung and thyroid cancer). Median time to secondary neoplasia was 34 months (range 5-94) from HSCT and 46 months (range 22-254) from diagnosis. In 4 out of 5 available cases cytogenetic analysis showed complex chromosomal aberrations, (45 XY –7) in one. Three out of 7 MDS evolved into AML after 4, 7 and 19 months. Nine of 12 pts. have died so far; 3 pts. (2 MDS, 1 SN) are still alive at 7, 8 and 18 months after secondary neoplasia.

The estimated cumulative probability of developing MDS/AL was 13% at 5 years. The actuarial incidence is significantly higher in pts. with HL (n=7/41, 17%) as compared to pts. with NHL (n=6/100, 6%) (p=0.044). Four out of 7 pts. with HL who developed MDS/AL had been previously treated with MOPP+ABVD, in three cases with additional radiotherapy.

Conclusions. Our data confirm the well-known risk of secondary neoplasia following HDT and HSCT in pts. with lymphoma. The incidence observed in HL was significantly higher than in NHL probably because of the combined effect of repeated chemotherapy and radiotherapy. Outcome after secondary neoplasia was generally poor.

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STEM CELL TRANSPLANTATION FOR SEVERE AUTOIMMUNE DISEASES A SINGLE CENTER EXPERIENCE

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Between 1996 and 2004, 26 patients with severe autoimmune diseases (SADs) were included in a stem cell transplantation (SCT) program. Of these 23 were in the autologous program, but 3 did not receive their SCT, 1 because of complete remission (CR) following mobilization, 1 because of failure to mobilize with a hypoplastic bone marrow, and 1 still in course of mobilization. Of the transplanted patients 13 were with multiple sclerosis (MS), 4 with systemic lupus erythematosus (SLE), 1 with autoimmune thrombocytopenic purpura (AITP), 1 with limited scleroderma (SSc), and 1 with Behçet's disease (BD). 10 patients were female and the age ranged from 15 to 53 years. SC source was bone marrow in the first 3 (up to 1998), and peripheral blood SC for the other 17. Mobilization was performed with cyclophosphamide (CY: 2-4 g/m^2) and G-CSF. Conditioning consisted of BEAM-ATG for the MS patients, and of Thiotepa (TT)-CY, 10 and 100 mg/kg respectively. There was no transplant-related mortality. All patients with MS had a marked reduction of gadolinium (Gd) enhancing lesions on MRI already following mobilization, which disappeared after ASCT, and have not reappeared after a 3-78 months except in 1 case. Clinical amelioration of Kurzke's Expanded Disability Status Scale (EDSS) was modest (mean 0.5 All the 4 patients with SLE enjoyed a dramatic CR, with almost complete negativisation of antinuclear immunity. However antinuclear (ANA) and anti-dsDNA antibodies have reappeared after 2-3 years, but the disease was controlled by small doses (5 mg) of prednisone.Three patients underwent allo-BMT. The first had Evans' syndrome, and is in CR after 3 years. The second had pure white cell aplasia (PWCA), obtained a good partial remission but is still receiving donor lymphocyte infusions (DLI) because of persistent mixed chimerism with recurrent neutropenia. The third will severe neuropsychiatric Behçet's disease is discussed in a parallel abstract.The treatment of SADs with SCT is little less than 10 years old, but is already considered an accepted therapeutic option. Autologous SCT is capable of halting the progression of disease but is not curative. Allo-SCT furnishes a Graft-vs-Autoimmunity (GVA) effect, and may, eradicate culprit antigens.

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KAPOSI'S SARCOMA ASSOCIATED TO HUMAN HERPES VIRUS 8 TRIGGERED BY CYTOMEGALOVIRUS INFECTION AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Incidence of Kaposi sarcoma associated HHV-8 infection is a possible complication after solid organ transplantation while its occurence in hematopoietic stem cells transplantation (HSCT) is rare and regards mostly patients undergoing allogeneic procedures. We report the first case of a CMV infection followed by an onset of Kaposi sarcoma associated HHV-8 infection in a patient who underwent autologous HSCT for a non-Hodgkin lymphoma. The patient was a 51 years old Caucasian man with a history of follicular B cell Lymphoma. The patient was first diagnosed in 1996 with a stage I A disease, he achieved a CR after 3 courses of CHOP and involved field radiotherapy. In November 2000 he relapsed with stage IV disease with latero-cervical, axillary and abdominal massive lymph

nodes involvement and with a PCR positive for bcl-2 (t 14:18)in his marrow. The patient was then treated according to a protocol of high dose sequential therapy and a second CR with a negative marrow PCR was achieved and autologous HSCT was performed. He received a total of 6.67 x 10^6 CD 34 cells/kg. Trilinear engraftment was rapidly achieved and he was discharged on day + 18. On day +47 he developed fever (38°C) with O2 desaturation. On admission his counts were: WBC 2910 /microL, Hgb 9.3 g/dL and PLTS 95000/microL. A high-resolution chest CT scan showed bilateral interstitial infiltrates. Bronchoalveolar- lavage fluid was negative for pathogens. Two days later his counts dropped with WBC 900/microL, Hgb 8.5 g/dL and PLTS 45000/microL; a core bone marrow biopsy showed no signs of lymphomatous infiltration but a severe hypocellularity with reduced hematopoiesis. The marrow aspirate was positive for CMV while other herpesviruses as well as bacteria or fungi were negative. Foscarnet was then started (60 mg/kg q 12 hrs as induction for 1 week) achieving fever control and a slow but consistent improvement in pulmonary infiltrates; after one week the counts started to slowly recover. On day + 54 many purple lesions appeared bilaterally on lower extremities. The skin biopsy showed rare rudimentary vascular-like spaces lined by mild atypical spindle cells dissecting the mid and lower dermis compatible with of a early phase of KS; the presence of HHV-8 genome was detected by PCR in a skin lesion. CMV reactivation was controlled by day +80 (normal blood counts). In the meanwhile KS skin lesions were stable. Only in the next 12 months a complete remission of KS skin lesions was observed. We suggest a possible relationship between the CMV reactivation, its consequent immunodepression and the flare-up of a HHV-8 positive KS. This relationship is consistent with the "spontaneous" long-lasting remission obtained after immunocompetence recovered.

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AUTOLOGOUS STEM CELL TRANSPLANTATION IN RECURRENT AND RESISTANT HODGKIN'S DISEASE

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About 80% of patients (pts) with Hodgkin's Disease (HD) can be cured with current treatment strategies, but pts resistant to induction therapy or who relapse early, have a poor prognosis. High dose chemotherapy and autologous stem cell transplantation (ASCT) is still the optimal strategy in early relapsed malignant lymphomas. However the potential role in chemoresistant disease is still controversial and optimal timing and indications are still to be assessed. The outcome of 41 pts with chemoresistant HD who underwent salvage chemotherapy with DHAP-IEV and BEAM plus ASCT was evaluated. Pts were classified as standard risk (if in partial remission or in sensitive relapse >12 months) or high risk (if in relapse <12 months or in induction failure). At transplant international prognostic score >2 was documented in 20 pts (48.8%), bulky disease in 18 (43.9%), extranodal disease in 12 (29.3%), B symptoms in 28 (68.3%) and stage 3-4 in 26 (63.4%). First line treatment was ABVD or ABVD like in 30 (73.2%), MOPP in 5 (12.2%) and EVE in 6 (14.6%). Median age was 45 yrs (16-62); median follow up was 50 mths (2-120 mths).

Results. After ASCT, complete remission was documented in 32 pts (78%). 8/32 pts (25%) relapsed in a median time of 9 mths (2-52). Main characteristics of the nine (22%) non responders pts were: B symptoms in 9 pts (100%), elevated LDH in 6 pts (66.6%) and stage 4 in 5 pts (55.5%). The 5 years overall and event free survivals were 52% and 44%; 3 (7.3%) toxic death (1 MOF and 2 septic shock) and 8 (19.5%) Hodgkin related deaths occurred. No myelodisplastic syndrome was observed. The five years overall survival and event free survival of standard risk subset of pts was superior, but not statistically better to high risk subgroup. In our analysis, high-risk pts, B symptoms, elevated LDH and stage 4 was associated with poor survival.

Conclusions. These results suggest that salvage chemotherapy followed by ASCT is an effective, feasible therapy for resistant and recurrent HD. Newer therapeutic approaches (doubles ASCT, reduced intensity allotransplantation and cellular immunotherapy) are needed to improve the outcome of pts with poor risk HD.

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LONG-TERM OUTCOME OF PATIENTS AUTOGRAFTED FOR ACUTE MYELOID LEUKEMIA: THE IMPORTANCE OF STEM CELL SOURCE

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Autologous stem cell transplantation (AuSCT) is one of the options for post-induction therapy of acute myeloid leukemia (AML) patients. It is considered as standard treatment for young patients lacking a HLA-identical sibling donor or those with low risk karyotypic abnormalities. In the last years, the feasibility of AuSCT has greatly improved with the use of peripheral blood stem cells (PBSC) rather than bone marrow (BM) as graft source. We reviewed the outcome of AML patients undergoing an AuSCT at our institution; fifty-two patients have been transplanted between 1990 and 2004. Twenty-eight were autografted from BM, and twenty-four received PBSC. All patients had achieved complete remission by standard cytarabine-antracycline-etoposide chemotherapy; autologous stem cells were collected after consolidation therapy from either BM or PBSC, and were tested for minimal residual disease by flow cytometry. Forty-seven patients were in first remission at the time of transplant, and five in remission >1; all but five cases were conditioned using a BuCy regimen. BM and PBSC groups did not differ for age, gender, time to transplant and FAB distribution; high risk patients with adverse karyotype or remission >1 were well balanced in the two groups. Infused total nucleated cells (TNC) and $CD34^+$ cells were significantly higher in PBSC compared to BM (median TNC 5,6 vs 0,6x10^8 and median $CD34^+$ 6,6 vs 0,8x10^6, in PBSC and BM recipients, respectively; p<0,001). The engraftment was faster in patients receiving PBSC, who required less supportive therapy and

shorter hospitalization; however, TRM and life-threatening complications did not differ in the two groups. The projected 13-years DFS was 54% in CR1 patients, and much lower (17%) in patients with more advanced disease. In CR1 patients, DFS was significantly better in the BM group compared to PBSC (72% vs 31%, p=0,03); OS showed a trend in favour of patients autografted from BM, but statistical significance was missed given the salvage therapy. Infused TNC and CD34$^+$ cells also significantly affected DFS: using the median as cut-off, patients receiving less TNC and CD34$^+$ cells showed a significantly better DFS compared to those grafted with higher cell doses. However, TNC and CD34$^+$ cell doses did not affect the outcome within either BM or PBSC group, separately, and Hazard Cox's regression model did not show any independent role for infused TNC and CD34$^+$ cells.

In conclusion, in this cohort of autografted AML patients the stem cell source was the principal factor affecting the long-term outcome; patients receiving BM showed a significantly better DFS compared to those transplanted to PBSC, due to a lower relapse risk. Other factors affecting the DFS were the numbers of infused TNC and CD34$^+$ cells, that were lower in the BM group. These results may be explained with a lower leukemic contamination of BM graft, due to the reduced cellularity. However, it cannot be excluded that some biologic differences intrinsic to the procedure, such as kinetic and type of immune reconstitution, may also play a role. In our opinion, these observations should lead to reconsider the broad application of PBSC, suggesting that the achievement of the largest stem cell dose is not the goal to be targeted; the entire procedure may need additional investigation in randomized trials comparing PBSC to BM as source for AuSCT.

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PEGFILGRASTIM IS HIGHLY EFFECTIVE IN ENHANCING HEMOPOIETIC RECOVERY IN PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANT

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Introduction. Chemotherapy-induced neutropenia associated with infectious complications may have a detrimental effect on overall survival and disease-free survival after stem cell transplantation. Granulocyte colony-stimulating factor (G-CSF) can reduce the duration of severe neutropenia and the incidence of febrile neutropenia. Filgrastim is effective for the prophylaxis and treatment of chemotherapy-induced neutropenia but its short half-life requires repeated daily subcutaneous injection. The addition of the PEG molecule to the filgrastim protein increases its serum half-life, thereby requiring less frequent dosing. Pegfilgrastim is comparable in safety and efficacy to filgrastim for decreasing the duration of severe neutropenia in patients with hematological malignancy.

Methods. Four patients submitted to high-dose chemotherapy (HDT) followed by autologous stem cell transplant (ASCT) were evaluated (see Table).

Table.

Patient	Sex/ Age	Diagnosis	Disease Status	Previous Therapy	Source of SC	Conditioning regimen	MNC x10^8/kg infused	Days of filgrastim/ pegfilgrastim	Days of ANC <500x10^9/L	Day of discharge
1	M/65	AML	CR	FLAG	Bone Marrow	BU+CY	3.34	27/1	10	33
2	F/37	Lymphocytic Lymphoma	PR	CHOP, RTX, EDX +RTX, FLU+ NOV	Peripheral	BEAM	3.27	19/3	22	40
3	F/17	ALL	2nd CR	L20,HAM	Bone Marrow	BU+CY	1.24	3/7	27	42
4	F/32	Burkitt Lymphoma	2nd CR	Previous ASCT, Hyper-CVAD	Bone Marrow	BEAM	2.94	14/2	23	30

Three patients were not eligible for stem cell mobilization. One patient had previously undergone HDC/ASCT. HDT was standard BU-CY or BEAM. All the patients received daily G-CSF (5 to 10 mcg/kg) after day +2 or +3 from stem cell infusion and subsequently were shifted to a 6 mg injection weekly of pegfilgrastim because of inadequate hematopoietic recovery or inadequate recovery plus infectious complications (case 2, complicated by CMV reactivation), until stable neutrophil recovery.

Results. All the patients engrafted neutrophils but not platelets at discharge. There were no toxic or infectious death in our study.

Conclusions. The duration of neutropenia and its clinical consequences following high-dose chemotherapy were significantly reduced by the addition of filgrastim, with no increased risk of death (second malignancy or relapse). Few single injections of pegfilgrastim have been shown to be comparable to continous daily injections of filgrastim in the management of high-dose chemotherapy-induced neutropenia. Subjects in the filgrastim treatment required a median of 16 daily injections followed by a median of three administration of pegfilgrastim. Pegfilgrastim is highly effective in enhancing leukocyte reconstitution after filgrastim administration, especially in those patients heavily pretreated with poor neutrophil recovery.

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EARLY AND LONG-TERM ENGRAFTMENT AFTER AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION IN ACUTE MYELOBLASTIC LEUKEMIA PATIENTS

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Autologous peripheral stem cell transplantation (PBSCT) has been increasingly adopted in acute myeloblastic leukemia (AML) patients without an HLA-matched related donor; the use of mobilized PBSC has resulted in more rapid and equally effective long-term engraftment compared with bone marrow stem cells. However, there has been considerable debate concerning the cells responsible for early and long-term hematopoietic reconstitution after PBSCT. This study aimed to identify which graft product subset of CD34$^+$ cells might be the most predictive of ear-

ly and long term hematopoietic recovery following autologous PBSCT in AML patients. The relationships between the number of mature subsets of $CD34^+$ cells ($CD34^+/CD33^+$, $CD34^+/CD38^+$, $CD34^+/CDDR^+$ and $CD34^+/CD90^-$) and immature subsets of CD34+ cells ($CD34^+/CD33^-$, $CD34^+/CD38^-$,$CD34^+/DR^-$ and $CD34^+/CD90^+$) and early and long-term hemoglobin, neutrophils and platelets count were studied in a homogeneous series of 26 AML patients (12 males and 14 females, aged 18-60 years, median 38) after autologous PBSCT. All patients received the same induction chemotherapy (with DNR, ARA-C and VP16), the same consolidation-mobilization chemotherapy (with ARA-C and DNR) and the same conditioning regimen (with busulphan and cyclophosphamide). The $CD34^+/CD33+$ cell number was found to be inversely correlated with the days to recovery of $0.5x10^9$/L neutrophils (r=-0.70, $p<0.05$); this correlation was similar to the total number of $CD34^+$ cells and the days to recovery of $0.5x10^9$/L(r=-0.71, $p<0.05$). The number of $CD34^+/CD38^-$ cells also correlated with the neutrophil (r=0.80, $p=0.005$) and platelets(r=0.67, $p<0.05$) counts at 12 months after PBSCT; the correlation between the total number of $CD34^+$ infused and the neutrophil and platelet counts at 12 months was lower than that for the CD34+/CD38- infused (r=0.44 and r=0.48 respectively). The number of $CD34^+/CD90^+$ cells was correlated to the platelets count at 6 (r=0.70, $p<0.05$) and 12 months (r=0.80, $p=0.005$) after PBSCT; this correlation was more predictive than the CD34 total dose and platelet counts at 6 and 12 months ($p=0.25$ and $p=0.54$, respectively). With regard to the threshold dose for long-term engraftment, all 12 patients who received more than $50x10^4$/Kg of CD34+/CD38- had a higher neutrophil and platelet count at 12 months than 100 x 10^9/L and $1.5x10^9$/L, respectively. As to $CD34^+/CD90^+$, 17/18 patients and 16/17 patients who received more than 75 x 10^4/Kg had a higher platelet count than $100x10^9$/L at 6 and 12 months, respectively. $CD34^+$ subset analysis suggests that a high number of $CD34^+/CD33^+$ committed peripheral blood stem cells may be associated with faster neutrophils recovery, and the immature subset $CD34^+/CD38^-$ and $CD34^+/CD90^+$ may be associated with sustained long-term engraftment. These findings could help to predict the repopulating capacity of PBSC in acute leukemia patients, especially when a relatively low number of $CD34^+$ cells is infused.

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AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PRIMARY AMYLOIDOSIS

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Primary systemic (AL) amyloidosis is a rare condition in which deposits of monoclonal immunoglobulin light chains cause damage to key tissue. Most common clinical manifestations of AL amyloidosis are proteinuria/nephrotic syndrome, cardiomyopathy, hepatomegaly, macroglossia and neuropathy. Prognosis of patients with primary systemic amyloidosis is poor with a median survival of only 12 months. Since AL amyloidosis is related to multiple myeloma (MM), treatment approaches of MM were investigated in amyloidosis. ASCT has been suggested for treatment of patients with AL. However, transplantation-related mortality (TRM) is much higher than in patients with hematological malignancies due to visceral organ involvement, that is commonly absent in patients with MM alone. Here, we report on our experience with high-dose melphalan followed by ASCT in patients with systemic AL amyloidosis . From 1997 to 2004 11 patients with primary systemic amyloidosis were treated in our Unit. 6pts were male and 5 female. Median age was 53 yrs (range 32-66). Two pts had only single organ involvement: one pt with renal and one with liver involvement. In 6/11 pts (54%) cardiac involvement was documented, 7 pts (64%) had renal amyloidosis, 4 pts (36%) had hepatic involvement, one pt with pulmonary involvement and 3 with neuropathy. Stem cell mobilization was performed with G-CSF in 7 and with cyclophosphamide (CY) plus G-CSF in 4. All patients underwent high-dose therapy with melphalan: 1pt with 3 organ involvement received 100 mg/sqm, 8 pts 140 mg/sqm and 2 pts without cardiac involvement received 200mg/sqm. None of pts died for transplant related complications within 100 days from the procedure. All patients were evaluable for treatment response. With a median follow-up of 23 months from ASCT, the overall survival is 64 %. Four pts (40%) had a disease progression and died; seven (64%) pts showed improvement or stable disease status with a median follow-up of 12 (range 3-21) months after ASCT. *Conclusions.* no TRM was observed; ASCT seems to be a feasible and effective procedure in patients with systemic AL.

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THIOTEPA, ETOPOSIDE AND CARBOPLATIN AS CONDITIONING REGIMEN FOR AUTOLOGOUS TRASPLANTATION IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Introduction. High-dose chemotherapy conditioning regimens followed by autologous stem cell transplantation (ASCT) generally give good results in non-Hodgkin lymphoma (NHL). We studied a new high-dose chemotherapy regimen based on Thiothepa, Etoposide and Carboplatin (TECA). This high-dose schedule is commonly used for the treatment of acute leukemia in children and this is the first evidence of its usefulness in NHL.

Methods. 41 patients (23 M, 18 F) with NHL at various stage of disease were included. The selection criteria included: age less than 70 years, creatinine less than 200 mg/mL, cardiac ejection fraction >50%, DLCO >50%, no active infection disease or other co-morbidity conditions.

The International Prognostic Index at diagnosis were: 1 for 10, 2 for 22, and 3 for 9 patients. The inclusion of patients with IPI 1 at diagnosis was related to: primary chemoresistance (2 pts), chemoresistant relapse (3 pts), and incomplete response to the primary chemotherapy (5 pts). The LDH level at transplantation was increased in 7 patients. All patients received previous debulking therapy with various treatment schedules, and mobilization with: Cyclophosphamide 4 (7 pts), 5 (2 pts) or 7 (21 pts) gr/m^2 and rHuG-CSF. 11 pts received other mobilization schedules (VP-16 2 g/m^2, IEV, DHAP). The median age at transplantation was 41 years (range 15-65), 29 patients (18M, 11 F) were diagnosed B-cell NHL and 12 patients (7 M, 5 F) T-cell NHL. At transplantation 22 patients were in CR, 12 were in PR, 4 showed a chemoresistant relapse, and 3 showed a primary chemoresistance. All patients were treated with Etoposide 250 mg/m^2 days 1-4, Thiothepa 166 mg/m^2 days 2-4 and Carboplatin 266 mg/m^2 days 2-4. The number of CD34$^+$ cells infused at day 7 range from 2.04 to 18.1x10^6/Kg (median 7.06x10^6/Kg). All patients received rHuG-CSF 10 µg/Kg b.w./die after reinfusion of PBSC. 1 patient received immunotherapy (Rituximab) after transplant, 3 received radiation therapy (1 abdominal and 2 mediastinic), and 1 received total skin irradiation.

Results. After ASCT, days to achieve engrafment for neutrophyls (> 500/µl) and for platelets (>20.000/µL) ranged from 8 to 13 days (median 10 days) and from 8 to 17 days (median 12 days) respectively. The incidence of transplant-related infective and non-infective complications were similar to other conditioning regimens. The overall response rate was 78% (30 CR, 73%, and 2 PR, 5%), 7 patients (17%) were not responders, and 2 patients (5%) died for transplanted-related complications, both for septic shock with acute distress respiratory syndrome. The 5-year overall survival was 69%; 12 patients (8 B-NHL, 27%, and 4 T-NHL, 33%; p<0.05) relapsed until the first 5-years follow-up. The overall response rate and the 5-years overall survival was better for patients who showed at diagnosis an IPI = 1 (92% and 89% respectively) than those with IPI = 2 (79% and 72%), and IPI = 3 (80% and 78%), p<0.005 for both. Gender, debulking therapy, response to previous treatment, LDH serum levels at transplant, bone marrow infiltration and Karnofsky status, were not associated with different response rate and 5-years overall survival.

Conclusion. The TECA conditioning regimen we used for ASCT in NHL, have a good anti-lymphoma effect and gave good results in terms of response to treatment and 5-years overall survival. It was well tolerated, and did not showed excessive toxicity, suggesting that TECA may be a very useful conditioning regimens for NHL.

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PEGYLATED GRANULOCYTE GROWTH FACTOR AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN 23 PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES.

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We studied the hematological recovery and the clinical outcome of 23 patients with haematological malignancies, receiving Pegylated Granulocyte Grow Factor (PEG-GCSF) after autologous stem cell transplantation (ASCT). PEG G-CSF was administered subcutaneously (SC) at the single dose of 6 micrograms, on day +1, after blood progenitor cells (BPC) infusion, in place of glycosilated G-CSF 5 micrograms/day SC from day +1 until the neutrophil recovery as reported in our previous study. Twentythree consecutive patients (8 Multiple Myeloma, 6 Non Hodgkin's Lymphoma, 4 Hodgkin Disease, 2 Acute Myeloid Leukemia, 2 Solid Tumor and 1 Cronic Lymphocitic Leukemia) underwent ASCT with conditioning regimens including High-Dose-Melphalan, and received a median dose of 5.5 (range 3.4-9.9) CD34$^+$ cells x10^6/kg; 20 are now valuable for the engraftment kinetics and the clinical outcome. All the 23 patients engrafted quickly, with a median time of 10 days (range 8-20) to achieve absolute neutrophil count (ANC) >0.5x10^9/liter and 11 days to reach ANC count >1.5x^9/liter (range 9-24). The median duration of severe neutropenia was 4 days for ANC count< 100/microliter (range 2-7) and 5,5 days for ANC count<500/microliter (range 2-13). The platelet count >20x^9/ liter and >50x^9/liter were achieved in median at day +13 (range 8-34) and +14 (range 11- never achieved) respectively. The median duration of hospitalisation, including the conditioning regimen, was 9 days (range 4-29) and 3 days (range 0-22) starting from the day of BPC reinfusion respectively; 16 out of the 23 patients could be discharged the day after the BPC infusion; 5 out of these 16 patients required a short second hospitalisation due to septic fever or to pneumonia. The median number of days with fever >38 °C was 1 (range 0-12) with a very low requirement of intravenous antibiotic therapy (median 0 days; range 0-15). Thirteen people developed neutropenic fever: in 9 patients (41%) with clinical-microbiological demonstration (sepsis, infection of central venous catheter and pneumonia) and FUO in 4 (18%). We did not observe transplant related deaths (minimun follow-up: 6 weeks from day 0). The engraftment kinetics and the clinical outcome of these patients did not substantially differ from those we observed in a matched group of patients receiving filgrastim from day +1. Our data suggest that the use of PEG G-CSF after ASCT is safe and doesn't substantially modify both the engraftment kinetics and the clinical outcome in terms of days of fever and days of i.v. antibiotic therapy, compared with the use of G-CSF. Moreover, a single administration of PEG G-CSF SC seems to be easier than multiple administrations of G.CSF (in median 13), in this way facilitating the outpatient management of patients receiving ASCT.

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CD133+ HAEMOPOIETIC STEM CELLS POSITIVE SELECTION AS A PURGING SYSTEM IN INDOLENT NON HODGKIN'S LYMPHOMA

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CD133 is a 120 kd glycosylated polypeptide that contains five transmembrane domains with C-residue. The CD133 antigen is expressed on CD34 (bright) hematopoietic stem and progenitor cells (HSC) from human fetal liv-

er, umbilical cord blood, and bone marrow. A small population of $CD34^-$ / $CD133^+$ HSCs has also been observed. CD133 antigen is not expressed on the malignant cells in lymphoma, chronic lymphocytic leukemia or multiple myeloma. In this study we evaluated the role of positive selection of $CD133^+$ cells as a purging system in ten patient suffering from indolent non Hodgkin's lymphomas. The mobilization regimen was cyclophosphamide (7 grams/square meter) followed by rh-G-CSF. Peripheral blood stem cells were collected after a median of ten days (range 9–12 days) using a continuous flow cell separator (Cobe SPECTRA). A mean of $32.3 \pm 12.2 \times 0^9$ total nucleated cells was collected; a mean of $909 \pm 360 \times 10^6$ $CD34^+$ cells was harvested. The mean number of the $CD133^+$ cells was $681 \pm 373 \times 10^6$. The CliniMACS device was used to select $CD133^+$ HSCs in accordance with the manufacturer's standard procedure.$CD133^+$ and $CD34^+$ cell recovery was 68% ± 35 and 43% ± 17 respectively (mean 19 procedures). Flow cytometry analysis showed that the mean percentage of CD19+ contamination in the apheresis product was 0.3% ± 0.6% ($0.8 \pm 1.6 \times 10^6$ CD19+ cells). The CD19+ cells in the graft were $1.4 \pm 2.9 \times 10^6$, which correspond to a B cell depletion of 3 logs.

The conditioning regimens were TBI-Thiotepa or Mitoxantrone-Melphalan. The graft contained a median of 9×10^6 (range 4 to 13×10^6) CD133+ cells per kg body weight (b.w.) and 8×10^6 (range 5 to13×10^6 $CD34^+$ cells per b.w. All patients engrafted promptly. Median time to 500/cubic millimetre neutrophils count was eleven days (range 9 to 12 days) and to 1,000/ cubic millimetre neutrophils count was eleven days (range 9 to 13 days). Median time to 20,000/ cubic millimetre and 50,000/ cubic millimetre were respectively thirteen days (range 10 to 13 days) and eighteen days (range10 to 21 days). No patients experienced acute infusion-related toxicity. Nine patients are alive and 7 in complete remission with a median follow-up of 37±7 months.

In conclusion this study shows that positive selection of CD133 stem cells using CliniMACS technology is able to remove three logs of contaminating B cells with rapid and sustained haematological reconstitution.

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NO-CROSS-RESISTANT ESCALATION THERAPY CUMLMINATING WITH AUTOLOGOUS STEM CELLS TRANSPLANT IN MULTIPLE MYELOMA: AN EFFECTIVE UP-FRONT STRATEGY FOR NEWLY DIAGNOSED PATIENTS

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In recent years the treatment of multiple myeloma (MM) has been intensely influenced by the results of high dose therapy and autologous stem cell transplantation (HDT/AHSCT), with a substantial improvement in the rate of patients (pts)achieving a complete remission(CR). Furthermore, HDT/AHSCT, compared with conventional chemotherapy, is known to improve progression-free survival (PFS) and overal survival (OS), so that the single or tandem AHSCT has become the standard of care for pts with symptomatic MM. We conducted a study to evaluate the feasibility and efficacy of HDT/AHSCT in 26 pts as upfront strategy.Between January 1994 and April 2001, 26 pts were enrolled in the HDT/AHSCT protocol. Pts were eligible if they had age<60y, clinical evidence of disease or multiple radiological lytic lesions,adeguate cardiopulmonary function,no previous treatment. Renal dysfunction or evidence of amyloidosis at diagnosis were not a controindication. The primitive cytoreduction was obtained with 2 courses of VMD (VCR, Mitoxantrone, Dexametasone). HDT was articulated into a sequence of growing therapeutic aggressiveness including three consecutive no-cross-resistant regimens(Cytoxan high dose, EDHAP, dexa-BEAM) adressed to obtain the greatest destruction of myelomatous bulk before transplant. Peripheral blood stem cells (PBSC) were collected after each consolidation regimen in order to gain $CD34^+$ enriched products having a progressively reduced plasma cells contamination. The myeloablative regimen consisted of Melphalana 140 mg/mq and fractioned TBI 1200 cGy, or Busulphan 16 mg/Kg and Melphalan 60 mg/Kg.

Pts enrolled included 14 men and 12 woman, median age 51y (range 32-60y),stage III for 20 pts and stage II for 6 pts,lytic lesion in 23 of 26 pts.20/26 pts were reinfused with PBSC collected after EDHAP. 5 pts achieved CR and the others obtained PR before transplant.PMN and PLT recovery occurred a median of 12 and 15 days after AHSCT,respectively. The median time from the beginning of the induction therapy to AHSCT was 5,5 months. There were not treatment-related deaths. Three months after transplant 14 pts received INF therapy. To date,12 pts are alive, of whom 6 in CR. 6 relapsed pts underwent a second auto- or allografting.At a median follow-up of 6,5 years the OS is 40%.

Thrombosis and Hemostasis II

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INDUCTION OF REMISSION OF RELAPSED IDIOPATHIC THROMBOTIC THROMBOCYTOPENIC PURPURA: ROLE OF FRESH FROZEN PLASMA. SIGLE CENTER EXPERIENCE

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Thrombotic thrombocytopenic purpura (TTP) is characterized by microangiopathic hemolytic anemia and thrombocytopenia, accompanied by microvascular thrombosis. Its pathogenesis could recognize an acquired inhibitor of a plasma metalloprotese called ADAMTS13. Plasma exchange can induce remission in about 80% cases of idiopathic TTP. But the underline alteration that causes the deficiency of ADAMTS13 is not reverted, so, relapse is frequent. Many treatment have been proposed to treat relapse and as front-line therapy, using drugs against immunocompetent cells, like vincristine, rituximab associated or not with corticosteroids, but therapy is still not codified. And relapse remains a challenge for haematologist. Here we report our experience in relapsed TTP.

From 2001 to 2004 7 patients received diagnosis of TTP. First line therapy was corticosteroids (1 mg/kg) and plasma exchange, obtaining complete remission. All patients were affected by idiopathic TTP. Five of them relapsed in a period of time variable from 6 months to 1 year. First therapy for first relapse was fresh frozen plasma and corticosteroids, 1 mg/kg. 3 patients obtained complete remission. Main characteristics were as follows: 2 females, aged 42 years old and 34 years old, and 1 male 22 years old. At relapse the only alteration was thrombocytopenia, 32000/mmc, 34000/mmc and 42000/mmc, without any other alteration of emocromocytometric parameters, slightly decrease of aptoglobin and mild increase of LDH. Treatment with daily fresh frozen plasma (FFP) was immediately started associated to corticosteroids 1 mg/kg. Normalization of platelets, aptoglobin and LDH was obtained in 7, 7 and 8 days respectively, and a total of 35, 23 and 30 units of FFP infused. After recovery treatment was continued for two weeks with FFP every other day, and corticosteroid were progressively decreased. Follow up is now 6, 7 and 16 months without relapse, and cortiscosteroids have been discontinued. Our experience suggest that FFP can induce rapidly remission of TTP, and can be used as a guide to propose other treatment in case of non response. Other relapses need other kind of treatment. And debate is still open.

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ROLE FOR CIRCULATING ENDOTHELIAL CELLS (CECS) IN ENDOTHELIAL INJURY IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Background. Several in vitro and *in vivo* studies have confirmed the presence of endothelial disfunction/injury in SLE patients. Recently, CECs have been proposed as useful tool for studying vascular injury. Aim of the study was to investigate the presence of CECs in SLE patients and evaluate its clinical associations and possible pathogenetic role. Material and methods. The study cohort included 50 healthy controls (HC) and 120 SLE patients (> 4 ACR criteria). For all SLE patients disease activity was assessed using the ECLAM score. Five-parameter, 3-color flow cytometry was performed with a FACScan. PB cells were labeled with anti-CD45, -CD31, -CD62, and -P1H12 monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), or peridinin chlorophyll protein (PerCP). By using the same method, we examined CECs for antigen tissue factor (TF) expression. In addition we evaluated the levels of soluble E-selectin, thrombomodulin and TF by commercial ELISA as established markers of endothelial damage

Results. The number of CECs (CD45-/CD31+/P1H12+) was significantly higher in SLE patients than in HC ($p<0.001$). As far as circulating endothelial phenotype is concerned, we found that CECs are microvascular in origin, as defined by the marker CD36. In addition, only a proportion of CECs expresses a pro-adhesive phenotype, as indicated by surface expression of CD62 and represented about the 25% of total CECs. In addition, we found that CECs from SLE patients abnormally express TF antigen. Total and activated CECs in SLE patients strongly correlated with plasma levels of TF, E-selectin and thrombomodulin. With respect to specific disease manifestations, patients with kidney disease showed significantly increased levels of CECs in comparison with SLE without renal involvement. There was a lack of correlation between CEC number and disease activity (ECLAM). There was a significant correlation between CECs and lupus anticoagulant positivity. In addition, a positive correlation was found between CECs and clinical manifestations of antiphospholipid syndrome.

Conclusions. Patients with SLE have increased levels of CECs that strongly correlated with parameters of endothelial damage and elevated TF levels. The presence of CECs in SLE may represent direct evidence of endothelial disease rather than a clinical marker for active SLE. Interestingly, the finding of a close association between CECs number and both renal disease and LA positivity suggest an important role for CECs in this disease subset with prominent vascular changes. The positivity of CECs for TF expression provides the most direct evidence that the hemostatic system of these patients is activated.

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RISK OF RECURRENT VENOUS THROMBOEMBOLISM ASSOCIATED WITH INHERITED DEFICIENCY OF NATURAL ANTICOAGULANTS

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The risk of recurrent venous thromboembolism (VTE) in patients with inherited deficiency of natural anticoagulants (antithrombin -AT, protein C -PC, protein S -PS) has been scarcely investigated. We studied a retrospective cohort of 912 patients with a first VTE objectively diagnosed, after preliminary exclusion of overt cancer or antiphospholipid antibodies. The inclusion criteria consisted of an antithrombotic treatment after the first VTE not longer than 6 months and an interval time between withdrawal of antithrombotic treatment and referral to our Centers longer than 1 year. After further exclusion of patients with factor V Leiden or prothrombin 20210A alone, we examined 618 patients: 582 with DVT of the legs, in 100 cases associated with pulmonary embolism – PE, and 36 with isolated PE. We compared 79 patients with AT (n=19), PC (n=32), or PS deficiency (n=28) and 539 patients without any known defect; 15 of the patients with deficiency of natural anticoagulants carried also FVL or PT20210A or both. The two groups did not differ in sex distribution (M/F 0.44 vs. 0.42), rate of first spontaneous VTE (38% vs. 41%), median time between the first VTE and recurrence or referral to our centers (4 years for both groups); the cumulative incidence of recurrent VTE in the whole cohort was 26.3% at 8 years from the first VTE. The patients with AT, PC, or PS deficiency had an increased risk for recurrent VTE in comparison with the patients with normal genotype (hazard ratio 1.5, 95% CI 1.1-2.2); exclusion from the analysis of the patients with multiple defects did not substantially change the results. Analysis was adjusted for other potentially confounding factors such as sex, age > 45 years at the first VTE event, first VTE event occurred spontaneously or after exposure to a circumstantial risk factor, and occurrence of pulmonary embolism at the first VTE event, confirming that inherited deficiency of natural anticoagulants was an independent risk factor for recurrence (adjusted hazard ratio 1.5, 95% CI 1.0-2.3, p=0.03). Separate analysis showed that the risk was particularly increased in the carriers of AT deficiency (adjusted hazard ratio 1.9, 95% CI 1.0-3.9, p=0.05); the carriers of PC or PS deficiency showed an increased risk too, yet not reaching the statistical significance (adjusted hazard ratio 1.4, 95% CI 0.9-2.2, p=0.14). In conclusion patients with inherited deficiency of natural anticoagulants have a moderate increase in risk for recurrence, which must be carefully weighed against the hemorrhagic risk during long-term anticoagulation. Further multicenter studies are warranted to evaluate the risk-benefit balance of long-tem anticoagulation after a first VTE in such subjects.

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INCIDENCE OF HEREDITARY THROMBOPHILIC FACTORS IN WOMEN WITH RECURRENT MISCARRIAGE

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Recurrent miscarriage is a multifactorial pathology. The mutations of II factor and V Leiden have been recognized to have a pathogenetic role in recurrent miscarriage, while the role of other mutations of some identified genes of hereditary thrombophilia (MTHFR-C677T, MTHFR A1298C, PAI-I 4G/5G, XIII factor-V34 L, beta Fibrinogen – 455G->A, HPA1-a/b) are still controversial.

The aim of the study was to evaluate and correlate the allelic and genetic frequencies of the mutations of the thrombophilic genes in a population of women with clinical history of poly-abortion and in a control group paired for age and sex.

Patients. 57 women with recurrent miscarriage and 80 healthy women with no history of abortion. Both groups underwent to molecular study by Reverse Dot Blot on solid phase for the following thrombophilic mutations: Factor V (G1691A, H1299R), Prothrombin (G20210A), Factor XIII (V34L), beta Fibrinogen (-455G>A), PAI-1 (4G/5G), HPA1 (a/b), MTHFR (C677T, A1298C)

Results. No statistical significant results have been found in genotypic and allelic frequencies of the examined molecular mutations between control group and group with recurrent abortion. We observed high frequencies of association in both groups of MTHFR (C677T, A1298C), PAI-1 (4G/5G), HPA1 (a/b) of about 50% although not significantly responsible of recurrent abortion.

Conclusions. These data don't show a significant correlation of single mutation or association of studied polymorphisms in control group and recurrent miscarriage group. These preliminary results need a wider casuistry for a better interpretation.

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TOTAL JOINTS REPLACEMENT IN HAEMOPHILIACS: 17 YEAR SINGLE INSTITUTION EXPERIENCE AT CASTELFRANCO VENETO

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End stage haemophilic arthropathy causes severe pain and disability: in this circumstance total joint replacement has recommended. We conducted a retrospective analysis on 79 arthroplasties in 66 haemophiliac patients performed at our institution between 1987 to 2005: 29 were total hip replacement (THR) in 25 patients, 48 total knee substitution (TKR) in 39 patients and 2 prosthesis of the ankle in 2 patients. The mean follow-up was 8 years (range 17 years - 12 month). 63 patients had severe FVIII deficiency (HA) and 3 severe FIX deficiency (HB). 4 showed high titre anti FVIII inhibitors and 5 low titre inhibitors. All but one

patient were HCV positive, 7 patients were HCV and HIV confected. The patients received plasma-derived replacement therapy via bolus injections (in 34 procedures) or continuous infusion (in 45 procedures). Patients with anti FVIII inhibitors received rFVIIa. The median age of haemophiliacs who underwent hip surgery was 36 years (range 23-50 years) and in knee replacement 43 years (range 22-62 years). The medium consumption of coagulation factor concentrates was 50.000 UI in hip surgery and 56.000 UI in knee total replacement. In 4 patients two hip replacements were performed during different sessions as well as 4 patients underwent two knee replacements. Total hip revision was necessary in 5 cases (17%) because of the mobilization of the prosthesis. In knee surgery 10 cases needed joint revision because of infections (20%): 5 patients received a replacement during the revision operation while 4 patients underwent removal of the components, and 2 patients underwent amputation of a portion of their leg. In our experience total hip replacement may be a safe therapeutic choice in patients with haemophilia and severe hip arthropathy, with an acceptable low rate of complications. In total knee replacement performed in our cohort of patients there were a high rate of late infections as some authors have already reported in the literature. Orthopaedic surgeons and haematologist should consider carefully risks and benefits of these procedures as they can carry an high rate of complications and they should be suggested on a very selected proportion of patients with haemophilia.

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CLINICAL FEATURES ARE POORLY PREDICTIVE CRITERIA FOR DIAGNOSIS OF INHERITED THROMBOPHILIA

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Laboratory screening for inherited thrombophilia is warranted in young patients, especially those having suffered from severe venous thromboembolism occurred spontaneously or recurrently. The opportunity to carry out the laboratory investigation in older patients is debated and sometimes discouraged, especially in the case of mild clinical manifestations or provoked events. However this policy could lead to miss a number of carriers of thrombophilia, leaving undiagnosed their kindreds. In order to check this hypothesis, we analyzed the clinical records of 1,165 patients (492 males and 673 females) referred to our center for laboratory investigation because of a clinical history of venous thromboembolism in the legs. The previous clinical history was taken at the admission blinded to the laboratory results.The median age of the first event was 37 years (range 0 to 84). All the events were objectively diagnosed. Inherited thrombophilia was defined as the presence of deficiency of antithrombin, protein C, and protein S, factor V Leiden, prothrombin G20210A. Patients were stratified according to the age of the first event (< 18 years, 18-45 years, 45-60 years, > 60 years), the type of clinical manifestation (defined as severe in the case of proximal DVT and/or pulmonary embolism and mild in the case of distal DVT or superficial vein thrombosis), the circumstances of the first event (defined as spontaneous or provoked by exposure to transient risk circumstances such as surgery, trauma, bed rest, pregnancy and puerperium, oral contraceptive intake), and the presence or the absence of recurrent events in the clinical history. The overall prevalence of inherited thrombophilia in the whole cohort was 34.8%, with no significant difference between the patient groups identified according to the age < 45 years at the first event (p=0.23), the severity of the clinical manifestation (p=0.42), the circumstances of the first event (p=0.31); thrombophilia was overrepresented in the patients with recurrences (38.7%) in comparison with the patients without (32.9%, p=0.05). In the subgroup of 50 individuals with the putative higher probability of inherited thrombophilia (age < 45 years, severe clinical presentation, spontaneous first event, history of recurrences) inherited thrombophilia was found in 52% of the cases, yet with no significant variance in comparison with the rate obtained in the subgroup of 29 individuals with the putative lower probability of diagnosis (age > 45 years, mild clinical presentation, provoked first event, no history of recurrences) (41%, relative risk 1.2, 95% CI 0.7-2.1, p=0.48). Subdividing inherited thrombophilia as severe (deficiency of natural anticoagulants or multiple defects) or mild (factor V Leiden or prothrombin G20210A alone) the prevalence of severe thrombophilia (6.7% of the whole cohort) was significantly higher only among the individuals with severe clinical manifestations (8.1% vs 3.6%, p=0.005) or with younger age (8.2% vs 2.9%, p<0.001); on the opposite, no significant variation was noticed in the distribution of mild thrombophilia among the different patient groups. In conclusion clinical criteria seem scarcely predictive of the presence of inherited thrombophilia in patients with venous thromboembolism, and admission to laboratory screening should not be restricted according to their presence or absence.

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THROMBOPHILIC GENETIC AND ACQUIRED FACTORS IN THE PATHOGENESIS OF THROMBOEMBOLISM IN GASTROINTESTINAL CANCER PATIENTS:LACK OF A ROLE

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Venous Thromboembolism (VTE) is a well known and feared complication in cancer; however its relationship with laboratory thrombophilic alterations in neoplastic patients is not yet clearly known. Aim of this trial was to evaluate changes in the levels of physiological coagulation inhibitors and some thrombophilic acquired and congenital factors, and to assess their impact on the development of VTE in gastrointestinal tumors patients. All patients > 18 years, not assuming anticoagulant therapy, with a new histopathologic diagnosis of gastro-intestinal cancer and no known hematologic or immunologic diseases observed in the University Hospital "Campus Bio-Medico" from October 2002 to June 2004 have been included in this trial. Patients were tested for: Protein C (PC), Protein S (PS),

Antithrombin III (ATIII), Lupus Anticoagulant (LAC) and Anticardiolipin Antibodies of IgG type (ACA IgG). Furthermore, we investigated the levels of homocysteine and annexin V as well as the presence of activated Protein C resistance. Tests were performed on samples taken just before and after surgery as well as before and at the end of the chemotherapy. In addiction, VTE's prevalence has been evaluated. A total of 102 patients (60 males and 42 females) have been included; median age was 67 years (range 28-92). Of these patients, the first 48/102 (47%) who undertook chemotherapy were also investigated by standard polymerase chain reaction techniques for the presence of Factor V Leiden, prothrombin G20210A gene mutation and the C677T mutation in the 5,10 methylenetetrahydrofolate reductase (MTHFR) gene. VTE's prevalence has been 7,8% (8/102). A DVT was diagnosed in 4 of 20 patients with metastatic disease (20%) and in 4 of 82 patients (4.8%) with a surgically treatable disease ($p<0.02$). A significant modification in the values of LAC of DRVVT type, ACA IgG, PS, ATIII has been observed in the samples taken just after surgery ($p<0.001$) and just before chemotherapy ($p<0.004$). Moreover, PC levels were significantly reduced only after surgery ($p<0.000$); LAC of KCT type was significantly prolonged before starting chemotherapy ($p<0.003$) while homocysteine levels were significantly reduced ($p<0.001$) just after the end of the chemotherapeutic protocols. Of the 48 patients tested for thrombophilic genetic mutations, a homozygous or heterozygous Factor V Leiden was present respectively in 1 and 3 patients, while the G20210A Factor II mutation was observed in 6 patients (all heterozygous). The allelic frequency of the C677T mutation in the MTHFR gene in the studied population was 46%. A VTE was observed in 7 of these 48 patients (14.5%) and none had Factor V Leiden or G20210A Factor II mutation, while 4 had a C677T MTHFR mutation (p=NS). 31 of these 48 patients undertook a 5-Fluorouracil (FU) based chemotherapy, and a grade 3-4 hematological or gastrointestinal toxicity was present in 25% (7/29) of patients with the C677T polymorphism of the MTHFR while none of the 11 patients without mutation had any toxicity (p=0.06).

From this study it appears that the main responsible of VTE pathogenesis in gastro-intestinal cancer patients is an advanced disease, whereas thrombophilic genetic and acquired factors seem to have no effect on its development. Moreover, patients carrier of the C677T mutation in the MTHFR gene subjected to antimetabolites-based chemotherapy have a tendency to a higher risk of grade 3-4 hematological or gastrointestinal toxicity (OR: 1.4; C.I. 95%: 0.88-2.24; p=0.06).

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ROLE OF CONGENITAL AND ACQUIRED THROMBOPHILIC FACTORS IN THE FAILURE OF THROMBOLYSIS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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In patients with acute myocardial infarction, a persistently occluded infarct related coronary artery, despite a correct thrombolysis, is associated with an unfavorable prognosis.Therefore, identification of variables predictive of ineffective thrombolysis is crucial to identify patients at higher risk of thrombolysis failure. To investigate whether or not acquired and congenital thrombophilic factors had a role in the ineffective thrombolysis we designed a study in which patients treated with intravenous thrombolysis for a ST segment elevation myocardial infarction were blind tested for the thrombophilic factors on the occasion of coronary angiography performed within 30 days from the thrombolytic treatment. A total of 101 consecutive patients were available for this study.All patient underwent, within 30 days from thrombolysis, a coronary angiography and of the 101 participating in the study, 40 resulted occluded while 61 had a patent artery. In these 101 patients we blind tested the levels of ATIII, PC, PS; moreover, we determined also the levels of homocysteine, the presence of Lupus Anticoagulant (by mean of DRVVT and Silica Clotting Time) and ACA of IgG type as well as the Plasminogen levels. Furthermore, blood samples were also analysed by PCR technique for the presence of Factor V Leiden, the G20210A factor II mutation and the C677T mutation in the MTHFR gene. Surprisingly, patients with MTHFR 677TT homozygosis had a significantly higher prevalence of occluded infarct artery (73%) vs those with MTHFR 677CT/CC genotype (30%, p=0.0008), frequency of MTHFR 677 TT homozygosis was 4-fold higher in patients with occluded vs those with a patent vessel (40% vs 10%, p=0.0008). MTHFR 677TT genotype predicted the risk of failed thrombolysis with a specificity of 90% and multivariate analysis showed that MTHFR 677TT homozygosis was indipendently associated with an occluded artery (odds ratio 3.8, 95% confidence interval 1.1-9.1; p=0.03). None of the other studied factors at multivariate analysis influenced the thrombolysis failure. Patients with occluded infarct vessel and MTHFR 677TT genotype had the highest homocysteine levels (p=0.011).Our findings indicate that in patients with acute myocardial infarction MTHFR 677 TT homozygosis is associated with a persistently occluded infarct-related artery after thrombolysis.

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ATYPICAL ACQUIRED VONWILLEBRAND DISEASE IN ESSENTIAL THROMBOCYTHEMIA: THERAPEUTIC INVOLVEMENT

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Background. Patients with essential thrombocythemia show a predisposition to both thrombosis and bleeding. Bleeding manifestations are often associated with acquired von Willebrand (vW) deficiency, due to the loss in plasma of intermediate and large multimers. We describe herein the case of a patient affected by thrombocythemia with decreased vW Ag and decreased vW function, related to a transitory inhibitor.

Case rerport. Male, 45 years old, initially observed for thrombocytosis with a platelet count of $700X10^3$ / mL. The

diagnosis of essential thrombocythemia was confirmed by bone marrow biopsy. Bone marrow cytogenetic analysis showed a normal 46xy karyotype. The patient was initially treated acetil salicilic acid (ASA) 100 mg daily. Forty day days after the beginning of ASA treatment a spontaneous bleeding (epistaxis) forced us to withdrawn the therapy with ASA. During the first three months of follow up, platelets increased from 700x10^3/mL up to 1.400x10^3/mL. Meanwhile the APTT became prolonged and both von Willebrand antigen determination and RIPA (Ristocetin 1,5 and 0.5mg/mL) decreased. It was then possible to document the presence of inhibitors directed to vW F, measured as Bethesda unit (0.55). Coagulation abnormalities were however transitory: all laboratory tests were repeated 30 days later while platelet count was unmodified at 1300x10^3/mL and ten days after ASA suspension related to bleeding episode: both vW Ag and RIPA came back to the normal range and vWf inhibitor was no more detectable.

Comments. Acquired Von Willebrand disease is described in patients with essential thrombocythemia caused by a platelet-dependent proteolysis of large von Willebrand multimers. Some authors showed the inverse relationship between platelet count and vW large multimers with increased bleeding risk. In our patient acquired vW deficiency was related to transitory inhibitors independent from platelet count. This data is atypical in essential thrombociytemia, even if it has been described in association with lymphoproliferative diseases. These findings suggest two clinical implication; the first is that in essential thrombocytemia laboratory vW evaluation before ASA therapy might be useful in order to prevent bleeding. Another question is the choose of chemioterapic agents in respect of presence of autoantibodies (interferon or anagrelide?).

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THROMBOTIC THROMBOCYTOPENIC PURPURA. THE EXPERIENCE OF AN INTERNAL MEDICINE UNIT

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Between 1997 and 2005 six patients with thrombotic thrombocytopenic purpura (TTP) were treated in our Medical Unit . M/F was 2/4 and mean age was 30 years (range 22-71). At presentation mean Hb level was 9.3 g/dL (8.2-11) , mean platelet count was 10.500/µL(6.000-15.000), mean creatinine 1.1 mg/dL (0.8-1.7), mean LDH 858 U/L (671-1173); neurological signs were present in 3 and fever in 5 patients. Diagnosis was made on the basis of clinical data and the relief of hemolytic microangiopatic anemia. In two cases a deficiency of ADAMS13 was documented, one patients had used ticlopidine for one month before and four patients presented a mild infection some days before the onset of the disease; no one had a history of familial disease. All patients were treated with metilprednisolone (1-2 mg/Kg/die) and plasma exchange, the mean number of procedures was 11.8 (3-41). Two patients relapsed, one of them for three times and finally underwent splenectomy. These two patients had severe skin rash with fever attributed to plasma so plasma exchanges were stopped; subsequently were treated with vincristine and could reach complete remission (CR). All the patients are now in CR (0-7 years of observation). The diagnosis of TTP must be suspected in the presence of the classic triad or pentad of signs and symptoms. The differential diagnosis is extensive and achieving a definitive diagnosis can be difficult; occasional patients with disseminated intravascular coagulation secondary to malignancy or sepsis presenting with occlusive microangiopathy of sufficient severity can be confused as having TTP. The treatment of TTP is based upon plasma-exchange; corticosteroids are often used as part of initial therapy; splenectomy in the plasma exchange era is reserved for refractory patients, with variable response rates; for antiplatelets agents a potential role in preventing relapse has been reported; vincristine , as in our two patients, can have favourable responses; anedoctal reports of others immunosuppressive therapies such as rituximab , cyclophosphamide and cyclosporine need more prolonged and controlled studies.

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PREGNANCY LOSS AND THROMBOPHILIA

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Introduction. Extensive research carried out during the last few years strongly suggests that a strict association exists between thrombophilia and recurrent pregnancy loss or other serious obstetric complications. The risk seems to increase throughout pregnancy and pathologic findings of placental infarction indicate that the pregnancy loss could be due to uterine-placental insufficiency and thrombosis. In this prospective study we screened a cohort of 130 women with history of pregnancy loss, without concomitant and/or previous thrombotic events and an equal number of control women, for a panel of congenital or acquired thrombophilic factors.

Patients and methods. We consecutively enrolled 130 women with a history of pregnancy loss of unknown cause (patients). Inclusion criteria were: one or more fetal losses during the 1st trimester or one or more intrauterine fetal deaths; no history of venous or arterial thromboembolic events and/or familial thrombophilia.

A group of 130 women who had at least one successful gestation, with negative history for fetal loss, intrauterine fetal death, intrauterine growth retardation or thrombotic events, was also studied (controls). The following thrombophilic factors were studied: Antithrombin (AT), Protein C (PC), free Protein S (f PS), Leiden FV mutation, G20210A Factor II mutation, Lupus Anticoagulant (LA) and IgG anticardiolipin antibodies (aCl Abs). At the time of the study,

all patients and controls were in apparent good health, not pregnant and not under oestrogen/progesterone therapy.

Results. Median age of patients and controls was 33 years (range 23-44) and 41 years (range 25-65), respectively. Fourteen/130 patients (10.8%) experienced one or more intrauterine fetal deaths; 17/130 (13.1%) one or more fetal losses and one or more intrauterine fetal deaths; 9/130 (6.9%) a single episode of fetal loss; 90/130 (69.2%) more than one fetal loss.

AT levels were in normal range both in patients and in controls; PC was reduced (< 70%) in 6 patients (4.6%) and in 4 controls (3%), f PS (< 64%) in 4 patients (3%) and in 2 controls (1.5%). Leiden FV mutation was found in 2 patients (1.5%) and in 2 controls (1.5%), G20210A Factor II mutation in 9 patients (6.9%) and in 3 controls (2.3%). LA was found in 3 patients (2.3%) and in 1 control (0.8%), aCl Abs in 12 patients (9.2%) and in 6 controls (4.6%). No statistically significant differences were found as concerns the presence of thrombophilic factors, separately considered, between patients and controls: only for Factor II mutation, a trend was noted (6.9% vs 2.3%, p=0.07, Mann-Whitney test). In summary, 30/130 patients (23%) showed one or more thrombophilic factors, as compared to 15/130 controls (11.5%), (p=0.015): a-single thrombophilic factor was recorded in 26/30 (86.7%) and two or more in 4/30 (13.3%). The presence of one or more thrombophilc factors resulted in an Odds Ratio of 2.28 (95% C.I. : 1.16-4.48, p=0.017).

Conclusions. Acquired and/or inherited thrombophilia is commonly considered as an important risk factor of pregnancy loss and, possibly, of other serious obstetric complications. In our study, only the presence of aCl Abs and of G20210A Factor II mutation were slightly higher in patients' group then in controls' group. These results suggest that further research is needed before stating a strict correlation between thrombophilia and recurrent pregnancy loss or other obstetric disorders.

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EFFICACY OF RITUXIMAB TREATMENT IN POST-PARTUM ACQUIRED HEMOPHILIA

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Selective B cell depletion with Rituximab, a chimeric anti-CD20 monoclonal antibody, has been shown to be quite effective in the treatment of immune disorders. Recent reports also suggest a role for Rituximab in the treatment of patients with acquired FVIII inhibitors. A 25 years old woman, with a previous diagnosis of post-partum acquired hemophilia A, came to our Institution on December 2002 because not responder to conventional prednisone doses (lmg/kg/day). FVIII:C level and inhibitor titre at diagnosis were not available. At admission, aPTT ratio was 2.59; FVIII:C <1%; inhibitor titre 621 BU/mL. Different treatments were tried: high-dose dexamethasone (40 mg/day for 4 days, then tapered to a maintenance dose), high-dose immunoglobulin (1g/kg for 2 consecutive days for 4 cycles, every fourteen days), cyclophosphamide (100 mg/day orally for two months), without any clinical improvement, but with a slight decrease of inhibitor titre (413 BU/mL). In the mean time, therapy with low-dose prednisone was administered. Intercurrent hemorragic events, (hematomas of arms and legs), were treated with recombinant FVIIa (rFVIIa) at a dosage of 90 micrograms/kg, every three-four hours until the resolution. On July 2004, a sudden abdominal shedding of blood was diagnosed. Treatment with rFVIIa, 90 micrograms/kg every 3 hours, was performed, and for severe anemia (haemoglobin level 6.5 g/dL) four RBC units were transfused. A laparoscopy was made and hemorragic corpus luteum was found and intraoperative coagulated. On August 2004, she began therapy with Rituximab at the dosage of 375 mg/m^2 for four doses, once a week, and she continued therapy with prednisone at a dosage of 70 mg/week. Before Rituximab therapy start, aPTT ratio was >3; FVIII:C <1%; inhibitor titre 206 BU/mL. A week after therapy stop, aPTT ratio was 2.9; FVIII:C <1%; inhibitor titre 75 BU/mL . One month after therapy stop, aPTT ratio was >3; FVIII:C <1%; inhibitor titre 50 BU/mL. At the second month after therapy stop, aPTT ratio was 2.96; FVIII:C <1%; inhibitor titre 30 BU/mL. At the third month after therapy stop, aPTT ratio was 2.31; FVIII:C 1.2%; inhibitor titre 9.7 BU/mL. At the fourth month after therapy stop, aPTT ratio was 1.8; FVIII:C 3.4%; inhibitor 5.5 BU/mL. At the fifth month after therapy stop, aPTT ratio was 1.7; FVIII:C 11%; inhibitor 2.1 BU/mL. Since start of Rituximab therapy the patient did not experience any hemorrhagic events; moreover, she progressively tapered prednisone therapy unti1 20 mg/week. Before Rituximab start, CD 19+ B cells were 205 x 10^6/L and T4/T8 ratio was 1.2. At the first week after therapy stop, CD19+ B cells were absent and T4/T8 ratio was 1.28; at the fifth month after therapy stop, CD 19+ B cells are still absent and T4/T8 ratio is 1.1. There were no changes of immunoglobulin (IgG, IgA, IgM) levels after Rituximab therapy. These results indicate that Rituximab induced a progressive slow decrease of inhibitor titre and a consequent increase of FVIII:C level. Until now, we obtained a partial response, characterised by the absence of bleeding symptoms, although a complete eradication of inhibitor was not still reached. Rituximab seems to be effective in the treatment of acquired hemophilia A, with high-titre inhibitor, resistant to other immunesuppressive therapy lines.

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SPLENECTOMY IN CHRONIC RELAPSING ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Thrombotic thrombocytopenic purpura (TTP) is a rare syndrome characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, renal failure and neurological manifestation. It is caused by a severe decreased of von Willebrand factor cleaving protease activity (ADAMTS-13), leading to persistence of unusually ultra-

large von Willebrand multimers (ULVWF) in the circulation that bind to platelets, causing platelet aggregates, microangiopathic hemolysis and thrombocytopenia. A lack of ADAMTS-13 activity can be caused by autoimmune inhibitors or may be due to a constitutional deficiency of this protein. Recently, the ADAMTS-13 gene that encodes for the ADAMTS-13 protein was found. It was mapped to chromosome 9q34 and consists of 29 exons. Several mutations has been identified in the ADAMTS gene in patient with the congenital form of TTP. Although TTP usually occurs as an acquired form due to autoantibodies against ADAMTS-13. The determination of the activity of ADAMTS-13 and of antibodies against ADAMTS-13 are important part in the workup of patients with TTP. Plasma exchange (PE) with fresh frozen plasma replacement is the standard treatment. The efficacy of PE is likely due to the removal of both antibodies and ULVWF and the infution of ADAMTS-13. Additional treatment modalities include glucocorticoids, splenectomy, vincristine, cyclophosphamide, azathioprine, cyclosporin A, combination chemotherapy, intravenous immunoglobulins and, recently, rituximab. We report a case of chronic relapsing acquired idiopathic TTP successfully treated with splenectomy. The patient, an 50–year old woman, developed her first episode of TTP in May 2001. Remission was achieved after 12 sessions of PE, four dose of vincristine at dose of 0,02 mg/kg/die, corticosteroids at dose of 1 mg/kg/die and increased dose of prociclide from 10 to 60 mg /kg/die. From 2001 to 2004, she had six relapses responding to treatment with PE, vincristine, and corticosteroids. The relapse in 2004 was followed by a protracted course despite the addition of cyclosporine A and she become dependent on PE. On May 2004 she was treated with splenectomy. The postoperative course was uneventful. ADAMTS-13 activity and inhibitor levels were monitored. ADAMTS-13 activity was initially, pre-splenectomy, low to 6% (n.v. 46-160%) and inhibitor's titre against ADAMTS-13 was 12 U/mL (n.v. low to 1 U/mL). After splenectomy, the inhibitor against ADAMTS-13 disappeared rapidly after one month, while ADAMTS-13 activity has remained very low (low to 6%). After six months from splenectomy there wasn't full recovery of ADAMTS-13 activity. Follow up is now 10 months. ADAMTS-13 activity has remained low to 6% and inhibitors have not reappeared. Our experience with splenectomy in a patient with chronic relapsing acquired TTP, suggest that splenectomy, by eliminating an important source of B-lymphocytes producing inhibitory ADAMTS-13 autoantibodies, may be useful treatment option in patient with chronic relapsing acquired TTP.

P402

ACQUIRED HEMOPHILIA IN PATIENT WITH INTERFERON-ALPHA TREATMENT FOR HEPATITIS C VIRUS INFECTION

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Acquired Hemophilia (AH) is a rare bleeding disorder, caused by an autoimmune depletion of Factor VIII C (F VIII:C), due to specific inhibitor. The inhibitor may occur in association with pregnancy or post-partum status, autoimmune diseases, medication, solid tumors, hematologic malignancies, infections and dermatological conditions. However, up to 46% of cases have no identificable underlying disorders. The association between formation of factor VIII inhibitors and interferon-alpha treatment in patients with chronic hepatitis C, is extremely rare, and only one case, to our knowledge, has been described. Treatment consists of two objectives: permanent inhibitor suppression and management of the acute bleeding episode, but no general consensus exist on the best therapeutic approach. Several investigations suggest that oral cyclophosphamide and prednisone, without FVIII therapy, may be useful in patients with high titre inhibitor. We report a case of AH associated with interferon-alpha treatment for chronic hepatitis C virus infection, treated with oral immunosuppressive therapy and activated recombinant FVII (rFVIIa NovoSevenR). A 58-year old man, with a 10-years hystory of chronic hepatitis C virus infection and six months PEGinterferon-alpha 2a and Ribavirine treatment, developed spontaneous soft tissue hemorrhages expecially on right leg and on tongue, with acute and severe anemia (Hb 8 gr/dL), although platelets number was normal (Plt $202x10^9$). His family history was negative for hemorrhagic diathesis. HCV-RNA was elevated (350.000 copy/mL). Coagulation assay showed a normal prothombin time and fibrinogen levels and a prolonged activated partial thromboplastin time (APTT 73''-n.v. 34''). FVIII C level was < 1% (n.v. 60-150); lupus anticoagulant's research was negative. An antibody direct against FVIII C was found at high titre (130 BU/mL). A diagnosis of AH was made and oral immunosuppressive therapy with prednisone 1mg/Kg/die, cyclophosphamide 100mg/die, and rFVIIa (NovoSevenR) at dose of 90 micrograms/kg every three hours for two days, was started. APTT, level of FVIII and inhibitor was measured every 1-week. APTT gradually returned to normal value, inhibitor level decreased, whereas FVIII levels increased and returned to normal value after 4 weeks (Table 1).

Table 1- Coagulation profiles.

	n.v.	At visit	1st wk	2nd wk	3rd wk	4th wk
APTT (sec)	34"	73"	62	54	45	33
F VIII (%)	60-150%	< 1	4	56	62	80
F VIII inhibitor (BU/ml)	< 0,01%	130	54	6	2	< 0,01

One month later, hemorrhagic diathesis disappeared and Hb increased (14,1 gr/dL) without blood transfusions. Cyclophosphamide was stopped after 4 weeks and pred-

nisone was gradually tapered off after 3 months. In patients with acute and chronic hepatitis C virus infection has been hypothesized a dysregulation of the immune system that may favour the development of an abnormal lymphoid clone and in our case, probably, autoantibodies against FVI-II. In conclusion our observation illustrates high titre inhibitor-AH associated with peginterferon-alpha 2a treatment for chronic hepatitis C virus infection, successfully treated with oral prednisone, cyclophosphamide and rFVIIa. Causal relationship between interferon-alpha treatment for chronic hepatitis C virus infection and AH remains speculative. Although the clinical course is not predictable and inhibitor may disappear spontaneously, in some cases, with high titre inhibitor associated, combined therapy with prednisone , cyclophosphamide and rFVIIa may be sufficient to suppress inhibitor and to arrest bleeding.

P403

THROMBOPHILIC SCREENING IN 23 THROMBOTIC THROMBOCYTOPENIC PURPURA PATIENTS. EXPERIENCE OF THE UNIVERSITY LA SAPIENZA ROMA

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Thrombotic thrombocytopenic purpura (TTP) is characterized by microangiopathic haemolytic anemia and thrombocytopenia, accompanied by microvascular thrombosis. 23 TTP patients in remission at different intervals from the onset of the disease, were studied to evaluate whether or not alterations possibly correlated to a thrombophilic status, were present. In our study we evaluated plasma homocysteine concentrations and mutations of Leiden Factor V, prothrombin gene and MTHFR C677T. Sixteen patients (69%) showed one or more alterations. Hyperhomocysteinemia, with a median value of 21.5 micrommol/L (range 15-23.3), was found in 7/23 patients (30%) and it was associated with a MTHFR C677T gene mutation in 5/7 patients (3 were heterozygotes and 2 homozygotes). MTHFR C677T gene mutation was found in 12/23 patients (52%): 10 patients were heterozygous, while 2 were homozygous. Leiden Factor V mutation was found in 3/23 patients (13%) and Factor II mutation was found in one patient. Two patients, with normal homocysteine plasma levels, were MTHFR heterozygous plus Leiden Factor V mutation in one case and Factor II mutation in the other. All patients with hyperhomocysteinemia received folate, vitamin B6 and B12 supplementation. One patient with Leiden Factor V mutation required oral anticoagulant therapy for the occurrence of two spontaneous episodes of deep venous thrombosis. In our experience on 23 TTP patients, it was observed an high frequency of alterations such as hyperhomocysteinemia with or without MTHFR C667T gene mutation or Leiden Factor V mutation, which represent risk factors for thrombotic disorders. As a matter of fact we recommend to investigate a thrombophilic status in all TTP patients in remission of the disease, to identify patients with an increased risk of thrombotic events who could take advantage from a close clinical ad laboratory monitoring and possibly from an adjuvant therapy. Prospective studies on a large cohort of patients will be necessary to confirm our data.

P404

HAEMOPHILIA A PATIENTS WITH INHIBITORS AND *"IN VITRO"* REACTIVITY VERSUS DIFFERENT COMMERCIAL CONCENTRATES

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A relevant aspect in the treatment of patients with haemophilia A presenting inhibitor against FVIII is the different antigenicity of factor VIII used for replacement therapy. In this study the reactivity of inhibitors from patients with haemophilia A and the reactivity of three commercial concentrates are compared. To date 8 patients with severe haemophilia A have been enrolled. Three commercial FVI-II concentrates have been used and compared: a plasma-derived heat purified concentrate (Haemate P, ZLB Behring) containing high amount of VWF; a plasma-derived chromatographically purified concentrate (Beriate P; ZLB Behring) containing low amount of vWF and a recombinant concentrate (Helixate NexGen; ZLB Behring) not containing vWF at all. The aim of this study was to assess the effect of different products, with variable vWF concentration, and their possible role in the binding of inhibitor to FVIII. This might be relevant in the treatment of clinical manifestations *in vivo* when concentrate show different reactivity *in vitro*. Products were reconstituted according to the manufacturer's instructions. Plasma samples were incubated with the different FVIII concentrates at 37°C for 2 hours. Then FVIII:C was measured by one-step coagulative test and inhibitor titre was calculated by the Bethesda method. In order to remove the variability due to patient inhibitor titre, the result obtained with different concentrates has been divided by the inhibitor titre of each patients. Results suggest the existence of higher inhibitor interaction with Beriate P which corresponds to a low level of vWF. Both concentrates with high amount of vWF and with complete absence of vWF (corresponding to Haemate P and Helixate respectively) inhibitor interaction decreases of ∽53%. Although statistically relevant conclusions cannot be drawn because of the small number of patients (the study is still in progress and other patients will be enrolled) a qualitative picture for inhibitor reactivity vs vWF amount can be suggested (see figure). Results show that Beriate P presents greater inhibitor interaction with respect to Haemate P suggesting that increasing amount of vWF may protect FVIII from inhibitors. For Helixate the results are not clear as they are quite similar to Haemate P. A possible interpretation is that its activity decades quickly and the experiment becomes less reproducible.

P405

CELLULAR DIFFERENTIATION AND PROCOAGULANT ACTIVITY ARE SIMULTANEOUSLY REGULATED BY SYNTHETIC RETINOIDS IN FRESHLY ISOLATED ACUTE PROMYELOCYTIC LEUKEMIA BLASTS

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The remission of human APL by differentiating therapy with all-trans-retinoic acid (ATRA) is associated with a rapid resolution of the life-threatening coagulopathy typical of this disease. We have previously demonstrated that during ATRA therapy, the expression of the two main tumor cellular procoagulants, i.e. tissue factor (TF) and cancer procoagulant (CP), is downregulated in blast cells of APL patients. In addition, synthetic retinoids, selective for each retinoic acid receptors (RAR) alpha, beta and gamma, differently downregulate the procoagulant activity of the APL cell line NB4. Aim of this study is to evaluate whether the same retinoids may affect the cellular procoagulants of blast cells freshly isolated from APL patients, and whether this modulation may occur in parallel with cellular differentiation. The following retinoids were used: ATRA (a pan-RAR agonist), Am580 (selective RAR-alpha agonist), CD2019 (selective RAR-beta agonist) and CD437 (selective RAR-gamma agonist). Promyelocytic blasts, isolated from bone marrow specimens of 8 consecutive patients diagnosed with APL, were incubated for 24h with increasing concentrations of each retinoid (0.01 to 1 micromol/L). TF and CP expression were then characterized and quantified in cell sample preparations by both functional (chromogenic assay) and antigenic methods (ELISA). Differentiation of blasts into neutrophils was evaluated as an increase in % of cells positive for CD11b expression on cell membrane (cytofluorometric method). The results show that ATRA treatment significantly reduced the expression of both TF (44±18% reduction; $p<0.005$) and CP (31± 15% reduction; $p< 0.05$) activities. These results were confirmed by the antigenic assays. The analysis of cell differentiation showed that ATRA significantly increased CD11b expression (control vs ATRA-treated cells: 12.5 ± 3.5 % vs 28.5 ± 4.3 % positive cells; $p<0.01$). Experiments with the three synthetic retinoids indicated that the RAR-alpha agonist significantly reduced both TF (27 ± 17 %; $p<0.001$) and CP expression (24 ± 20 %; $p<0.001$. This modulation occurred simultaneously with cell differentiation (CD11b: control cells vs Am580: 12.5 ± 3.5 % vs 39 ± 3.9 % positive cells; $p<0.01$). The RAR-beta agonist was ineffective in modulating both the procoagulant activities and cyto-differentiation. Finally, the RAR-gamma agonist did not affect TF, but significantly reduced CP expression (20±7 %; $p<0.001$). This effect was not associated to cell differentiation. In summary, our data indicate that in freshly isolated APL cells, ATRA down-regulates TF and CP expression, as previously observed *in vitro* in the APL NB4 cell line and *in vivo*, in APL patients during ATRA therapy. Similarly to the NB4 cells, the modulation of the two procoagulants appears to be mainly mediated by RAR-alpha and occurs together with signs of cellular differentiation. An additional role for RAR-gamma in CP modulation is suggested in fresh APL of cells. This study on freshly isolated blast cells might offer a model for testing *in vitro*'the cell sensitivity to retinoids more selective than ATRA (with less side effects) for the control of cellular procoagulant activities.

Infections II

P406

DIAGNOSTIC VALUE OF C-REACTIVE PROTEIN IN DISCRIMINATING FUNGAL FROM NON-FUNGAL PULMONARY INFILTRATES IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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We recently published a risk group stratification aimed at predicting the outcome of hematologic patients with fever and pulmonary infiltrates. Our model was based on three baseline risk factors including serum C-reactive protein (CRP) levels, serum albumin and trend in WBC count (*Offidani et al, Cancer 2004*). However, it is also substantial to recognize factors predicting the etiology of pulmonary infiltrates. The goal of the current study was to evaluate the usefulness of CRP level in discriminating between fungal and non-fungal pulmonary infiltrates occurring in 143 adult patients (77 male, 66 female) with hematologic malignancies. The median age was 54 years (range 20-75) with 40% of them older than 60 years. Seventy-one (50%) of patients had AML, 20 (14%) ALL, 34 (24%) lymphoma, 6 (4%) multiple myeloma, 6 (4%) CML and 6 patients (4%) were affected by other malignancies. One hundred and four patients (73%) received chemotherapy, 24 (17%) underwent transplant while to 15 (10%) no treatments for underlying disease were administered. Antibacterial and antifungal prophylaxis were given to 57% and 78% of patients, respectively. At onset of fever with pulmonary infiltrates, neutropenia was present in 66% of patients. In sixty-eight episodes (48%) of pulmonary infiltrates a microrganism (51 bacteria, 10 fungi, 7 virus) was isolated premortem. According to EORTC/MSG criteria, 26 patients (18%) were classified as having fungal pneumonia (5 proven, 3 probable, 18 possible). Serum CRP level was significantly higher for patients with fungal pneumonia than in those with non-fungal pulmonary infiltrates (median 22.3 mg/dL vs 7.3 mg/dL; $p<0.0001$). Particularly, a significantly greater proportion of patients with fungal pneumonia had a CRP level higher than 10 mg/dL (81% vs 39%; $p<0.0001$). A trend in the statistical significance was also found when group of patients developing proven and probable fungal pneumonia was compared with that of possible infection ($p= 0.085$). Of note, all patients with proven infection had a CRP level higher than 10 mg/dL. Then we tried to assess clinical parameters able to predict fungal aetiology of pulmonary infiltrates. Univariate analysis showed that CRP level higher than 10 mg/dL, presence of neutropenia, previous neutropenia longer than 10 days, induction as treatment phase, not achievement of a complete remission, no antibiotic prophylaxis, previous hospitalization longer than 16 days were related to development of fungal pneumonia classified according to Ascioglu et criteria. At multivariate analysis only CRP level higher than 10 mg/dL, previous neutropenia longer than 10 days and not achievement of a

complete remission retained their prognostic value.

Our results suggest that CRP level may facilitate the differential diagnosis of pulmonary infiltrates in hematologic patients. Since invasive fungal infections remain a leading infection-related cause of death in hematologic patients and their early diagnosis is difficult, new approaches such as the generation of predictive models may be essential for identifying precisely those patients at highest risk for the development of fungal infections. Our experience suggests that in the design of any prospective risk model for fungal infections CRP level should be taken into consideration.

P407

CLUSTERS OF INFECTIONS DUE TO STENOTROPHOMONAS MALTOPHILIA IN A SINGLE INSTITUTION

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From a period of predominance of gram-positive pathogens as infecting organisms in neutropenic patients the etiological pattern is changing with increase in the proportion of gram-negative bacteriemias. It is unclear if it is associated to a decrease in the use or an increase in resistance to quinolones. Moreover the emergence of unusual organisms, as Stenotrophomonas maltophilia, has been found in many institutions. We report 6 cases of documented infection by Stenotrophomonas maltophilia seen at our unit. Patients were hospitalized and affected by ALL (1), AML (2), marginal zone NHL (1), high grade NHL (1), CLL (1); they were all given therapy (standard induction therapy for ALL, fludarabine for marginal zone NHL, R-CHOP for CLL, MEC regimen for AML, mitoxantrone plus cytarabine and rituximab for high grade NHL) when infections appear at except one patient with AML who develops infection before starting induction therapy. Only ALL and high grade NHL were receiving 1st line therapy when infection was diagnosed. Median count of neutrophils was $< 1000/mm^3$ (<100-<1000). 2 infections were documented on august 03 (blood colture and phlegm respectively); 2 between november and december 03 (both phlegm); 2 between june and august 04 (blood colture and phlegm respectively). Three cases of pneumonia, one of pneumonia plus cellulitis and two cases of febrile bacteraemia were recorded. All the patients were receiving prophylaxis with levofloxacin; 3 patients had central venous catheter. Antibiotic therapy was immediately started (moxifloxacin in one patient, cotrimoxazole in one, meropenem in two and piperacillin/tazobactam plus amikacine in two). Two patients died by pneumonia (one during aplasia from induction therapy), two had their infections (pneumonia) improved, two became afebrile. So far two patients (one in pneumonia group) are alive in CR. Some reports stated that ceftazidime plus levofloxacin (which achieves excellent penetration in the lungs) or the quinolone moxifloxacin could be useful in this setting.

Despite this, the frequent use of old quinolones and carbapenems is associated with the selection of multidrug-resistant isolates of Stenotrophomonas maltophilia. To overcome this problem antibiotic cycling (usually every 6 months) and antibiotics heterogeneity (that is equal use of multiple classes of drugs) might be useful alternatives; however no study to compare these approaches has been conducted. We need targeted drugs with activity against drug-resistant organism, but the market for such drugs appears too small for most pharmaceutical companies.

P408

GASTROINTESTINAL COMPLICATIONS DURING CHEMOTHERAPY IN ADULT ACUTE MYELOID LEUKEMIA

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In acute myeloid leukaemia (AML) patients, cytotoxic regimens, especially those including high dose cytarabine (HD Ara-C), have been frequently associated with the development of gastroenteric complications, characterized by abdominal pain and diarrhoea. We retrospectively analyzed the clinical course of 160 adult patients with AML, from January 1999 to December 2004, who received antineoplastic drugs as induction, consolidation or salvage regimens, to determine the incidence of gastroenteric complications, such as neutropenic enterocolitis (NE) and Clostridium difficile associated diarrhoea, and to evaluate clinical characteristics and treatment outcome. Gastroenteric complications were observed in 25/160 patients (15.6%), median age 53 years (range 24-64), after the completion of chemotherapy, during the neutropenic phase (median duration of neutropenia $<0.5x10^9$: 17 days, range 7-60). Most patients (80%) developed gastrointestinal complications during induction (52%) or salvage therapy (28%), whereas only 5 patients (20%) during a consolidation regimen including HD Ara-C.

Ten patients developed signs and symptoms of NE, characterized by abdominal pain, diarrhoea and fever. To support the clinical diagnosis, imaging techniques were performed when possible, in 7 of these 10 patients. The results showed bowel abnormalities such as wall thickening, mucosal enhancement and bowel dilatation. Microbiological diagnosis of C. difficile associated diarrhoea was obtained in 14 patients, who were treated orally with metronidazole or vancomycin obtaining a good response. One patient with C. difficile infection developed clinical signs of NE, confirmed also by radiologic evidence. All 25 patients received medical and supportive treatment, including broad-spectrum antibiotics, bowel rest, nasogastric decompression and total parenteral nutrition. Only two patients needed intensive care unit support, without surgery. NE did not result fatal in any patients. We conclude that in AML patients the development of infectious gastroenteric complications, above all during induction and salvage chemotherapy, is probably related to the presence of an underlying active leukaemia rather than to the administration of antineoplastic regimens containing HD Ara-C and/or Etoposide; it is also possible to obtain a favourable outcome with conservative measures until the resolution of neutropenia.

P409

PARVOVIRUS B19 INFECTION: AN UNDERDIAGNOSED DISEASE OFTEN FATAL IN HEMOPATHIC PATIENTS. A REPORT OF TWO CASES.

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Parvovirus B19 is a common source of infection with a seroprevalence of 60-70% in the adult population. The infection can give rise to a multifaceted clinical picture and is probably underdiagnosed, particularly in individuals at risk (with haemolytic anaemia, pharmacologic or constitutional immunosuppression, hemopatic patients and fetuses). We present two cases of fatal Parvovirus B19 infections occurred in two immunocompromised hosts.

Case 1. An 81 years-old male patient presented to our hematoncology division with diagnosis of acute lymphoblastic leukemia and complaining fever and dyspnoea. Its temperature was 39°C and physical examination showed hepatosplenomegaly. Empirical antimicrobial therapy was begun. Complete blood count showed hemoglobin 7.5 g/dL, platelets 4000/mcl, leukocytes 12040/mcl, (lymphocytes 4700/mcl). Hepatic and renal function were preserved. Electrophoresis showed hypergammaglobulinaemia (53%), with albumin 2.1 g/dL, total proteins 9.4 g/dL with IgG 4890 mg/dL (polyclonal). Peripheral blood smear showed 27% of large activated lymphocytes, without circulating blast. This morphological feature was confirmed also at peripheral and bone marrow immunophenotype and at bone marrow smear. Only serology for Parvovirus B19 showed an high IgM serum title. Consequently immunoglobulin infusion was started (0.4g/kg/ day for 6 days). Immediately PLT count and Hb value increased up to 110.000/mmc and 9.5 g/dL respectively. Two days later, after sudden onset of acute lung injury and progressive worsening of mental status, patient died.

Case 2. A 65 years-old man underwent to our observation with fever (Tc 39°C) and diagnosis of acute monoblastic leukemia. Immediately empirical antimicrobial therapy was started and 48 hours after, respiratory function worsened. Chest X rays showed a ground glass image. At the same time, patient developed an acute hepatitis. Subsequently an overt ARDS developed. Patient required invasive ventilatory support, but without benefits. Two days later patient died. Serological tests performed at hospital admission were ready after patient death and showed only positivity for Parvovirus B19 IgM. Parvovirus B19 is a common cause of communitarian infection. Transmission occurs by respiratory route. However infection can be transmitted also by blood and blood products. The most frequent clinical manifestations are: cutaneous erythema, especially in children (fifth disease), transient aplastic anemia, persistent anemia (especially in immunodeficient and immunocompromised patients), adult arthropathy and hydrops fetalis. Other infrequent manifestations are self limited hepatitis (rarely fulminant), myocarditis, necrotizing vasculitis, giant cell arteritis, pneumonia, acute lung injury, meningitis, encephalitis and a variety of neurologic complications. Parvovirus B19 infection is rarely severe in immunocompetent patient, but might be fatal in immunocompromised hosts, as described in our cases. Serology is the simplest and the most useful diagnostic tool in this disease. IgM antibody is detectable within 3 days of the onset of disease in over 90% of cases and it remains detectable for 2 to 3 months later. PCR for Parvovirus B19 DNA may be of diagnostic value in serum and respiratory secretions in specific situations. Actually commercial immune globulins are a good source of antibodies against Parvovirus. A 5 or 10 day course of Ig at a dose of 0.4g/kg/day is the main therapeutic tool and is promptly followed by viral DNA decline in serum and increased Hb and PLT levels.

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SUCCESSFUL TREATMENT OF PNEUMOCYSTIS CARINII PNEUMONIA WITH CASPOFUNGIN IN AN ACUTE T-LYMPHOBLASTIC LEUKEMIA PATIENT UNDERGOING BONE MARROW TRANSPLANTATION FROM UNRELATED DONOR

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A 45-year-old male T-ALL patient scheduled to receive an allogeneic bone marrow transplantation (BMT) in second CR from a matched unrelated donor (MUD) in October 2004 was admitted in September because of acute Pneumocystis carinii pneumonia (PCP) and concomitant ALL relapse. Despite high-dose cotrimoxazole in combination with methylprednisolone and a significant clinical improvement, his CT results did not normalise. Because of the concomitant infectious complication, he was treated with weekly vincristine and daily oral 6-mercaptopurine, which led to a stable bone marrow blast count and normal peripheral hemometry. The patient was discharged on October 7 and, two weeks later, a further lung CT scan showed the persistence of significant residual disease. On November 11, a PET scan revealed the presence of diffuse active disease, and the patient was admitted to our Unit to continue anti-PCP therapy (initially with high-dose cotrimoxazole) and to undergo BMT. A thiotepa-CTX conditioning regimen was started on November 22, and standard-dose caspofungin was imbricated with high-dose intravenous cotrimoxazole. On November 29, the CT scan findings were unchanged and cotrimoxazole was discontinued; BMT was performed on the following day. The subsequent course was complicated by mixed Enterobacter/Gram+ bacteremia followed by low-grade fever, which spontaneouly disappeared over the next two weeks. A CT scan on December 22 showed the complete resolution of the PCP lesions. PMN recovery occurred on post-BMT day 25, and platelet recovery on day 34, a delay that was attributable to mild microangiopathic hemolytic anemia responsive to defibrotide. Caspofungin was discontinued on January 5, and low-dose cotrimoxazole was started; the bone marrow aspirate showed complete remission and full donor chimerism. The patient was discharged on January 12. High-dose cotrimoxazole is the reference treatment for PCP but contraindicated in a proportion of patients. Moreover, it is suspected that the widespread use of cotrimoxazole in PCP prophylaxis and therapy has led to the development of cotrimoxazole resistance. Clindamycin, dapsone and pentamidine are well-known alternatives to cot-

rimoxazole, but have some disadvantages in terms of activity and toxicity; atovaquone is a promising agent that is not widely used in clinical practice. Caspofungin has proved to be an active and little toxic antifungal agent with promising in vitro activity against Pneumocystis carinii. This anecdotal report suggests that it may be an effective alternative for PCP patients in whom cotrimoxazole is contraindicated.

P411

MONITORING CMV P65 ANTIGEN IN RECIPIENTS OF AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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The risk of developing CMV infection after autologous hematopoietic stem cell transplantation (AHSCT) is evaluated in various ways, and monitoring post-AHSCT CMV reactivation is not a universally accepted practice. In our Centre, we did not routinely monitor of post-AHSCT CMV antigenemia until 2002, and all autografted patients received intravenous acyclovir starting on day +5; between 1992 and 2001, 3/212 AHSCT recipients (1.41%) died of CMV pneumonia within 100 days of transplant, and this also enabled us to monitor CMV. Between November 2001 and February 2005, CMV p65 antigenemia was measured twice a week from the day of admission until discharge in 102 consecutive AHSCT recipients (57 males and 45 females, with a median age of 52 years, range 19-70); the diagnoses were NHL in 48, HD in seven, AML in nine, systemic sclerosis in two, and MM in 36 cases. Acyclovir prophylaxis was administered as before. Three patients were p65 positive, one during the conditioning regimen, one on post-AHSCT day +15 and one on day +31. The first and second cases were treated with foscarnet and the third with gancyclovir (both administered at standard doses for 14 days), and all three became promptly and lastingly p65 negative; none of these patients experienced any recurrence and no case of CMV infection was recorded in the whole series. CMV infection is a generally infrequent complication after AHSCT, although various therapy- and disease-related conditions may influence its incidence and outcome, thus accounting for some of the differences reported in the literature; furthermore, the use of AHSCT in increasingly older and immunosuppressed patients may increase the relevance of CMV infection in the future. In our experience, routine CMV monitoring revealed a low rate of reactivations, which were easily managed by pre-emptive therapy. This and the fact that there was no case of CMV infection in the series as a whole does not mean that monitoring eliminated the problem. Nevertheless, comparison of the 2.9% reactivation rate with the previous incidence of CMV pneumonia in our Institution (1.41%) suggests that only a few patients may have been overtreated as a consequence of p65 positivity, and indicates a favourable risk/benefit ratio. Our experience certainly does not fully address the question of CMV in AHSCT, but it does support the policy of routine CMV monitoring.

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VORICONAZOLE TREATMENT OF ACUTE INVASIVE ASPERGILLUS RHINOSINUSITIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES OR APLASTIC ANAEMIA

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Acute invasive Aspergillus rhinosinusitis (AIAR) is a life-threatening fungal infection which appears almost exclusively in leukaemia patients following cytotoxic chemotherapy and/or bone marrow transplantation or in aplastic anaemia. Mortality is high, ranging from 20% in patients with leukemia in remission to up to 100% in patients with relapsed leukemia or those undergoing blood stem cell transplantation. The drug of choice is amphotericin B (AmB) which appears to be superior to itraconazole although the data are scanty. Many authors recommend a combination of medical and surgical therapy. However, radical surgery may be associated with major complications and appears to offer no survival advantages. Voriconazole is a new broad spectrum triazole that proved to be more active and less toxic than conventional AmB in the primary therapy of invasive aspergillosis, however, in the larger series of voriconazole therapy of invasive Aspergillus infections only a minority of patients were affected by AIAR. We retrospectively analyzed the outcomes for 13 consecutive patients with hematologic diseases who received first line therapy with voriconazole for confirmed (proven or probable) AIAR from June 2003 through January 2005. These patients were compared to a historical group of 21 patients with AIAR who received other antifungal treatments (conventional AmB, 16 cases; liposomal AmB, 3 cases; itraconazole, 2 cases) from 1987 to May 2003. The two groups of patients were well matched with regard to age, sex, underlying hematologic disease, type of chemotherapy, corticosteroid therapy and extension of Aspergillus infection. No patient in the voriconazole group vs 5 patients (24%) in the historical control group underwent radical surgery. Only 1 patient in the voriconazole group (8%) and 6 in the historical group (29%) received a salvage therapy with another antifungal drug. First line treatment with voriconazole was associated to better responses (improvement of clinical signs at day 7 of therapy, 62% vs 24%; OR= 5.12, 95% CI= 0.92-29.94, p=0.03) ; complete/partial response 69% vs 38%; OR= 3.66, 95% CI= 0.69-21.31, p=0.07) and improved 3-month survival rate (69% vs 38%; OR= 3.66, 95% CI= 0.69-21.31, p=0.07) compared to other treatments, and resulted in fewer severe side effects. These data support the use of voriconazole in first line treatment of hematologic patients with AIAR, but given the retrospective nature of the study and the small number of patients, a randomized trial will be necessary to determine whether voriconazole will be superior to other antifungal drugs for primary therapy of AIAR. Our experience is not able to evaluate the possible role of surgery in the management of AIAR, however, considering the early clinical responses observed in most patients treated with voriconazole it seems that the invasive surgical procedure could be avoided in the early phases of the infection and eventually reserved to patients with severe signs of chronic rhinosinusitis

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CLEARANCE OF HEPATITIS B VIRUS INFECTION FOLLOWING ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION WITH PRE-EMPTIVE USE OF LAMIVUDINE

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HBV reactivation after HSCT may occur not only in known HbsAg carriers undergoing Allogeneic Hematopoietic Stem Cell Transplantation , but also in patients with HBV antibodies (antiHBc and/or antiHBs) before transplant. Several mechanisms, such as chemotherapy-enhanced viral replication, steroids, restoring of immunocompetence, may significantly concur to HBV flare-up, with clinical manifestations ranging from asymptomatic viraemia to anicteric hepatitis up to progressive or fulminant liver failure.Among 180 patients undergoing autologous or allogeneic HSCT in our Unit between 2001 and 2004, 6 (3.3 %) pts , positive at transplant for antiHBs and/or antiHBc , developed HBV viraemia during follow-up. None of these patients was antiHBcIgM positive at transplant and all had normal transaminase values. The underlying disease was Acute leukemia in 3, Chronic Leukemia in 2, and Aplastic anaemia in. 1. All underwent allogeneic HSCT from HLA identical sibling donor . As part of infectious diseases surveillance protocol carried out in our institution, HBV-DNA, HCV-RNA, HbsAg, HbeAg, HBV antibodies and anti-HCV were tested in serum every month after transplant during the first year follow-up, and twice a year thereafter. Evidence of HBV viraemia (>200.000 copies in all cases) occurred between day +180 and +790 (median day + 348) and was concomitant to CSA tapering and withdrawal . All patients had ALT flare up concomitant to HBV-DNA appearence and in all cases we observed also seroconversion to HbsAg , HbeAg, and antiHBcIgM, while antiHBs disappeared. Lamivudine treatment (100 mg/day) was started as soon as viraemia was detected. No patient progressed to severe hepatitis, and ALT soon normalized .The duration of therapy varied according to the time of serological response, which occurred after 30-190 days (median 90 days). All patients showed seroconversion to HBV-DNA and HbsAg negative tests , and were treated for 3 additional months from seroconversion and then monitored monthly. All patients seroreverted to antiHBs + while antiHBc(IgM-) is still detectable in 3/6 cases ;HBV-DNA remains negative at latest follow-up (1-3 years from therapy withdrawal). HBV-related liver disease disappeared accordingly and to date all patients have normal transaminases. 1/6 patients showed overt hepatitis concomitant to HBV reactivation, but transaminases returned to normal after 30 days of treatment. Lamivudine treatment was well tolerated and we did not observe marrow or other relevant organ toxicity . In conclusion, the impact of Lamivudine pre-emptive therapy on HBV -related liver disease seems remarkable and efficacy on HBV infection is promising , as all the patients cleared HBV viraemia , which was mantained after therapy withdrawal, and developed stable protective antibodies.

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HEPATITIS B VIRUS REACTIVATION AND ALEMTUZUMAB THERAPY

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Reactivation of hepatitis B virus infection in subjects receiving cytotoxic treatment for haematological malignancies occurs in 21–53% of chronic HBsAg carriers and in an unknown number of HBsAg negative subjects harbouring occult HBV infection. Immunotherapy with alemtuzumab, a humanized monoclonal antibody against CD52 epitopes on lymphocytes cells, produces deep immunosuppression. We describe two subjects with chronic lymphocytic leukaemia and occult HBV infection who developed a virological and biochemical flare of hepatitis B following immunotherapy with alemtuzumab. One of them developed a full blown hepatitis with seroreversion from anti-HBs to HBsAg after four weeks of alemtuzumab therapy. Lamivudine (100 mg die) achieved a complete clinical recovery and HBV-DNA clearance from blood within 8 weeks. The second patient (HBsAg and HBV-DNA seronegative, anti-HBs and anti-HBc positive before treatment) was kept under prophylaxis with lamivudine up to three months after alemtuzumab. Two months after withdrawal of lamivudine, clinical and laboratory features of acute hepatitis B developed. Lamivudine therapy was restarted and obtained a prompt recovery with HBsAg and HBV-DNA clearance.

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MONITORING OF HEMOCULTURES POSITIVE FOR BACTERIA AND FUNGI IN PATIENTS ADMITTED IN A HEMATOLOGIC UNIT

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Background. Prophylaxis with quinolones is exclusively used in patients affected by Acute Leukemia or undergoing bone marrow transplantation, since these patients, because of the long and severe neutropenia, are at the highest risk of infection. Prophylaxis with trimethoprim sulfamethoxazole is used in patients treated for lymphoma. Empirical antimicrobial therapy is still considered a good approach but a better knowledge of the microbiology of the unit is needed. Microbiologic surveillance with repeated cultures has not given good results and has a too high cost benefit rate. The present approach consists in monitoring with blood cultures the microbiology of the environment and the risk of infections in the in-patients.

Purpose. To perform the best empirical antimicrobial therapy, according to the epidemiology observed in our institution and to evaluate the risk of resistance to the drugs most commonly used in prophylaxis.

Material and Methods. Our observation extends during 15

months (1/10/2003-31/12/2004). During this period we admitted to our institution 824 patients. Blood cultures for aerobics, anaerobics and fungi were performed when the temperature was >38°C (in some cases the fever had appeared before the day of admission, in other cases after it), with three samples taken at 30 minutes intervals. When the patients had a central venous catheter (CVC), the samples were taken from the CVC and from a peripheral vein.

Results. We performed 316 hemocultures, 46 of which were positive (15%). The percentage of positivity was less than the one observed in other italian institutions. 63% of infections are from GRAM + bacteria, 26% from GRAM – and 11% from fungi. Out of the 29 hemocultures positive for GRAM +, 25 were positive for Staphylococci, 3 for Streptococci, 1 for Clostridium Butyricum. Only in one case we observed resistance to Vancomycin, in one case resistance to Teicoplanin and in one case an intermediate susceptibility. Out of the 12 GRAM -, 10 are E.Coli, 1 Salmonella, 1 Acinetobacter. All the isolated GRAM– were sensitive to Amikacin and to Imipenem. 60% of E.Coli were resistant to Ciprofloxacin, 70% resistant to trimethoprim sulphamethoxazole. In five cases the hemocultures were positive for fungi: 3 Candida Albicans and 2 Candida Parapsilosis. In all the cases there was susceptibility to Amphotericin B and Fluconazole. The hemocultures were positive mainly in patients with Acute Leukemia.

Conclusions. Monitoring the blood cultures in our institution allows us to draw some considerations. In 15% of the blood cultures we made because of fever, either a fungus or a bacterium was isolated. GRAM+ prevail over GRAM-. Among GRAM+ 87% are Staphylococci and 10% only Streptococci. GRAM+ infections have a good response to Vancomycin or Teicoplanin while GRAM- respond well to therapy with Amikacin and Imipenem, then these drugs can be used as empirical therapy. All the fungi identified in our institution are Candida sp. and respond to Amphotericin B and Fluconazole. Care must be used when using Ciprofloxacin or Trimethoprim Sulphamethoxazole in prophylaxis due to the high percentage of resistances observed.

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COUNTING ANTIGEN-SPECIFIC T CELLS BY ELISPOT: A NEW APPROACH FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS

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Invasive aspergillosis has become a major cause of infection related mortality either in neutropenic patients with hematologic malignancies, especially after allogeneic stem cell transplantation, or in non neutropenic patients after solid organ transplantation. Early diagnosis is extremely difficult and often fulfils only the criteria of a possible or a probable infection, so that antifungal therapy is empiric in the majority of the cases. Culture has a poor sensitivity, and cannot discriminate between invasive disease and colonization, so that the diagnosis of a proven infection relies only on histopathology without culture confirmation. A positive Aspergillus antigen testing would support a probable diagnosis. An ELISA technique for galactomannan has demonstrated high sensitivity, but the test should be considered a true-positive result only when more than 1 sample is positive, because of the frequent occurrence of false positive results (Ascioglu, *et al.*, CID 2002; 34:7-14).

The aim of the present study was to develop a new approach to diagnosis of invasive Aspergillosis based on rapid detection and enumeration of Aspergillus-specific T cell by the ex-vivo enzyme-linked immunospot (ELISPOT) assay (single-cell interferon gamma release ELISPOT assay). We hypothesized that T cells from individuals with invasive aspergillosis become sensitized to Aspergillus antigens *in vivo*; when the T cells re-encounter these antigens *ex vivo* in an overnight ELISPOT assay, they would release a cytokine, the interferon-gamma. Each such T cell gives rise to a dark spot, which is the footprint of an individual Aspergillus fumigatus-specific T cell, and the readout is the number of spots. Either heat-killed Aspergillus fumigatus conidia or water-soluble cellular extracts (ECSAB) from conidia of Aspergillus fumigatus, were used to stimulate the peripheral blood mononuclear cells (PBMCs) from two liver transplant patients with biopsy-proven invasive aspergillosis of the lower respiratory tract (pt. 1 and pt. 2) as well as from four control patients with evidence of pulmonary infiltrates on CT, namely three neutropenic acute leukaemia patients with a microbiologically proven bacterial pneumonia occurred during the induction phase of chemotherapy (pts. 3, 4 and 5) and one patient with a marginal zone lymphoma of the lung (pt. 6). Aspergillus fumigatus specific T cell response was documented both in pt.1 (35 spot forming cells-SFCs-/1 million PBMCs), and in pt. 2 (60 SFCs/1 million PBMCs and 115 SFCs/1 million PBMCs, using the ECSAB and the heat-killed conidia, respectively). No SFCs were detectable in any of the four control patients, upon aspergillus antigen stimulation, although a vigorous T cell response was detectable in all these patients, upon phytohaemoagglutinin stimulation. Further experiments with single-cell interleukin-4 release ELISPOT assay are undergoing to study the interferon gamma-interleukin-4 ratio, which is known to influence the resistance to infection or disease progression in experimental murine apergillosis.

This study confirms the important role of Th-1 type cellular immune response for the control of invasive aspergillosis in immunosuppressed transplant and provide a new, rapid and specific tool for the early diagnosis of invasive aspergillosis, which warrants further investigations on a larger series of patients.

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BLASTOSCHIZOMYCES CAPITATUS INFECTION IN TWO ACUTE LEUKEMIA PATIENTS HOSPITALIZATED IN THE SAME PERIOD

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Blastoschizomyces capitatus, formerly known as Trichosporon capitatum or Geotrichum capitatum, is a rarely described yeast, responsible for invasive disease in immunocompromised patients. We report two cases of fungal septicaemia caused by B. capitatus. in two patients with acute myeloid leukemia (AML), who were hospitalized in the same period. The first patient was treated with induction therapy according to AML-12 Gimema protocol (random high dose Cytarabine), whereas the other (56-year-old woman) was treated with a three-drugs-schedule (daunorubicin, etoposide. standard dose cytarabine) for relapsing leukemia eleven months after the achievement of first complete remission. Fever arose during chemotherapy-induced neutropenia, while they were receiving prophylaxis with oral itraconazole. Lyposomal amphotericin was started empirically after five days of fever resistant to wide spectrum antibiotics. Some days later, B. capitatus was isolated in blood coltures in both patients. Total body CT scan showed multiple hypodense hepatic and splenic abscess in both patients whereas pulmonary infiltrates were seen in one of them. No encephalic involvement occurred. Severe clinical conditions and fever persisted although ANC had recovered up to > 1e 9 per liter and caspofungin had been added to amphotericin. For this reason voriconazole was given at a dosage of 6 mg/kg bid for two days and 4mgr/kg bid thereafter. After some days the fever regressed and clinical improvement was observed. The hepatic and splenic lesions persisted for three months but gradually disappeared. B. capitatus infection has been recently described by Martino *et al.,* in a series of 26 patients with leukemia (Clin Inf Diseas. 2004; 38:335) studied retrospectively from 1992 through 2002. Although this infection is rare and interhuman transmission is not described, it is remarkable that B. capitatus sepsis occurred in two patients hospitalized in our unit during the same period. In our experience B. capitatus infection in neutropenic patients may be resistant to amphotericin and caspofungin and voriconazole seems to be a suitable agent for this lifethreatening condition.

P418

INCIDENCE OF SEPSIS DUE TO ENTEROCOCCUS IN PATIENTS WITH HAEMATOLOGICAL DISEASE: AN EPIDEMIOLOGICAL STUDY

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The emergence of glycopeptide-resistant gram+ strains represents a relevant problem in haematological clinical practice. Nosocomial infections from vancomycin-resistant enterococcus (VRE) are very interesting because empiric antibiotic therapy for neutropenic fever does not cover those germs. We retrospectively evaluated the prevalence of enterococcus sepsis in patients with acute leukaemia and lymphoproliferative disorders and its association with rectal microbiological swab examination (representing enterococcus carrier status). Medical charts from February 2001 to February 2005 were examined. Rectal swabs were obtained in 153 patients. Positive enterococcus spp.rectal swabs were obtained in 32 patients (24.5%) of which 11 (7%) were VRE - positive. One hundred and ninety-eight sepsis occurred: 64% were gram-positive, 34% gram-negative, 4% were other (fungus, other germs). Enterococcus infections were reported in 17 cases: 10 were due to E. Faecalis (58%), 6 of which VRE, and 7 cases were due to E. Faecium (42%), 5 of which VRE. Almost all (90%) of VRE infections in patients with acute myeloid leukaemia were those who received many cycles of chemiotherapy, with prolonged hospitalization and antibiotic therapy. Colonizations of enterococcus is associated with the possibility to have a sepsis (p<0.0001). Among the 11 cases of VRE sepsis, 9 cases studied, all of them presented the gene vanA. Enterococcus VRE sepsis were associated with neutropenia, cephalosporine (>45 g) and number of hospitalisations. Incidence of E. Faecalis VRE infections are more elevated in our experience. Also mortality due to sepsis is high reaching 50% of cases. In conclusion, in our epidemiological study, the enterococcus spp represents the second isolate (with a higher proprotion of E. Faecalis) after staphylococcus coagulase negative (38%), while in other studies it is third or fourth place. The association between carrier status and sepsis may be useful to anticipate antibiotic therapy before blood cultures have isolated the specific germ.

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ENTEROCCUS CARRIER PATIENTS AFFECTED WITH HAEMATOLOGICAL DISEASES: EPIDEMIOLOGICAL SURVEY

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Introduction. In the last few years the treatment of haematological malignancies has become more aggressive and has included several new drugs producing an improvement of therapeutic results. In the same time, an increase of hospitalization due to infections has been registered, because of immune- suppression related to a longer survival in spite of the persistence of active disease. In addition, an excessive use of antibiotics induces the emergence of antibiotic-resistant bacterial strains. In the last decade, the increased prevalence of gram positive sepsis has caused a larger use of glycopeptide antibiotics either for the treatment of microbiologically documented infections or for empiric therapy. The emergence of glycopeptide-resistant gram+ strains represents a relevant problem in haematological clinical practice. Also cephalosporin of third generation play a very important role to produce resistance to glycopeptides. The aim of the present study is to identify enterococcus carriers in patients with malignant diseases and to

recognize the cases with vancomycin resistant enterococcus (VRE) carrier status. On this background, we evaluated the prevalence of enterococcus carriers.

Methods. For this purpose rectal tampons of 153 patients (76 males and 77 females), hospitalized in 3 year period (January 2002 to January 2005), were analyzed. 77 patients were affected by acute leukaemia (7 in progressive disease), 56 by lymphoproliferative disorders, 20 by non-neoplastic hematological diseases.

Results. 36 out of 153 patients (24.5%) were enterococcus carriers;. among these 36 patients positive for enterococcus, 11 were VRE carriers (30%), corresponding to 7% of 153 patients in study. Specifically, 24 E.Faecalis (9 VRE) and 12 E.Faecium (2 VRE) were isolated. Thus, enterococcus colonization was represented by E.Faecalis for 66% and by E. Faecium for 34%. This finding is apparently different from the prevalence figures reported in the literature. A higher incidence of VRE carrier status was directly correlated with the diagnosis of acute leukaemia, with cephalosporin therapy at a dose higher than 42 g and with a number of hospital admissions higher than 4.

In addition, the statistical analysis demonstrated a correlation between the rectal tampon enterococcus positivity and the development of sepsis. Our data on prevalence are different from the literature since in our series we found 24% carriers as compared to 57% of a published experience (Kolar 2002). Moreover, VRE among enterococcus Faecium in our experience is 20% as compared to 50% in the literature (Pfaller 1997) and among enterococcus Faecalis VRE is 40% as compared to 2-3% in the literature. Since the carrier status is related to the onset of sepsis, these data are very important for the epidemiology of the disease, because VRE sepsis is associated with a high morbidity and mortality, up to 50% of patients.

Conclusions. Taking into account the high morbidity and mortality of this infection, the knowledge of the colonisation status of haematological patients may be useful to use extended prophylaxis procedures of nursing and to start earlier specific treatments with new molecules.

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FATAL CEREBRAL CYSTICERCOSIS IN PEDIATRIC PATIENT AFTER MUD ALLOGENIC TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA REPEATEDLY RELAPSED

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Introduction. Cysticercosis is the Taenia larval form which, in humans, penetrates the intestinal wall and invades subcutaneous tissue, brain, eye, muscle, heart, liver, lung and peritoneum. In Europe neurocysticercosis is a rare complication of the parasitic infection, while it is endemically present in other areas such as south-east Asia and Latin America. So far, in hematological malignancies neurocysticercosis has never been reported. Here we describe a case of pediatric patient with acute myeloid leukemia (AML) who died because of neurocysticercosis after MUD allogenic bone marrow transplantation.

Case Report. In August 2002 patient F.A., male, 11 yrs old was diagnosed with AML, FAB M4 with hyperleukocytosis and inv 16 as the only karyotypic abnormality. He received induction therapy according to the current pediatric trial (AIEOP LANL 2002) achieving the hematological remission. In August 2003 he developed a hematological relapse and obtained the second remission after FLAG chemotherapy. In December 2003 the patient showed the second hematological relapse with low bone marrow blast cells (12%) and received salvage chemotherapy according to the pediatric trial for relapse (AIEOP REC 2001), followed, in March 2004, by MUD allogeneic bone marrow transplantation. The conditioning consisted of BuCy2 schedule with the addition of thymoglobulin; methotrexate and cyclosporine were used as prophylaxis for GVHD. The engraftment of PMN > $0.100x10^9$/L occurred at day 21 and of platelets > $30x10^9$/L at day 27. In May 2003 the patient showed a reactivation of CMV and started gancyclovir therapy. In the same time the patient presented WHO grade 2 intestinal and cutaneous GVHD. On June 15th the patient was admitted for somnolence, headache and rigors, but he was awake without focal symptoms. The MRI with contrast showed several focal lesions of variable dimensions (1-6 mm) in the cerebellar cortex, in the left cerebral stalk and hemispheres which are typical lesions for cysticercosis, characterized by focal hypodensity with target morphology after contrast administration.

In the spinal fluid neither bacterial nor fungal contamination was demonstrated, glycorrhachia was 63 mg/dL, proteinorrhachia 50 mg/dL. In addition it was excluded a mycobacterial infection. ELISA immunoenzymatic text was positive for cysticercus SP antibodies at 1.98 O.D. units (normal values < 0.9 O.D. units). The patient started Albendazol (600 mg/day) therapy, but, after an apparent initial clinical improvement, the neurological conditions worsened with the occurrence of tonoclonic contractions and coma; the patient was then admitted in intensive care unit where he died after few days of irreversible coma.

Conclusions. The present case suggests that immune suppression after several lines of chemotherapy and GVHD intense prophylaxis can induce neurocysticercosis in a patient possible carrier of tapeworms. In this contest such a possibility should be taken into account in children receiving bone marrow transplantation especially in endemic areas.

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METASTATIC MUSCULAR ABSCESSES DUE TO WIDESPREAD INFECTION BY E.COLI IN ALL NEUTROPENIC PATIENT

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Bacteremia is a major cause of morbidity and mortality in patients with hematological disorders during chemotherapy-induced neutropenia. We describe a case of recurrent fever with bloodstream infection by E. coli, cellulitis and muscular metastatic abscesses. CASE REPORT: A young woman (37 y) received at hour institution a diagnosis of

high risk B-ALL with MLL/AF4 rearrangement, WBC 128.540/ul (blasts 100%), marked splenomegaly, high LDH. Hyperleucocytosis disappeared at 6th day of steroid pre-treatment (WBC 1200υ/L) and, then, induction chemotherapy with VCR, DNM and L-Ase was started (ALL GIMEMA Protocol). Oral levoxacin was used as antibacterial prophylaxis. On day +9 from chemotherapy, the patient, severely neutropenic, developped chills, hyperpirexia (T 39,4°), painful cramps on the right leg; soon, empirical antibiotic therapy with ceftazidime, teicoplanine and amikacine was started. After three days, the patient became afebrile, but myalgias, painful swelling of right leg and painful oedema on the back right hand worsened day by day; cellulitis and a large ulceration on the hand, painful deep tumefactions on the right calf developped, then fever relapsed. Multiple previous blood cultures were positive for E.coli (levoxacin resistant). I.V. antibiotic therapy was modified according to in vitro antimicrobial susceptibility tests; the new antibiotic regimen (meropenem, amikacine, metronidazole and clindamicin) + G-CSF provided progressive clinical improvement. At the recovery from neutropenia on day +34 no more chemotherapy was administered because blood cultures and pus from necrotic lesions of right hand continued to be positive for E.coli. Ultrasound scan and MRI of the right leg showed multiple deep muscolar abscesses (until 5 cm of diameter). The important cellulitis on the right hand was cured by plastic surgery. After removal of a muscular leg abscess by the orthopedic staff (resulted microbiologically sterile), the patient completed planned antileukemic treatment. In the clinical setting of the chemotherapy of the acute leukemias, the widespread bacterial infections by Gram-ve pathogenic germs are still a frequent cause of morbidity and they often require a multidisciplinary approach. Emergent Gram-ve enterobacterial strains fluoroquinolones-resistant require an innovative strategy for antimicrobial resistance control and treatment of infectious complications during the neutropenic phase.

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TOXOPLASMOSIS AFTER T-CELL-DEPLETED HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM EITHER HLA-IDENTICAL SIBLING OR ONE-HAPLOTYPE MISMATCHED DONORS

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Toxoplasma infection after allogeneic hematopoietic stem cell transplantation (HSCT) is infrequently reported and the incidence may be underestimated. Toxoplasmosis (2 pulmonary, 10 cerebral) was observed in 12/ 192 (6.2%) consecutive patients (160 acute leukemia, 22 chronic myeloid leukemia, 7 myeloma, 3 non-Hodgkin's lymphoma) we transplanted between January 1999 and January 2005. After our standard TBI-based conditioning regimen all patients received a T cell-depleted hematopoietic stem cell transplant from a HLA-identical sibling (n=44) or a mismatched family donor (n=148). None was given post-transplant immunosuppressive treatment for GvHD prophylaxis. Pre-transplant serology was positive in 7/12 cases of toxoplasma infection and negative in 5. Toxoplasmosis (1/44 matched,11/148 mismatched) occurred at a median of 48 days after transplantation (range 10-240) with fever in 11/12 patients. Other major clinical symptoms were seizures (2), motor deficits (3), somnolence or obtundation (7), headache (2), disorientation (1), cerebellar syndrome (1), personality change (1) and bilateral pneumonia (2). According to EBMT-IDWP 7 patients had probable (5 MRI of the brain, 2 CT scan of the lungs) and 5 possible CNS Toxoplasma diseases (PCR positivity at spinal fluid and/or serum). At onset, only 6/12 patients were on pneumocystis carinii prophylaxis with standard trimethoprim/sulfamethoxazole. At the median time of toxoplasma infection, the CD4+ cell counts ranged from 0 to 100 cells/mmc with a median of 10 cells/mmc. Pyrimethamine and sulfadiazine were administered for a median of 90 days (range 30 to 180). Toxoplasma infection was fatal in 3 patients (25%). Four of the 9 patients who survived Toxoplasmosis are alive disease-free 15-75 months after transplantation (median 65 months). Three died of leukemia relapse (medianly 264 days after the onset of toxoplasma infection) and 2 of overlapping infections (1 adenovirus, 1 CMV) which occurred 68 and 66 days after toxoplasmosis was diagnosed. In conclusion, an opportunistic infection with toxoplasma should be suspected in patients at risk, i.e. those who are severely immunodepressed such as T-cell-depleted transplant recipients, when they develop fever, neurological symptoms and/or pneumonia.

Chronic Lymphocytic Leukemia and Lymphoproliferative Syndromes II

P423

REPORT OF ONE CASE OF CHRONIC LYMPHOCITIC LEUKAEMIA WITH VASCULITIC MANIFESTATIONS RELAPSING AFTER RITUXIMAB THERAPY

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We present a case of CLL associated with vasculitic and thrombotic symptoms and experiencing an uncommon side effect following R therapy,consisting of a flare of vasculitic manifestations. A 63y old woman admitted with purpura, episodic dyspnea, mildly enlarged cervical nodes and spleen, had WBC 20.000/mm3,with prevalence of small mature lymphocytes(kappa Blymphocytes CD20,38,FMC7+,CD5+in nearly half of the cells, CD23,11c,22,25,10,103-),Hb13,4 gr/dL, platelets 234.000/mm^3,anti-neutrophil cytoplasmic antibodies (cANCA) 93,8 U/mL(n.v.<10 U/mL),creatinine1,5 mg/dL with 30 mL/min clearance,normal immunoglobulin level with IgM lambda monoclonal component and lambda chains in the urine 30mg/dL.Skin biopsy showed perivascular infiltrates of T-cells and neutrophils and fibrinoid necrosis of the capillary wall. At bone marrow biopsy CLL was diagnosed. FISH analysis showed a t(11;14)(q13;q32) in 80%cells. CTscan detected pulmonary embolism; LMWH and oral anticoagulant resolved the dyspnea. After 2 weeks of chlorambucil 6mg/m^2 normalization of leucocyte count and disappearance of purpura was achieved. R 375 mg/m^2/week was introduced. After the 3rd administration, notwithstanding normalization of cANCA, we observed a relapse of purpura involving the trunk, face and conjunctivae. Creatinine rose to 1,8 mg/dL. Prednisone was not active, but skin lesions and creatinine normalized after a 1st course of CVP. On the basis of FISH results, we introduced again R after the 4thCVP. After 3 days there was relapse of purpura and mild rise of creatinine, which resolved after a new course of CVP.A bone marrow biopsy showed complete CLL remission. The rare vasculitic syndromes associated to CLL are mainly due to T-cell reactive infiltrates. Presence of ANCA in CLL is described in one case with kidney and lung vasculitis.In our patient indolent CLL was accompanied by immunologic alterations and by vascular damage involving skin and probably causing kidney lesions and a thrombophilic condition. This case was particular also for a t(11;14), which in CLL not always heralds poor prognosis. R and chemotherapy, induced complete remission of CLL, but was followed by flare of vasculitic symptoms, possibly attributable to a worsening of T-cell infiltrates.

P424

ONCE WEEKLY GANCICLOVIR IS AN EFFECTIVE AND SIMPLE CMV PROPHYLAXIS IN HEAVILY PRETREATED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS DURING SUBCUTANEOUS ALEMTUZUMAB THERAPY

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Intravenous (i.v.) Alemtuzumab therapy is almost invariably associated with reactions, sometimes of severe grade, or intercurrent infections, which proved to discourage the routinely use. We explored the efficacy/safety of subcutaneous (s.c.) Alemtuzumab in 8 heavily pre-treated chronic lymphocytic leukemia (CLL) patients and the role of i.v. ganciclovir 7.5 mg/kg once a week as prophylaxis of CMV infection, comparing to oral acyclovir.

Methods. Alemtuzumab was given as s.c. dose escalated from 3 mg up to 30 mg thrice a week for up to 12 weeks followed by 30 mg weekly for 4 weeks. All patients had been previously treated with at least two lines of chemotherapy including fludarabine, chlorambucil at standard (8 cases) or high doses (3 cases). Starting from February 2003, 8 patients (5 males, median age of 63, range 49-64) with relapsed (5 cases)/not responder (3 cases) CLL were treated. Two patients performed the protocol for two times for progressive disease after initial PR. Thus, response to alemtuzumab is based on 8 cases but toxicity data, CMV prophylaxis, CMV reactivation are assessed on 10 cases. At baseline, all patients had negative CMV antigenemia. Prophylaxis with oral acyclovir 800 mg tid (in the early 6 patients enrolled in the trial) or i.v. ganciclovir 7.5mg/kg once a week (in the following 4 cases) was given during therapy and for 1 month after alemtuzumab therapy.

Results. Two patients achieved a CR, two a SD, five a PR and one progressed. The two CR patients were treated with 10micrograms/kilograms/die G-CSF following the last dose of Alemtuzumab to obtain a PBSC mobilization. The harvest was successful and they were transplantated without complications and rapid haemopoietic engraftment and are still in CR. With a median follow-up of 21 months, 2 patients are in CR, 1 PR, 1 SD, 2 died and 2 alive with progressive disease. Alemtuzumab-infusion-related reactions occurred in virtually all patients but were manageable (grade 1-2). Grade 3 neutropenia occurred in 7 cases, requiring G-CSF; transient fever in 4 cases. CMV reactivations were observed in 5 cases; 4 in the acyclovir group (6 patients) and one in the weekly ganciclovir prophilaxis setting (4 cases). Alemtuzumab was discontinued in all patients with CMV reactivation and the patients were treated immediately with i.v. ganciclovir 7.5 mg/kg/day. Only one patient required hospitalization for fever. After a median of 15 days of daily ganciclovir therapy all patients had achieved negative CMV antigenemia. Weekly s.c. 7.5 mg/kg ganciclovir prophylaxis/pre-emptive therapy and alemtuzumab treatment were resumed and no further CMV reactivations were observed.

Conclusions. In conclusion, weekly i.v. ganciclovir is effective and safe as CMV prophilaxis in CLL patients during alemtuzumab administration, allowing an easy management of a therapy previously difficult to be routinely used.

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P425

PURGING *IN VIVO* WITH CAMPATH-1H AND AUTOLOGOUS TRANSPLANTATION IN CHRONIC LYMPHOCYTIC LEUKEMIA NON RESPONDING TO FLUDARABINE: A CASE REPORT

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Recent observation suggest that monoclonal antibodies can have might a role in lymphoprolyferative disorders, the prognosis for not responder patients at fludarabine is poor, and high dose chemotherapy with autologous bone marrow transplantation can check better and for a longer time the disease.

Case report. 48 years-old man with diagnosis of chronic lymphocytic leukaemia (CLL) stage IV B in November 2001. Has received 6 cycles of fludarabine e.v. getting a haematological partial remission but not nodal remission. The patient has received other two cycles of chemotherapy and in December 2002 therapy with Campath-1H (30 mg s.c. for 3 days the week for a total dose of 313 mg) has started. Medullary immunophenotype before campath it showed an infiltration (cells CD5/CD19^{+}) of 8%, at after therapy with Campath such marrow infiltration was 1,9%, but smaller nodal response has been. In March 2003 the patient received therapy with high dose ciclophosphamide (7 g/m^2) and G-CSF for mobilization CSP. The patient in the week mobilization has received other 90 mg of Campath and with 2 leucoapheresis picks up 5x10^6 of CD34/Kg, harvest has showed a 0.5% infiltration and lymphomegalies were completely regress. In April 2003 the patient has been conditioned according to BEAM scheme and has been infused with 3x10^6 CD34/Kg. From day +1 at +5 the patient has received other 90 mg of campath s.c. has not been late effect during hospitalisation and haematological recovery (n>500 and Plts> 20000) has been documented in the day + 16. At day +30 medullary control has showed complete remission and not present lymphomegalies. Hematologic recovery in the patient has been slow (at day + 60 Plts: 28000) and has needed blood support (1 blood bag weekly). Infectious episodes are not documented and continuous prophylaxis with Bactrim, itraconazolo and valaciclovir has effected. At +24 months after transplantation the patient is in CCR and medullary infiltration (CD19/CD5+) is 0%, and TC not lymphomegalies here observed. In conclusion, high dose therapy with Campath-1h for purging *in vivo* has been a very good treatment for complete remission in a patient with CLL to poor prognosis. Currently the patient is in continuous complete remission after two years post-transplantation. This treatment scheme could be useful in first line treatment in patients with poor prognosis.

P426

CONVENTIONAL AND MOLECULAR CYTOGENETICS ANALYSIS IN B-CLL PATIENTS: CORRELATION WITH ZAP-70 AND CD38 EXPRESSION AND IGVH MUTATIONAL STATUS

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Major advances have been made in the biology and treatment of CLL in the last decade. The prognosis and clinical course are eterogeneous even in those patients with RAI or Binet early stages. Genetic abnormalities may contribute, together with other diagnostic features, to a risk assessment for individual patients, thus giving the opportunity for a risk-adapted management. Particularly fluorescence in situ hybridization (FISH) provides a more reliable assessment of the incidence of all chromosomal abnormalities, because of the low proliferative index of CLL. In addition ZAP-70, a cytoplasmic protein tyrosine kinase normally expressed in normal T and NK cells, is preferentially expressed in the group of CLL patients with unmutated IgHV genes and correlates with an aggressive disease. We studied 37 B-CLL patients with conventional cytogenetics analysis (CCA) and FISH and correlate results with IGHV mutational status, ZAP-70 and CD38 expression. Sixteen (43%) of 37 patients studied were in RAI stage 0, 12 (32%) were in stage I-II and 11(45%) were in stage III-IV. Fifteen patients (40%) were at diagnosis. CCA was performed on stimulated peripheral blood lymphocytes, cultured for 72h at 37C in RPMI medium supplemented with 15% of fetal calf serum. FISH was done according to manufacturer instructions utilising commercially available probes: utilizing the D13S319 locus (LSI D13S319, Vysis),for the 13q14 region, one for chromosome-12-specific α-satellite DNA probe (CEP12,Vysis), one for the P53 gene at 17p11(LSI P53, Vysis), LSI ATM for 11q22-23, and one probe for 3'-5'IgH at 14q32. Two hundred interphase nuclei from patients and controls were analyzed and scored for each probe by three different observers. The cut-off for positivity (mean of normal control) was determined for each probe on 5 PB samples from normal individuals. ZAP-70 was measured with a direct immunofluorescence technique by flow cytometry (FACSCAN, Becton Dickinson) in CD19+ gated cells using a cut off of 20%.Tumor VH gene sequence and mutational status was assessed by RT-PCR. CD38 was considered positive if present in more then 15% of the cells.

CCA: 17/37 (45%) had valuable metaphases; 13 had a normal karyotype (NKa) and 4 patients had a complex karyotype (3 or more abnormalities) (AKa) involving chromosomes 6q, 11q22, 17p11 or 12.

FISH analysis: 28/37 patients (76%) had a genomic abnormality revealed by FISH. In particular we observed del (13q) in 10/37 (27%); del (17p) in 10/37 (27%), +12 in 5/37 (13%), del 11q in 3/37 (9%) in a median of 55% of the cells (range 10-100%). 7 patients had more than one abnormality simultaneously. We considered as relevant the abnormality carrying the worse prognosis.

IGVH status, ZAP- 70 and CD38: 13/19 (68%) patients

had an unmutated IGHV status; 20/28 (71%) patients tested showed ZAP positivity, while CD38 was positive in 12/30 (40%) patients.

Correlations of CCA and genomic aberrations with IGVH mutational status ZAP-70 and CD38: we did not find at CCA any particular correlation with ZAP positivity or VH genes mutational status, while we found that all 4/4 (100%) patients with AKa had an aggressive disease. Twenty of 27 patients (75%) with a FISH abnormality showed progression, while only 3/9 (33%) without FISH abnormalities progressed. In particular 8/10 (80%) patients with del 17p progressed, 8/8 (100%) of patients with 11q, and +12 progressed, while only 1/10 (10%) with del 13q showed an aggressive disease. Importantly, there was a strong correlation between patients having FISH abnormalities, ZAP positivity, and VH unmutated status (76%, 80%, 70%, respectively). In addition interestingly the sole patient carrying a del 13q and those 3 patients without FISH abnormalities that had clinical progression showed ZAP positivity. In conclusion we found a clear correlation between patients carrying del 11q, del 17p and +12 by FISH, ZAP positivity and VH unmutated status. Larger studies are warranted to better define risk-assessment in CLL patients and to identify those patients that could have a more aggressive disease and benefit from early treatment.

P427

THREE CASES OF BICLONAL CHRONIC LYMPHOCYTIC LEUKEMIA: RELEVANCE FOR CLINICAL PRACTICE AND PATHOPHYSIOLOGICAL IMPLICATIONS.

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Clonality is classically considered a sine-qua-non condition for the diagnosis of chronic lymphocytic leukemia (CLL) and other lymphoproliferative disorders (LPD); it represents the most important argument for the differential diagnosis of lymphocytoses. Clonality assessment may be performed by different techniques, such as flow cytometry (FC), karyotype analysis and molecular biology, alone or in combination. Flow cytometry is usually the standard screening method, and is based on the demonstration of a lymphocyte population with a particular surface molecule expression pattern, or with unbalanced expression of surface immunoglobulin light chain (SIg-L).

Here we present three cases where routine FC failed in demonstrating clonality, and additional tests were needed for an appropriate diagnosis. These additional studied included: (i). larger immunophenotype analysis by multiparameter FC; (ii). molecular analysis of the Ig-heavy chain (IgH) locus. This latter study was performed by PCR amplification of VDJ and DJ rearrangements, using specific VH or DH primers against a JH consensus; clonality of the PCR amplicons was assessed by heteroduplex analysis performed by denaturing-high performance liquid chromatography (D-HPLC). Molecular analysis was done on both total lymphocytes and cell fractions enriched/depleted of lymphocytes expressing a single light chain, obtained using a magnetic microbeads technology. The first patients was a 84 year-old man with anemia and moderate lymphocytosis in peripheral blood (4,000/uL), with no kappa/lambda restriction by FC; however, given a CD5/CD19 co-expression and a massive marrow infiltration by these B-cell, the patient received diagnosis of CLL. Molecular analysis of the IgH locus documented two complete VDJ (VH1/VH7 and VH4/VH6) and two incomplete (DH2 and DH7) rearrangements. When the same analysis was performed on separated SIg-kappa and SIg-lambda lymphocytes, the presence of two distinct clones was demonstrated: the SIg-kappa+ harbored a VH1/VH7 and a DH7 rearrangement, while the SIg-lambda+ utilized VH4/VH6 and DH2. The second patient was a 63 year-old female with an absolute lymphocytosis (21,000/uL) and mild liver and spleen enlargement; again FC showed a typical CLL population (CD5+/CD19+), with no restricted usage if SIg-light chains. The molecular study demonstrated multiple complete and incomplete rearrangements, consistant with the presence of at least two distinct pathological clones, as confirmed by analysis on lymphocyte subpopulations. The last patient was a 60 year-old male, with a moderate CD5- lymphocytosis and significant organ involvement; FC did not show unbalanced kappa/lambda surface expression, and molecular biology revealed an oligoclonal pattern, with at least two distinct clones. Interestingly, after multiple courses of chlorambucyl performed to contain the lymphocytosis, FC analysis showed the predominance of the SIg-kappa+ population; at the same time, molecular analysis documented an unique rearrangement, suggesting a shift from an oligo-biclonal to a more typical monoclonal LPD. All the three patients reported harbored at least two distinct clonal B cell populations, both neoplastic. The biology of Ig rearrangement excludes that this phenomenon is due to a clonal evolution of one original mature B-cell tumor or to a leukemic transformation of an immature B-cell progenitor; rather it raises the idea of two genetically independent oncogenic events, eventually playing in the context of a propensity to develop clonal B-cell expansions, derived from antigenic triggering or impaired T-cell surveillance. In conclusion, we have documented that the occurrence of biclonal lymphoproliferative disorders is possible and possibly underestimated (since two clones harboring the same light chain can be overlooked). Patients with features of LPD should be studied in details even if routine FC fails in demonstrating clonality by SIg-light chain restriction. In these cases, molecular analysis is mandatory for a correct differential diagnosis; on the other hand, the presence of multiple neoplastic clones may raise intriguing issues on the pathogenesis of LPD.

P428

ABERRANT EXPRESSION OF TRAIL IN B CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Chronic lymphocytic leukemia (CLL) is a quintessential example of human malignancies that are caused primarily by defect in apoptosis, as quiescent G(0) phase CD19/5+ lymphocytes accumulate. Defects in apoptotic pathways contribute also to chemoresistance and can promote resistance to cellular immune responses. TNF-related apoptosis inducing ligand (TRAIL), a member of the TNF family of cytokines, interacts with four high affinity membrane receptors (TRAIL-R1 – R4): R1 and R2 are thought to transducer apoptotic signals, while R3 and R4 have been proposed to act as "decoy" receptors, protecting cells from apoptosis. Despite its potential anti-cancer activity, TRAIL alone has a low/moderate cytotoxic activity on B-CLL, and no data are available on the expression of TRAIL and its biological potential function in B-CLL.

We have examined the expression of TRAIL in peripheral blood (>85% CD19+/CD5+) B lymphocytes obtained from 44 patients affected by CLL at diagnosis, the susceptibility of B-CLL cells to recombinant TRAIL and the role of endogenous membrane-bound TRAIL on autologous cell survival.

Surface TRAIL was expressed in all 44 B-CLL samples, at variable intensity but at higher levels with respect to normal unfractionated lymphocytes and purified CD19+ B cells obtained from 15 normal blood donors. Of note, in a subset of B-CLL samples, the addition to B-CLL cultures of a TRAIL-R1-Fc chimera, which binds at high affinity to surface TRAIL, significantly decreased the percentage of viable cells with respect to untreated control B-CLL cells, suggesting that surface TRAIL may play an unexpected role in promoting B-CLL cell survival.

In spite the majority of B-CLL lymphocytes expressed variable surface levels of "death" receptors TRAIL-R1 and TRAIL-R2, the addition in culture of recombinant TRAIL increased (>20% versus controls) the degree of spontaneous apoptosis in only 11/44 of the B-CLL samples, had no effect in 19/44, while it significantly increased leukemic cell survival in 14/44. The different apoptotic/survival response to recombinant TRAIL among the three groups did not depend on significant difference in "death" or "decoy" receptor patterns of expression. A progressive increase of surface TRAIL expression (reported as MFI) was noticed from group 1 (the B-CLL samples susceptible to TRAIL cytotoxicity) to group 3 (the samples showing a pro-survival response to TRAIL).

Taken together, these findings suggest that an aberrant expression of TRAIL might contribute to the pathogenesis of B-CLL by promoting the survival in a subset of B-CLL cells.

P429

B-CLL AND CAPSAICIN: APOPTOTIC EFFECT IN LYMPHOCYTES AND GRANULOCYTES

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Introduction. Capsaicin is the active content of the dried mature fruit of Capsicum (Solanaceae): this substance carries on its pharmacological actions either interacting with specific receptors called vanilloid or by mechanisms indipendent of the activation of vanilloid receptors. The fact that capsaicin can lead apoptosis in B-lymphocytes induced us to verify its action on healthy subjects lymphocytes and on lymphocytes of B-cronic lymphoid leukaemia (B-CLL) patients, in which there is their progressive accumulation because of a maturative stop of cell cycle in G0-G1 phase with prolonged survival owing to an apoptosis inibition, in consequence of an expression increase of bcl2 gene. We proceeded to evaluation of the eventual apoptotic effect of capsaicin in different concentrations in leukocytes (lymphocytes and granulocytes) of blood donors healthy subjects and in leukocytes of B-CLL patients.

Methods. 5 patients, 2 men and 3 women, 55-75 years old, affected by B-CLL, and 5 blood donors healthy subjects, 3 men and 2 women, in variable age, have been examined. We proceeded to leukocytic isolation from 3 milliliters blood samples in EDTA of each examinated subject by erythrocytic lysis with NH4Cl and centrifugation at 1200 rpm per 7 minutes. The obtained pellet has been suspended in PBS 100 microliters and stained with capsaicin 10 microliters (Sigma) reconstituited in DMSO at 10^{2} M and 10^{3} M concentrations. Finally the effect of capsaicin has been verify by propidium iodure and annexin V-FITC in flow cytometry after three different incubation stages at the dark: after 6, 12 and 24 hours.

Results. There are two experiences led staining leukocytes of examinated subjects with two different concentrations of drug: the first experience underlines that 10(e)-2 M capsaicin is an apoptotic inductor in normal subjects and treated leukaemic patients lymphocytes with a different profile (Figure 1).

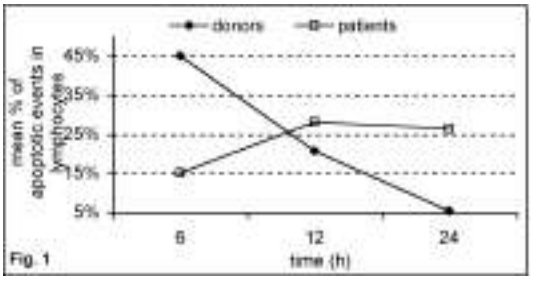

Fig. 1

The second experience, in which we used capsaicin at a

lower concentration (10^3M), underlines that the drug doesn't induce apoptosis in normal subjects and patients lymphocytes, but in granulocytes of all the examined subjects with a different profile: apoptosis percentage increases more in patients than in blood donors with the incubation time (Figure 2).

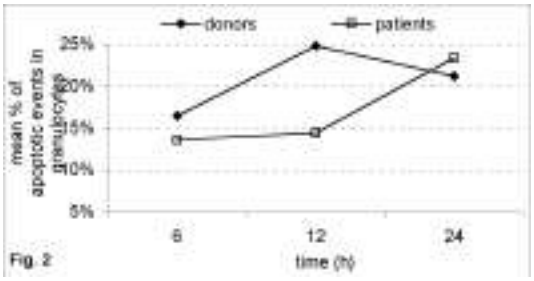

Fig. 2

Comparision of mean percentages of leukocytic apoptotic events after assay with 10^2 M (Figure 1) and 10^3 M (Figure 2) capsaicin:

Discussion Obtained data are in accordance with what found in literature about LMC patients. The molecular mechanism at the basis of this process isn't explained yet: it's possible to suppose that the principal effects induced by capsaicin are an increase of intracellular levels of O2 reactive species and phosphorilation of p53 protein in Ser-15 residue. The different apoptotic profile observed in the two examinated subjects groups (blood donors and B-CLL patients) can find a possible explanation in the diverse background in that healthy donors granulocytes are situated in comparision with those of patients, in which these cellular elements would be more susceptible to the action of the drug. The comforting finding emerging from this experience is the significant percentage of apoptosis induced by capsaicin in leukaemic cells at 10^2 M concentration and in neutrophil granulocytes at 10^3 M concentration, that could prelude a development of vanilloid receptors agonists (even more selective), in view of an antileukaemic therapy.

P430

HETEROGENEITY OF GENE EXPRESSION PROFILING IN CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL) is the most prevalent adult leukemia in Western countries, accounting for about 25% of all leukemias. CLL results from clonal accumulation of long-lived mature looking B lymphocytes characterized in the immunophenotype by positive CD19, CD5 and CD23 antigens, low or absent CD22, FMC7, CD79b and surface immunoglobulin (IgM and IgD). The relentless accumulation of this small mature B-cells, which have escaped programmed cell death and have undergone cell cycle arrest in the G0 phase, is the hallmark of B-CLL. Despite the apparent uniformity of the leukemic cell phenotype, there is considerable heterogeneity in clinical outcome in individual patients, some having an indolent disease that never requires therapy, whereas others showing a rapidly progressive disease requiring prompt treatment. B-CLL cells have been shown to have somatically mutated immunoglobulin variable region genes in at least half of the cases. Thus, B-CLL patients can be divided into 2 subgroups according to the mutational status of the immunoglobulin VH genes. The mutated CLL cases have a more favourable prognosis and require less treatment than the unmutated ones. Here, we report large scale gene expression analysis of 29 well-characterized B-CLL patients using Agilent Human 1A Oligo Microarray comprising 20,173 (60-mer) experimentally validated oligonucleotide probes. The status of immunoglobulin genes was assessed analysing the nucleotide sequence of rearranged heavy chain variable region (IgVH) obtained by direct sequencing. 14 of 29 patients harboured somatic mutation in immunoglobulin VH gene. Mutational status well correlated with progression-free survival showing a rapid disease evolution in patients with unmutated VH genes.

Resultant microarray profiles were first analysed using an unsupervised, agglomerative hierarchical clustering with average link as heuristic criteria and correlation with mean subtraction as similarity metric to discover the mutual relationships among B-CLL cases on the basis of the gene expression pattern. The resultant matrix view clearly evidenced the high grade of heterogeneity in gene expression in this form of leukemia that can be divided in two clusters (designed I and II), comprising 16 and 13 patients. Clustering to the first group was mainly due to high expression of a large group of genes comprising ribosomal and other translational-associated genes, as well as large number of genes involved in mitochondrial respiratory chain and ubiquitin-proteasome system. Importantly, some genes involved in apoptosis regulation as FKSG2, PDCD6 and TPT1 were over-expressed in this group I . Moreover, to identify those genes distinguishing the mutated leukemic cells from unmutated ones, we performed a supervised analysis. 82 genes, significantly different among classes based on t-tests, were selected. Among genes up-regulated in unmutated group, we found LPL, FLNA, ADAM29, ZAP70 and CASP3. Our data reveal a clear gene expression heterogeneity among B-CLL patients that can be divided in two main subtypes independently of immunoglobulin mutational status. Furthermore, our results suggest a role of translation-associated genes, mitochondrial respiratory chain and ubiquitin-proteasome system in the pathogenesis of B-CLL.

P431

SEQUENTIAL HIGH-DOSE THERAPY AND ALEMTUZUMAB (CAMPATH-IH) AS PURGING *IN VIVO* IN HIGH RISK CHRONIC LYMPHOCYTIC LEUKEMIA; AN ONGOING REPORT

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Background. High risk CLL exerts a mean survival of about 6 to 8 years in affected patients with conventional therapies. In relatively young patients (< to 65 years) this couldn't be acceptable and new and possibly safe and more effective approaches are warranted.

Patients and methods. In order to value the efficacy of a sequential and more aggressive approach to high risk CLL we investigated a combination of chemotherapy, specific immunotherapy and high dose therapy sequentially given. A first phase included CHOP classic chemotherapy, four cycles. A II phase was devoted to mobilise CD34 positive cells with two administrations of Cytarabine at 8gr/sqm over two days in 4 doses followed by Alemtuzumab at 30 mg x 2 at each course and then collection of CD34 cells. The final phase included the administration of BEAM scheme as conditioning regimen to autologous reinfusion of CD34 cells. Evaluation of purging efficiency was done at each phase of therapy and at the end of the entire procedure by morphology, immunophenotype and molecular biology. In an ongoing study 7 patients, stage III to IV of RAI classification, of 50 to 70 years old, are now under treatment and 6 of them completed the procedure while one is under therapy.

Results. After the first phase of therapy all patients had a residual disease detectable by morphology. After the second phase with high-dose Cytarabine and Alemtuzumab, of six valuable patients 3 had residual disease detectable at immunophenotype, 2 had morphology positive and one is in molecular CR. One patient did not mobilise an acceptable number of CD34 positive cells. At the end of therapy, of the 5 valuable patients 2 had molecular CR, 2 immunophenotypic CR and 1 with residual disease detected at the immunophenotype. At the follow-up 3 patients remain in CR, one is still in PR and one showed progressive disease. Concerning side effects we recorded a Gram-sepsis during citopenia following high-dose therapy and autologous CD34 transplantation.

Considerations. The procedure including sequential high-dose chemotherapy and *in vivo* purging with Alemtuzumab is feasible with acceptable toxicity in patients with CLL of age up to 70 years. The efficacy of the procedure is almost good in all patients although a precise definition of response and duration is under evaluation.

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PROGNOSTIC SIGNIFICANCE OF IMMUNOPHENOTYPE IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Recently, several studies have been developed to identify Chronic Lymphocytic Leukaemia subtypes with different prognosis. Prognostic factors avaible to help predict the outcome of patients with B-CLL include clinical stage, lymphocyte doubling time, cytogenetic abnormalities immunoglobulin heavy chain variable region mutation and, in particular cellular phenotyping. Recent reports indicate immunoglobulin mutational status and CD38 expression level as new prognostic markers. However, whether any of these assays would act as indicators for progression of early stage disease remains to evaluate. In a search for markers that could define biological activity of different B-CLL subsets we investigated 75 patients with typical CLL ($CD5^+$, $CD23^+$, $CD19^+$). Patients were staged according to Rai and fourty one were at 0-1 stage, twenty six at II and eight at III. We analysed also CD38, CD20, CD10, CD14, CD25, CD11c antigens with direct fluorescence (Becton Dickinson). The fluorescence was detected with flow cytometry Partec Pas IV after calibration with fluorescent microspheres (Dako Fluorospheres). All B-CLL lymphocytes were CD10 and CD14 negative. CD20, CD11c and CD25 were positive in some patients but often they were not coexpressed and not correlated with the stage and the disease progression or aggressivity. CD38 antigen was positive (>25%) in 16 patients (10 at I Rai's stage, 5 at II and 1 at III). Six patients presented a coexpression of CD20 and CD38 antigen and one of them presented also CD11c antigen all with a bright fluorescence; this was a patient at I stage with a rapid outcome (eight months) to III stage. Our preliminar results show that different subtypes of B-CLL, with a different prognosis and outcome, can be demonstrated by differentia coexpression of a variety of surface markers.

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CYTOFLUORIMETRIC ANALYSIS OF ZAP-70 IN B LYMPHOCYTES OF B-CLL: CORRELATION WITH MUTATIONAL STATUS OF VH GENES AND PROGRESSION OF THE DISEASE

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ZAP-70 is a Syk family protein tyrosine kinase expressed in normal T and NK cells and it is a critical enzyme required for successful T lymphocyte activation. Following antigenic stimulation ZAP-70 rapidly associates with CD3 complex and zeta-homodimer of the T cell receptor and contributes to the activation of multiple biochemical pathways leading to cell differentiation and proliferation. Recently, it has been demonstrated that ZAP-70 partecipates in early B-cell differentiation in animal models, in addition it has been identified also in B neoplastic cells from Chronic Lymphocytic Leukemia (B-CLL) frequently correlated with unmutated IgVH genes and with aggressive disease. In previous

studies in B-CLL ZAP-70 has been detected using different techniques with different cut-off percentage of positivity, meaning a wide range of posive cases in literature. In our study we analyzed the presence of ZAP-70 in B cells of 47 patients with B-CLL using two different approaches, a direct immunofluorescence technique by flow cytometry (FACSCAN, Becton Dickinson) and a standard Western blotting procedure. We measured ZAP-70 in both fresh and previously frozen cells obtaining concordant results. Briefly, to detect ZAP-70 protein by flow cytometry, mononuclear cells, obtained from peripheral blood by ficoll gradient centrifugation, were stained with CD3 PerCP and CD19 PE and then were fixed with paraformaldehyde 0,5% for 10 minutes at 4°C. Subsequently the samples were washed twice with 2% BSA, 0,05% Tween 20 in physiologic solution and than incubated 1 h at 4 °C with ZAP-70 FITC–conjugated antibody (Upstate Biotechnology) or isotipic control in presence of 50 µL of 0,02% saponin. In all cases ZAP-70 expression was evaluated in $CD19^+$ gated cells using the cut-off of 20%, because this represents the mean value detected in CD19+ B cells obtained from healthy donors. To assess ZAP-70 protein biochemically, CD19+ B cells from 34/47 B-CLL patients were purified using a CD19 magnetic beads system (purity greater than 95%) and analyzed by SDS-PAGE and ZAP-70 immunoblotting. The results obtained by the two methods were 100% concordant, however flow cytometry analysis resulted to be more useful to measure ZAP-70 protein in samples containing different cellular populations, and so more applicable in the clinical routine. Forty seven B-CLL patients entered the study, 20/47 patients were males and 20 females, 19/47 of them were at the onset of the disease, 15/47 were on untreated follow-up and the remaining 13 were previously treated; 15/47 (32%) were stage 0, 11/47 (23%) were stage I, 11/47 (23%) were stage II and III, 10/47 (22%) were stage IV. Overall results showed that ZAP-70 was positive in 33/47 (70%) cases with a lower frequency (52%)in patients at the onset of the disease. The median percentage of positive cells was 51% (range 22%-94%)and no difference among the different clinical stages was recorded: in fact, 8/33 (24%) ZAP-70+ case were on stage 0, 7/33 (21%) were on stage I, 9/33 (27%) were on stage II-III and 9/33 (27%) were on stage IV, while most (50%) of B-CLL ZAP-70- were on stage 0. As expected, a significant correlation with presence of ZAP-70 and mutational status of VH immunoglobulin genes was recorded, in fact 10/12 (84%) ZAP-70+ positive cases showed unmutated VH immunoglobulin genes, while in 4/4 ZAP-70- cases VH immunoglobulin genes were mutated. Moreover, our results demonstrated a significant correlation between presence of ZAP-70 protein and expression of CD38 antigen on B cells, in fact CD38 was found in 20/28 (71%) ZAP-70+ , while only 3/14 (21%) ZAP-70- cases expressed CD38 antigen (p=0,0032).ZAP-70 positivity significantly correlate with progression disease, in fact progression of disease was recorded in 5/14 (21%) ZAP- cases and 22/33(67%) ZAP+ cases (p=0,049). In conclusion, our results confirm the correlation of ZAP-70 expression with VH mutational status in B-CLL, and its usefulness as a prognostic marker of disease outcome. Full concordancy of Western blotting and flow cytometry strengthen the reliability of the analysis and offers two different methods for ZAP-70 evaluation. In addition, strong correlation with stage and outcome makes ZAP-70 expression a powerfull and a simple laboratory tool to predict outcome in B-CLL.

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SERUM SYNDECAN-1 IS NOT ELEVATED IN PATIENTS WITH EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA BUT IS STILL A PROGNOSTIC FACTORS FOR DISEASE-PROGRESSION

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Syndecan-1 is a transmembrane proteoglycan generally not expressed in mature B-cell neoplasias like chronic lymphocytic leukemia (CLL). Given the syndecan-1 relevance in cell-microenvironment interactions we measured its concentrations in serum drawn at the time of diagnosis from 67 B-cell CLL patients (Binet stage A, 46; stage B, 7; stage C, 14). To this purpose a syndecan-1 enzyme-linked immunosorbent assay (ELISA, Diaclone, Besancon, France) was used. Although detectable levels were found in all patients, serum concentrations of syndecan-1 were significantly lower in CLL patients in comparison to age- and sex-matched controls (p=0.02; Mann-Whitney test). As far as clinico-hematological variables reflecting tumor mass are concerned, a significant correlation was found only with absolute peripheral blood lymphocytosis (PBL) (p=0.01) and LDH (p=0.05). Serum levels of syndecan-1 did not parallel those of several angiogenic cytokines such as VEGF (p=0.963), FGF-2 (p=0.216), angiogenin (p=0.478), MMP-9 (p=0.125) as well as bone marrow (BM) microvessel density (p=0.110). The same applied with adhesion molecules such as ICAM-1 (p=0.233), V-CAM1 (p=0.799), CD44 (p=0.816) and PECAM-1 (p=0.508). Interestingly, the inverse correlation (r=-0.539; p=0.01) between serum concentrations of syndecan-1 and plasma levels of stromal derived growth factor-1 (SDF-1) is in keeping with the different function, respectively pro- and anti-apoptotic, of these molecules. In 46 Binet stage A patients serum levels of syndecan-1 were further evaluated as a dicothomous variable with respect to progression-free survival (PFS), an end-point surrogate for overall survival in early B-cell CLL. The best separation of curves was seen with a cut-off point at the median value of syndecan-1 (i.e., 36.5 pg/mL). Median PFS was not reached in the patient group with low syndecan-1, compared to a median of 34 months observed in the remaining patients (p=0.01; HR=0.208; 95% CI= 0.115-0.816). At the multivariate analysis performed including variables significant in the univariate analysis [i.e., PBL (p=0.03) and syndecan-1 (p=0.01)], only a trend for syndecan-1 (p=0.08) was retained. Despite the pro-angiogenic activity of syndecan-1 which mediates FGF-2 binding and activity no correlation with either angiogenic cytokines or the extent of BM angiogenesis was found in CLL. The inverse correlation with plasma levels of SDF-1 suggests an involvement in the processes leading to apoptosis. Finally, our results highlight the involvement of syndecan-1 in the mechanisms of disease-progression of early CLL.

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DEATH-ASSOCIATED PROTEIN KINASE PROMOTER METHYLATION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. A PRELIMINARY STUDY BY USING MS-PCR AND SEMI-QUANTITATIVE CAPILLARY ELECTROPHORESIS

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The expression of the Death-associated protein kinase (DAPK), a pro-apoptotic serine/threonine kinase located on chromosome 9q34.1, is frequently lost in human tumors. DAPK counteracts oncogene-induced transformation by activating p53 in a p19ARF/p53-dependent apoptotic checkpoint. Moreover, DAPK partecipates in the execution of IFNgamma-induced cell death and TNFalpha and Fas-induced apoptosis. Analysis of the methylation status of promoter DAPK's 5' untranslated region (UTR) in DNA showed a high frequancy of methylation in several human carcinomas and B cell malignancies. Aberrant methylation of gene promoter regions represents the most widely studied epigenetic abnormality in human malignancies. This event is associated with the loss of tumor suppressor gene function and it acts as an alternative to mutations and deletions. The aim of this preliminary study was to determine the methylation patterns of the gene promoter region of DAPK in circulating cells of patients with B-CLL. Moreover, we tried to explore a possibile correlation between aberrant methylation and immunoglobulin heavy-chain variable region (VH) mutational status. Up to now, 35 B-CLL cases were studied. We employed MS-PCR (Methylation specific polymerase chain reaction) where the 98 bp sequences upstream of the traslation start site were targeted for methylation analysis (bp 5-102 of X76104). DNA methylation patterns in the CpG island of DAPK were determined by standardizated chemical treatment with sodium bisulfite and subsequent PCR with methylation specific primers. The primers specific for unmetilated and metilated alleles were togheter present in the same PCR, but coniugated with a different fluorocrome (Fam and Tet). After capillary electrophoresis of the PCR products on an automatic sequencer (ABI Prism 310), the ratio of the underpeaks areas of methylated/unmethylated alleles were determined. Choosing as cut-off value 0.25, corrisponding to 25% of methylated alleles, the gene promoter region of DAPK was found to be methylated in 11 B-CLL cases (31.4%). Notably, 84.6% (11/13) of muted B-CLL cases clustered in the group of unmetylated DAPK cases, while 40.9% of VH unmutated cases are metylated (p=0.1). In conclusion, these data, although very preliminary, show as the VH unmutated B-CLL cases could further dissect into two groups, one with a methylated and the other with an unmethylated DAPK promoter. Analysis on a higher number of cases is in progress, with the aim to both confirm these preliminary results and explore a possible different clinical outcome between metylated and unmetylated B-CLL cases.

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CORRELATION OF GENOMIC ABERRATIONS WITH THE MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION, ZAP-70 AND CD38 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chromosomal abnormalities have been detected in over 80% of chronic lymphocytic leukaemia (CLL) patients by fluorescence *in-situ* hybridization (FISH). We analysed the most common chromosomal abnormalities associated with CLL using a panel of probe specific for 13q14, 11q22, 17p13 deletions and for the detection of chromosome 12 trisomy. Several studies reported the importance of the prognostic impact of these abnormalities in CLL: del(13q14) is the most favourable, followed by no detectable abnormalities and by chromosome 12 trisomy, while 11q22 and 17p13 deletions are associated with a dismal outcome. These evidences lead to the proposal of stratifying patients into low (del(13) and normal), intermediate (chromosome 12 trisomy) and high (17p13 and 11q22 deletions) risk. In order to gain insights into the role of biological prognostic indicators, we investigated by FISH the occurrence of such chromosomal abnormalities in a representative panel of 58 CLL patients and correlated them with the levels of CD38 (cut off value 30%) and Zap-70 expression (strong: 40%-100% positive cells; weak: 20%-40% and negative: <20% of positive cells) and the mutational status of immunoglobulin heavy-chain variable region (VH) (samples in which <2% of base pairs differed from those of the consensus sequence were considered unmutated). The FISH analyses revealed that 34 of the 58 patients (58.6%) showed one or more abnormalities. In particular, 13q14 deletion was found in 11 (19%), 11q22 deletion in 5 (8.6%), chromosome 12 trisomy in 7 (12.1%) and 17p13 deletion in 11 (19%) cases. All the 11 cases showing 13q14 deletion (low risk group) were CD38 negative, while only 6 had a VH mutated status and Zap-70 either negative or weak. Notably, among the 15 cases with deletion of either 17p13 or 11q22 (high risk group), strong expression of Zap-70 was observed in 10 patients (66.7%), unmutated VH in 11 (73.3%) and CD38 expression in 8 (53.3%). Finally, among the 19 cases with no detectable lesion, 52.6% were CD38 negative, 47.9% showed a mutated status of VH and 38.9% Zap-70 a negative or weak expression. Our preliminary analysis suggests that the molecular cytogenetic could represent an independent indicator of clinical outcome in CLL patients.

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REVERSAL OF BONE MARROW ANGIOGENESIS IN CHRONIC LYMPHOCYTIC LEUKEMIA FOLLOWING FLUDARABINE THERAPY

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We evaluated bone marrow (BM) microvessel density (MVD) in 12 Binet stage B patients at diagnosis and following at least 4 courses (median, 8; range, 4-12) of conventional chemotherapy [fludarabine (F) , n=7; or intermittent chlorambucil (CLB) + prednisone (PDN), n= 5]. According to National Cancer Institute (NCI) criteria 7 patients could be considered in complete remission (CR) and 5 in good partial remission (G-PR), without differences as to the degree of response between the two therapeutic sub-groups (p=0.890). MVD at diagnosis was significantly different from that of 45 stage A patients used for statistical comparison (p=0.001), but such a difference disappeared when comparison was carried out after therapy (p=0.644). Assessment of minimal residual disease (MRD) carried out in flow cytometry and expressed as percentage of CD5+/CD19+ B-CLL cells reflected the type of therapy: CLB + PDN, 21%, range, 11-29.9%; F, 9.1%, range, 0.1-11% (p=0.017; Mann-Whitney test). Responders experienced a significant decrease in MVD. Specifically, median microvessel area was 2.616 mm^2x10^{-2} (range, 0.545-4.126) before therapy and 0.644 mm^2x10^{-2} (range, 0.383-1.914) after therapy (p=0.03; Mann-Whitney test). Finally, we wondered whether different therapies could affect the size of MVD reduction. A separate comparison carried out before and after therapy in patients treated with F or CLB+PDN showed a significant decrease of MVD only in patients who received the former (p=0.01). These results provide the first evidence that chemotherapy is accompanied by a significant decrease of BM angiogenesis in B-cell CLL. F, which leads to a more consistent reduction of MRD, produces the greater decrease of MVD.

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PROGNOSTIC VALUE OF SERUM THYMIDINE KINASE LEVEL AND ZAP-70 EXPRESSION DETECTED BY IMMUNOHISTOCHEMISTRY ON BONE MARROW BIOPSIES IN B-CLL: COMPARATIVE ANALYSIS

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In B-CLL serum thymidine kinase (s-TK) levels, that are probably related to the proliferative activity of the disease, and ZAP-70 expression on leukemic cells were both recently proposed as relevant biological prognostic factors, associated to the mutational IgVH gene status. In order to compare the prognostic significance of s-TK and of ZAP-70, we evaluated 83 patients affected by B-CLL in whom both s-TK levels and ZAP-70 expression at presentation were available. They were 53 males (64%), aged 34 to 83 years (median 60). At diagnosis 57 (69%) were Binet stage A, 20 (24%) stage B and 6 (7%) stage C. The median follow-up period from diagnosis was 51 months (range 7–197 months).The levels of TK activity in the sera, obtained at diagnosis and stored at –20°C until analysis, were measured by a commercially available radioenzyme assay (Immmunotech; Beckman Coulter), and expressed in units per liter. Control values of s-TK, as determined in 24 healthy adults, were 3.1 + 2.2 U/L (mean + SD; range 0.3-.7.2 U/L). The cytoplasmic expression of ZAP-70 protein in leukemic cells was evaluated by immunohistochemical methods on bone marrow trephine biopsies taken within 6 months from presentation, using a mouse anti-human monoclonal antibody to ZAP-70 (clone 2F3.2, 1/200, Upstate, Lake Placid NY). s-TK levels were elevated (i.e.>= 8 U/L) in 39 patients (47%). In 44 cases (53%) cytoplasmic expression of ZAP-70 was present; 27 of them (60.4%) had elevated s-TK levels. Conversely, in 27/39 ZAP-70 negative cases (69%) s-TK was normal. Overall, in 54/83 cases (65%) s-TK and ZAP-70 data were concordant. Mean s-TK levels of ZAP-70 positive and ZAP-70 negative patients were 14.8 U/l (+/- 6.8 SD) and 7.6 U/L (+/- 7.1 SD) respectively (p<0.01). At univariate analysis both elevated s-TK levels and ZAP-70 expression were associated with inferior progression free survival (PFS) (p=0.0007 and p=0.005 respectively). Combining the 2 parameters we found that PFS at 5 years was significantly better (61% +/- 9%) in ZAP-70 negative patients with normal s-TK as compared to patients with either positive expression of ZAP-70 or elevated s-TK levels (28%+/- 9%; p=0.01), or both ZAP-70 positivity and elevated s-TK levels (17%+7%; p=0.0006).

In conclusion, our findigs suggest that the combined assessment of s-TK levels and ZAP-70 expression can improve the prognostic evaluation of B-CLL at diagnosis.

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PATTERN OF CD38 EXPRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Since the early report of Damle *et al* (Blood 1999), several studies confirmed the independent prognostic significance of CD38 expression in B-cell chronic lymphocytic leukemia (B-CLL). However, some controversial issues are still matter of debate. Among them, the exact threshold of CD38 positivity is not well established. Some Authors have used 30%, while some others 20% or even 7% as cut-off level to better discriminate among prognostic subgroups. More recently, Ghia et al (Blood 2003) showed that the pattern of CD38 expression (homogenously positive, homogenously negative, and bimodal profile), more than the percentage number of neoplastic B-cells co-expressing CD38 antigen, is use-

ful to identify B-CLL patients at risk of disease progression. Fifty-two patients with B-CLL were investigated (30 male and 22 female; mean age 66 years; range 37-91 years) aiming to evaluate the prognostic impact of CD38 expression in B-CLL patients according to Ghia's suggestions. A flow-cytometric evaluation on immunologically gated B-cells from peripheral blood samples by means of a three-color fluorescence (CD45-PerCP/CD19-FITC/CD38-PE) was performed. The expression pattern of CD38 was correlated with clinical features at diagnosis, treatment requirement and survival. Twenty-five (48%) patients showed a omogenously negative pattern of CD38 expression, 12 (23%) a omogenously positive, and 15 (29%) a bimodal profile. No differences were found between the three groups with respect to Rai stages (low, intermediate, high risk) and peripheral blood lymphocytosis. At a mean follow-up of 25 months from diagnosis (range 0 – 84 months), 29 patients (56%) started therapy (12/25 omogenously negative, 9/12 omogenously positive and 8/15 with bimodal profile). Moreover, a shorter interval time from the diagnosis and the start of therapy was recorded in the bimodal profile group of patients (mean time: 12 months; range: 0 – 27 months) with respect to omogenously negative (mean time: 33 months; range 0 – 84 months) and omogenously positive (mean time: 23 months; range 0 – 67 months). Finally, CD38 homogenously negative patients have a significantly longer survival (median survival 120 months) than homogenously positive (median survival 85 months) and bimodal profile (median survival 51 months). The differences among the three curves were statistically significant (p 0.00196; Figure 1). Significantly different curves (Figure 2) were also found when patients were stratified according to 30% cut-off of positivity (p 0.0383), while no differences were found using a cut-off of 20% (p 0.1142). In conclusion, in our hands also, the analysis of B-CLL patients according to the expression pattern of CD38, instead of a numerical cut-off, has shown to identify groups of patients with different prognosis.

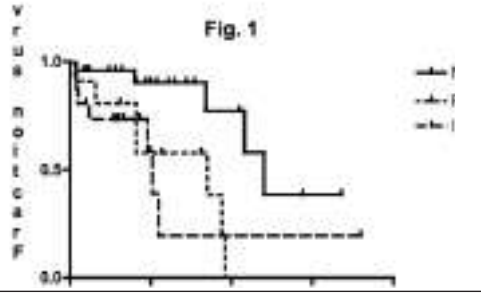

Figure 1.

Figure 2.

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FAND-CAMPATH-1H FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH ADVERSE CLINICAL AND BIOLOGICAL PROGNOSTIC FEATURES

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On the basis of the efficacy in CLL of FAND, a Fludarabine combining therapy, and of Campath-1H (MabCampathÒ), a humanized anti-CD52 monoclonal antibody, we conducted a clinical study to determine the feasibility, safety and efficacy of a FAND-Campath-1H (FAND-Cam) combination schedule given to 17 previously treated CLL patients with adverse clinical and biological features. Median age was 55 years (range: 43-64), median CLL duration 54 months (range: 4-134), median number of prior treatments 2 (range: 1-6). Fifteen patients (88%) were refractory to prior therapy which included Fludarabine in 59% of the cases (Fludarabine: 2 patients; Fludarabine-Cyclophosphamide: 7; FAND: 1). A IgVH germline configuration was present in 77% of patients. Six patients showed marked enlarged nodes and/or spleen.

FAND schedule included: Fludarabine (F: 25 mg/m^2 i.v. daily, D1-3, at 0, 24 and 48 hours) combined with ARA-C (A: 700 mg/m^2 i.v. daily, D1-3, at 4, 28 and 52 hours), NovantroneÒ(N: 10 mg/m^2 i.v., D1 at 6 hours) and Dexamethasone (D: 20 mg i.v. daily, D1-3).

FAND courses were followed by 3 administrations of 30 mg of Campath-1H given intravenously over 2 hours on 3 consecutive days (D4-6). Before the first FAND-Cam course, a dose escalation of Campath-1H (3 mg, 10 mg and 30 mg) was given intravenously on 3 consecutive days. Patients received chlorphenamine and acetaminophen 30 minutes before Campath-1H infusion. G-CSF was given in case of severe neutropenia. Oral trimethoprim/sulfamethoxazole (160/800 mg twice a day, 3 times a week) and fluconazole (100 mg daily) were given in all patients. Antiviral prophylaxis consisted of acyclovir (200 mg three times a day) for the first 9 patients and of valacyclovir (2 gr three times a day) for the last 8 patients. Infection prophylaxis was given after completion of therapy as long as CD4 lymphocytes were less than 200/mm^3. Quantitative assay for cytomegalovirus (CMV) viremia was performed weekly. A clinical, cytometric and molecular evaluation of response was assessed after therapy and, thereafter, every 3 months. The first 6 patients received, as initial debulking, 2 FAND courses followed by 2 FAND-Cam courses. All the 6 patients obtained a response which was a CR in 5 cases (cytometric CR, cyCR: 3 patients, molecular CR, molCR: 1). However, a high infection rate was observed (CMV reactivation: 2 patients; CMV reactivation and nocardia pneumonia: 1; pneumonia: 2; sinus infection: 1). In the aim of reducing the infection risk, the last 11 patients received 2 courses of FAND-Cam without a prior debulking with FAND therapy. A response was obtained in 6/7 evaluable patients treated with 2 FAND-Cam courses (CR: 2 patients; cyCR: 1; PR: 3). Infections were observed in 4 patients (pseudomonas sepsis: 1 patient; pseudomonas sepsis + CMV reactivation: 1; aspergillus: 1; CMV reactivation: 1).

After the introduction of valacyclovir phrophylaxis the rate of CMV reactivation decreased from 55% to 25%; ganciclovir cleared CMV viremia in all cases. Despite the considerable high infection rate, no infection related deaths were observed. In conclusion, in a subset of patients with a low chance of response to therapy, FAND and Campath-1H combination proved effective in the majority of the cases. Taking into account the severe immunodeficiency state related to the refractory disease and to the treatment, a vigorous surveillance and an extended infection prophylaxis are required.

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EPIDEMIOLOGY, CLINICAL FEATURES AND OUTCOME OF FAMILIAL CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: A SINGLE INSTITUTION STUDY

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The aims of this study were to define the clinical characteristics of familial Chronic Lymphocytic Leukemia (CLL) patients and of their affected relatives, to evaluate whether familial and sporadic CLL patients share the same clinical features and whether the presence of a familial history of hematologic malignancy (HM) has a prognostic impact on survival. The familial histories of 1449 CLL patients diagnosed at a single institution were retrospectively evaluated. The clinical features and outcome of sporadic patients, without a familial history for HM, and familial patients with a familial history for HM were analyzed and compared. One hundred and eighty-one patients (12.5%) reported a familial history of a HM which was a lymphoproliferative disease (LD) in 9% of cases and a CLL in 6%. In 78% of cases, the affected relative was a first degree relative (FDR). Among 10,145 FDRs, the rates of LD and CLL cases were 1.15% and 0.8%, respectively. A higher proportion of females than males showed a familial history for CLL ($p<.05$) and female familial cases showed an earlier diagnosis ($p<.05$). Sporadic and familial cases showed a similar treatment-free duration and survival probability. In conclusion, a familial history of a LD, which was a CLL in the majority of cases, was recorded in 9% of CLL patients and was more frequently observed among female patients. The presence of a familial trait did not imply an adverse prognosis. This suggests, that if an inherited gene predisposing familial CLL/B-LD cases exists, it could play a role in promoting the disease development while it is probably independent from other genetic factors involved in CLL progression.

P442

THE PROGNOSTIC VALUE OF CD38 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS: A SINGLE INSTITUTE EXPERIENCE

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The purpose of this study was to assess if there is an association between CD38 expression (according to the 7%, 20% and 30% positivity cut-offs) and clinical characteristics, treatment-free survival (TFS), survival of CLL patients, and if this parameter changes over time. CD38 expression was evaluated in 242 consecutive and untreated CLL patients at diagnosis. The median age of the patients was 67 years. Fifty-five% were male and 45% females, 146 (60%) showed a Rai stage 0. The median follow-up of the patients was 31 months (range 3-62). At diagnosis all the three subgroups of CD38+ patients (CD38 expression: >=7%, >=20% and >=30%) showed a significantly higher male/female ratio and rate of intermediate and high-risk disease(Rai I-IV), a significantly lower Hb value and platelet count, and a significantly more elevated lymphocyte count than the respective CD38- patients (CD38 expression: <7%, <20% and <30%). Furthermore, all subgroups of CD38+ patients showed a significantly lower TFS probability than the respective CD38- patients (TFS probability at 3 years, patients with CD38 >=7% vs patients with CD38 <7%: 31% vs 78%, $p<0.0001$, patients with CD38 >=20% vs patients with CD38 <20%: 25% vs 76%; $p<0.0001$; patients with CD38 >=30% vs patients with CD38 <30%: 25% vs 76%; $p<0.0001$). When 146 Rai stage 0 patients were separated according to 7% threshold for CD38 positivity also a significantly different TFS probability was observed (TFS probability at 3 years: 87% vs 59%; $p=0.0005$). At multivarate analysis the CD38 positivity emerged as a significative and independent prognostic parameter on TFS, either in all 242 patients or in the subset of Rai stage 0 patients. Nevertheless, at multivariate analysis CD38 expression, analyzed according to the 20% and 30% cut-offs did not result an independent parameter predictive for survival duration. In 31 out of 242 (13%) patients a second determination of the CD38 expression has been performed after a median time of 24 months (range: 12-45) from the first evaluation. In all patients but one no relevant variations of CD38 expression were recorded. In conclusion, the results of this study suggest that the 7% may be a useful threshold for CD38 positivity to discriminate at diagnosis CLL patients with adverse clinical features that will early require treatment. Moreover, since this parameter is stable over the time, the prognostic value of CD38 expression is maintained during the clinical course of the disease.

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DETECTION OF MINIMAL RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA BY SYBR GREEN I DYE BASED REAL-TIME QUANTITATIVE PCR

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In B-Chronic Lymphocytic Leukemia (B-CLL) and in others B-cell malignancies Minimal Residual Disease (MRD) is usually evaluated by qualitative nested-PCR using Immunoglobulin Heavy (IgH) chain gene rearrangement as patient-specific tumor marker. With the advent of more effective therapeutic modalities, Real-Time Quantitative-Polimerase Chain Reaction (RQ-PCR) methods could provide relevant prognostic information. We have developed a novel and feasible strategy for MRD detection with a RQ-PCR based technique, the DNA intercalating Sybr Green I dye and clone-specific IgH gene sequences. To assess the clinical utility of the approach, we quantified MRD in four CLL patients receiving a second line treatment with the monoclonal antibody Alemtuzumab (Campath 1H) after a combination therapy with Fludarabine phosphate and Cyclophosphamide (FC).

For each patient clonal rearrangements were identified by a single-step PCR using Variable Heavy chain region (VH) family consensus forward primers and a Joining Heavy chain region (JH) consensus reverse primer positioned in the germline IgH gene segments. VDJ rearrangements were identified by direct sequencing method and compared with germline data in the IMGT/V-QUEST database. Patient-specific oligonucleotide primers were generated from the Complementary Determining Region 2 (CDR2) and 3 (CDR3) of IgH gene and used to quantify residual tumor cells in follow up samples in a nested RQ-PCR approach (RQ-N-PCR) using iQ Sybr Green Supermix (Bio-Rad). RQ-PCR standard curves were performed by cloning patient specific VDJ regions and GAPDH gene fragment into plasmid vectors. Melting curve analysis allowed the optimisation of RQ-N-PCR conditions and the control of specificity of PCR products. RQ-N-PCR Sybr Green I dye results were expressed as copy number/ug of genomic DNA after normalization with the GAPDH housekeeping gene. CLL-1 and CLL-3 were highly positive at the time of re-stadiation of the disease ($5{,}7x10^6$ and $1{,}8x10^4$) and showed a reduction of tumor cell load over one and two log respectively after FC chemotherapy. The patients became PCR negative after Campath-1H administration, but turned PCR positive during 5-10 months follow-up period ($2{,}4x10^4$) and $1{,}5x10^5$ for CLL-1; $1{,}3x10^2$ and $6{,}1x10^2$ for CLL-3). In CLL-2 ($2{,}0x10^4$), PCR negativity was not obtained after alemtuzumab therapy, since RQ-N-PCR technique revealed the persistence of the disease ($6x10^{-1}$). CLL-4 ($1{,}4x10^4$), became PCR negative after alemtuzumab therapy and remained PCR negative during follow up period. No clonal amplification was observed with all the patient-specific primers using DNA extracted from reactive B-lymphocytes. The sensitivity of the method was slightly higher (10^{-5}-10^{-6}) as compared to published RQ-PCR techniques based on patient-specific primer and probe sets, like IgH Allele-Specific Oligonucleotide (IgH-ASO) RQ-PCR. Our results suggest that the Sybr Green I dye is a sensitive, simple and cost-effective approach to evaluate MRD alternative to traditional fluorescent probes; in our initial group of patients, we demonstrated its feasibility for IgH gene rearrangements detection and quantification in routine screening.

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REGRESSION OF LOW-GRADE NON-HODGKIN'S LYMPHOMA AFTER TREATMENT WITH PEGILATED INTERFERON PLUS RIBAVIRIN OF HEPATITIS C VIRUS INFECTION

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Background and Aims. Previous epidemiological studies suggest a link between hepatitis C virus (HCV) infection and B-cell non-Hodgkin's lymphomas (NHL). Furthermore, some authors reported the efficacy of antiviral therapy in patients with NHL after HCV eradications. We decided to treat twelve consecutive NHL HCV-positive patients with different interferon-based therapies.

Methods. Twelve HCV RNA-positive patients presenting low grade lymphomas (No. 11 B-cell NHL with small lymphocytes and lymphoplasmocitoides elements and No. 1 splenic lymphoma with villosus lymphocytes) were treated, all for 12 months, with interferon alfa-2b monotherapy (No. 7 subjects) 3MIU/TIW, interferon alfa-2b 3MIU/TIW + ribavirin (RBV) 1,000-1,200 mg/day (No. 2 subjects) and peginterferon alfa-2b 1-1.5 mcg/QW + ribavirin 1,000-1,200 mg/day (No. 3 subjects).

Results. Two out of seven patients treated with IFN monotherapy attained a complete lymphoma remission after HCV eradication, three subjects showed a partial response, two patients did not show a response. One interferon alfa-2b monotherapy partial responder was retreated with peginterferon alfa-2b + RBV, showing an end-of-treatment complete response that was followed seven months later by a virological relapse and NHL reappearance. One out of 2 patients receiving IFN + RBV obtained complete lymphoma remission after HCV suppression; One out of three patients treated with peginterferon alfa-2b + RBV obtained complete lymphoma remission after HCV eradication, one subject showed a partial responce and one patient did not show a response. The patient who had a response to antiviral treatment showed the complete disappearance of all clinical manifestations of the disease(purpura, arthralgias and weakness)and either reduction of the serum cryocrit level. Follow-up of this patient at 11 months showed maintenance of complete remission of B-NHL and HCV-RNA.

Conclusions. From our experience we can conclude that NHL regression can be obtained following HCV eradication. This is a new indirect evidence confirming the pathogenetic role of HCV on low-grade lymphoma.

P445

CAELYX PLUS FLUDARABINE AS INITIAL TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

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The purine analog Fludarabine is a very effective drug to treat chronic lymphocytic leukemia (CLL). It was first established as a salvage agent capable of inducing responses in refractory disease, and today is the treatment chosen in the majority of cases of CLL. The combination of Fludarabine and anthracyclines or cyclophpsphamide induces a higher response rate and a longer duration of remission of the disease. Pegylated liposomial doxorubicin (PLD-Caelyx), compared to the conventional doxorubicin, is characterized by an improved pharmacokinetic and therapeutic index, thus a reduced toxicity profile. Based on these suppositions, the aim of this study was to evaluate the efficacy and toxicity of the combination regimen fludarabine plus PLD as a first-line therapy of CLL. We treated 10 patients with B-cell CLL (Binet stage B or C) with an association of Caelyx 30 mg/m^2 day1 plus fludarabine 25 mg/m^2 day 1-4 every four weeks for a maximum of 5 cycles. All patients were evaluated for the prognostic factors CD38 and ZAP 70 and cytogenetic aberrations are under study in all cases. The cardiac toxicity was evaluated by echocardiography performed at the beginning and at the end of the treatment. Cotrimoxazole profilaxis was started after the third cycle of therapy, and filgrastim (5 µg/Kg/day) was administered in case of severe neutropenia.. According to National Cancer Institute (NCI) criteria 3 patients achieved the complete remission (CR), 4 patients a partial remission (PR), and 3 showed a stable disease (SD). In the cases of PR, the treatment was very effective on the peripheral and marrow lymphocytosis while it was less active on the lymphadenopaties. According to NCI criteria, Grade III neutropenia and thrombocytopenia were observed in 1 case, and Grade III/IV and infections were never observed. Cardiotoxicity and alopecia were not observed in any case. The correlations between prognostic factors and response to treatment will be evaluated. These preliminary results suggest that Caelyx-Fludara is an effective, low-toxic first treatment approach in patients with CLL, representing another possible Fluda-based association in the treatment of this disease. We have the intention to continue the study with additional patients in order to obtain a better evaluation of this combination.

Acute Myeloid Leukemia II

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ACUTE LEUKEMIA SECONDARY TO 5Q- SYNDROME PRESENTING CHRONIC MYELOPROLIFERATIVE DISORDERS-LIKE MANIFESTATION: A CASE REPORT

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5q- syndrome is characterized by interstitial deletion of long arm of chromosome 5, macrocytic anemia, female predominance, normal or high platelet count, bone marrow erythroid hypoplasia, megacaryocytic hypolobulation, bone marrow blasts less than 20% and relatively good prognosis. We present a case of 5q- syndrome with chronic myeloproliferative disorders-like manifestation. A female patient 78 years-old underwent to our observation complaining left side pain and with CBC showing WBC 12000/mmc, Neutrophils 10000/mmc, Basophils 200/mmc. Physical examination revealed severe hepatosplenomegaly. First diagnostic hypotesis was chronic myelogenous leukemia. However morphologic examination of peripheral blood smear showed erythroid and granulocytic dysplasia, 15% of blasts with single Auer rod and many dakriocytes. Bone marrow smear revealed increased number of small, hypolobulated megakaryocytes, erythroid and granulocytic dysplasia and 30% of blasts. Abdomen CT confirmed severe hepatosplenomegaly. Karyotype showed 5q- deletion. Then diagnosis of secondary acute myeloid leukemia was posed. In consideration of patient poor performance status, age and cohexistent chronic heart failure, therapy with hydroxyurea and 6-mercaptopurine was started with good control of organomegaly, blast count and leukocytosis. One month later patient showed huge increase of organomegaly with persistent normal peripheral CBC. Bone marrow biopsy showed severe myelofibrosis. Dosage of performed therapy was increased with normalization of organomegaly. However cytopenia persisted and after fulminant sepsis due to pseudomonas the patient died. Autoptical examination evidenced heterotopic splenic hemopoyesis. Such case, apparently rare, suggest the existence of a subgroup of acute leukemia secondary to 5q- syndrome with proliferative characteristics. Basophilia, thrombocytosis, erythroid and granulocytic dysplasia, leukocytosis and often myelofibrosis with hepatosplenomegaly are the main clinical and laboratory findings of this particular condition. Our case support the concept of mixed myelodysplastic-myeloproliferative syndrome and underline the possible existence of a new pathological entity.

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ACUTE MYELOID LEUKEMIA IN THE ELDERLY, INTENSIVE THERAPY OR OF MAINTENANCE THERAPY? OUR EXPERIENCE IN PATIENTS OVER 65 YEARS

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The treatment of acute myeloid leukemia in elderly with age > 65 years is still debated. in literature numerous studies have valued the feasibility of intensive chemotherapy in these patients. the aim of the our study is to value the difference in DFS and OS among 2 groups of patients with elderly AML treated with intensive chemotherapy (IC) or maintenance (M). From June 2001 to March 2005 we have treated in our Division 44 AML, 24 male and 20 female with median age of 75 years (67-86 years). 21 patients (11 M and 10 F with median age of 71 years) have received intensive chemotherapy (I.C. Flag and MICE)and 23 (13 M and 10 F with median age of 78 years) have received maintenance (low dose cytarabine and/or support). In IC group 11 patients (52%) have obtained to complete remission (CR) with to DSF and OS media of 6,5 and 6 months respectively, the rate of TRM has been of 19%. In the M group the CR has been documented in 5 patients (22%) with to DFS and OS media of 6,7 and 4 months respectively. This results have shown a best rate of CR in the IC group but the OS and DFS difference is not statistically significant in the two groups (p: 0.5). In conclusion the Intensive chemotherapy has not improved the survival in AML elderly patients. New therapeutics strategy is necessary for to improve the DFS and OS in these patients. Interesting is the use of specific monoclonal antibodies (anti CD33) in this poor disease especially in maintenance after a CR obtainable with a intensive or low dose chemotherapy.

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CLINICAL OUTCOME AND THERAPEUTIC OPTIONS FROM A SERIES OF 576 CONSECUTIVE PATIENTS WITH ACUTE MYELOID LEUKAEMIA AGED OVER 60 YEARS

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Acute myeloid leukemia (AML) is a prevalent disease of elderly people, more than half of the cases in western countries being diagnosed in patients aged over 60 years. In the elderly, the actual benefit of aggressive chemotherapy (aCHT) is still debated because of higher morbidity and mortality, lower complete remission (CR) rate, and higher incidence of relapse as compared to young/adults. Notwithstanding, the achievement of CR is an absolute prerequisite to obtain long-term disease control and cure. In this study we did retrospectively analyze treatment results from a series of 576 consecutive patients with nonM3 AML aged over 60 years, observed from 1985 to 2004. The median age was 69 years (61-91) and in 278 patients (48%) AML was preceded by an antecedent hematologic disorder (95% myelodysplasia). 314 (54%) patients were treated with aCHT aimed at CR achievement, while 262 (46%) were given supportive therapy and/or hydroxyurea in the case of leukocytosis; in a minority of cases low dose ARA-C was employed. Intensive induction therapy consisted of either a combination of anthracyclines with conventional dose ARA-C and etoposide or of fludarabine, ARA-C, and G-CSF (FLAG). There was no statistically significant difference between the two induction schedules in terms of CR achievement and duration. Performance status, severe concomitant disease and age by itself were the main reasons of exclusion from intensive induction regimens. In particular, the rate of inclusion into aCHT was 68 % within the age group 61-70 and 38% in patients aver 70 years (p:0.001). Patients receiving CHT had lower median age (67 vs 71, p:0.0001), but higher white blood cell count at diagnosis ($13.8x10^9$/Lvs $8.1x10^9$/L, p:0.002). The median survival of the whole patient population was of 125 days. Following aCHT, CR was obtained in 140 out of 314 patients (45%) and was significantly related to survival duration (p:<0.0001). Overall, patients aggressively treated had a better survival as compared to the opposite group (219 days vs 69 days, p<0.001). Of note, when we compared patients managed with palliation (n= 262) to those given CHT and not achieving CR (n=174), we did not found any statistically significant difference (69 days vs. 83 days, p:0.24). Finally, for a more adequate evaluation, we did also compare patients from palliation group to those aggressively treated by separately considering two distinct age subgroups, i.e. 61-70 and over 70 years. The advantage of aCHT was found in both subgroups, but it was more relevant in the 61-70 years subset (median 271 days vs 102 days p:<0.0001) as compared to those over 70 (135 days vs 58 days, p: 0.01). Once again, the difference in survival was not statistically significant even when patients of aCHT group achieving CR were excluded (p:0.78 for 61-70 and 0.19 for those over 70, respectively). We conclude that elderly AML patients who are not eligible for intensive induction treatment should be in turn accrued into clinical trials, based on novel agents with mechanism of action alternative to conventional chemotherapy.

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INTENSIVE CHEMOTHERAPY WITH GENTUZUMAB OZOGAMICIN, HIGH DOSE CYTARABINE P44 ± FLUDARABINE FOR REFRACTORY/RELAPSED ACUTE MYELOID LEUKEMIA OF ELDERLY PATIENTS(>60 YEARS

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High doses Ara-C (HidAC), Fludarabine (FL) and Gentuzumab Ozogamicin (GO) are highly effective drugs for the treatment of patients with high risk MDS and acute myeloid leukemia (AML). In particular, GO, an anti CD33 monoclonal antibody conjugated with calicheamicin, as single agent may reinduce a second complete response in approximately 30% of elderly patients with relapsed AML. Moreover, the preliminary results of the MRC group indi-

cate that induction treatments with GO combined to intensive chemotherapy may increase the response rate in untreated AML patients younger than 60 years. Therefore, aims of the present study were to evaluate the feasibility and efficacy of a chemotherapy combining GO with high doses Ara-C ± fludarabine for elderly patients with refractory/relapsed AML.

Fifteen patients >60 years of age with refractory/relapsed CD33+ AML were treated at the Hematology Section of the University "La Sapienza" of Rome, between February 2003 and December 2004. Seven cases received GO (6mg/m^2 IV,d 1) combined with Fludarabine (25mg/m^2 IV, d 2-6), Ara-C (2gr/m^2 over 3hr infusion IV, d 2-6) and G-CSF (300 micrograms subcutaneously from day 0 up to neutrophil > 1.0x10^9/L) (GO-FLAG group). Due to an excessive extra-medullar toxicity, Fludarabine was withdrawn by the treatment given to the additional 8 cases (GO-AC group). Prior to these salvage treatments patients received the FLAG regimen (2 cases), Daunorobicin + Ara-C (11 cases) and Daunoxome+Ara-C (2 cases). At the entry of the present study 4 patients, in the GO-FLAG group, and 5 cases, in the GO-AG group, were resistant to prior chemotherapy. Two patients in each group were in first early relapse (< 6 months), while only one case of the GO-FLAG group presented a late relapse (> 6 months). The clinico-biologic characteristics of patients according to the two types of treatment were reported in table1.Overall, a complete remission (CR) of disease, defined as less than 5% blasts on a bone marrow aspirate of adequate cellularity showing evidence of trilineage regeneration with a peripheral ANC to 1.0x10^9/L and platelet count to 100x10^9/L was achieved in 6 out of the 15 cases (40%). Respect to the two treatment groups, a CR was obtained in 4/7 patients and 2/8 patients of the GO-FLAG and GO-AG groups, respectively. Resistant disease, defined as the persistence of >20% blasts in the BM, was observed in 2 and 5 patients, respectively. Deaths during induction occurred in 1 patient of each group. A relapse of disease was documented in 3 of the 4 responders to the GO-FLAG (at 2, 6 an 12 months, respectively) and in both the 2 responders to the GO-AG group (at 4 and 6 months). In addition, 1 patient of the GO-FLAG died in CR at 3 months for a disseminated Varicella Zoster infection. Overall the median survival periods were 8 months (range: 2 – 24 months) and 2 months (range: 1 - 7) for patients in the GO-FLAG and GO-AC groups, respectively. According to the NCI criteria, a myelosuppression grade 3-4 occurred in all the 14 evaluable patients, whereas, in the GO-FLAG and GO-AG groups, infections (grade 3-4) occurred in 6 and 7 patients, hemorrhages in 3 and 1 cases, and a reversible AST/ALT increase (grade 3-4) occurred in 5 and in 1 patients, respectively. None patients developed VOD/SOS syndrome.

In conclusion, the present findings demonstrate the efficacy of our combined treatment with GO and HidAC ± fludarabine inducing a 2nd CR rate of 40% even in an extremely unfavorable leukemic subtype such as that of our patients. In addition, although limited by the low number of patients allocated in each of the two treatment groups, our data may suggest that the withdrawn of the fludarabine, reducing the therapy related toxicity, decrease the anti-leukemic efficacy resulting in a shorter overall survival of patients. Moreover, the hepatic toxicity of these GO based therapeutic combinations was limited to a reversible grade 3-4 transaminitis with no patients developing a VOD/SOS syndrome.

Table 1. Patients' clinic characteristics according to types of treatment.

Characteristics	GO-FLAG (no. pts. = 7)	GO-AG (no. pts.= 8)
Sex M/F	3/4	5/3
Median Age (range)	67 (61-69)	67.5 (64-72)
Median WBC count 1 x 10^9/L(range)	6.8 (0.7-44)	2.5 (0.7-50)
Karyotype: normal	2	1
-7	1	-
-1	-	1
complex	4	4
not evaluable	-	2
Disease Status: resistant	4	5
1st relapse (≤ 6 mos.)	2	2
1st relapse (> 6 mos.)	1	1

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PML-RARALFA, FLT3 AND HTERT GENE EXPRESSION ANALYSIS IN ACUTE PROMYELOCYTIC LEUKEMIA WITH MULTIPLE RELAPSES

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PML-RARalfa is the molecular marker of APL. An internal tandem duplication (ITD) of the FLT3 gene is observed in about 20-40% of APL patients and is associated with a poor disease outcome. hTERT, the catalytic subunit of telomerase involved in telomere length maintainance, plays a still undefined role in haematological malignancies. The present study analysed three APL patients with multiple relapses focuses on the possible correlations between FLT3 ITD and FLT3 expression and on whether FLT3 and hTERT genes may be used as additional prognostic molecular markers in APL patients. Gene expression analysis was conducted on total RNA samples, previously isolated from mononuclear cells routinely separated by density gradient centrifugation from bone marrow or peripheral blood. All the samples were also submitted to qualitative RT-PCR for the detection of PML-RARalfa rearrangements and FLT3 ITDs mutation. Relative quantifications of PML-RARalfa (BCR1 and BCR3 isoforms), FLT3 and hTERT transcripts were performed by real-time RT-PCR using SybrGreen Master Mix (Applied Biosystems). ABL mRNA expression was used to normalize target genes expression. For each assay, amplification efficiency was evaluated by standard curve analysis of serial dilution of total RNA isolated from samples with known high espression of the gene of interest. DDCt method was applied to calculate target genes mRNA levels, expressed as number fold in relation to a calibrator sample. Specificity of each amplification assay was demonstrated by dissociation curve analysis. FLT3 and hTERT expression analysis was also conducted on a control group of normal sample. Two of the three patients studied were diagnosed at our Institution

and presented with hyper-leucocytosis. On diagnosis a PML-RARalfa BCR1 fusion transcript, an ITD of the FLT3 gene, a high expression of PML-RARalfa and FLT3 genes were detected in both the patients. The first patient achieved a complete haematological remission (CHR) never followed by a molecular remission (MR). During CHR, which lasted fourteen months, he showed the disappearance of ITD, while FLT3 and hTERT expression were respectively low-medium and high. This patient relapsed two times and on both circumstances ITD was newly detected. In addition, on first relapse PML-RARalfa and FLT3 expression were markedly increased, while on the second relapse it was the expression of hTERT gene to be increased. The second patient entered a CHR associated with a MR of fourteen months duration. During CHR, ITD was no more discovered, FLT3 expression was normal and hTERT expression remained high. When relapse occurred, ITD remained undetectable, but FLT3 expression increased and hTERT expression was further augmented. Since then, the patient remained PML-RARalfa positive and showed a persistent high expression of FLT3 and hTERT genes. The third patient was diagnosed and treated in another Centre. He reached our observation when he has already entered a CHR and a MR, which was lost three months later. This patients relapsed three times and on all circumstances he developed an ITD of the FLT3 gene. In addition, he presented a high PML-RARalfa BCR3 and FLT3 expression always preceded by a progressive rise in hTERT expression. In conclusion, our data indicate that: i) in all the 3 patients, PML-RARalfa and FLT3 expression variations during patients follow-up show a similar course; ii) an increased expression of FLT3 gene may be caused by other mechanisms in addition to ITD, as suggested by our second patient who relapsed with high PML-RARalfa and FLT3 expression but without ITD; this event did not occur in the other two patients who showed ITD reappearance in corrispondence with similar or lower FLT3 levels; iii) hTERT increase seems to precede the increments of both PML-RARalfa and FLT3 expression: this observation is particularly evident in the third patient on each of the three relapses, in the first patient on second relapse and in the second patient with persistently high levels of hTERT expression despite PML-RARalfa and FLT3 ITD negative tests during molecular remission and before clinical relapse. Undoubtedly, additional patients are required to confirm these observations.

P451

A CASE OF ACUTE MYELOID LEUKEMIA AND PYODERMA GANGRENOSUM

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Pyoderma gangrenosum (PG) is an uncommon ulcerative cutaneous condition of uncertain etiology. It is classified as neutrophilic dermatosis as it exhibits intense dermal inflammatory infiltrates composed of neutrophils with little evidence of a primary vasculitis. Aetiology is believed to represent a manifestation of altered immunologic reactivity. It responds to immunosuppressives. Ulcerations of PG may occur after trauma or injury to the skin in 30% of patients: this process is termed pathergy. Approximately 50% of the cases of PG are associated with a specific systemic disorder. These include inflammatory bowel disease, rheumatoid arthritis, non-Hodgkin's lymphoma and myeloproliferative disorders. We report the case of a 25 years old male patient who came to our observation in January 2004 because of anaemia,thrombocytopenia, fever, fatigue and a violaceous skin nodule on the left side of the thorax. We diagnosed AML M4 according to FAB classification. Molecular biology was positive for FLT3-ITD. The nodule became wider and in a few days it reached a diameter of 13 cm with a vaste ulceration in the center. The fever (max 40°C) was not controlled by paracetamol and low doses of steroids. Cultures of the skin lesion and blood were negative. Other similar lesions appeared on the lips, ear and right arm, where a venous access was located, all with a fast evolution from vescicule to ulceration. Total body C.T. scan did not show any involvement of other organs.

The dermatologist suspected a PG and the histology of the skin confirmed his diagnosis. The patient was treated according to GIMEMA protocol DCE and with steroids (hydrocortisone 2g/day). Topical therapy was local wound care and dressing and tacrolimus. On the fourth day from the beginning of therapy we observed a decrease of the fever; about five days later a slow regression of the skin lesions started. The widest one (see picture), that had been the first to appear, healed completely only after two months. The chemotherapy was well tolerated and the recovery started on the 22nd day. The patient underwent a consolidation cycle. A skin biopsy performed after the evaluation of bone marrow remission did not show any sign of PG. The patient underwent bone marrow transplantation from HLA identical sibling. He is in complete remission 10 moths after the BMT. Our case underlines that PG should be included in the differential diagnosis of any nodular, pustular, ulcerative or necrotic cutaneous eruption in patients with acute leukaemia.

P452

LOW DOSE GEMTUZUMAB OZOGAMICIN MAINTENANCE MAY PROLONG REMISSION DURATION IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AND HIGH RISK MYELODYSPLASIA

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From may 1999 to february 2003, a cohort of 30 consecutive elderly (mean age 69 y; range 65-76) patients with Acute Myeloid Leukemia *de novo* (AML=23) or secondary to antecedent hematological disorder (sAML=4) or with high risk Myelodysplasia (RAEB=3) received an induction chemotherapy with Fludarabine 30 mg/sm and Cytarabine 1 g/sm for four days plus Idarubicin 10 mg/sm delivered as a continuous iv perfusion on days 1,3 and 5. G-CSF was given from day 7 to granulocyte recovery. After the induction course 15 patients (50%) entered complete remission (CR), 3 patients died during the induction phase and 12 (40%) were resistant. One patient died while in CR for invasive fungal infection and one patient discontinued chemotherapy because of toxicity. Fourteen patients proceeded to an outpatient consolidation with Fludarabine 30 mg/sqm and Cytarabine 1 g/sm for two days plus Idarubicin 10 mg/sm on day 2. Three patients relapsed after consolidation. Maintenance therapy with Cytarabine 100 mg/sm once a week and Thioguanine 50 mg/sm for five days a week was given to 11 patients. The median overall (OS) survival was 4.7 months and the remission duration in CR patients calculated from the start of maintenance was short (median 7 months). Due to these unsatisfactory results, the protocol was modified in march 2003 and maintenance with low dose Gemtuzumab Ozogamicin (GO) (3 mg/sm iv every three months for a maximum of three doses) was used instead of chemotherapy. A second cohort of 28 consecutive patients (mean age 68.5 y, range 65-79) was treated with the same induction and consolidation therapy: 16 patients (57.4%) obtained CR, 11 were resistant, 1 early death, 1 off therapy because of toxicity. The consolidation course was given to 15 patients. Three relapsed after consolidation, two refused GO therapy and 1 is too early. Nine patients received a minimum of one GO course. The remission duration analysis shows a better remission duration in patients treated with GO maintenance compared to patients treated with chemotherapy (15.7 months vs 7 months; p=0.04). Toxicity of GO was mild and minimum transfusion support was required. No infectious complications were observed. Infusion reactions were generally mild. A severe hypotension occurred in one patient during the first infusion. All infusions were given on an outpatient setting. These preliminary results are encouraging and the study is ongoing. Updated results will be presented.

P453

LONG TERM MOLECULAR REMISSION BY IMATINIB MESYLATE IN PH-POSITIVE AML M6

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A 52-year old woman was admitted to our Hospital in August 2000 for cutaneous bleeding. Laboratory testing revealed thrombocytopenia (plt 16x10^9/L), anemia (Hb 11 g/dL), and leukocytosis (35x10^9/L). Bone marrow biopsy and aspirate were consistent with a AML-M6 FAB. Immunohistochemistry showed blast cells CD20-, CD79a-, PAX5-, CD68(KP1)+, MPO+, (PGM1)-, MLF1-, CD34$^+$. The immunophenotype was CD13+, CD33+, CD34$^+$, CD117+, MPO+, HLA-DR+. Cytogenetic analyses presented a 46, XX, add(9)(q34), der(21), der(22) kariotype and molecular analyses showed a AML1/ETO negative, CBF-beta/Mhy11 negative, DEK/CAN negative, flt3-ITD negative, b2/a2 BCR/ABL positive leukemic population. The patient was enrolled in the GIMEMA 99P protocol and achieved a first complete remission. After consolidation therapy, she presented hyperpyrexia due to tubercular mycobacterium pulmonary infection. Eight months after diagnosis the patient had a clinical relapse. Cytogenetic analyses presented a 46, XX, t(9;21;22) (q34;q21;q11.2) kariotype in 18 metaphases and a 46,XX,t(9;10;21;22) (q34;p14;q22;q11.2)+Ph kariotype in 2 metaphases, instead the molecular analyses did not reveal difference in the bcr/abl fusion transcript. In June 2001 the patient underwent therapy with Glivec (dose 600 mg/day) and after four months went into a clinical and molecular second remission. At present she is in continuous molecular remission with a good physical condition and under molecular monitoring. Conclusion: Bcr/abl molecular rearrangement is uncommon in acute myeloid leukemia, particularly in AML-M6 FAB. However the presence of bcr/abl fusion transcript gives an important therapeutic possibility, by means of the tyrosin kinase inhibitor imatinib mesylate (Glivec). We report a case in which the standard chemotherapy, with infectious complications, failed but a therapy targeted to molecular damage seems to be successful, giving the patient a good quality of life perspective.

P454

EFFICACY AND TOXICITY OF FLAI-G-CSF AND MYLOTARG FOR INDUCTION/CONSOLIDATION OF POOR-RISK AML PATIENTS

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Twenty poor-risk AML patients were treated with an induction regimen including fludarabine (25 mg/sqm/day for 3 days), cytarabine (1 g/sqm/day for 3 days), idarubicine (5 mg/sqm/day for 3 days), mylotarg (3 mg/sqm day +4)

and G-CSF (5 µg/day starting on day +9, until hematological recovery). An identical consolidation course was administered if at least partial remission (PR) was achieved. Twelve males and 8 females patients were treated. Median age at diagnosis was 62 (range 33 – 75). Out of 20 pts, 10 had a secondary AML, 8 had an unfavourable cytogenetics, 14 were older than 60 years, 2 had peripheral blast count higher than 30 x 10^9/L. Three patients were relapsed after standard induction chemotherapy; all the other patients were at diagnosis. Fourteen (70%) out of 20 patients responded to single induction course (12 CR and 2 PR). The median time to PMN recovery (> 1.0x10^9/L) was 20 days (range 17-100). The median time to PLT recovery (> 100x10^9/L) was 34 days (range 19-100). Four patients developed fever of undetermined origin (FUO) and 6 patients a Gram+ bacteremia. Three patients had radiologically documented fungal infection (lung) and 1 patient had microbiologically documented aspergillosis of maxillary sinus. Gastro-intestinal toxicity (anorexia, nausea, vomiting, mucositis) was really mild and only one patient developed a grade III WHO hemorragic syndrome (melena). Up to now, 10/20 patients completed the second course of therapy. Eleven patients out of 20 died (4 progressive disease resistant to the therapy, 5 progressive disease after relapse, 1 multi organ failure after allogeneic bone marrow transplantation, 1 myocardial stroke while in CR) and 5 (36%) patients out of 14 are in continuous complete remission (median follow-up: 6 months). These preliminary results suggest that FLAI-G-CSF-Mylotarg may be an effective regimen for poor-risk AML patients. Non-hematological toxicity, especially on gastro-intestinal tract, appeared to be mild or moderate. The high incidence of infections was probably due to the characteristics of patients and/or severe hematological toxicity. Longer follow up and larger series of patients are necessary to confirm these data.

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P455

LONG SURVIVORS AMONG AML ELDERLY PATIENTS NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY: DO THEY EXIST?

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Survival of elderly patients with Acute Myelogenous Leukemia (AML) not eligible for Intensive Chemotherapy (IC) is generally very poor (<4-6 months): however, episodic reports exist on patients with longer outcome. In order to assess the true incidence and clinical characteristics of this particular subset of long survivors, we examined 18 patients (M/F 10/8, median age 68.5 yrs, range 61 – 80) who survived more than 24 months (median survival 41.5 months, range 24.5 - 109). They represent the 7.3% of a whole population of 244 consecutive AML patients aged > 60 yrs diagnosed at our Institution from 1/89 to 12/98 and unfit for IC. The marrow and peripheral smears of the 18 patients at disease onset of were retrospectively revised and AML diagnosis confirmed in all cases. Median WBC, PLTS, peripheral and marrow blasts at diagnosis were 3.5 x 10^9/L, 125 x 10^9/L, 10% and 45%, respectively. Twelve patients (66%) had a previous documented myelodysplastic syndrome (MDS), 13 patients (72%) a good PS (0 - 1 according to WHO). The exclusion criteria from IC were age > 70 years (4 patients), a cardiological disease (4 patients), a previous MDS lasting > 6 months (4 patients), a PS > 2 (1 patient) or other organ failures (5 patients). Clinical characteristics of long survivors at diagnosis were compared with those of the whole cohort of 244 patients: there was a significative difference (p<0.01) as to median WBC (3.5 vs 7.5 x 10^9/L), median PLTS (125 vs 76 x 10^9/L), previous MDS (66 vs 36.5%) and 0 - 1 PS (72 vs 39%). Ten patients (55.5%) received only supportive care and did not require any chemotherapy; 8 patients (44.5%) received palliative chemotherapy with Hydroxyurea (5 patients) or 6Thioguanine + Cytarabine (3 patients) to control disease progression. The "hospital stay ratio" (hospital stay duration/days of survival x 100) was < 5% in 16/18 long survivors patients (89%). In conclusion, the incidence of long survivors among AML elderly patients conservatively treated is small but measurable (7 - 8%); clinical parameters at onset seem different from those of general AML elderly population and could be useful to recognise these patients at better prognosis, thus avoiding toxic, potentially life-threatening chemotherapies. However, a biological characterization is warranted to better define this subset.

P456

HYDROXYUREA + VINDESINE IN THE TREATMENT OF ELDERLY AML PATIENTS NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY

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In order to improve the dismal results obtained in elderly patients with Acute Myelogenous Leukemia (AML) not eligible for Intensive Chemotherapy (IC), we tested the addition of Vindesine (VND) to the standard palliative chemotherapy with Hydroxyurea (HU). From 12/2002 to 10/2004, 23 elderly patients (M/F 18/5, median age 76.2 yrs, range 62.8-90.3) with newly diagnosed AML and not eligible for standard IC were enrolled in to the study. Four patients had a previously documented myelodysplastic phase, 7 patients had a PS (WHO) > 2 and 4 patients had a documented infection at onset. Median Hb, WBC, PLTS and marrow blasts were 9.3 g/dL, 12.7x10^9/L, 65x 10^9/Land 48%, respectively. Clinical criteria for exclusion from IC were age > 75 yrs (7 patients), a cardiological disease (6 patients), a COBP (3 patients), a renal disease (2 patients) or other organ failures (5 patients). After diagnosis, patients were observed weekly and the association of HU (initial dose 1500 mg/day) and VND (5 mg iv every 15 days) was started in the presence of WBC > 10 x 10^9/l or a doubling time shorter than a week. Five patients (21.7%) did not

require chemotherapy: 3 died from infective or haemorrhagic complications while in stable disease after 1.5, 9.5 and 12 months, respectively, and 2 are still alive after 5 and 7.5 months. After a median time from diagnosis of 6 days (range 0 - 93), 18/23 patients (78.3%) started HU + VND: 2 patients achieved a Complete Remission (CR), 3 an haematological improvement, 6 a stable disease (WBC < 10 x 10^9/l for > 2 months during treatment) and 7 showed a disease progression. As to the 2 patients in CR, 1 with an abnormal karyotype (47 xy,+13) at diagnosis, achieved both morphologic and cytogenetic CR that lasted 14 months and 1 is still in CR after 3 months. Documented infections (5 broncopneumonic episodes, 4 abscesses and 1 bacterial sepsis) were the most common complications during treatment. Median survival of patients who received chemotherapy was 3.5 months (range 0.5-19). Median overall survival was 4 months (range 0.5 - 19), with 8/23 patients (%) surviving > 6 months. After a minimum observation period of 5 months, 4 patients are still alive, 3 were lost to follow-up and 16 died, 10 from disease progression and 6 in stable disease from infections (3), haemorrhage (2) and acute myocardial infarction (1). The addition of VND to HU seems to ameliorate the response rate (CR + haematological improvement) as compared to HU alone, but it does not seem to prolong survival. The role of VND in this subset of frail patients remains unclear and further studies with different doses and associations might be tested.

P457

ACUTE MYELOID LEUKAEMIA IN UNSELECTED OLDER ADULTS (>55 YEARS): ROLE OF HD-ARA-C-BASED MULTI-CYCLE CONSOLIDATION WITH G-CSF/AUTOLOGOUS STEM CELL SUPPORT

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Introduction. The managenent of AML in older adults (>55 years) is problematic due to reduced treatment compliance and high incidence of risk factors. We are evaluating a flexible risk-adapted consolidation program for unselected patients (FAB M3 excluded), consisting of (1) three consecutive IDR/HD-ara-C courses supported by a limited amount of autologous blood stem cells or (2) stem cell allotransplantation (SCT).

Methods. Patients are stratified into standard-risk (SR) and high-risk (HR) according to MRC/ECOG-SWOG cytogenetic classifications: F, favourable (SR), U, unfavourable (HR), and intermediate/normal (I/N). Additional risk features are used to sub-classify the I/N cytogenetic risk group (WBC >50.000, FAB M0/6/7/granulocytic sarcoma (GS), sec/MDS-AML, FLT-3+, hepato/splenomegaly, late CR: SR, none present; HR: any one present). After CR induction (cycles 1-2: ICE + IC or high-dose cycle in nonresponders), stem cell mobilization is attempted (cycle 3: Ara-C 1g/sqm./bd dd 1-4 + G-CSF). Thereafter, SCT is offered to HR cases. Those deemed unfit for SCT or without donor and all SR patients receive the supported HD-ara-C program (cycles 4-6: ara-C 2 g/sqm./bd dd 1-5, IDR 8 mg/sqm./d dd 1-2, $CD34^+$ cells 1-2x10e6/kg d 6, G-CSF from d 7) or, in the absence of $CD34^+$ cell mobilization, one-two unsupported intermediate-dose courses (cycles 4-5: ara-C 1 g/sqm./bd dd 1-5, IDR 10 mg/sqm. d1).

Results. So far, 131 patients aged 55-68 years (median 60) were entered on trial, of whom 34 (26%) and 97 (74%) exhibited SR and HR characteristics, respectively (F/I/N/U/unknown cytogenetics: 4/22/56/40/9; FLT-3+: 15; FAB M0/6/7/GS: 8/5/2/1; sec/MDS: 2/26; WBC >50.000: 23; hepato/splenomegaly: 22/14; late CR: 9). Overall, 96 patients entered CR (73%), of whom 10 underwent an allo-SCT (out of 71 HR in CR, i.e. 14%), 45 commenced the HD-ara-C phase and 24 (non-mobilizers, 28%) out of 86 patients evaluable after cycle 3 received intermediate-dose consolidation. Considering cytogenetics only and excluding the very few F cases (n=4), overall survival at 4 years was 12% in U, 36% in N, and 46% in I groups, respectively. Eventually, by combining cytogenetics and additonal risk factors, 4-year DFS was 47% in SR group (n=25) and 12% in HR group (n=71), with a 4-year survival of 52% and 28%, respectively. No treatment-related mortality occurred during multiple HD-ara-C cycles, in keeping with study objectives. Projected survival after allo-SCT was 53%.

Conclusion. These results imply that older adults with AML at intermediate-low cytogenetic risk and without additional clinical risk factors may benefit from HD-ara-C consolidation as herein employed. In the remainder, attempts to perform an allo-SCT are justified but, owing to advanced age, co-morbidity or early relapse, only a minority will be able to undergo the procedure.

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PROGNOSTIC ANALYSIS AFTER SEQUENTIAL HIGH-DOSE ARA-C, IDARUBICIN AND G-CSF IN REFRACTORY AND RECURRENT ACUTE LEUKAEMIA

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Introduction. SHAIG regimens were used as salvage therapy for refractory AML (after ICE) or ALL (after IDR-VCR-ASP-$PDN^{+/-}$-CY), or recurrent disease, in view to proceed, in responsive patients, to further high-dose Ara-C consolidation and SCT from related/unrelated donors or an autologous source. Here we analyze the prognostic factors correlating with a favourable treatment response.

Methods. SHAIG consisted of Ara-C 3 g/sqm. bd on dd 1-2 and 8-9 (2 g if age >60), IDR 12.5-20 mg/sqm. (phase I study: MTD 17.5, extended to phase II) on dd 3 and 10, cyclosporin (CS) 13.5 mg/kg over 12 h along with IDR, and G-CSF from d 11; modifications included omission of CS and/or prolongation of IDR infusion time (cellular pharmacodynamic study). Responders were given a second similar or reduced-intensity cycle and were transferred as soon as possible to SCT phase.

Results. Eighty-nine patients were treated: AML 72, ALL 17; M/F 46/43; age 46 (16-65) years; refractory 58, 1st recur-

rence 27, >1st recurrence 4; prior CR <12 mos. 25; prior SCT 7). Cumulative results were CR 50.5%, NR 27% and ED 22%. No significant prognostic difference was noted comparing AML vs. ALL, high-risk (CR 52%) vs. other cytogenetics, relapse vs. recurrence state, length of prior CR, age < vs. >55 years, CS (CR 61%) vs. no CS, and IDR over 30' vs. 180'. The significant factors for CR were performance status 0-1 on ECOG scale (CR 58% vs. 38%, p=0.007) and hypocellular, blast-free bone marrow on day 11 (BMd11-: CR 76% vs. 0%, p=0.000). By combining ECOG 0-1 and BMd11- in patients <61 years (full-dose Ara-C), CR was 23/32 (83%) vs. 0/9 in those with ECOG >1 and/or BMd11+ (p=0.000). Fourteen out of 45 CR patients are still in CR (31%). Median and 3-year overall survival and DFS are 9 mos. and 13%, and 8 mos. and 21%, respectively. For CR patients, median survival is 13 mos. and 25% at 3 years. Postremission treatment significantly affected survival: median 21 mos. (49% at 3 years) in 18 patients having SCT (auto 3, allo 15) vs. 13 mos. (none at 3 years) in 18 patients having chemotherapy only and 6 mos. (none at 3 years) in 8 patients transplanted with active disease (p=0.024).

Conclusion. SHAIG regimens were remarkably active in this setting, even in cases with refractory AML/ALL and/or high-risk cytogenetics. Prognostic factors were identified for both CR and survival (ECOG 0-1, BMd11-, post-SHAIG SCT), the projected leukaemia-free rate being around 50% at 3 years in the subset identified as favourable. Thus, an early therapeutic shift may be crucial at failure of first induction or, alternatively, SHAIG could be used first-line in selected risk groups. Evaluation of BMd11 may represent an early surrogate end-point for survival, calling for immediate therapeutic intervention in patients with residual leukaemia (SCT, experimental treatments).

P459

NPM1 GENE MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Nucleophosmin (NPM1, B23, numatrin) is a nucleolar phosphoprotein which regulates the ARF-p53 tumor-suppressor pathway and it is the translocation partner of MLF1 (Myeloid Leukemia Factor 1) in acute myeloid leukemia with t(3;5)(q25;q35), of RARA (Retinoic Acid Receptor Alpha) in acute promyelocytic leukemia with t(5;17)(q35;q12), and of ALK (Anaplastic Lymphoma Kinase) in non-Hodgkin lymphomas with t(2;5)(p23;q35).

Falini et al (NEJM 2005, 352:254-266) identified a NPMc+ AML subgroup characterized by cytoplasmic localization of NPM protein. This subgroup includes around 60% of AML with normal karyotype. By direct sequencing we showed that exon 12 NPM1 mutations are the genetic lesion leading to altered -COOH terminus in the NPM cytoplasmic protein.

Aims. Characterization of NPM1 mutations in acute myeloid leukemia with cytoplasmic NPM.

Materials and methods. For the NPM coding region analysis, 1ug of RNA was retrotranscribed with use of the Thermoscript RT-PCR System (Invitrogen).Then cDNA sequences were amplified with primers NPM1_25F (5'-GGTTGTTCTCTGGAGCAGCGTTC-3') and NPM1_1112R (5'-CCTGGACAACATTTATCAAA-CACGGTA-3') with use of the Expand Hight-FidelityPLUS PCR System (Roche, Applied Science) . To amplify the sequence of NPM1 exon 12 from genomic DNA, two oligonucleotides were designed to anneal to the flanking intron sequences (NPM1_F [5'-TTAACTCTCTGGTG-GTAGAATGAA-3'] and NPM1_R [5'-CAAGAC-TATTTGCCATTCCTAAC-3']. PCR products, purified by standard methods, were sequenced directly from both strands.

Results. All mutations detected in NPMc+ AML were heterozygous and occurred at exon 12 of the gene resulting in an alterated -COOH terminus for the NPM protein. Sofar, we identified fourteen different mutations, all of them producing a +4 nt frameshift and thus originating the same COOH terminus.

Conclusions. NPM mutations represent the most frequent and specific genetic lesion sofar identified in AML with normal cytogenetics (around 60% of cases). Mutation A (>75% of cases)is due to a TCTG tetranucleotide duplication at position 956 through 95: 956_959 dup(TCTG). Other less frequent mutations, i.e B, C and D (around 20% of cases) are due to different insertions: 959_960 ins(CATG); 959_960 ins(CGTG); 959_960 ins(CCTG), respectively. Based on mechanisms of mutations we have already identified new types of mutations in single cases, and additional sporadic variants can be predicted.

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CLINICAL AND BIOLOGICAL FEATURES OF SECONDARY ACUTE MYELOID LEUKEMIAS

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Modern treatment regimens for many malignant diseases now result in a significant number of prolonged and durable remissions. Nevertheless, a number of agents that contribute to the treatment of cancer have the potential to induce a second malignancy. Secondary acute myeloid leukemias (sAML) are usually related to: a) previous chemotherapy; b) documented environmental exposure to various different agents such as benzene, petroleum, organic solvents and arsenic pesticides; c) antecedent myelodysplasia. We reviewed the cytological and clinical features of sAML observed after antecedent solid neoplasia. Between January 1988 and December 2004, 767 new cases of adult AML were diagnosed and 35 cases of sAML (4.6%) were

observed. The median age of patients with sAML was 62 years (range 28-79). In this group the prior neoplastic malignancies identified were: colon (5), breast (6), thyroid (5), lung (3), uterus (2), skin (3), ovary (1), prostate (1), pharynx (1), rectum (2),testis (1), bladder (1), stomach + prostate (1), uterus + breast (1), breast + uterus + ovary (1), seminoma + thymoma (1); 5 patients (14.2 %) had undergone chemotherapy (CT), 11 had been treated with radiotherapy (RT) alone, 3 had received CT plus RT and 16 had developed AML after surgical treatment only; all 19 patients were treated with alkylanting agents. The median latency between diagnosis of the primary neoplasia and the development of AML was 65.2 months (range 6-324). According to FAB criteria the AML cases were classified as M0 (1), M1 (3), m^2 (7), M3 (3), M4 (11), M5 (8), M6 (2). Conventional cytogenetic analysis was performed in 24 (68.5%) cases: t(15;17) (3 cases); del(5) (2 cases); del(5),del(17p),del(20q),del(22q) (3 cases); +8 (4 cases); t(X;4),del(10p) (1 case); del(7q34) (3 cases); inv16 (2 cases); inv16,del(9) (2 cases); del(5),del(17p),del(20q) (1 case) and a normal karyotype in 3 patients. Phenotypic evaluation of the blast cells revealed a high incidence of CD34 positivity in 24/35 (69%), CD7 in 15/35 (42.8%) and MRK16 in 28/35 (80%) patients evaluated. According to FAB classification there was a high incidence of M4/M5 FAB subtype (52%). In all patients morphological evaluation revealed dysplasia of the granulocytic (28), erythroid (24) and megakariocytic (18) lineages. Twenty-two patients received induction treatment including anthracyclines plus cytosine arabinoside (ARA-C). We obtained complete remission in 9 patients (40.9%), in whom median disease-free survival was 4 months and overall survival 12 months. Four patients refused treatment and 9 patients, not elegible for intensive chemotherapy, were treated with standard dose ARA-C plus Etoposide.

Secondary AML represents a distinct subgroup with high frequency of cytogenetic abnormalities and poor clinical outcome, according to data reported in literature. The therapeutic strategy should be defined considering the conventional risk factor combination, similar to the de novo AML cases. In our study there is a group of patients in whom the occurrence of secondary acute leukemia is not related to previous chemo e/o radiotherapy. However the hypothesis that these patients could develop two or more malignancies because of familial predisposition, possible due to the presence of genetic factors, must be taken into account.

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A CASE OF HYPERCALCAEMIA ASSOCIATED WITH ALL-TRANS RETINOIC ACID THERAPY FOR ACUTE PROMYELOCYTIC LEUKEMIA

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We report a case of a 37-year-old woman with hypercalcaemia caused by all-trans retinoic acid (ATRA) therapy for acute promyelocytic leukemia (APL). M.G., a 37-year-old woman, was diagnosed of APL in April 2004. Peripheral blood evaluation showed: white blood cells 50000/mmc (80% promyelocytes, 5% neutrophils, 10% lymphocytes), haemoglobin 9.2 g/dL, platelets 12000/mmc. Fibrinogen was 109, D-dimer was over 20, coagulation tests were in the normal range. Bone marrow evaluation showed 76% atypical promyelocytes with Auer Rods. Chromosomal analysis identified a (15;17) translocation in 15 out of 20 metaphases, and molecular biology confirmed a pml-rar-alpha transcript. The patient was treated according to Italian guidelines Gimema AIDA 2000. The patient received one course of induction chemotherapy with idarubicine 10 mg/mq/day (total 25 mg/day) for 4 days, and ATRA 45 mg/mq/day (total 70 mg/day) for 30 days, followed by three consolidation courses, respectively with idarubicine 12 mg/mq/day (total 18 mg/day) for 1 day, Ara-C 450 mg/mq/day (total 690 mg/day) for 5 days, all of them combined with ATRA 45 mg/mq/day (total 70 mg/day) for 15 days. The patient obtained a hematological and cytogenetical remission at the end of the induction, and a molecular remission at the end of the consolidation courses. On day 10 of the third consolidation course, hypercalcaemia (2.78 mmol/l, normal 2.1-2.6 mmol/l) was observed; the patient was asymptomatic. Conventional supportive therapy against hypercalcaemia (hyperhydration and diuretics) was performed, but the serum calcium levels continued to rise until 3.56 mmol/l. The concentration of intact parathyroid hormone (PTH) was 4 pg/mL (normal 10-65pg/mL) and phosphate levels were normal. Because of the persistence of high serum calcium levels, ATRA was stopped on day 14 and biphosphonates (Pamidronate 90 mg intravenously) were administered. Then, calcaemia was normalized within 3 days. The mechanism of ATRA-induced hypercalcaemia is still unclear. Other cases are described in literature; in few cases hypercalcaemia was normalized with biphosphonate without discontinuation of ATRA. Our observation suggests that hypercalcemia is occasionally associated with ATRA administration, and that regular biochemical examination including calcaemia is needed during the systemic ATRA therapy in APL.

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EXTRAMEDULLARY INFILTRATES OF ACUTE MYELOID LEUKEMIA: BIOLOGICAL AND CLINICAL FEATURES

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Acute myeloid leukemia (AML) is a hemopoietic neoplasm that may be associated with extramedullary infiltrates (EMI) of leukemia blasts at diagnosis. We evaluated the frequency, the biological features and the outcome of adult AML patients with EMI at diagnosis. From January 1997 to December 2002, 200 patients with de novo AML(median age 42 years, range 15-60) were studied; patients with APL were excluded because they received different treatment. Of 200 cases with de novo AML, 29(14.5%) had EMI at diagnosis. Eleven patients (37.9%) had gum hypertrophy, 12(41.3%)had skin infiltrates, 5 (17.4%) had CNS involvement and 1 (3.4%) had pericar-

dial infiltration. No significant differences in term of sex, age, median Hb level and platelets count were found between patients with EMI and patients without EMI. The patients with EMI had higher median WBC count (27 x10^9/L) than patients without EMI (8.5x10^9/L)(p=0.05). The patients with EMI had higher incidence of M4/M5 FAB subtype (62%) than patients without EMI (27.4%)(p=0.005). The cytogenetic analysis was performed in patients with and without EMI; none of the abnormal cytogenetic findings was associated with EMI. We evaluated the relationship between the AML blasts surface antigen expression and EMI using a large panel of MoAb; no significative differences in term of single expression antigen were found between the two groups. The coexpression of CD56/CD4 and CD56/CD14 was more significantly found in patients with EMI (35% and 29% respectively) than without EMI (10.4% and 6.9 respectively)(p=0.004 and p=0.003). All patients had been treated with induction therapy according to the GIMEMA Protocols including Ara-C, etoposide and idarubicin (20 pts), mitoxantrone (25 pts) and daunorubicin (155 pts). The overall CR rate was 65%; the NR rate of patients with EMI (75.8%) was significantly higher than patients without EMI (33.9%)(p=0.0001). The median DFS was 8,5 months (range 3-25) and 12 months (4-83), while the median OS was 9 months (range 3-70) and 47 months (range 3-84) in patients with and without EMI, respectively. Our data demonstrate that a high WBC count, M4/M5 FAB subtype, CD56/CD4 and CD56/CD14 expression are associated with extramedullary infiltrates of adult AML at diagnosis and that the presence of EMI adversely affects the complete response rate to induction chemotherapy. Analysis of the clinical and biological features in a larger series of adult AML patients is needed to evaluate the allocation of this subgroup in different or more intensive treatment arm.

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THE UNBALANCED RATIO BETWEEN KTS- AND KTS+ ISOFORMS OF WT1 MAY PROBABLY BE RESPONSIBLE FOR THE DYSFUNCTION OF ITS PHYSIOLOGICAL ROLE IN LEUKEMIC CELLS.

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WT1 is a tumor-suppressor gene coding for a zinc-finger transcription factor located on chromosome 11p13, which was originally identified for its involvement in the pathogenesis of the Wilms' tumor. In normal bone marrow and peripheral blood WT1 expression is extremely low. By contrast WT1 expression is increased in AML, ALL, MDS, CML and PH negative Chronic myeloproliferative disorders (CMPD) such as CMML and MMM. In spite of his well established role as a molecular marker of leukemic cells, little is known at present regarding the role of WT1 in the leukemic process, in particular regarding his hypothetical role as oncogene rather than tumor suppressor gene. The WT1 protein is predominantly nuclear and contains four zinc fingers at the C-terminus, suggesting that its function as a transcriptional factor. Over the past decade a great deal of evidence has been accumulated to show that WT1 can indeed function as a transcriptional regulator: as an activator, a repressor or a co-activator. However, it has also become clear that the function of WT1 is more complex and that it is likely to be involved in different processes depending on isoform differences. There are two major WT1 isoforms, which differ by the insertion of three amino acids, KTS between zinc fingers 3 and 4 as the result of alternative splicing. The KTS- WT1 isoform binds to DNA with higher affinity and appears to be more active in transcriptional regulation. There is evidence that the two different isoforms of WT1 (KTS+ and KTS-) are involved in different steps of gene expression control and may have different functions. Moreover the two isoforms may possess different shuttle capacity between the nucleus and cytoplasm. In the present study we analyzed and quantified the two isoforms in normal and leukemic cells. We analyzed KTS+ and KTS- isoforms by RT-PCR using a fluorescent forword primer. The fragment analysis was performed by capillary electrophoresis on the ABI PRISM 3100 ABI Genetic Analyzer. We analyzed 116 samples (81 BM and 35PB) collected from 68 AML and 48 CML patients. Blast cell population has been purified from 6 AML BM samples. In addition, 20 BM and PB samples collected from healthy volunteers have been study. We found that in normal BM and PB samples the KTS- isoform is prevalent a ratio KTS-/KTS+ ranging from 7,2 to 2. By contrast in 65% of AML and 70% of CML samples and in all the purified blast cell population the KTS+ isoform is mainly represented with a ratio KTS-/KTS+ ranging from 0,12 to 0,5. We conclude that in leukemic cells there is an unbalanced ratio between the two WT1 isoforms, and this may probably cause a dysfunction in WT1 physiological role of transcription factor. The ongoing studies of genes whose expression is regulated by WT1 suggest a close relationship between WT1 KTS- isoform amount and the expression levels of these latter genes.

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NOT PUBLISHED

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ACQUISITION OF A RAS POINT MUTATION IN A PATIENT WITH CHRONIC MYELOMONOCYTIC LEUKEMIA AFTER PROGRESSION FROM DYSPLASTIC TO PROLIFERATIVE VARIANT

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Chronic myelomonocytic leukemia (CMML) represents a disease entity with high heterogeneity of clinical and hematological characteristics, classified into a new category of myelodysplastic/myeloproliferative diseases by the last World Health Organization classification of myeloid malignancies. Biological knowledge on CMML is very limited, and molecular abnormalities related to disease devel-

opment and progression remain largely to be clarified. Among hematological malignancies, CMML has the highest frequency of point mutations of the RAS gene family (20% to 35% of patients). Patients with proliferative variant of the disease (MP-CMML, WBC > 12 x 10^9/L) have significantly higher frequency of RAS point mutation than patients with the dysplastic variant (MD-CMML, WBC < 12 x 10e9/L). In facts, RAS mutations have been shown to associate with features of cell proliferation and with monocytosis rather than with features of dysplasia. However, the importance of RAS mutations in the pathophysiology of CMML is unknown.Ras family members are small G-proteins bound to the inner plasma membrane; here, by switching from the active GTP-bound to the inactive GDP-bound conformation, they transduce extracellular signals that regulate proliferation, differentiation and apoptosis. Ras proteins possess a weak intrinsic GTPase activity that is regulated by distinct classes of proteins. Switching between the active and the inactive state is associated with a conformational change of two regions, designated as switch I (residues 30-38), overlapping with the core effector region, and switch II (60-76). Activating point mutations of codons 12, 13, 59, 61 and 63 have been shown to decrease GTPase activity, thus leading to constitutive activation of Ras.

In April 2003 a patient with diagnosis of CMML, myelodysplastic variant (WBC = 6,5x10^9/L) was admitted at Ospedale Maggiore of Milan. Genomic DNA extracted from peripheral blood mononuclear cells was amplified with primers for exons 1 and 2 of N- and K-RAS by PCR, and subsequently sequenced. At this stage of the disease, no mutation was detected. In January 2005, the patient experienced a rapid progression to myeloproliferative subtype, with WBC counts constantly over 30 x 10^9/L. Molecular analysis was repeated and a mutation GGA->GAA in codon 60 of N-RAS was found, leading to the substitution of Glycine with Glutamate in the corresponding aminoacidic position. PCR amplification and direct sequencing of both forward and reverse strands from the genomic sample were repeated twice and the presence of the point mutation confirmed.

Mutations of codon 60 were rarely reported in human cancers. Although transforming potential of such aberrations is unknown, detection of Gly60Glu substitution after progression of MD- to MP-CMML suggests a pathogenetic event. In fact, the replacement of the neutral aminoacid glycine with the negatively charged glutamate could effectively alter the conformational changes necessary for Ras activation. To the best of our knowledge this is the first time that a RAS point mutation is detected concomitantly with progression of CMML from the dysplastic to the proliferative variant. Further studies are warranted to clarify the exact role of this novel acquired mutation.

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OSTRUZIONE MECCANICA DI NEOVESCICA ILEALE ORTOTOPICA IN UN PAZIENTE AFFETTO DA LEUCEMIA ACUTA MIELOIDE M0 IN TRATTAMENTO CHEMIOTERAPICO

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Il paziente S.D., di anni 65, ricoverato presso la nostra U.O. per Leucemia Acuta Mieloide M0 (LAM M0) diagnosticata nel 2004, in fase di aplasia secondaria alla chemioterapia di induzione (Daunorubicina 50mg/mq al dì per tre giorni e Citosina Arabinoside 100 mg/mq al dì per sette giorni) ha presentato peggioramento delle condizioni cliniche con febbre, ipotensione, anuria persistente, in assenza di dolore in sede pelvica. Il paziente, per una pregressa diagnosi di carcinoma a cellule di transizione della vescica, nel 1997 si era sottoposto ad intervento di cistectomia ed impianto di neovescica ileale ortotopica. La persistenza dell'anuria, malgrado trattamento con diuretici e dopamina, ha indotto alla cateterizzazione vescicale con Foley 18Ch con successiva irrigazione, che non è stato possibile realizzare per la presenza di un ostacolo meccanico. Si è quindi proceduto ad aspirazione mediante siringa, che ha dato esito alla fuoriuscita di muco in quantità pari a circa 4,5 cc. La formazione del tappo mucoso, che ostacolava la fuoriuscita di urina, potrebbe essere messa in relazione con una flogosi mucipara dell'epitelio di rivestimento della neovescica ileale; tale condizione poteva avere eziologia infettiva e/o essere conseguenza di un danno da chemioterapici responsabile di incremento della componente mucipara. Tale circostanza è stata evidenziata per la prima volta in occasione del trattamento chemioterapico. Ciò può essere considerato come un raro effetto collaterale che può verificarsi in corso di trattamento di leucemia acuta.

Gene and Cell Therapy

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PHASE I-II STUDY TO EVALUATE THE SAFETY AND THE EFFICACY OF GRANULOCYTE COLONY STIMULATING FACTOR IN MOBILIZING HEMATOPOIETIC STEM CELLS IN PATIENTS WITH ADVANCED LIVER CIRRHOSIS

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Bone marrow-derived hematopoietic stem cells (HSCs) contribute to tissue regeneration after acute or chronic liver damage. In this study, we assessed whether G-CSF can be safely administered to patients with liver cirrhosis in order to expand and to mobilize HSCs. Seventeen patients with advanced liver disease [16 male, mean age: 59 years; 12 HCV, 1 HBV, 1 HDV, 3 alcohol abuse; median Child-Turcotte-Pugh (CTP) score: 7 (range 5-9), median MELD (Mayo End Stage Liver Disease) score: 11.5 (range 7-17)] were consecutively treated with r-huG-CSF (Lenograstim®). Increasing doses of G-CSF were administered subcutaneously for 7 consecutive days to five cohorts of three patients each, starting from 2 microg/kg/daily. The dose of G-CSF was escalated according to Fibonacci's increment rule until the successful mobilization of 10 CD34(e)+ cells/microl in at least 2/3 patients. Fifteen patients were enrolled in the phase I study. G-CSF mobilizing dose was found at 15 microg/Kg/day when mobilization of HSCs occurred in 2/3 patients on day 5 from the first administration (mean $CD34^+$cells/microl: 49,2 ±2,7). Two out of 4 patients have been enrolled so far in the phase II study with the mobilizing G-CSF dose, and all of them showed a successful mobilization and collection of HSCs. Overall, the median peak value of CD34(e)+ and CD133(e)+ cells in mobilizing patients were 41.5±13.5 cells/microl and 26.5±3.2 cells/microl, respectively. No severe adverse events were observed at any dosage. From the 6.6 microg/kg dose of G-CSF or higher, the side effects observed were bone pain (10/17), fever (4/17) and alkaline phosphatase increase (10/17). Up to 30 days after treatment with G-CSF, no significant modification of liver function parameters has been observed with the notable exception for the significant decrease of alpha-foetoprotein in all but one patients and the decrease of transaminases in mobilizing patients. In conclusion, the administration of G-CSF to patients with liver cirrhosis is safe and feasible and is capable of mobilizing HSCs at the dosage of 15 microg/kg/day. The decrease of alpha-foetoprotein and transaminases may suggest a positive effect of G-CSF and/or HSCs in these patients. Our study represents the first step for evaluating the role of G-CSF and mobilized HSCs to improve liver function in patients with liver cirrhosis.

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THE PROTEASOME INHIBITOR PS-341 INDUCES APOPTOSIS OF CD14+ DENDRITIC CELL PRECURSORS AND OF IMMATURE AND MATURE DC

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Downregulation of NF-kB activity has been shown to prevent the differentiation and maturation of DCs. As PS-341 promotes the degradation of NF-kB, we have investigated whether PS-341 affects DC differentiation and/or maturation. Immunomagnetically purified CD14+ monocytes were cultured with GM-CSF (50 ng/mL) and IL-4 (800 U/mL), in the presence or absence of PS-341 at 0.1 to 100 ng/mL. Addition of PS-341 at doses as low as 10 ng/mL significantly reduced the recovery of viable cells already after 24 hours (7≥5% vs 59±26% in control cultures) (n=6 experiment, $p<0.05$). To test whether the reduced recovery of viable cells was due to apoptosis induction, the expression of annexin V was determined by flow cytometry. Upon addition of 10 ng/mL PS-341, apoptotic cells increased at 24h (87±10 vs 37±13%) (n=6 exps) ($p<0.05$), whereas no significant difference was observed with 1 ng/mL. Kinetic experiments showed no effect of PS-341 on cell survival for up to 16 hrs of culture (n=3), whereas annexin V appeared on the cell surface after 18 hrs. Microscopical analysis of cytospins showed nuclear fragmentation and massive cytoplasmic vacuolization, features suggestive of apoptosis. To test whether PS-341 induced apoptosis of immature and mature dendritic cells as well, monocytes were cultured for 6 days with GM-CSF and IL-4 then 100 ng/mL LPS was added for the last 48 hours. PS-341 was added to the culture either at the same time or following LPS administration. Addition of PS-341 at the time of LPS administration strongly reduced day 8 cell recovery as compared to control cultures (3±1% vs 17±3%) (n=4 experiments). PS-341-cultured DC were mostly apoptotic (97±2% as compared to 31±7% in control cultures). Moreover, addition of 10 ng/mL PS341 following LPS-induced DC maturation also strongly increased the number of apoptotic mature DC ($61^{+/-}29$ vs 32±20, n=2). Moreover, PS-341 at 10 ng/mL inhibited the upregulation of the costimulatory molecules CD80, CD86 and CD40 on immature (by 90±4%, $90^{+/-}3$% and 84±11%, respectively, n=4 exps, $p<0.05$) as well as on mature (by 60±35%, 57±31% and 57±32%, respectively, n=2 exps, p=n.s.) DC. Finally, PS341 at the dose of 10 ng/mL did not induce apoptosis of either resting or proliferating T lymphocytes, suggesting that cells belonging to the DC lineage may be selectively sensitive to proteasome inhibition. Thus clinically relevant doses of PS-341 (in the range of 10^{-8} M) may effectively and rapidly inhibit the survival pre-DC and DC in vitro, suggesting its potential utility in the prophylaxis of DC-triggered immunologic diseases, such as GVHD.

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IL-7 ENGINEERED MESENCHYMAL CELLS HASTEN IMMUNOLOGICAL RECONSTITUTION IN NOD/ SCID MICE

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Interleukin-7 (IL-7) is a cytokine produced by multiple stromal tissues, including epithelial cells on thymus and bone marrow that plays a pivotal role in human T-cell survival, development and homeostasis. We engineered human stromal cells with the IL-7 gene and studied the effects on T-cells in vitro and on immunological reconstitution in NOD/SCID mice. Transduced mesenchymal cells were negative for CD45 and CD14, positive for CD90 (98.15%), CD105 (87.6%) and STRO-1 (86.7%) and stably produced IL-7 (16.37±2SD picograms/mL). In co-cultures a) with T cells, IL-7 engineered stromal cells inhibited PHA-induced T cell proliferation. (Proliferation Index: 3.6 vs 8.0 in untransduced cells and 65.8 in PHA alone); and b) with immunoselected naive T cells, they maintained the CD45RA+CD45RO- naive phenotype (Resting Naive Cell Count: 4.2 times more than controls). $5x10^5$ engineered stromal cells were injected intraperitoneally after 3.5 Gy total body irradiation. Engraftment was evaluated by NeoR based PCR in all murine tissues and immunohistologically in bone marrow, spleen and thymus by human stromal-specific stainings with anti-vimentin and anti-endoglin (CD105) monoclonal antibodies. NeoR/based PCR showed transduced mesenchymal cells homed to all organs (highest percentages in liver and lung; overlapping signals in spleen, thymus, bone marrow, heart, kidney, skin and gut; traces in brain). Immunohistological analysis detected stromal cells in bone marrow, spleen and thymus. Staining with anti-vimentin was stronger than with anti-endoglin. Positivity was more evident in bone marrow than in spleen or thymus with both antibodies. The cells had engrafted in these organs and were not phagocytised by macrophages. Compared with transplantation of $1x10^6$ $CD34^+$ cells alone, co-transplantation of $5x10^5$ untransduced mesenchymal cells and $1x10^6$ $CD34^+$ cells and co -transplantation of 5x10 (e) 5 IL-7 engineered stromal cells with $1x10^6$ $CD34^+$ cells, the last improved engraftment in terms of CD45+ cells and significantly increased the CD3+ cell count in peripheral blood (7.4±3 vs 1.4±1.6 $p<0.05$), bone marrow (5.5±2 vs 0.8±1 $p<0.05$) and spleen (6.2±2 vs 0.08±0.1 $p<0.05$). No significant differences emerging in CD19+ cell recovery compared with controls. These data demonstrate IL-7 transduced stromal cells maintain the naive T phenotype (CD45RA+/CD45RO-) *in vitro* and *in vivo* home to lymphoid organs and produce sufficient IL-7 in loco which, through presentation by means of the extracellular matrix complex, could support T-cell development. This is a novel approach, that could have important clinical implications in the treatment of immunocompromised patient.

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EX VIVO GENERATION AND FUNCTIONAL CHARACTERISATION OF CYTOKINE INDUCED KILLER CELLS IN GMP CONDITIONS

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Background. Cytokine induced killer cells (CIK) are $CD3^+/CD56^+$ cells that can be expanded in vitro following stimulation of peripheral blood mononuclear cells with interferon-gamma (IFN-γ) on day 0, anti-CD3 antibody (OKT3) on day 1, followed by expansion in interleukin-2 (rhIL-2) for 18-21 days. These cells have been shown to be cytotoxic against tumour cells with little *in vivo* toxicity. We are planning a phase I study with CIK cells in acute myelogenous leukaemia patients relapsed after allogeneic bone marrow transplantation. CIK cells from the peripheral blood of the donors will be generated in vitro in order to administer $1x10^7$ $CD3^+/CD56^+$ cells/kg/ infusion for up to 3 infusions.

Aims. In order to fulfill the cell therapy requirements by the italian authorities, we have set up clinically feasible conditions to generate CIK cells in vitro under GMP rules. *Results.* CIK cells were expanded in vitro from a mean of $160x10^6$ PBMC (range 20-525 x 106) from 22 consecutive donors. CD3+/CD56+ cells were 4% of the total mononuclear cells on day 0 (range 1-9%) and reached a mean of 56% after 18-21 days of culture (range 13-82%). An average 328 fold expansion of $CD3^+/CD56^+$ cells was obtained (range 29-1300). Thus a mean of $980x10^6$ CIK cells could be obtained (range 150-2600 x 10^6) after 18-21 days culture. In 18/22 donors, expanded CIK preparations satisfied the established criteria for lot release (at least 40% CD3+/CD56+ cells). They showed an average of 46% cytotoxic activity against the K562 erythroleukemia cell line at a 30:1 E:T ratio (range 20-100%), and 42% cytotoxicity against freshly isolated patients' leukemic cells (range 11-40%). CIK cells could also expanded from autologous PBMC in patients having still up to 80% of blasts cells in te periphery at day 0. These CIK cell populations at the end of culture had the same phenotype as CIK cells derived from normal donors and were free of leukemic cells, as determined by phenotypic analysis. Finally quality controls assays were performed on 6 consecutive CIK preparations with validated tests (viability, colony assays, measurement of contamination by aerobic and anaerobic bacteria and fungi, endotoxin and mycoplasma), confirming that the preparations were adequate (6/6) for *in vivo* use. *Conclusions.* An adequate number of CIK cells can be expanded in vitro in GMP conditions, starting from 200-300x106 leukocytes obtained by leukapheresis. These cells satisfy all criteria of viability, functionality, phenotypic characteristics and sterility required for *in vivo* use in more than 80% of cases.

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NUCLEOFECTION IS AN EFFICIENT NON-VIRAL TRANSFECTION TECHNIQUE FOR HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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To date, the most efficient systems to deliver DNA into stem cells (SC) are viral-based techniques which show high gene transduction and transgene expression in many cellular models. These systems, unfortunately, present some disadvantages mainly involving safety risks. On the contrary, non-viral methods have proved to be inefficient for most primary cells. The NucleofectorTM technology, a new non-viral electroporation-based gene transfer technique, has proved to be an efficient tool for transfecting hard-to-transfect cell lines and primary cells. However, little is known on the capacity of this technique to transfect adult SC. In this study, we applied the NucleofectorTM technology for engineering human bone marrow-derived mesenchymal SC (hMSCs). Using a green fluorescent protein reporter vector, we demonstrated a high transgene expression level using two different pulsing programs (median average over 50%; range 42.53±3.46%-73.66± 2.93%). Total cell recovery after nucleofection was 28% (range 12.65±7,2%-44.55±3.98%) and viable cells represented more than 80% (range 81.20±7.78%-94.31± 0.85%). By comparison, other non-viral transfection systems as FUGENE6 and DOTAP showed a much lower efficiency: 4.45±22% and 6.83±4.2%, respectively. Nucleofection did not affect either the immunophenotype of hMSCs or their normal adipogenic and osteogenic differentiation potential and their ability to inhibit T-cell alloreactivity. Moreover, the interleukin-12 gene could be successfully transfected into hMSC and the immunomodulatory cytokine was produced in great amount for at least 3 weeks without impairment of its biological activity. In conclusion, nucleofection is an efficient non-viral transfection technique for human bone marrow-derived SC and nucleofected hMSCs can serve as cellular vehicles for the delivery and local production of biological agents.

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CONTROL OF GRAFT-VERSUS-HOST DISEASE INDUCED BY CENTRAL MEMORY TK+ HUMAN T LYMPHOCYTES

Bondanza A, Valtolina V, Magnani Z, La Seta Catamancio S,[1] Benati C,[1] Toma S,[1] Traversari C,[1] Radrizzani M,[1] Bonyhadi M,[2] Ciceri F, Bordignon C, Bonini C

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Suicide gene therapy is a promising approach for the safe exploitation of the graft-versus-leukemia effect. Retroviral transduction of T cells to express the Herpes Simplex Virus thymidine kinase confers an inducible suicidal phenotype upon ganciclovir (GCV) administration, thus enabling the selective elimination of T lymphocytes causing graft-versus-host disease (GvHD). Despite clinical and experimental studies substantiating the efficacy of the strategy, CD3-mediated activation of T cells, utilized to generate genetically modified cells (TK+) has been shown to reduce alloreactivity. In this study we demonstrate that TK+ cells generated with anti-CD3 antibodies are mainly CD45RA-CCR7- "effector memory" cells. These cells produce high levels of IFN-_ and perforin but fail to up-regulate CD40L upon stimulation. In order to improve "T cell fitness" and restore alloreactivity, we utilized Xcyte Dynabeads, which are anti-CD3 and anti-CD28 antibody-coated paramagnetic microbeads (bCD3/CD28) to sustain T cell proliferation and retroviral transduction. We observed that TK+ cells generated with bCD3/CD28 display a normal CD4/CD8 ratio, a CD45RA-CCR7+ "central memory" phenotype and co-express CD28 and CD27. They produce high levels of IL-2 and strongly up-regulate CD40L upon stimulation. To analyse the alloreactive potential of bCD3/CD28-TK+ cells, we set up an *in vivo* model that aims at evaluating the ability of TK+ human lymphocytes to engraft and cause GvHD. By conditioning NOD/scid mice with sub-lethal irradiation and NK-depleting TM-?1 antibody, we were able to significantly decrease the threshold for engraftment of human lymphocytes and for development of GvHD. In this model, we observed a significant higher incidence of GvHD in NOD/scid mice infused with bCD3/CD28-TK+ cells than in mice receiving TK+ cells generated with anti-CD3 antibody. *in vivo* elimination of TK+ cells with GCV resulted in the complete abrogation of GvHD. These results validate a tool for the generation of human TK+ lymphocytes with high alloreactive potential, to be utilized in clinical trials of allogeneic hematopoietic stem cell transplantation for the cure of tumors.

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MODULATION OF GRAFT-VERSUS-HOST DISEASE INDUCED BY CENTRAL MEMORY TK+ HUMAN T LYMPHOCYTES

Bondanza A, Valtolina V, Magnani Z, Perna SK, Ponzoni M, La Seta-Catamancio S,[1] Benati C,[1] Toma S,[1] Traversari C,[1] Radrizzani M,[1] Bonyhadi M,[2] Ciceri F, Bordignon C, C Bonini

Cancer Immunotherapy and Gene Therapy Program, [1]MolMed SpA, S. Raffaele Scientific Institute, Milano, Italy, [2]Xcyte Therapies, Inc., Seattle, WA, USA

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IN VITRO EXPANSION OF CORD BLOOD DERIVED ENDOTHELIAL PROGENITOR CELLS

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Background. Human umbilical cord blood has been shown to contain abundant EPCs that can be isolated and expanded in culture. These cells participate in endothelial network formation in vitro and are incorporated *in vivo* into sites of active postnatal neovascularization. This study is aimed to evaluate EPCs expansion capacity in vitro in view of their possible utilization as a source of endothelial cells for clinical application (diabetes, cardiovascular diseases).

Methods. 5 samples from CB were processed as follows: MNCs were plated on Fibronectin coated flasks in endothelial basal medium plus supplements. Cells were incubated at 37°C in 5% CO2 and medium was completely changed every 4 days. Clones appeared in culture from day 10 to day 30, then cells were passaged up to ten folds. Phenotype analysis, endothelial gene expression profile (RT-PCR) and limiting dilution assay were performed at passage 1,3,5,7 and 10 to evaluate whether EPCs maintained the specific lineage profile and the clonogenic capacity during the expansion procedure.

Results. Median frequency of EPC clones/10^6 MNCs was 0.25 (range 0.13-0.36). After a median of 60 days (4-5 passages) cells were expanded a median of 80 folds. Over this period, median number of collected cells ranged from $7x10^8$ to $2x10^9$. Expanded EPCs showed specific endothelial phenotype: CD45 and CD14 negative, CD34, KDR, vW and CD133 dim, CD31, CD144 and CD146 positive. The same pattern of expression of endothelial-specific makers was screened and confirmed by RT-PCR. Limiting dilution assay showed no significant variation in the percentage of clonogenic cells during the expansion.

Conclusion. EPCs from CB, under the culture conditions previously described, can be expanded in vitro on a large scale without loss of lineage specific features and proliferative potential at least up to ten passages. Thus human umbilical cord blood seems to be a suitable source for EPCs. Indeed, even if we did not observe a difference in EPCs clones frequency, a greater number of cells developed from CB MNCs than from a similar number of peripheral blood MNCs (data not shown). At present a clinical use of expanded EPC seems difficult because cord blood transplantation is currently only allogeneic. Thus, futher studies are needed to assess the feasibility of EPCs transplantation.

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CHEMOKINES RECEPTORS EXPRESSION DURING CIK CELLS EXPANSION

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Chemokines are small, chemotactic cytokines that direct migration of leukocytes through endothelial cells into surrounding tissues. This navigation allows lymphocyte localization to be determined by the combination of the chemokines receptors (CKRs) they express. An immunotherapy program in advanced malignancies is currently underway at our institution based on the ex-vivo generation of cytotoxic effector cells, termed cytokine-induced killer (CIK) cells, that are highly efficient cytotoxic effector cells capable of lysing tumor cell target.. In an attempt to define patterns of migration for *in vivo* immunotherapy with these cells, we studied the modification of chemokines receptors (CKRs) expression during CIK cells expansion. The CIK cells were produced by culturing peripheral blood mononuclear cells, from 10 normal donors and 10 leukemia patients, with Interferon gamma, OKT3 and inteleukin 2. After 21 days in culture, with the addition of fresh media and interleukin 2 every 2 to 3 days, the median culture was: 98.8% CD3, 21% CD4, 80.6% CD8, 30% CD3+56+ and 78% CD3+69+. At the end of CIK cells expansion we show a significant increase of CXCR3 expression, a slight decrease of CCR4 expression and a significant decrease of CCR7 expression. The values were for donors CD4 and CD8 respectively 89.9% (p=0.018) and 98.6% (p=0.04) CXCR3, 25.8% and 7.5% CCR4, 41.6% (p=0.012) and 27.6%(p=0.03) CCR7 and for patients CD4 and CD8 respectively 88.6% (p=0.05) and 98.0% (p=0.05) CXCR3, 19.1% and 6.5% CCR4, 16.7% (p=0.001) and 16.5% (p=0.01) CCR7. Many reports show that the coreceptors for CXCR3 Mig and IP-10 (both induced by Interferon-gamma) are critical in mediating inibition of tumor growth in presence of IL-12 while CCR4 is preferentially expressed by Th2 producing inibytory cytokines (IL-4 and IL-10) lymphoid cells. These patterns of CKRs expression may reflect the same patterns presented by effector cells in inflammed tissues because Mig, IP-10 and IL-12 are largely induced by inflammatory cells. The combinatorial expression of CD69 by these population indicates the high level of activation of CIK cells at the end of culture. These preliminary data may suggest an hypothetical role of inflammatory cytokines for a succesful *in vivo* targeting of these expandend population in cancer immunotherapy.

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EX-VIVO EXPANSION OF CORD BLOOD MESENCHYMAL STEM CELLS FOR A POTENTIAL CLINICAL USE

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Introduction. The MSCs seems to have a promising role in the allogenic transplant setting. It has been reported how they decrease the incidence of graft-versus-host disease and increase the speed of myeloid engraftment. The aim of this study was to analyze the growth kinetics of MSCs from full-term CB

Methods. CB were separated by negative lineage-depletion immunoselection (RosetteSep). The cell suspensions were seeded at $1x10^6$ cells/cm^2 in alphaMEM with 20%FCS and 2mM L-glutamine. On reaching confluence, the adherent cells were resuspended by using 0,25% trypsin-EDTA and replated at 2500 cells/cm^2. The CB-MSCs growth kinetics was evaluated calculating the cell number at each passage up to 17 passages

Results. As showed in Figure 1, CB-MSCs growth curves presented an initial lag phase of 25-30 days (5 passages) followed by exponential growth. At passage 6 (30-40 days of culture) the average amount of expanded MSCs was $>120x10^6$ cells enough for an adult patient of 60kg ($2x10^6$ MSCs/kg). CB-MSCs grew in culture for up to 17 passages, almost 10 log of expansion rate (Figure 2).

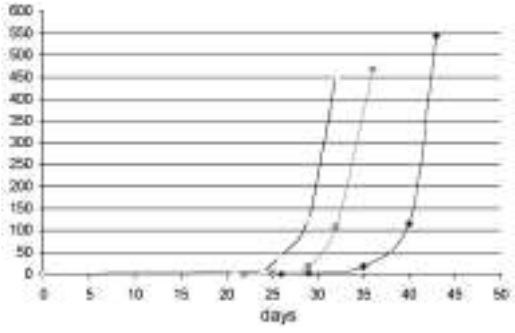

Figure 1. Examples of 3 growth curves of human MSCs cultures from CB from passage 0 to passage 7.

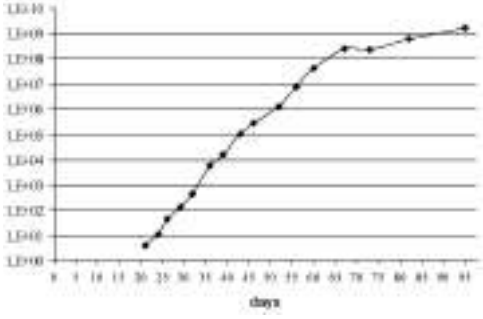

Figure 2. Growth curve of human MSC culture from CB from passage 0 to passage 17.

These cells were CD45, CD34, CD31, HLA-DR negative, CD105, CD73, CD29, CD44, HLA-ABC positive. Moreover, they were capable to differentiate in osteogenic, adipogenic and myogenic lineage and kept this capacity even after 17 subcultivations.

Conclusions. Our results suggest that CB-MSCs ex-vivo expansion is possible and a suitable number of MSCs for adult patients can be achieved. Although further studies are needed to shorten the lag fase of CB-MSCs growth curves, CB could represent an alternative source of MSCs for clinical needs.

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EFFICIENT AND REPRODUCIBLE GENERATION OF ACUTE LEUKEMIA SPECIFIC CYTOTOXIC T LYMPHOCYTES SHOWING CONCOMITANT HIGH LEVEL EXPRESSION OF CD45RA AND CD45RO MOLECULES.

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Relapse of acute leukemia after allogeneic hematopoietic stem cell transplantation remains a major clinical problem and reflects the failure in achieving an effective graft versus leukemia (GvL) effect. One possible reason for a poor GVL effect against leukemia is the down regulation of cell surface accessory molecules on the blasts that is essential for the T cell response. Many efforts have been made to find clinical grade protocols to optimally induce a rapid differentiation of leukemic cells to mature dendritic cells (DCs) using clinically tested reagents and controlled culture conditions. We examined the feasibility to find a rapid and effective method that reproducible modify leukemia cells into professional antigen presenting cells (APC) capable to drive the expansion of reactive cytotoxic T cells (CTLs) in HLA matched and unmatched allogeneic setting Leukemic blasts from acute myeloid (AML) and lymphoid (ALL) patients were cultured 5 days in vitro with Stem Span (Life Technologies, Vancouver, Canada) culture medium, GM-CSF (100 ng/mL, Schering Plough), IL-4 (50 ng/mL, Euro-Clone, U.K.) and calcium ionophore (100 ng/mL, Sigma–Aldrich, Italy). All B precursor ALL (N22) and all AML (N12) differentiated into LD-APC, acquiring a mature phenotype (CD40+, CD83+, CD86+ and CD80+) and dextran-FITC uptake capacity. No differentiation was possible when T-ALL (N3) were studied. All LD-APCs tested (5 ALL and 4 AML) generated anti leukemia CTLs from adult HLA-identical (N8) or unrelated donors (N2). After 14 days of in-vitro culture in the presence of IL-2 (120 U/mL, Proleukin), growing cells were found in a median of 73% (range 55-99) of initially plated wells. They were CD3+CD4+ double positive cells in 53% of cases, CD3+CD8+ in 10%, while a mixed population of CD4+ and CD8+ cells was present in remaining wells. Upon in vitro culture, adult donor derived CTL acquired an effector memory T cell profile (CD45 RO+, CCR7-, CD11a high, CD62L+, CD27+, and CD28+), but interestingly they maintained CD45RA expression. Cytofluorimetric intracytoplasmatic analisys showed they were granzyme positive (median expression 70%), variable perforine positive (median expression 21%) and that they were able to produce IFN gamma upon aspecific stimulation (median expression 34%). CTL were cytotoxic against patient leukemic blasts (median 18% at E:T ratio of 10:1 after 4 hours) but not against autologous or donor derived PHA blasts. LD-APC from 4 ALL patients were used to generate leukemia reactive CTL also from cord blood naive T cells. As from adults, growing cells were found in a median of 54% (range 45-66) of initially plated wells, but a mixed population of CD4+ and CD8+ cells was present the majority of wells. T cells changed their phenotype from naïve into classical effector memory cells, became all perforine, granzyme and IFN-g positive and more interestingly showed a higher cytotoxicity against leukemia blasts as compared to adult CTL (median 45% at E: T ratio 10:1). T cell receptor (TCR) cytofluorimetric analysis of both adult and cord blood derived CTL showed a skewing from a complete repertoire to an oligo-clonal /clonal pattern. The possibility to generate in vitro CTLs cytotoxic against leukemia may offer new innovative therapeutic opportunities to treat the clinical or molecular leukemia relapse after allogeneic transplantation.

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SCALING UP PROTOCOL FOR IN-VITRO GENERATION AND EXPANSION OF HLA IDENTICAL DONOR DERIVED CYTOTOXIC T LYMPHOCYTES AGAINST MYELOID AND LYMPHOID LEUKEMIA

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During the past few years several in vitro protocols have been described aimed to manipulate donor T cells in order to improve graft versus leukemia (GvL) while diminishing graft versus host disease (GVHD) potential. However only few adoptive immunotherapy trials have been reported so far, due to the complexity and the low reproducibility of such approaches, and moreover the low rate of expansion of cytotoxic cells. In this study we describe a simple, efficacious and reproducible method that allows the generation and expansion of donor derived cytotoxic T cells (CTL) directed toward different types of myeloid (AML) and lymphoid (ALL) leukemia using clinical grade culture conditions. Lymphocytoaphereses were performed in 8 HLA identical donors (3 familiar and 5 unrelated donors). 5-10x10^9 total nucleated cells were processed under elutriation using an USFDA approved closed system (Elutra cell separation System, Cobe, Gambro BCT), obtaining a reproducible recovery of 99% of cells: the positive fraction contained monocytes with a purity above 90% in all cases (contaminating cells were granulocytes, that were therafter removed by ficoll), while the negative fraction was composed of pure CD3 positive cells. Monocytes and CD3 cells were than separately aliquoted and frozen. 30 x 10 6 monocytes were thawed and cultured in a T75 flask for five days in the presence of GM-CSF (100 ng/mL, Schering Plough) and IL-4 (50 ng/mL, Euro-Clone, U.K.). Thereafter TNF-α (100 ng/mL, Euro-Clone, U.K.) was added, and immature dendritic cells were loaded with 25 Gray irradiated leukemic blasts at a ratio of 1:2. After two days, CD3 positive cells were thawed and added to the culture at a ratio

of 4:1. The same procedure was repeated one week after, when cultured lymphocytes were added to T75 flask containing the new generation of dendritic cells. Cells were maintained in culture in RPMI supplemented by 10% FCS (Euro-Clone, USFDA approved), with the only addition of IL-2 (120 U/mL, Proleukin). After 21 days we obtained a median number of 1,370x10^9 total nucleated cells (range 0,8- 1,665x10^9 cells). Phenotypic analysis showed they were almost all CD3 positive (median 96%, range 95-99%), of which a variable proportion was CD4 positive (median 65%, range 32-87%) and CD8 positive (median 55%, range 20-61%). Lymphocytes showed a phenotype of memory cells, being CD45 RO+, CCR7-, CD11a high, CD62L+, CD27+, and CD28+. Moreover, cultured lymphocytes were in all cases able to lyse leukemic cells from the patient (we documented a median cytotoxicity of 30% after 4 hours at a 30:1 E:T ratio). T cell receptor (TCR) cytofluorimetric analysis showed a skewing from a complete repertoire to an oligo-clonal pattern. The description of a simple, reproducible, effective and clinical grade method to generate and expand in vitro CTL cytotoxic against leukemia may offer the possibility of designing clinical protocols of adoptive immunotherapy.

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CASE REPORT-BURGER'S DISEASE: THERAPEUTIC ANGIOGENESIS AFTER INFUSION OF BONE MARROW MONONUCLEAR CELLS IN END-STAGE REFRACTORY ISCHEMIC LIMB

Console G, Irrera G, Martino M, Messina G, Morabito F, Pucci G, Cuzzola M, Dattola A, Callea I, Pontari A, Condemi A, Marcuccio D, Iacopino P

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Burger's disease is a clinical syndrome characterized by the development of segmental thrombotic occlusions of the medium and small arteries of the extremities. The conventional therapeutic procedures are palliative and not are changed the history of this disease. Recently, japanese authors have been reported a preliminary experiences with the limb infusion of BMMC in patient with diabetic foot. We investigate a male patient, 48 years, smoker, with critical limb ischaemia and rest pain with distal ulcer. This patient was not candidate for other non surgical or surgical procedures of revascularization. Baseline digital subtraction angiographic studies showed the severe reduction or absence of foot microvascularization .

Procedure. we proposed to patient the BMMC infusion and, obtained informed consent, we started the program. Primary endpoints were safety and feasibility of the treatment and total healing of the most important lesion. Secondary endpoints were total relief of rest pain without the need for analgesics, change in peak walking time (PWT) at 12 weeks, improvements in ankle-brachial pressure index (ABI) angiographic evidence of new collateral vessel formation we harvested bone marrow (500 mL) from the posterior iliac spine. After red cell depletion and BMMC concentration, we have obtained a final volume of 30 mL that we have infused a total of 250x10^6 BMMC in gastrocnemius muscle of ischemic limb with site

Results. the patient was followed for monitoring with instrumental iconography and biological features of endothelial cell at one week, one month, three months, six months, one year, respectively. Clinically, we documented a complete healing of ulcer two months after BMMC implantation ABI have, dramatically, improved (baseline 0, 1 vs 0, 6 score at three month).The PWT shown an improvement and limb pain intensity had a reduction after 24th hour to infusion and have been relieve after two week without analgesic drugs. Digital angiography showed at tree months from BMMC implantation, the presence of a new angiogenesis with revascularization, quite complete, of ischemic foot. During the follow up nothing alteration of metabolic parameters was documented.

Conclusion. This procedures, at six months of follow up, represent a valid alternative therapeutic program in this patients refractory to other treatments.

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AUTOLOGOUS ANTI TUMOR ACTIVITY OF T LYMPHOCYTES FROM CLL PATIENTS IN NOD/SCID MICE: A PRECLINICAL MODEL

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CLL (Chronic Lymphocyte Leukemia) is a good candidate for immune-based therapeutical strategies. CD154-based gene therapy may, by activating the CD40 pathway, increase malignant cell capacity to present tumor antigens. We transferred the hCD154 molecule by co-culturing CLL B lymphocytes with the HFL-1/hCD154+ cell line. Cytofluorimetric analysis was performed on B cells before and afterwards. After stimulation with hCD154+ B lymphocytes, autologous T cell proliferation was assesssed by thymidine incorporation and cytolysis of CLL B lymphocytes by the annexin V assay. Control experiments were based on autologous CLL B lymphocyte stimulation of T cells. NOD/SCID mice were injected intraperitoneally with 40x10^6 hCD154+ B lymphocytes (group 1) or 40x10^6 T + 40x10^6 hCD154+ B lymphocytes (group 2). After 1 month flow cytometry checked intraperitoneal lavage fluid for engraftment and Minimal Residual Disease (MRD).

Values are expressed as mean percentages of positive cells. CLL B lymphocyte co-culture transferred hCD154 protein (from 0.1 to 73 after co-culture) (p<0.000036). Immune accessory molecules were significantly upregulated: CD80 from 3.4 to70; CD86 from 8 to71, CD54 from 8 to 72 and CD95 from 5.7 to 81. Mean stimulation index of autologous T lymphocytes cultured with CD154+ B cells was 56.9 vs 10.6 in controls. Mean T cell cytotoxicity against autologous B-CLL was 42.6% vs 7.3% annexin V positive cells in controls. Mean percentage of engrafted human CD45+ cells in mice was 13.11% (group 1) and 7.39% (group 2). Mean percentage of MRD (as evaluated by CD19+/CD5+ cells) was 44.9% in group 1 and 9.5% in group 2 (p<0.007). Intercellular transfer of hCD154 protein induces CLL B lymphocytes to become efficient APCs. Sig-

nificant upregulation of co-stimulatory, adhesion and pro-apoptotic molecules is associated with enhanced autologous T cell proliferation and cytotoxic activity against autologous B lymphocytes in vitro and *in vivo*, significantly reducing the leukemic burden in mice. These findings suggest intercellular CD154 transfer on to B CLL lymphocytes, might provide a clinical cellular vaccine.

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EVALUATION OF ENDOTHELIAL PROGENITOR CELLS MOBILITATION IN PERIPHERAL BLOOD AFTER INJECTION OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION.DOUBLE-BLIND RANDOMIZED PHASE-II CLINICAL TRIAL.

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Background. Recent studies suggest that bone marrow–derived cells co-expressing CD34, CD117 and VEGFR-2 antigens may represent a population containing circulating endhotelial progenitors cells (EPCs). Moreover, EPCs have been demonstrated to induce both vasculogenesis and angiogenesis in experimental models of myocardial infarction.

Aim of the study. We analyzed the expression of some humoral factors and the trafficking of early and late EPCs in a setting of acute myocardial infarction (AMI) patients (pts) underwent intramyocardial injection of autologous bone marrow mononuclear cells (ABMNC) according to a double-blind randomized phase-II clinical trial,

Patients and Methods. From March 2004 to February 2005, 22 patients were enrolled in this study. These pts experienced an acute transmural myocardial infarction less than 6 months before. Pts were randomised 1:1 to receive ABMNC + conventional surgery treatment, or placebo + conventional surgery ABMNC were obtained by density gradient centrifugation from 50 ml of bone marrow, aspirated from the iliac crest on the same day of surgery. The final cell suspension contained a mean value 30.0 x 10^6 of mononuclear cells and of 1,43%±0,43 of CD34/CD45-positive cells. Cell viability was greater than 96%. Samples of peripheral blood mononuclear cells, serum and plasma were collected to evaluate stem cell subpopulations (FACScalibur; Becton Dickinson), cytokines (R&D System) and colony forming units at the time of enrolment (T0), 24 hours, 7days and 30 days post-surgery. We herein reported results on a series of 16 cases.

Results. The longitudinal detection of CD34 positive cells showed a progressive increase during the study period. The number of circulating CD34 positive cells coexpressing either CD133 or CD31 was significantly higher at + 24 h as compared with the baseline value ($p<0.005$, $p<0.0001$, respectively). Similarly, we found a significantly higher number of $CD34^+$ cells coexpressing CD117 ($p< 0.003$) at same period than T0. Whereas, the $CD34^+$/ VEGFR-2+ cell subset didn't show significantly variation at the different time points. Moreover, circulating mononuclear cells expressing ICAM-1 and E-selectine antigens increased already at +24 h ($p<0.0001$) mantaining this over-expression for the entire period of observation. Notably, SDF1-alpha plasma concentration showed persistent elevation in the longitudinal study. On the contrary, VEGF-2 serum level showed a peak value at day 7, returning to baseline at 30 day. CFU-GM, BFU-E, CFU-EPC followed the same behaviour.

Conclusion. In line with previous studies, our experience demonstrated that intra-coronary injection of ABMNC or placebo could regulate progenitor cell mobilization and homing factors. Of interest, 3 cases showed no substantial modification of this parameters. Since this random study is still blinded, we are not able to provide any definitive conclusion on the association between biological results with clinical outcome.

P482

CLINICAL SCALE EXPANSION OF MESENCHYMAL STEM CELLS ACCORDING TO GMP REGULATION

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It is now well established that bone marrow (BM) contains pluripotent mesenchymal stem cells (MSC) that are capable to differentiate in various tissues, are capable to inhibit in vitro the proliferation of alloreactive T cells and seem to be immunogenically privileged. Preliminary clinical experiences of co-transplantation of MSC with haemopoietic stem cells showed a potential role of MSC in modulating Graft versus host disease (GVHD). (1, 2)

Our GMP facility has initiated preclinical expansion of MSC from BM cells with aim to utilise these cells for the treatment/prophylaxis of GVHD in the setting of allogeneic hematopoietic cell transplantation.

After informed consent, bone marrow aspirate was obtained from posterior iliac crests of 6 donor volunteers (median age 32, range 29-47). Mesenchymal stem cells were expanded in a sterile class II biologic safety cabinet, according to GMP regulation. Briefly, mononuclear cells (MNC) were isolated by Ficoll (density 1,077grams/milliliter Eurobio) and washed twice in Dulbecco's phosphate buffered saline (D-PBS, Euroclone UK). Cells were plated at density of 10^6 cells/milliliter in 75 square centimeter flasks in MesenCult medium (StemCell Technologies Inc., Vancouver, BC-Canada) and incubated at 37 Celsius with 5% humidified CO2 atmosphere. After 24-48 hours, non-adherent cells were discarded and fresh medium was added. When cultures reached 90% confluence, adherent cells were detached with accutase and replated at a density of 10^6 cells in 75 square centimeter flasks with MesenCult medium. After at least 2 passages, confluent MSC were washed twice with D-PBS and incubated with Medium 199 (Gibco) for 1 hour at 37 Celsius to eliminate protein of the bovine serum. Adherent cells were detached with accutase, resuspended in D-PBS + 1% HSA and

washed four time. Cells were resuspended in D-PBS + 1% HSA to a final concentration of $2x10^6$/milliliter and cryopreserved with a rate-controlled freezer in a final concentration of 10% dimethyl sulfoxide (Wak-Chemie Medical GMBH, Li StarFISH, Carugate, Milano, Italy) and 5% HSA in (Fresenius HemoCare Gmbh, Germany) freezing bags. *Ex vivo* expanded MSC were tested for morphology, surface expression, karyotype, potential microbiological contamination (aerobic and anaerobic bacteria, mycoplasma, bacterial endotoxins) and viability. Expanded MSCs must meet the following criteria: >95% viability; negative bacterial cultures; >90% cells expressing CD29, CD44, CD105, CD166, and less than 5% cells expressing CD14 and CD45; normal kariotype and absence of detectable mycoplasma or endotoxin. MSCs were isolated from a median of 75millilter (range 60-120 millilter) of bone marrow aspirate from 6 donors and culture-expanded up to II or to IV passages (median 21 and 37 days respectively). Results from *ex vivo* MSC expansion documented a significant individual variability as shown in the table below.

Donor	RM	CP	EW	BP	FN	VG	
Age	31	30	47	37	29	33	Median 32
Gender	F	M	M	F	F	M	
BM aspirate (mL)	80	120	90	70	60	60	Median 75
MNC $x10^6$	255	200	200	620	300	107	Median 227.5
MSC $x10^6$ at I passage	50	18	20	54	10	7	Median 19
MSC $x10^6$ at II passage	50	30	30	18	22	9	Median 26
MSC $x10^6$ at III passage			50		26.5	11	Median 26.5
MSC $x10^6$ at IV passage					70	24	Median 47

After the second passage, morphology and phenotype met the criteria for clinical infusion. In one MSC expansion (BP) there was a marked decrease of the total MSC yield due to a bacterial contamination of several flasks which occurred between the I and II passage.

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Allogeneic Transplantation II

P483

HEMOPOIETIC PROGENITORS ARE TRAPPED IN MARROW FILTERS: RECOVERING CELLS FROM THE FILTERS, REDUCES GVHD AND TRANSPLANT MORTALITY

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Background. Cell dose is a crucial determinant of outcome in allogeneic bone marrow transplantation. Bone marrow is filtered either in the operating room, in the laboratory, or during infusion of bone marrow to the patient. Filters are usually discarded. Little is known of hemopoietic stem cell trapping in the filters.

Aim of the study. Evaluate hemopoietic progenitor cell content in the filters; assess patient outcome.

Methods. Before january 2001 bone marrow was filtered and filters were discarded. This program started in january, 2001. Bone marrow from donors (related or unrelated donors) was harvested without filters. Marrow was filtered in the lab, the filters were flushed and cells and progenitors counted, and added back to the marrow suspension. The suspension was then infused without further filters in the patient. Progenitors counted were CFU-GM, BFU-E, CFU-GEMM, LTC-IC. We also compared the outcome of patients receiving BM grafts in the years 1998-2000 (before compared with 2001-03. Hemopoietic progenitors: the median proportion of cells recovered from the filters of sibling marrow (harvested in our Unit) (n=9) was as follows: nucleated cells 3% , $CD34^+$ cells 4%, CFU-GM 6%, LTC-IC 23%, CFU-F 16%.

Patient outcome. Patients selected for outcome were leukemias in first remission, prepared with TBI 10 Gy and allografted from an HLA identical sibling. 31 patients allografted with filter-recovered marrow (2001-2003) were compared with 35 patients who received filter-discarded marrow 1998-2000. Age was comparable (33 and 32 years) Filter-recovered patients had significantly less grade II-IV GvHD (15% vs 44% , p=0.004), less TRM (2% vs 12%, p=0.07), significantly higher platelet counts on day +50 (147 vs $117x10^9$/L; p=0.01), and significantly higher cholinesterase serum levels (a surrogate marker of GvHD) (4080 vs 3560; p=0.001).

Conclusions. This study suggests that (1) approximately 20% of LTC-IC are lost in the filters together with 15% of CFU-F and possibly many adipocytes; (2) recovery of these cells may lead to improved outcome.

P484

LOW TRANSPLANT MORTALITY IN ADULTS WITH ACUTE MYELOID LEUKEMIA IN REMISSION: SHOULD MORE PATIENTS RECEIVE AN ALLOGENEIC TRANSPLANT?

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Background. The role of allogeneic bone marrow transplant (BMT) as post-remission treatment for acute myeloid leukemia (AML) is still a matter of debate, mainly because of transplant related mortality (TRM).

Aim of the study. The aim of this study was to analyse TRM in 100 patients with AML in 1st or 2nd remission undergoing an unmanipulated allogeneic bone marrow transplant (BMT) from an HLA identical sibling, between 1991 and 2002 in our Unit.

Patients. The median age was 36 years (14-62) and the median follow up 5 years (range 0.2-12 years). 80 patients were in 1st CR and 20 patients were in 2nd CR, with a median interval diagnosis BMT of 153 and 466 days respectively. Cyclophosphamide (CY) and total body irradiation (TBI) 3.3 Gyx3 (total 9.9 Gy) was the conventional conditioning regimen in 86 patients and 14 received a reduced intensity conditioning regimen (RIC) with CY and thiotepa. The latter were significantly older (median age 50 vs 33 years , p=0.00001). The median age of Graft vs host disease prophylaxis was cyclosporin with (n=86) or without (n=14) short course methotrexate.

Results. The actuarial 10 year TRM is 10% both for patients in 1st or 2nd CR, the actuarial relapse rate 25% and 47% (p=0.03)) , the overall survival 75% and 50% respectively (p=0.01). In 1st CR patients TRM is 14% and 3% before and after 1996 (p=0.06). RIC transplants produced similar TRM (10%) and survival (75%) but were associated with a higher relapse rate (47% vs 21% , p=0.03).

Conclusions. This study shows that TRM is overall 10% for adults with AML in 1st or 2nd CR undergoing an HLA identical sibling transplant. However , delaying transplant to 2nd CR significantly reduces the success of the procedure, mainly because of a significantly higher risk of leukaemia relapse. We take these results as an argument in favour of allogeneic transplants in first remission AML.

P485

GVHD-LIKE REACTION WITHOUT A GVL EFFECT AFTER SYNGENEIC TRASPLANT FOR A MYELODISPLASTIC SYNDROME

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A 25 years female was referred to our Centre for pancytopenia (Hb 10 g/dL, WBC 2969/mm^3, Plt 36000/mm^3) in October 2003 and a diagnosis of MDS (RAEB, intermediate II risk according to IPSS) was made. The percentage of blasts at diagnosis was 11%. Cytogenetic analysis was normal. Family study revealed a HLA identical twin sister and no other HLA identical sibling. Extensive DNA study demonstrated an identity for 12 DNA polimorphic loci. Review of mother's obstetric file showed the presence of a single placenta for both twins, so the status of monochorial twins was confirmed. On Jenuary 2004 patient received Hematopoietic Stem Cell Transplantation from her identical twin. Conditioning regimen consisted of busulphan 16 mg/Kg, etoposide 30 mg/Kg and cyclophosphamide 120 mg/Kg. A total dose of 6.34x10^6/Kg CD34 was infused (CD3 20% of total MNC). No GVHD prophylaxis was given. On day +4 a GVHD-like syndrome occurred with the appearance of an inguinal skin rash, diarrhoea, abdominal pain and a substantial increase of serum transaminases (ALT over 1000 mg/dL). Within few days the rash progressed to a nearly total body erythema involving the face, arms, trunk, hystologically recognized as Grade II cutaneous GVHD. Then, patient was treated with methylprednisolone 0.5mg/Kg up to 2 mg/Kg and cyclosporine 1 mg/Kg/day per continuous infusion.

After two days erythema and diarrhoea improved, while high levels of aminotransferases persisted until day +18. Patient was discharged on day +29 in good clinical conditions with full engraftment. Gradually patient tapered the immunosuppressive treatment until complete discontinuation after 9 months. On February 2005, in spite of good clinical conditions and normal haematological parameters, bone marrow evaluation revealed 4% of blast cells. This case report underlines that a GVHD-like syndrome can occur in a syngeneic transpant setting, but the risk of relapse remains high because of the poor GVL effect.

P486

NONMYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH METASTATIC SOLID TUMORS

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A graft-versus-tumor effect mediated by nonmyeloablative allogeneic SCT has been reported for patients with refractory, metastatic solid tumors. The aim of this study was to evaluate the feasibility and efficacy of a nonmyeloablative regimen of fludarabine and TBI to achieve complete donor chimerism after allogeneic SCT in patients with metastatic solid tumors. Between January 2002 and august 2004, 7 patients with refractory renal cell carcinoma (RCC), 3 with colorectal carcinoma and 1 with soft tissue sarcoma received an allogeneic SCT after a nonmyeloablative regimen including fludarabine 30 mg/m^2 for 3 days and TBI 200 cGy, with cyclosporine and mycophenolate as post-transplant immunosuppression. Patients received a median of 7x10^6 G-CSF mobilized CD34$^+$ cells/Kg (range 2.7-9.6) from an HLA-identical sibling donor (n=10) or one antigen mismatched related donor (n=1). Only 1 patient experienced neutrophils < 0.5x10^9/L. At day 30, median donor chimerism was 94%. One patient rejected the graft and had autologous recovery. Seven patients (64%) had acute GVHD, which was scored as grade I in 4 cases and grade II in 3 cases; 4 of 8 (50%) evaluable patients

had chronic GVHD which was graded as limited disease in 1 case and as extensive disease in 3 cases. Regression of all tumor metastases has been observed in 1 patient with RCC. Two patients had stable disease, while tumor progression was noted for 8 patients. Two patients received DLI with no objective responses. Median progression-free survival was 3.7 months (range 1-29 months). Overall 8 patients (73%) died from progressive disease and 1 (9%) from nonrelapse treatment-related complication. At the time of last follow-up, 2 patients were alive (n=1 in complete remission; n=1 with stable disease) 152 and 862 days after transplantation. In conclusion, our preliminary results suggest that nonmyeloablative allogeneic SCT for metastatic solid tumors is feasible, although may lead to durable responses in a minority of patients. Careful patient selection seems to be mandatory in this transplant setting.

P487

URSODEOXYCHOLIC ACID THERAPY IN CHRONIC GRAFT-VERSUS-HOST DISEASE OF THE LIVER

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Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid that is effective in dissolving cholesterol gallstone. It has promising effect in several other cholestatic liver diseases, such as cystic fibrosis and intrahepatic cholestasis of pregnancy. UDCA improves biliary secretion of bile acids, ameliorates the damage of cell membranes caused by retained toxic bile acids. It has immunomodulatory properties that may reduce immune-mediated liver damage.

We tested the safety and efficacy of UDCA in the long-term treatment of chronic graft-versus-host disease (cGvHD) of the liver in a open-label study in which each patient served as his or her own control. Thirteen patients were diagnosed with cGvHD of the liver after related stem cells transplantation for malignancies. Diagnosis of liver GvHD had been made on the basis of biochemical abnormalities of liver function (ALT, AST, alkaline phosphatase, GGT, total serum bilirubin values higher than 2,5 times the upper limit of normal) in 6 patients who had GvHD at other sites (group I)and on liver biopsies in 7 patients with isolated liver abnormalities (group II). At study entry all patients were receiving appropriate therapy for cGvHD. UDCA was added and given concomitantly with CSA in 2 patients; CSA and PDN in 3 patients; CSA, PDN and ECP in 3 patients; FK and PDN in 1 patient; CSA and MMF in 1 patient; CSA, MMF, PDN and ECP in 1 patient; FK, PDN and ECP in 1 patient. Patients received UDCA at the dosage of 12 to 15 mg/kg body weight per day, until the signs of liver cGvHD were cleared. The median time of UDCA therapy was 13 (range, 3-39) months. One patient of group I died due to fungal lung infection. Nine of 13 patients obtained a complete response after 13 (range, 3-24) months of combined therapy. The median time to obtain the response was of 5 months in group I versus 16 months in group II. This difference was not statistically significant. The trend of a faster response in group I argue for inappropriate clinical diagnosis of liver cGvHD in this group of patients. Three patients are actually in therapy, after 3, 29 and 39 months, respectively, due to persistence of liver damage. After discontinuation of UDCA therapy, 2 of 9 patients showed significant worsening in liver function test results, and a further cycle of UDCA therapy needed to maintain the liver function enzyme value in the normal range. The treatment with UDCA was well-tolerated and no clinical signs of toxicity were registered during the entire cycles of therapy.

Conclusion. The therapy with UDCA was safe, well-tolerated, and efficacious in the long-term treatment of cGvHD. Probably a maintenance therapy with UDCA should be considered in selected cases.

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GAMMA-DELTA T LYMPHOCYTE SPECIFIC CLONES ARE INVOLVED IN THE GRAFT-VERSUS-MYELOMA AND IN THE GRAFT-VERSUS–HOST DISEASE IN MULTIPLE MYELOMA PATIENTS AFTER NON-MYELOABLATIVE TRANSPLANTATION

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After allogeneic stem cell transplantation, the reconstituting immune system seems to play an important role in the eradication of malignancy, the most convincing evidence coming from the demonstration that infusion of donor lymphocytes could induce satisfying haematological and molecular responses. Analysis of T-cell receptor (TCR) rearrangement by fluorescent PCR has proven to be an available and useful tool for the *in vivo* characterization of immune response. Changes of TCR spectratype due to the relative expansion or regression of clonal T-cell population can be detected by this molecular technique and correlated with events occurring during follow-up, in particular with virus infections, acute and chronic graft-versus-host disease (GVHD) and graft-versus-tumor effect.

We analyzed T cell receptor delta (TCR) subfamilies in 20 patients who underwent allogeneic non-myeloablative transplant (NMT). In the normal spectratypes, PCR products were typically distributed in a Gaussian fashion, with an average of 7-9 different peaks. In our patients, TCR shapes frequently deviated from this normal pattern by having few predominant peaks, in the 60% of cases the pattern being biclonal. With a median follow-up from the graft of 12 months, all patients presented a skewed T-cell pattern. Nine patients developed acute GVHD (aGVHD); in 83% of them TCR analysis concomitantly showed new T-cell peaks. On the other hand, average score of TCR complexity did not differ between patients with or without chronic GVHD. Cytomegalovirus (CMV) reactivation was detected in 31% of patients; in no case TCR spectratype changed concomitantly to the CMV antigen positivity. Even the chimerism status was not significantly correlated with the evolution of TCR profiles. Of the 10 patients that showed detectable changes of TCR spec-

tratypes during follow-up, 4 were persistently mixed chimeras and 3 retained their full donor pattern. As surrogate of minimal residual disease (MRD), the IgH rearrangement was monitored; 61% of patients had at least one PCR-negative BM sample during follow-up. Interestingly, the eradication of MRD had a favorable impact either on OS or progression rate. In the 61.5% of patients a new clonal predominant TCR peak preceded or appeared concomitantly to the achievement of the IgH-negativity. On the other hand, 4 cases converted back to the IgH-positivity during follow-up; interestingly, in two of them the area of a clone previously characterized as recipient-specific resulted significantly increased. These results suggest that different T populations sustain GVM and GVHD and that analysis of TCR spectratype could be a useful tool for monitoring patients who underwent non-myeloablative allogeneic transplant.

P489

REDUCED CONDITIONING INTENSITY AND BONE MARROW TRANSPLANTATION IN HIGH RISK MALIGNANCY; A SINGLE CENTER EXPERIENCE

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Background. Many hematological tumors or other malignancies primary resistant or relapsing to two or more lines of therapy lose their chances of cure with conventional therapy. TMO with conventional conditioning regimen could be proposed as alternative salvage therapy in some patients, however the high toxicity due to the previous therapies and the old age of patients warrants to test the possibility of cure with RIC regimen.

Patients and methods. Twenty patients, 4 AREB-t, 3 MM, 3 HD, 4 NHL, 3 AML, 2 CML in BC and 1 Metastatic Breast Cancer (MBC) represent the basis of the present study. The mean age of patients is 55 years (extremes 36-70 years) and all patients had disease in progression at the time of the procedure. Lymphomas, MM, AML and MBC underwent a minimum of two lines of therapy before RIC-BMT, 3 mean (extremes 2 to 5), AREB-t and CML in BC underwent the procedure as front line therapy (2 pts) or following a failure of induction therapy (4 pts). The conditioning regimen to RIC-TMO was Fludarabine 50 mg x 3, 4 days ATG, CTX 300 mg/sm or Thiotepa 10 mg/Kg and Melphalan 100 mg/sm.

Results. Engraftment was recorded in all patients. Fifteen patients responded to RIC-TMO, 10 CR and 5 more than 80% reduction of disease. Complete responding patients were 2 MM, 3 AML, 2 HL, 1 NHL and 2 AREB-t. Partial responding patients were 1 HD, 2 NHL, 1 MBC and 1 MM. In lymphomas and myelomas the response was achieved in a period ranging from 1 to 6 months. The two CML in BC, 1 NHL and 1 AREB-t did not responded and early progressed. aGVHD was recorded in 11 patients. One patient with AREB-t died for grade IV aGVHD 2 months from RIC-TMO. The duration of response was 3 to 20 months with 4 patients still in CR. Six patients died for progression of disease, 3 patients died for late aGVHD complications due to CMV activation and one for Gram- sepsis 3 to 6 months following RIC-TMO. Comment and conclusions. RIC-TMO is a feasible procedure in high risk patients for age and disease with haematological tumors. Engraftment is the rule. The incidence of aGVHD in almost 50% of patients give a risk of peritransplant mortality of 25% of patients including early and late complications. The efficacy of the procedure seems to be mostly correlated to Graft Versus Tumor expecially in those patients with late response however long lasting observation is still under evaluation. The recognition of CR in lymphomas and myelomas already resistant to many lines of therapy warrants consideration and further studies.

P490

LATE EFFECTS IN SURVIVORS OF THALASSEMIA MAJOR TREATED WITH HEMATOPOIETIC STEM CELL TRANSPLANTATION

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The purpose of this study was to analyze medical late effects among patients with thalassemia major treated with hematopoietic stem cell transplantation (HSCT). Overall, 111 patients (M56, F55) were given 115 transplants from HLA identical related donors between May 1983 and February 2004. Ten patients died from transplant related causes in the first 7 months post-HSCT. As of March 2005, 99 patients are living after a median follow-up of 14 years (1-22) and are included in our analysis. Ninety five patients are cured free of thalassemia and 4 are under transfusion treatment following autologous reconstitution.

Late deaths. Two patients died from septic shock and disseminated intravascular coagulopathy 54 months post-HSCT and from parotitis carcinoma 138 months post-HSCT, respectively.

Chronic graft-versus-host disease (cGvHD). The actuarial probability of developing cGvHD was 17% (7% limited, 10% extensive). Only two patients are under immunosuppressive therapy for active cGvHD with lung involvement and pulmonary insufficiency.

Secondary malignancies. One patient showed parotitis carcinoma 134 months post-HSCT and died 4 months later. An other patient was diagnosed with carcinoma of cervix and is now living and doing well following surgical treatment. Pulmonary complications. Pulmonary function tests were done in 48 patients. The mean value expressed in liters for vital capacity, forced vital capacity and forced expiratory volume in one second was 3.34 + 2.29 (85% of predicted value), 3.98 + 0.76 (82%) and 3.01 + 0.71 (86%) respectively. The pulmonary function was normal in 39 patients. A mild restrictive defect was present in 7 patients, a mild obstructive defect in 1 patient and a mild combined defect in 1 patient.

Endocrine dysfunction. The thyroid function was studied in 50 patients. The mean value of TSH was 2.97 + 2.89 mU/L. The mean value of FT3 and FT4 was 4.5 + 1.85 and 14.7 + 7.09 pg/mL respectively. Five patients are under treatment for hypothyroidism, which was present before HSCT in 4 and de novo in 1. The parathyroid function evaluated by PTH and the adrenal function evaluated by ACTH and cor-

tisol levels were normal in all patients. Fertility. Eight pregnancies were observed in 4 female patients and in partners of 2 male patients and resulted in 5 normal live births. Three pregnancies are currently coming. The median age of these patients at time of HSCT was 15.06 years (13-24). Following HSCT a careful follow-up is required to monitor and treat late transplant-related and thalassemia complications.

P491

CLINICAL BENEFIT IN PATIENTS SUFFERING FROM RESISTANT SCLERODERMIC CHRONIC GVHD AFTER ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION AND TREATED WITH SYNTHETIC PGI-2

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Background. Scleroderma is a late manifestation of cGVHD, presenting as progressive tightness of the skin, hampering patients'quality of life. In recent times Iloprost, a synthetic PGI-2, has been proving effective in the therapy of idiopathic systemic sclerosis, which has a similar pathogenesis to sclerodermic cGVHD.

Patients and methods. From 2001 to 2005 we observed 4 pts. affected by sclerodermic cGVHD.

A classical HSCT from sibling donors had been performed, with myeloablative conditioning and bone-marrow cells. Chronic GVHD evolved 18 months after HSCT (median time, range 10-27), presenting as diffuse skin changes (tightness and thickening of the face, neck, hands and trunck), cutaneous dischromia, digital pitting scars and functional joint impairment. In two patients these alterations were linked with itch and pain. In all cases cGVHD severely affected quality of life. It was limited to the skin in two cases, and involved internal organs in the other ones (liver and lungs). In three cases it presented as de novo cGVHD. All pts. had already been treated as first-line with various regimens (CSA, azathioprine, mofetil mycophenilate, steroids, repeated cycles of PUVA) with small or no effect on the skin. They were then treated with Iloprost at median time 11 months from appearance of cGVHD (range 9-29), 50 microgr a day, over 8 hours iv continous infusion for 5 days a month, on repeated courses in our Outpatient Department.

Results. The drug was well-tolerated with little or no side effects: hypotension and cardiac arythmias, considered the main side effects of the therapy, have not been observed. We saw a significant clinical benefit after 2 cycles in three pts., after 5 cycles in one patient. In all cases this improvement was progressive over the subsequent courses until a stable but not complete recovery. We started therapy with monthly cycles until a satisfactory clinical and personal response was obtained (median 5 courses), and then continued Iloprost as mainteinance therapy 3-4 times a year. At present, one patient has died because of relapse of acute leukemia; the other three pts. are alive and well, with satisfactory quality of life and stable skin clinical status on maintainance therapy.

Conclusions. In our four pts. failing prolonged previous treatment, Iloprost has been successful in reverting sclerodermic diffuse cGVHD and reducing the extension of the disease, with re-gaining of skin tenderness and disappearance of pain.

P492

MYCOPHENOLATE MOFETIL IS A VALID ALTERNATIVE TO CYCLOSPORINE IN PATIENTS TREATED WITH ALLOGENEIC HEMATOPOIETIC STEM CELLS TRANSPLANT

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Background. In the field of post-transplant immunosuppression, little is known about the role of MMF, and no prospective study comparing this to other immunosuppressive drugs has been conducted so far.

Patients characteristics. We retrospectively considered 32 patients (pts.) from 2000 onwards, treated with MMF after HSCT. They represent 40% (32/79) of the pts transplanted at our Institution in the same period. The group was highly heterogeneous for age, disease, type of HSCT (peripheral/marrow stem cells; sibling/MUD donor) and reasons requiring the use of MMF. The schedule was 15 mg/Kg, and the total dose ranged from 500 mg twice daily to 1 g three times a day. Clinical response was generally observed within the first 2 weeks after the start of treatment (range 1-20). We could divide our population into 3 major groups: 1) 11 pts. who used MMF as treatment for steroids resistant acute Graft Versus Host Disease (GVHD); 2) 10 pts. who used it for second line therapy of chronic GVHD, resistant to cyclosporine (CSA) and prednisone; 3) 11 pts. who substituted CSA with MMF because of renal (9) or vascular (2) toxicity, regardless to presence of GVHD.

Results. 1) in all 11 pts. MMF was added to previous treatment in the attempt to ameliorate aGVHD in progress; we experienced 5 complete and 4 partial responses, and no response over GVHD in two cases (response rate >80%). 2) in all 10 pts. with cGVHD, MMF was added to other immunosuppressive drugs (CSA and prednisone) because of clinical progression; we observed 3 complete regressions of cGVHD, 6 partial responses and only one overt progression (response rate >85%); 3) worth to note, among the last group of 11 pts, in all cases MMF was able to maintain or ameliorate the clinical conditions, and proved a valid alternative to CSA (effective in 100% of cases).

Side effects. MMF was well tolerated, main side effects being gastrointestinal (10/32 = 31%); these were generally mild and did not require suspension of the drug. We observed also a mild decrease in neutrophilic blood counts in 4 pts.

Conclusions. The data collected show that MMF has an effective role in both treatment of overt acute as well as chronic GVHD and as a substitute of CSA in those pts. who experience toxicity, but still need further immunosuppression. Prospective controlled trials are desirable.

P493

DOUBLE UNRELATED UMBILICAL CORD BLOOD TRANSPLANT: REPORT OF TWO CASES AT THE BMT UNIT, OSPEDALE S.CAMILLO, ROME

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Umbilical cord blood (UCB) represents a convenient hematopoietic stem cell (HSC) source that may significantly extend the HSC donor pool. UCB has the advantage of prompt availability and low risk of severe GVHD despite donor-recipient HLA-disparity. However UCB has the limitation of low cell dose. UCB transplantation (UCBT) has become a standard practice in the treatment of pediatric malignancies, but remains investigational in adults. To augment the graft cell dose, we have attempted to combine 2 partially HLA-matched UCB units in two young patients with no available unrelated HLA-matched marrow donor. The first is a 24 y-old male with 2nd remission T-ALL. He received 2 UCB units (both 4/6 HLA-A,B,DRB1 matched) following a conditioning regimen with TBI 1200 cGy, cyclophosphamide 2 x60 mg/kg, and ATG (rabbit, IMTEX) 2x15 mg/kg. Total cell dose was $4.9x10^7$ NC/kg. GVHD prophylaxis was the combination of cyclosporin 2 mg/kg and prednisolone 1 mg/kg for 30 days. Neutrophil engraftment (>$0.5x10^9$/L) occurred on day +28, platelet engraftment (>$20x10^9$/L) on day + 55. The patient suffered an episode of Stenotrophomonas Malthophilia pneumonitis responding to trimetoprim/sulfametoxazolo. Severe haemorrhagic cystitis occurred early after transplant but resolved completely with forced hydratation and intensive platelet support. No acute GVHD occurred. He is on day +70 of follow-up, in fair condition and transfusion-independent. While at day + 30 he had a mixed chimerism (recipient plus double donor), at +60 he shows a complete combined donor chimerism.

The second patient was a 17 y-old girl with high-risk ALL in 1st remission who had previously failed an unrelated HLA-identical donor bone marrow transplant (NC $2.5x10^8$/kg) with a standard TBI-Cy-ATG conditioning and cyclosporin-methotrexate GVHD prophylaxis. In this case the double UCBT was offered as salvage from irreversible aplasia, due to second donation refusal. UCBT conditioning was reduced-dose with TBI 200 cGy, cyclophosphamide 50 mg/kg and fludarabine 200 mg/m^2. Total cell dose was $7.4x10^7$/kg and GVHD prophylaxis was carried out with cyclosporin and mycophenolate mofetil up to day +30. The patient was critically ill at the time of transplantation, with probable invasive fungal infection and concurrent renal impairment. She suffered from a severe stomatitis and was given parenteral nutrition for two months. She never engrafted, and died of multi-organ failure at day +40. At day +30 she had a single-donor UCB complete chimerism. We confirm that first transplant with double partially-matched UCB is feasible and safe, with no GVHD, though engraftment is delayed in comparison with bone marrow. Conversely, there are no published data of double UCBT as salvage from previous graft failure, and the case reported here is not encouraging. Interestingly, in our second patient we demonstrated a full single- (not double) donor chimerism despite profound marrow aplasia. Based on this preliminary observation our current policy in first transplants is to search for multiple-UCB samples for adults or >50 kg patients lacking an unrelated HLA-matched bone marrow donor or in need of a prompt stem cell transplantation.

P494

POOR GRAFT FUNCTION FOLLOWING ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION TREATED WITH UNMANIPULATED OR CD34 SELECTED STEM CELLS WITHOUT CONDITIONING

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Background. we have shown in a previous study that approximately 30% of patients may experience poor graft function (PGF) following an allogeneic hemopoietic stem cell transplant (HSCT), risk factors being graft versus host disease (GVHD), donor type, CMV infections and cell dose.

Methods. we have attempted to rescue PGF using stem cells from the same donor in 32 patients affected by hematologic malignancies: 12 received unmanipulated and 20 received CD34 positive selected stem cells after a median of 132 (25-1162) and 153 (67-1188) days from a sibling or MUD transplant. The median age was 33 years in the first group and 37 in the second group. Peripheral blood count was similar at the time of second infusion in the two groups and the median value were respectively: Hb 10 g/dL, neutrophil $1,5x10^9$/L, platelet $15x10^9$/L and Hb 9,4 g/dL, neutrophil $1,5x10^9$/L, platelet $17x10^9$/L. At the time of second infusion GVHD was absent or limited in all patients, CMV antigenemia was positive in 19/32 patients and 29/32 patients had complete donor chimerism. The median number of infused unmanipulated cells was 3,75 x10^8/kg in the first group and $2,39x10^6$/kg CD34 selected cells of recipient body weight in the second group.

Results. Recovery of hematopoietic function in term of median platelet count at days +30, +50 and +100 was 21, 27, $63x10^9$/L and 50, 53, $77x10^9$/L respectively.

7/12 pts (58%) in the first group and 5/20 (25%) in the second group died for transplant related complications. The actuarial survival at 3 years is 37% vs 80%.

Discussion: infusion of CD34 selected donor cells without conditioning results in prompt and sustained hematologic recovery in patients with poor graft function and produces encouraging long term survival.

P495

ALLOGENEIC BONE MARROW TRANSPLANTATION FOR REFRACTORY BEHÇET'S DISEASE WITH SEVERE CNS INVOLVEMENT HAVING FAILED AN AUTOLOGOUS TRANSPLANT

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Behçet's disease (BD) with neurological involvement is a very severe condition. Autologous peripheral stem cell transplantation (ASCT) following high dose immunosuppressive chemotherapy has been attended by remissions, but FU is short. Two cases of BD with coincidental diseases have undergone allogenic BMT. The first patient died on day +60. The second received a cord blood SCT and attained a remission of both diseases. No long-term FU is recorded.A 32 years old (in 2004) lady started suffering with severe headaches, vertigo, aphthous oral, vaginal and intestinal bleeding ulcers in 1992. In 2001 the patient went into grade 1 coma. On August 28, 2002 $CD34^+$ cells were mobilized, and in October, 2002 she was conditioned with BEAM and received her own $CD34^+$ cells ($3.2x10^6$/Kg). She also received ATG. Haematologic recovery was normal, and all symptoms receded. Three months later she had a severe relapse. On May 22, 2003 she underwent BMT from her HLA-identical brother after conditioning with Thio-Tepa 10 mg/Kg and CY 100 mg/Kg. Bone marrow cytogenetics were 100% 46,XY on day +75, and STR analysis showed full chimerism on day +120. Acute gastrointestinal and liver grade IV GVHD developed, with bilirubin peaking at 564.3 mmol/L at day +120. Liver biopsy was consistent with GVHD. Treatment consisted of corticosteroids, extracorporeal PUVA, CY 3 g on day +100 and $50x10^7$ mesenchymal cells (MSC) on day +140. GVHD resolved and all liver parameters were within normality by ay +360. After a 2-year CR mucosal ulcers, intestinal bleeding and severe headache reappeared. The relapse was treated with a 4 weeks infusion of anti-CD20 (Rituximab) with regression (temporary?) of all symptoms.Five SPECT examinations were performed along a 14-month period (from May12, 2003 to July 2, 2004). The first SPECT disclosed several areas of asymmetry. These areas disappeared following transplants and haven't reappeared since.Although BD's target organ damage is certainly immune mediated, this case proves that it is not a "primary" autoimmune disease. The cytokine CNS fluid pattern has been shown to be consistent with an infectious rather than with an autoimmune pattern. Intense immunosuppression is helpful, but even allogeneic SCT was not curative.

P496

NON MYELOABLATIVE ALLOGRAFTING VERSUS AUTOGRAFTING IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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Autologous hematopoietic cell transplantation (HCT) is regarded as the standard first line treatment for myeloma patients up to the age of 65-70 years. However, patients are at continuous risk of relapse with few disease-free long-term survivors. In contrast, allogeneic HCT provide both tumor-free grafts and allogeneic graft-versus-myeloma effects which may lead to long-term molecular remissions. Novel allogeneic approaches, which employed low dose total body irradiation (TBI) based non myeloablative conditioning regimens in newly diagnosed myeloma patients, reported a dramatic reduction of transplant-related mortality (TRM) compared to conventional high dose conditionings (Maloney *et al,* Blood, 2003, Bruno *et al,* ASH meeting, 2003, Garthon et al, personal communication, 2005). Importantly, response rates were high and eligible age for allografting was increased by almost a decade. Nonetheless, the role of allografting compared to double autologous HCT remains to be determined. Between September 1999 and July 2004, 166 consecutive, stage IIA-IIIB, myeloma patients under 65 were diagnosed at the Divisions of Hematology at the University of Torino, and at the Hospital of Cuneo, Italy. One-hundred-five /135 (78%) patients with natural siblings were HLA typed. Thirty patients/135 (22%) were not typed for the following reasons: patients not eligible for high dose chemotherapy (n. 14); siblings not eligible for peripheral hematopoieitic cell (PHC) donation (n. 7); refusal to high dose chemotherapy (n. 9). Forty-six patients (46/105, 43%) had an HLA identical sibling and were offered a non myeloablative allogeneic HCT. Four potential donors (4/46, 9%) were not eligible for donation and nine patients refused an allogeneic HCT. Thirty-three patients (auto-allo group) were enrolled on a tandem auto-allo protocol and 31 have completed both transplant phases at the time of this analysis, while 41 patients (double-auto group), without HLA identical siblings or after refusal to allografting, have completed a planned double autologous HCT. Briefly, after induction chemotherapy, based on VAD like regimens, patients underwent G-CSF mobilized autografting with high dose melphalan (200 mg/sqm). In the auto-allo group, the autologous HCT was followed, 2-4 months later, by low dose (2.0 Gy) TBI, PHC infusion from an HLA-identical sibling, and immunosuppression with mycophenolate mofetil (15 mg/kg BID) for 28 days and cyclosporin (6.25 mg/kg BID) for a minimum of 80 days. In the double-auto group, patients received a second autologous HCT after a conditioning regimen consisting of high dose melphalan or high dose melphalan and mitoxantrone. Demographics of the two biologically randomised groups were as follows: age: 53 (range 34-65) vs 53 (range

37-65); stage III myeloma: 75% vs 73%; beta 2 microglobulin > 2,5 mg/dL, 73% vs 71%, in the auto-allo group and in the double-auto group, respectively. After a median follow up of 28 months (range 8-76), overall survival was 76% versus 66% (p=0.13); overall TRM was 18% (day 100 TRM= 3%) vs 5% (p=0.06) in the auto-allo group and in the double-auto group, respectively. Clinical complete remission, defined as the disappearance of the monoclonal paraprotein by immunofixation, was achieved in 41% (13/31) of the patients vs 12% (p=0,005); progression free survival was 76% vs 41% (p=0.002); event free survival was 52% vs 37% (p=0.08) in the auto-allo group and in the double-auto group, respectively. Even though longer follow up on a larger series of patients is required to draw definitive conclusions, the data suggest: i) overall survival of patients undergoing non myeloablative allogeneic HCT was not inferior to double autologous HCT though TRM was not negligible ii) the allogeneic graft-versus-myeloma effects determined significantly higher complete remission rates with significantly longer progression free survival. In summary, non myeloablative allogenic HCT appears an important treatment option for newly diagnosed myeloma patients.

P497

CD 8 DEPLETED DONOR LYMPHOCYTES INFUSION AFTER NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION IN REFRACTORY MALIGNANCIES

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Background. Donor lymphocyte infusions are an effective therapy for patients with many hematological malignancies who relapse after an allogeneic-hematopoietic stem cell transplantation(HSCT). However, lymphocyte infusions are frequently associated with adverse effects including graft-versus-host disease (GVHD) in approximately 60% of recipients. These complications have been the major causes of treatment-related mortality, which has occurred in up to 20%. Nonmyeloablative conditioning regimens have been established as alternative to conventional allogeneic HCT in order to achieve engraftment of donor hematopoietic cells. The toxicity of the procedure was significantly reduced while a potent graft versus tumor effect (GVT) was retained. Recent reports on the use of DLI after non-myeloablative allo-HSCT demonstrated the retained capability of inducing a GVT effect while the lower toxicity was registered. In humans, selective CD8+ T-cell depletion of donor marrow significantly reduced the rate of acute GVHD compared with unmodified marrow in a randomized controlled trial.

Patients, methods and Results. We evaluated the infusion of depleted of CD8 donor lymphocytes cells as a treatment for patients with hematological and solid tumors, who relapsed after non-myeloablative allo-HSCT. We treated 9 patients (2 with Multiple Myeloma, 2 with colorectal cancer, 2 with renal cell carcinoma, 1 with breast cancer,1 with thymic cancer and 1 with Hodgkin disease) with CD8 depleted DLI. All patients were conditioned with the Seattle regimens that includes Fludarabine 30 mg/m^2 i.v. given on days -4, -3 and -2 and total body irradiation, given at a dose of 2 Gy on day 0. Graft versus Host Disease prophylaxis consisted in a combination of Cyclosporine (CSA) and Mycophenolate Mofetil (MMF). All the patients at the moment of transplant as well as at the time of progression had a high tumor burden. The first DLI was given at a median of 320 days after transplant(260-440). A median of 1x10^7 CD3+/CD8 (0.5-2.5) cells/kg were infused, containing less than 0.5 x 10^6 (0.07-0.84) CD3+/CD8+ cells/kg. Dose escalation was possible in all the patients treated for a total of 15 courses of DLI. Two patients received four infusions without any toxicity. No patients developed > grade II acute GVHD, 1 patient developed limited chronic GVHD; mild hematological toxicity (grade 1 thrombocytopenia) was registered in 2 patients. No serious infectious complications were observed and all patients were followed up in the out-patient department. Among the 8 assessable patients, one achieved a PR (MM), five patients achieved SD that lasted a median of 2 months (1-5).

Conclusion. The demonstration that CD8 depleted DLI are safe and feasible in patients progressing after non-myeloablative allo-HSCT will allow us to consider such a strategy earlier in the course of transplant in order to evoke more robust GVT especially in those patients who underwent allo-HSCT for unresponsive diseases.

P498

MRD EVALUATION BEFORE ALLOGENEIC TRANSPLANTATION IN ADULT ACUTE LINFOBLASTIC LEUKEMIA

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Background. Leukemia relapse is the major cause of treatment failure after allogeneic stem cell transplantation (Allo-SCT) in adult patients with acute lymphoblastic leukaemia (ALL). The aim of this study was to evaluate whether the presence or the amount of Minimal Residual Disease (MRD) before Allo-SCT correlates with the clinical outcome.

Methods. MRD was analyzed before and after Allo-SCT in 32 adult patients homogeneously treated in the context of prospective Phase II clinical studies of the North Italian Leukemia Group (NILG). MRD was determined by quantitative PCR (RQ-PCR) using molecular probes derived either from fusion chimeric genes (19 patients, including 17 BCR/ABL and 2 MLL/AF4 positive cases) or T-cell receptor (TCR) and Immunoglobulin (Ig) gene rearrangements (11 patients). In all cases in which a TCR or Ig derived probe was used, the minimal reproducible sensitivity was at least 10-3 and the maximal sensitivity was never below 10-4. One log more sensitive was the PCR analysis performed using BCR/ABL or MLL/AF4 probes.

Results. Before the conditioning regimen was started, a negative RQ-PCR analysis was demonstrated in 9 cases while a positive results was found in 19 patients. None of the MRD negative patients relapsed after transplantation. In these patients, the molecular follow-up confirmed a PCR negative status in all but one patient who showed only transitory evidence of minimal residual disease not confirmed in subsequent controls. Among the 23 patients showing MRD evidence before transplantation, 12 were found to have a high leukemia level (10-2 to 10-3) while 11 patients showed a lower level of residual disease(<10-3). Among the 23 MRD positive patients, 9 had relapsed (39%) within 2 years and a durable molecular conversion to a negative status after transplantation was demonstrated in only 6 patients.

Conclusions. MRD evaluation before allogeneic transplantation is a powerful tool to predict the clinical outcome of adult ALL patients. Patients remaining PCR positive before transplantation are at high risk of leukemia relapse. Innovative strategies to increase the rate of molecular remission before transplantation as well as to induce a more efficacious response of donor immune system against leukaemia cells are needed.

P499

LATE EFFECTS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THALASSEMIA MAJOR

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The purpose of this study was to analyze medical late effects among patients with thalassemia major treated with hematopoietic stem cell transplantation (HSCT). Overall, 111 patients (M56, F55) were given 115 transplants from HLA identical related donors between May 1983 and February 2004. Ten patients died from transplant related causes in the first 7 months post-HSCT. As of March 2005, 99 patients are living after a median follow-up of 14 years (1-22) and are included in our analysis. Ninety five patients are cured and free of thalassemia and 4 are under transfusion treatment following autologous reconstitution.

Late deaths. Two patients died from septic shock and disseminated intravascular coagulopathy 54 months post-HSCT and from parotitis carcinoma 138 months post-HSCT, respectively. Chronic graft-versus-host disease (cGvHD). The actuarial probability of developing cGvHD was 17% (7% limited, 10% extensive). Only two patients are under immunosuppressive therapy for active cGvHD with lung involvement and pulmonary insufficiency. Secondary malignancies. One patient showed parotitis carcinoma 134 months post-HSCT and died 4 months later. An other patient was diagnosed with carcinoma of cervix and is now living and doing well following surgical treatment. Pulmonary complications. Pulmonary function tests were done in 48 patients. The mean value expressed in liters for vital capacity, forced vital capacity and forced expiratory volume in one second was 3.34 +/- 2.29 (85% of predicted value), 3.98 +/- 0.76 (82%) and 3.01 +/- 0.71 (86%) respectively. The pulmonary function was normal in 39 patients. A mild restrictive defect was present in 7 patients, a mild obstructive defect in 1 patient and a mild combined defect in 1 patient. Endocrine dysfunction. The thyroid function was studied in 50 patients. The mean value of TSH was 2.97 +/- 2.89 mU/L. The mean value of FT3 and FT4 was 4.5 +/- 1.85 and 14.7 +/- 7.09 pg/mL respectively. Five patients are under treatment for hypothyroidism, which was present before HSCT in 4 and de novo in 1. The parathyroid function evaluated by PTH and the adrenal function evaluated by ACTH and cortisol levels were normal in all patients. Fertility. Eight pregnancies were observed in 4 female patients and in partners of 2 male patients and resulted in 5 normal live births. Three pregnancies are currently coming. The median age of these patients at time of HSCT was 15.06 years (13-24). Following HSCT a careful follow-up is required to monitor and treat late transplant-related and thalassemia-related complications.

P500

REDUCED INTENSITY CONDITIONING REGIMEN FOR ALLOGRAFTING FOLLOWING AUTOGRAFTING IS FEASIBLE AND HAS A STRONG ANTI MULTIPLE MYELOMA ACTIVITY

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The development of reduced intensity conditioning regimens (RICT) has renewed interest in allografting for patients with multiple myeloma (MM). Taking advantage of this new approach, we firstly postulated that combining maximal tumor reduction achieved with autografting and the benefits of RICT, we could achieve more cures of multiple myeloma (MM) with acceptable toxicity. Sixteen patients, 51 years of age (range, 36-63) with previously treated stage III MM were given melphalan 140 mg/m^2 and autologous peripheral blood progenitor cells (PBPC) reinfusion. The regimen-related toxicities were moderate with a median of 8 and 11 days of neutropenia and thrombocitopenia, respectively. Forty-six to 156 days later (median, 79 days), the patients received fludarabine 30 mg/m^2/d x 3 days plus 2 Gy TBI and HLA-identical donor mobilized PBPC. Postgrafting immunosuppression consisted of cyclosporine and mycophenolate mofetil. Donor lymphocyte infusions were given to eight patients with stable mixed chimerism or progressive disease who did not show signs of aGVHD. Engraftment occurred in 14 patients (87%). Ten patients (62%) are alive with 9 of them in continuous complete remission 11-36 months (median, 30 months) after transplants. All remitters patients achieved full chimerism and developed GVHD. Grade II-III acute GVHD occurred in 7 patients (43%) but no patient died of aGVHD. Three patients (18%) developed extensive chronic GVHD requiring intensive therapy. Six patients died; five of them of progressive disease and one of progressive disease combined with extensive cGVHD and interstitial pneumonitis. In conclusion, this 2-step approach is feasible and demonstrated to have a strong antimyeloma activity with reduced deaths due to acute toxicities.

P501

CONVERSION OF LOW DONOR T-CELL CHIMERISM IN THE SETTING OF NON-MYELOABLATIVE STEM CELL TRANSPLANT USING ONE DOSE OF FLUDARABINE AND DLI

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A 53 yers old patient with myelofibrosis with myeloid metaplasia was referred to our Center because of increasing splenomegaly, LDH and anemia. The patient received a NMST from an HLA matched sibling donor. The conditioning regimen included fludarabine 30 mg/m^2 on days –4, -3, -2 and 2Gy TBI on day 0, and post grafting immunosuppression with mycofenolate mofetil (MMF) and cyclosporine (CSA). A total of 7.0x10^6 CD34/Kg were infused. The patient developped skin aGVHD grade I. Day +28 and +56 analysis showed 60% and 66% donor CD3 respectively and CSA and MMF were tapared off by day 60. On day +96 chimerism showed a decline in donor CD3 compartment, confirmed by a second assessment two weeks later:45% donor CD3. Our previuos experience approching rejection using withdrowal of immunosuppression with subsequent DLI was disappointing, we then used a different approach and CSA and MMF were again initiated. Day +128 and +159 chimerism showed respectively 48% and 36% donor CD3. There was, even if slowly, but still a trend toward rejection. On day +177 (in the absence of GVHD) the patient received Fludarabine single dose 30 mg/m^2 and 2 days later DLI with 1x10^6 donor CD3/Kg; after DLI CSA and MMF were suspended. Two weeks post- DLI the patient developped (byopsy-proven) skin and GIT aGVHD grade III and was treated with CSA+PDN 2mg/Kg. Three days later, because of worsening diarrhoea, infliximab was added. Twentyone days post DLI chimerism analysis showed a drammatic donor increase (CD3= 88%). The patient developped hepatitic aGVHD post DLI (byopsy proven) at day +46 after DLI and ATG was added. Improvement was in skin and GIT but not in liver aGVHD with bilirubin stable at 20 mg/dL and the patient died of S. Aureus pneumonia , with chimerism showing donor CD3 to be 90%. In conclusion: in this patient, in the attempt to slow rejection, immunosuppression was restored. In order to create an imballance between host T cells and residual donor T cells Fludarabine and DLI were delivered. This approach resulted in a drammatic increase in donor peripheral CD3 compartment. One issue is the severe aGVHD developped post DLI performed with a relatively low dose of donor CD3. This might mean that with this approach a lesser dose of donor lymphocyte might be needed, eventually followed by excalating doses of donor lympocyte.

P502

IMMUNE RECOVERY AFTER REDUCED INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH LOW DOSE ALEMTUZUMAB

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In vivo administration of alemtuzumab is effective to decrease the incidence of graft-versus-host disease (GVHD) after allogeneic stem cell transplantation (allo-SCT). However, post-transplant immune reconstitution is impaired. The aim of this study was to investigate the effect of *in vivo* administration of low-dose alemtuzumab on the kinetics of immune reconstitution and on the incidence of infections. Twenty-seven patients entered a pilot study employing reduced-intensity conditioning (thiotepa /fludarabine/cyclophosphamide) and low-dose alemtuzumab 15mg/ms (n=10) or 7.5 mg/ms (n=17) before allo-SCT from HLA identical or one-antigen mismatched sibling donors. Thirteen of 27 patients (48%) received post-transplant donor lymphocyte infusions (DLI) for a total of 23 infusions (median 2). All lymphoid subsets, T-cell receptor (TCR) spectratyping and T-cell receptor excision circles (TRECs) were analyzed at various time points after transplantation. The median time to achieve more than 200 CD4+/uL and 500 CD8+/uL were 6 and 9 months, respectively. The dose of alemtuzumab influenced the early immune recovery: median value of CD4+/µL and CD8+/µL at day +90 were 170 and 450 in 7.5mg group versus 100 and 119 in 15 mg group, respectively (p<0.02 and p<0.007). There was not a significant difference in the recovery pattern of CD4+ and CD8+ at day +180 between the two groups (p=0.6, p=0.3, respectively). In addition, there is not a significant difference in the recovery of CD4+ and CD8+ at day +90 and +180 between patients with or without any GVHD or receiving DLIs. Natural killer cells remained between the value of 300/µL and 500/µL throughout the first year whereas the median time to reach CD19+ blood concentrations of >200 cells/µL was 9 months. TCR spectratyping was performed in 17 patients (62%). Significant higher percentages of Vbeta families with normal complexity score (≥ 5 bands) were observed at +270 days as compared to previous control at +120 days: median value 65% (range: 23%-77%, median value normal donors 84%) versus 40% (range: 15%-69%), respectively (p<0.02). In 21 patients (77%), TREC counts per microgram of DNA were analyzed. Blood from 27 healthy donors (median age 52) contained a median value of 9x10^2 TREC per microgram of DNA. Before allograft, the median value of TREC per microgram of DNA was 1x10^2. TREC counts were significant higher at 180 days (median value 1.7x10^2, mean value 3.7x10^2 TREC per microgram DNA) and at 270 days (median value 2.1x10^2, mean value 7.6 x10^2 TREC per microgram of DNA) as compared to day +60-90 (median value 0, mean value 30 TREC per microgram of DNA) (p<0.002, p<0.001). There was a trend versus higher TREC counts at day 180 in patients with less than 55 versus more than 55 years (median 2.2 x10^2, mean 5.4 x10^2 versus median 1.1 x10^2 mean 2.5 x10^2 TREC per microgram DNA) (p=0.06). In these patients we also analyzed the pattern of infections: i) 24 patients (88%) experienced CMV

reactivation with a median time to first CMV reactivation of 29 days; ii) 14 of 26 patients (53%) developed one or more infectious complications after hospital discharge (n=10 bacterial, n=3 Herpes Zoster, n=1 HH-V6, n=2 Aspergillosis). In three cases infection was the cause of death with an actuarial probability of TRM at one year of 11%. The cumulative incidence of grade II-IV acute GVHD at day 100 was 11%. The overall incidence of chronic GVHD was 28%.

In conclusion, 1. the time of immune reconstitution is shorter than with higher doses of alemtuzumab (100 mg), but still delayed; 2. the risk of fatal infections is low; 3. the low dose is still effective in prevent GVHD.

P503

EFFICACY AND SAFETY OF ULTRASOUND-GUIDED CENTRAL VENOUS ACCESS IN ONCOLOGICAL AND HAEMATOLOGICAL PATIENTS: RESULTS OF A MONOCENTER SERIES OF 519 CONSECUTIVE PATIENTS

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Background. The management of the cancer patients frequently relies on the ability to deliver a variety of intravenous agents over a prolonged period. A central venous catheter (CVC) currently represents the most frequently adopted intravenous line for patients undergoing infusional chemotherapy and/or high-dose chemotherapy with concomitant blood stem cell transplantation and parenteral nutrition. The aim of this study was to explore the feasibility and safety of CVC insertion under ultrasound (US) control. US offers the advantage of real-time, multiplanar imaging as well as Doppler analysis, which makes it very practical and improves safety by allowing the operator to identify major vessels.

Methods. The patient is placed in Trendelenburg's position. After sterilization, local anaesthesia is applied and a 7.5 MHz puncturing US probe is placed in the right supraclavicular site and a 16 gauge needle is advanced into the last portion of internal jugular vein nearly innominate vein under US-control; after insertion of the guidewire the catheter (Sekalon Seldy, Becton Dickinson) is advanced into the superior vena cava until insertion to right atrium. At the end of the procedure an upright chest X-ray is carried out to confirm CVC position and to rule out a pneumothorax.

Results. From June 2001 to June 2004 this procedure has been performed 600 times in 519 consecutive patients: 350 with solid tumors and 169 with hematological malignancies. Some patients underwent CVC insertion more then one time along their clinical course (complete remission, relapse, stem cell collection and transplantation, parenteral nutrition). The procedure was successful in 595/600 time (99.1%), mean time of CVC permanence was 151 days (range 7-701 days). Four cases of deep vein thrombosis were detected successfully treated with low molecular weight heparin followed by oral anticoagulants. Infections episodes were 1.7/1000 days of use, successfully treated with antibiotics. No increase of infections was registered in the transplantation setting. No pneumothorax occurred.

Conclusions. This procedure is safe, cheap with high accuracy and success rate, and above all US-guidance avoids pneumothorax.

P504

FLAG IS A FEASIBLE TREATMENT IN RELAPSED ACUTE MYELOID LEUKAEMIAS PATIENTS AFTER SINGENEIC AND AUTO/ALLOGRAFT

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The risk of relapse after autologous (A-BMT) and singeneic bone marrow transplantation is high. The outcome for the patients relapsing after an autologous transplant is poor, on mayority this is for the relapses occurring after allogeneic bone marrow transplantation (ALLO-BMT). The achievement of a second complete remission (CR) may offers a second opportunity for transplantation. We evaluated the outcome of 32 patients (14 males and 18 females; median age: 39 years - range 4-62) with Acute Myeloid Leukemia (AML) treated with FLAG (n=24) or FLAG-Ida (n=8) after failing an autologous (n=21), an allogeneic (n=9) and singeneic. All pts received an induction regimen consisting of anthracycline, cytarabine (ARA-C) and etoposide in a 3+5+10 schedule. Consolidation therapy included intermediate dose ARA-C plus the same anthracycline used in induction. The median time between CR and transplantation was 4 months (range 3-7). Conditioning regimen included TBI in 69% of cases. Transplantation was performed in first CR in 31 pts and in second CR in one patient. The relapses occurred after a median of 11 months (range 4-30). Overall, After FLAG or FLAG-Ida regimens, CR was obtained in 23 pts (72%), all remitters needing a single corse. There were four deaths in induction due to cerebral hemorrhage (2) or infection (2) while 4 pts (18%) were resistant. Following CR achievement 2 pts underwent a second A-BMT, 2 pts a second ALLO-BMT, 3 pts a first ALLO-BMT, 3 pts received haplotype-mismatched stem cell from related donors, one patient matched-unrelated donor (MUD) transplant and finally 3 pts received DLI. Nine patients, in second CR, did not undergo any furher transplant; one of them died for relapse before MUD transplant, 3 patients relapsed and died and finally five pts are alive in second CCR (+88,+23,+2,+2,+1 months); of these, three pts are waiting for a MUD transplant. Five pts in CR died after a second transplant: 2 pts for transplant-related mortality; 3 pts for relapse. Median overall survival and disease free survival were 11 and 24 months, respectively. Fifteen (47%) are alive and 14 (44%) of them are in second CCR. In conclusion, our results showed that FLAG regimen is an effective therapeutic option for patients with AML who relapse after transplantation procedures. The toxicity has been acceptable enabling most patients to receive further transplant treatment.

P505

EFFECT OF CD34+ CELL DOSE ON THE KINETICS OF DONOR CHIMERISM OR REJECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED CONDITIONING REGIMENS

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Objective. We have investigated the correlation between $CD34^+$ cell dose and kinetics of engraftment or rejection after transplantation. METHOD: Chimerism analysis performed using short tandem repeat-polymerase reaction (STR-PCR) was carried out in 73 patients (42 male and 31 female) with different malignancies (10 HD, 17 NHL, 22 MM, 3 CML, 5 AML 1 CLL, 1 PTI, 8 Kidney and 6 Breast cancer) that received different non myeloablative conditioning, followed by allogeneic SCT: 22 Flu/Mel, 26 Flu/TBI and 25 Flu/Cy. The median age was 47 years (range 22-60). All but three received unmanipulated PBSC grafts from HLA-identical siblings donors mobilized with G-CSF. GVHD prophylaxis consisted of CSP/MTX or CSP/MMF (mainly used in patients with MM. Donor engraftment was evaluated at day +15, +30, +90 and so on in three subgroups of patients: $CD34^+$ cell dose $<2x10^6$ /kg; $\geq 2 \& \leq 8$ x 10^6/kg and >8.0 x 10^6/kg. At day +15 kinetics of engraftment was significantly related to $CD34^+$cell dose (p=0.028), while from day +30 the percentage of donor chimerism and rejection did not differ significantly in the three groups (p>0.5). On the contrary previous observation showed that kinetics of engrafting donor cells was different in the three conditioning regimens. The rate of complete donor chimerism (CDC=>95% donor cells) in FLU/MEL, FLU/TBI and FLU/CY at day +30 were 76%, 22%, 0%, respectively; at day +90 were 95%, 60%, 0%.

Conclusion. In stem cells transplantation with reduced conditioning regimens , $CD34^+$ cell dose does not significantly influence donor cells engraftment or rejection but it has a noticeable effect in the early kinetics of donor chimerism (1 to 15 days); while conditioning regimen has a greater impact on long term engraftment kinetics.

P506

T-CELL-DEPLETED HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR SECONDARY LEUKEMIA AND MYELODYSPLASIA FROM EITHER MATCHED OR MISMATCHED RELATED DONORS

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Purpose. With the aim of offering allogeneic hematopoietic stem cell transplantation to all patients with secondary leukemia or myelodysplasia, we started including them not only in our T-cell-depleted matched transplant programme which was started in July 1986 but also in our T-cell-depleted mismatched transplant programme begun in 1993. Here we report the results in 42 patients with secondary leukaemia or myelodysplasia who received haploidentical transplants and in a matched group of 21 patients who were candidates for HLA-matched sibling transplants.

Patients and Methods. Median ages (range) were 44 (17-65) and 37 (7-62) years for matched and mismatched recipients, respectively. Leukemia was secondary to MDS in 46 and radio-chemotherapy-related in 8. Median time from original disease to transplant and from leukemia diagnosis to transplant were 9 (range 2-168) and 10 months (range 2-60), respectively. At transplant, 30 patients were in CR, 23 in relapse and 10 had never been treated before transplantation. Cytogenetics were unavailable in 14 patients, normal in 20, abnormal in 29. TBI-based conditioning (14.4 Gy fractionated in matched or 8 Gy single fraction in mismatched transplants) was used in 56 patients and a chemotherapy-alone based protocol in 7 patients who had been previous irradiated because of prior cancer. TBI was followed by thiotepa, rabbit ATG and cyclophosphamide in the first 12 patients or fludarabine in the others. Melphalan was given instead of TBI in 7. GvHD prophylaxis consisted only of *ex vivo* T-cell depletion.

Results. One matched and one mismatched recipient died too soon after transplant leaving 61 patients to be evaluated for engraftment. Primary engraftment was achieved in 20/20 matched and in 40/41 mismatched recipients. Grade II-IV acute GvHD occurred in 3 cases and chronic GvHD in 2. Five of 21 matched transplant recipients and 17/ 42 mismatched died of non-leukaemic causes. The cumulative incidence of non-leukaemic mortality depended upon disease status at transplant and ranged from 0 to 40% (95% CI 20%-80%) in the matched group and from 30% (95% CI 15%-59%) to 52% (95% CI 33%-80%) in the mismatched group. Infections were the most common causes of non-leukemic deaths (CMV 4, EBV-related lymphoma 2, candida 2, fusarium 1, pn. carinii 1, bacteria 3, toxoplasma 1, mycobacterium tuberculosis 1). Other causes of death were GvHD (2), idiopathic pneumonia (3), chirrosis (1) and rejection (1). Eleven patients relapsed (6 were in CR and 5 in relapse at transplant). The cumulative incidence of relapse in patients transplanted in CR from a matched or mismatched donor was 40% (95% CI 16%-97%) and 16% (95% CI 5%-46%), respectively. For patients transplanted in relapse, the incidence of leukemia relapse was 0% in the matched group and 26% (95% CI 12%-56%) in the mismatched. Median follow-ups (ranges) for survivors 13/ 21 matched transplants and 17/ 42 mismatched were respectively 77 (8-233) and 48 (2-145) months. Probability of EFS was 0.60±0.18 and 0.50±0.11 for the patients transplanted in CR from a matched or mismatched donor, respectively. For those already in relapse at transplant, EFS was 0.59±0.14 in the matched and 0.21±0.09 in the mismatched group.

Conclusion. This study shows transplant should be recommended for patients with secondary leukaemia or myelodysplasia, independently a matched sibling being available. Extensive T cell depletion ensures no GvHD in matched transplants and an extremely low incidence in the mismatched. Most important, considering the median age of myelodisplasia cases, patients between 40 and 60 years of age are not excluded from the transplant programme.

P507

DONOR ORIGIN OF MESENCHYMAL STEM CELLS AFTER ALLOGENEIC STEM CELLS TRANSPLANTATION IN PEDIATRIC PATIENTS

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Bone marrow mesenchymal stem cells (MSC) remain host-derived years after allogeneic BMT despite successful hematopoietic engraftment. Little is known about the origin of recipient's MSC in case of cord blood (CB) transplantation. In the present study we have analyzed whether MSC are of host or donor origin depending on stem cells source (BM or CB).

MSC from patients – both adult and paediatric and with different diseases – who had undergone allogeneic transplantation were grown from bone marrow aspirates. The stem cell source was bone marrow for all adult patients, while in pediatric patients 45% was cord blood, 50% bone marrow and 5% mobilized peripheral blood. Cells were expanded and identified using immunophenotypic markers. Chimerism studies were performed at DNA level using polymerase chain reaction of amelogenin gene (AMG on chromosome X) and amelogenin-like gene (AMGL, on chromosome Y) in sex-mismatched transplants and short tandem repeat (STR) loci. Analyses were carried out at different time-steps after transplantation over a total of 20 pediatric samples and 10 adult ones. The results show that while in adults 100% of MSC confirmed to be host derived, in the paediatric cases a MSC mixed chimerism was identified; in detail, in case of BMT, 15% of patients showed MSC mixed chimerism (the chimerism range was 40%÷75%) while the percentage in CBT patients raised up to 62.5% (the chimerism range was 20%÷70%); the single case of PBSCT resulted in a MSC chimerism rate of 60%. These findings confirm that after allogeneic stem cells transplantation in adults MSC are always host derived while in pediatric patients donor MSC can engraft in bone marrow. This suggests that the bone marrow soil of pediatric patients is more favourable for the engraftment of transplanted MSC. Moreover, in case of cord blood transplantation MSC chimerism is significantly more evident suggesting a potential different engraftment mechanism of cells that, in the direct assessment from CB cells in vitro, are currently difficult to be grown.

Non-Hodgkin's Lymphoma III

P508

PRIMARY CHEMOTHERAPY WITH HIGH-DOSE METHOTREXATE (HD-MTX), CYTARABINE, THIOTEPA, AND IDARUBICIN (MATILDE REGIMEN) FOLLOWED BY WHOLE-BRAIN IRRADIATION FOR PRIMARY CNS LYMPHOMAS (PCNSL): FINAL RESULTS OF A MULTICENTER, ITALIAN PHASE II TRIAL

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Background. A phase II trial was designed to assess MATILDE chemotherapy regimen in pts with newly diagnosed PCNSL. This combination includes drugs active in different phases of the cell cycle, with demonstrated efficacy against aggressive lymphomas and able to cross the blood-brain barrier.

Methods. From May 2000 to January 2004, 41 HIV-negative pts with PCNSL (age ≤70 ys, PS<4) received 3 courses of MATILDE regimen, every 3 weeks, followed by response-tailored whole-brain irradiation. MATILDE regimen consisted of MTX 3.5 g/m^2 day 1; cytarabine 2 g/m^2, twice a day, day 2; idarubicin 15 mg/m^2, day 2; and thiotepa 25 mg/m^2, day 3. Following a preliminary tolerability analysis performed after 19 pts [Ferreri AJM, *et al.,* ASCO 2002], doses of cytarabine, idarubicin and thiotepa were reduced to 1.7 g/m^2, 13 mg/m^2 and 20 mg/m^2, respectively.

Results. Pt Characteristics: All pts (median age 57 ys) but one had brain lesions, with ocular and meningeal disease in 3/34 (9%) and 7 (17%) pts, respectively. According to the IELSG score [Ferreri AJM, *et al.,* J Clin Oncol 21: 266, 2003], risk was low in 10 (24%) pts, intermediate in 23 (56%) and high in 8 (20%). Activity: Response after chemotherapy was complete (CR) in 18 (44%) pts and partial in 13 (32%), with an ORR of 76% (95%CI: 63%-89%); 6 (15%) pts experienced progression and 4 died of toxicity. ORR after chemo-radiotherapy was 83% (95%CI: 71%-95%). Five of the 7 pts with meningeal disease achieved CR, one achieved PR but died early; among CRs, three pts are relapse-free at 13, 17 and 30 months, and two patients experienced relapse without meningeal involvement after 16 and 50 months. Efficacy: Seventeen patients are failure free, with a 3-yr FFS of 41±8%; 16 pts are alive at a median follow-up of 31 months, with a 5-yr OS of 37±7%. Tol-

erability: G4 neutropenia, thrombocytopenia and sepsis were reported in 54%, 45% and 14% of the 97 delivered courses. Seven (17%) pts did not complete chemotherapy due to toxicity. MATILDE doses reduction after the first 19 enrolled patients was associated with a relevant tolerability improvement (lethal toxicity was reduced from 16% to 4.5%), without a negative impact on efficacy. Complete data on MiniMental Status Examination were available in 13 survivors (median age 49), neurological deterioration was not observed at a median follow-up of 26 months. Prognostic factors: The IELSG score was the sole predictor of response and survival; with a ORR after MATILDE of 100%, 74% and 50% (p= 0.01) and a 3-yr OS of 70±12%, 37±10% and 0% (p= 0.0002), respectively for pts with low, intermediate and high risk.

Conclusions. MATILDE regimen is a new active combination against PCNSL. Myelosuppression is the main dose-limiting toxicity. Meningeal disease can be controlled without intrathecal drug delivery. Therapeutic results are especially good in patients with low-intermediate risk according to the IELSG score, whose prognostic value was confirmed.

P509

PROGNOSTIC IMPORTANCE OF INTEGRATED POSITRON EMISSION TOMOGRAFY/COMPUTED TOMOGRAFY IN MALIGNANT LYMPHOMAS MANAGEMENT

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Positron emission tomography (PET) with 2-[fluorine-18]-fluoro-2- deoxy- d-glucose (FDG) plays an important role in the evaluation and management of malignant lymphomas (Hodgkin and non Hodgkin). New technology of integrated PET/CT (computed tomography) results in further improvments in staging accuracy. We have described the value of chemotherapeutic effects on malignant lymphomas by use of PET/CT as early as a few courses after the initiation of chemotherapy (CHT) and the therapeutic response at the end of treatment.

Methods. Five patients with emblematic non Hodgkin's and Hodgkin's lymphomas (3 non HL; 2 HL) were enrolled in this study; all they have an important tumor burden with nodal and/or extranodal involvement (bone, lung, gastric, perirenal localization). PET/CT was performed before therapy to determine baseline stage; then it was repeated just after the second course of treatment and at the end of chemotherapy. Image findings were verified by clinical follow up and by other imaging modalities, when necessary.

Results. Four patients (2 HL; 1 Burkitt L. ; 1 High grade non HL.) were pratically disease free yet after the secod course of CHT and mantained PET/CT negativity at the end of CHT. On the contrary, one patient (Malt L.; gastric and pulmonary localization) had evidence of disease after the second course and had only partial remission at the end of CHT (also with standardized uptake value SUV- cor for a semiquantitative analisis).

Conclusion. Even if the optimal timing of PET/CT has yet to be clarifed we can say that:

1) PET/CT has proven useful in accurate staging of lymphomas; due to the exact anatomic localization, equivocal or false positive PET findings are avoided.

2) Negative results at second course of CHT may play an important role as early predictor and exellent indicator of good prognosis (while PET/CT positivity, at the same time, seems be a strong predictor of poor prognosis or of only partial remission).

Our results need a wider case report and, in particular, a long term follow up in negative findings may be hopeful. PET/CT accuracy and predictivity might help the clinicians, not only for a precise report, but for more correct treatment planning (additional treatment, radiaton therapy or other).

P510

HCV-RELATED INDOLENT NON-HODGKIN LYMPHOMAS ARE RESPONSIVE TO ANTIVIRAL THERAPY

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Backround. An association between Hepatitis-C Virus (HCV) infection and Lymphomas has been demonstrated by several studies from Italy and other country. A possible pathogenic role of the viral infection on lymphoma development is supported by experimental results. The possibility of obtaining clinical and instrumental response of the hematological disease following antiviral treatment with Interferon ± Ribavirin has been shown in limited series and isolated cases.

Objectives. We report a monocentric experience on 7 patients affected by HCV-related indolent non-Hodgkin lymphomas treated with alpha-Interferon ± Ribavirin.

Results. 8 patients were treated: 2 of them affected by Small Lymphocytic Lymphoma/CLL, 3 by Lymphoplasmocytoid Lymphoma, 1 by Marginal Zone – Splenic Lymphoma, 1 by MALT-type Lymphoma, and 1 by B-cell indolent lymphoma unspecified. Median age was 57.5 years (range 33-77 years) with 3 males and 5 females. Treatment consisted of alpha 2-IFN at the dose of 3 MU x 3/week in 2 cases, Pegylated - IFN (Peg-IFN) alone at the dose of 80 mg/week in 2 cases and Peg-IFN associated to Ribavirin in 4 patients. Treatment duration ranged from 6 to 18 months. A virological response, evaluated by HCV-RNA negativization was observed in 6 patients while 1 patient could not be evaluated and discontinued treatment due to lymphoma progression. Clinically evident hepatitis with transaminase elevation was present before treatment in 5 patients. A hematological response was observed in 7 patients (4 CR and 3 PR). All patients who achieved a virological response had also a hematological response.

Discussion. Antiviral therapy can induce complete or partial hematological response in HCV-related indolent lymphomas of different histologic subtypes.

P511

PERIPHERAL BLOOD LYMPHOCYTIC PROFILE IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Introduction. Follicular lymphoma is the most common adult low-grade non-Hodgkin's lymphoma. The influence of this disease in peripheral blood lymphocytes is not evident. Indeed it is generally known that while lymphocytic arrangement can be altered as a result of the leukaemia form (although thus occurs in a limited number) on the other hand the cause of occasional anomalies can be the involvement of the immune system against neoplasm. In order to better understand this condition we have analyzed, at the moment of the diagnosis, the lymphocytic immunophenotype in the peripheral blood in a group of patients followed up at our institution.

Patients and Methods. We have studied the peripheral blood of 34 patients with histologic diagnosis and grading of follicular lymphoma according to the WHO criteria. The medium age was 61,5 years (39-88). In all blood samples we tested total lymphocytes and, by flow cytometry, T, B and NK subsets (CD3, CD4, CD8, CD19, CD20, SmIg+, SmIg-, CD56, and the expression of CD11a molecule on T CD8 lymphocytes).

Results. Total number of lymphocytes was reduced in 29% of the subjects and a similar course had CD3. T CD4/CD8 ratio was altered in 44% (12% decreased, 30% increased). CD19 coincided exactly with CD20 marker and was abnormal in 26% of subjects. But 20% of all patients showed light chains clonal restriction mostly in histological grade 3. CD56 was high in 20% of the cases. Finally the CD11a molecule was over-expressed on CD8 in 68% of subjects. Selecting the patients according to age in 2 groups one composed by 14 subjects until 60 and the other by 20 over 60 years was obtained for the three parameters that showed the greater variability for the first group: lymphopenia in 7%, clonal restriction in 29%, over-expression CD11a in 50%; for the second group: lymphopenia in 50%, clonal restriction in 15%, over-expression CD11a in 80% .

Conclusions. Follicular lymphoma is a lymphoproliferative disease hardly ever with peripheral lymphocytosis but in some cases with lymphopenia more frequently in advanced age. Clonal restriction of B cells is present in a small proportion mostly in patients under 60 years. The parameter more frequently altered is the over-expression of CD11a on CD8 and especially in patients over 60 years. Therefore in subjects under 60 leukaemic form is more frequent while in those over 60 prevails the compromise of the immune system (lymphopenia, over-expression CD11a). These observations can help clinicians to interpret an eventual persistent lymphoadenopathy.

P512

DIAGNOSIS OF NHL BY THE COMBINED USE OF HISTOLOGY AND FLOW CYTOMETRY ON CELL SUSPENSIONS FROM LYMPH NODES, GASTRIC MUCOSA, CUTIS AND LACHRYMAL GLAND

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The aim of this study was to assess the diagnostic impact of the combined use of flow cytometry (FCM) and histology (HI) in non-Hodgkin lymphoma (NHL) patients. We analyzed 48 biopsies, from 42 patients with suspected lymphoma; specimens were from lymph nodes (22), gastric mucosa (21), cutis (4) and lachrymal gland (1). In order to obtain cell suspensions for FCM, we performed lancet fragmentation of tissue specimens in a Petri dish, followed by two washings in PBS with 0.2% bovine serum albumin. Cells were adjusted to $5x10^6$/mL, and 50 microL of the cell suspension were mixed with MoAbs combinations. The panel of MoAbs was the following: CD3, CD5, CD10, CD19, CD20, CD22, CD23, CD37, CD79b, FMC7, CD103, CD25, HLA-DR, CD38, CD11c, CD35, CD43. Pathologist and flow cytometrists were blind to patient characteristic and to each other results.

Of the 22 lymph node biopsies analyzed, 4 were classified as reactive hyperplasia (RH) and 8 as NHL according to both HI and FCM; five cases were defined as Hodgkin diseases (HD) by HI, and as RH by FCM; one case was diagnosed as HD by HI and as NHL by FCM; one case was diagnosed as NHL by HI but was not evaluable by FCM for dead cells excess. Finally, 2 cases were diagnosed as NHL by FCM and RH by HI: after careful revision, pathologists agreeded with diagnosis of NHL. One case was diagnosed as NHL by HI and not by FCM: after careful list-mode revision, a few large NHL cells were detected also by FCM. As for gastro-intestinal biopsies (21 specimens), in 15 cases RH was concordantly diagnosed by both HI and FCM. One case was classified as maltoma and another as follicular lymphoma by both HI and FCM. One patient had three biopsys samples taken, in which HI was unable to identify neoplastic cells, while FCM detected clonal expansion of marginal zone NHL B cells. Finally, one case was initially diagnosed as NHL by FCM and as RH by HI: a second biopsy allowed to diagnose the case as maltoma by HI. As for cutis biopsies (4 cases) one was diagnosed as NHL and another case as RH by both methods. The remaining two cases were diagnosed as NHL by HI, while FCM did not detect clonal lymphocytes. Finally, in the lachrymal gland biopsy, FCM identified neoplastic T cells (peripheral T-NHL), while HI suggested reactive picture. In conclusion, in the blinded analysis 31/48 (65%) cases showed complete concordance between FCM and HI even. Three cases, in which FCM (and not HI) had diagnosed NHL, became concordant after histological revision. One case in which HI (and not FCM) diagnosis had been NHL was reclassified as NHL after FCM revision. The case in which HI evidenced HD and FCM suggested NHL after revision was finally considered as composite lymphoma. When considering these 5 revised cases, the concordance between

the two methods reached 75% (36/48). Among the remaining 12 discordant cases, 5 were HD, 2 were cutaneous NHL, 1 was not evaluable by FCM and 4 are still under revision (these results of the latter will probably become concordant, due to unequivocal FCM results). In conclusion, if we exclude HD and cutaneous NHL, in which HI remains the best diagnostic approach FCM and HI are complementary tools for diagnosing nodal and extranodal lymphomas; the combined use of both methods strikingly improves the sensitivity of diagnostic process in NHL.

P513

ASSESSMENT OF THE FREQUENCY OF ADDITIONAL MALIGNANCIES IN PATIENTS WITH SPLENIC MARGINAL ZONE LYMPHOMA

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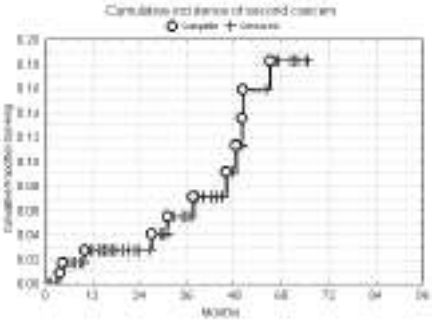
Purpose. The purpose of this study was to assess the frequency of additional cancers in patients with Splenic Marginal Zone Lymphoma (SMZL), and to estimate the risk of second primary cancers (SPC).

Methods. We investigated the incidence of additional cancers in 129 consecutive SMZL patients diagnosed in three Italian haematological centres, asking the cooperating physicians for additional data on initial and subsequent therapies and on onset and type of second cancers.

Results. Twelve SPC were recorded (9.3%); the 3- and 5-year cumulative incidence rates were 5.5% and 18.3%, respectively. Five more patients had a diagnosis of cancer prior to that of SMZL.



The frequency of SPC was higher than that expected in the general population. The standardized incidence ratio (SIR) was 2.03 (95% confidence interval [CI], 1.05 to 3.56; $p<0.05$). Of the twelve SPC observed, four were genitourinary neoplasms (SIR, 3.70; 95% CI, 1.01 to 9.48; p <0.05), four were lung cancers (SIR, 9.09; 95% CI, 1.41 to 13.25; $p<0.05$) and the other four were: hepatic carcinoma, endometrial cancer, breast cancer, and colo-rectal cancer.

Conclusion. Our findings evidence a high frequency of additional cancers in patients with SMZL and suggest that the incidence rate of SPC is significantly different from that expected in the general population. The frequency of cases with genitourinary tract and lung malignancies in our series is higher than expected. Although confirmatory data are needed, it is our opinion that SMZL patients are at risk of second cancer and should be carefully investigated on diagnosis and monitored during the follow-up.

P514

INTENSIFYING, ESCALATING OR DENSIFYING DRUG DOSES IN LARGE-CELL LYMPHOMAS ?

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Introduction. It is still unclear the actual contribute of dose density (DD), dose size (DS) and dose density (DD) in the conventional chemotherapy of large-cell non-Hodgkin lymphomas.

Methods. A prospective, randomized trial compared the original cyclic schedule of ProMECE-CytaBOM chemotherapy (c-PC, 6 cycles) with a modified version of it, which administered the same drugs sequentially (s-PC), within the same time frame (113 days) and with the same planned cumulative DI, but with three times higher DS of all the drugs except vincristine. Drug doses were as follows (mg/sm): c-PC (q 21 d x 6 cyles), CTX 650, Epi 40, and VP16 120, i.v. d 1, Pred 60 p.o. d 1-14, ARA-C 300, Bleo 5, VCR 1.4, and MTX 120 i.v. d 8; s-PC, CTX 1950 i.v. d 1, 64, VCR 1.4 i.v. d 15, 43, 78, 106, MTX 360 i.v. d 15, 78, VP16 360, and Epi 120 i.v. d 29, 92, Bleo 15 i.v. 43, 106, ARA-C 900 i.v. d 50, 113, Pred 50 p.o. for 5 days after every i.v. administration. Folinic acid recsue was performed in both regimens, mesna uroprotection only in the sequential one. Growth factors was utilized on demand, not prophylactically.

Results. Fifty-six patients received c-PC and 52 s-PC. Clinical and prognostic characteristics were well-balanced in both groups. The actual mean cumulative DI of all the 7 antitumoral drugs was 0.78 ± 0.15 with c-PC, 0.81 ± 0.14 with s-PC. Clinical response was complete in 59 and 52%, partial in 20 and 21%, null in 5 and 6%, respectively. Progression was recorded in 14 and 13%. Four toxic deaths (2 per arm) were recorded, all due to severe neutropenia. With a median follow-up of 53 months, relapses occurred in 36 and 37% of completely responders, respectively. Toxicity was quite similar in both arms except for heavier neutropenia and thrombocytopenia with s-PC. Overal, failure-free, progression-free and disaese-free survival of the two groups were statistically indifferent, with a slight advantage for the c-PC arm, more clear in the overall survival comparison.

Conclusions. The identical short- and long-term results recorded in both arms suggest that the very similar DI actu-

ally delivered was the main determinant, much more important than the higher drug DS administered in one arm. It proves that increasing dose size – at least within the limits clinically attainable without stem cell rescue – does not improve results. The slight survival advantage recorded in patients treated with c-PC might be related to its lower toxicity and/or its little higher DD (10 vs. 12 administrations within 113 days).

P515

PRIMARY HEPATIC BURKITT'S LYMPHOMA IN PATIENT WITH HEPATITIS C INFECTION: CASE REPORT

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The sporadic adult Burkitt' s lymphoma (BL) is a variant of aggressive Non Hodgkin Lymphoma, distinguished for higher proliferate activity and preferential extra nodal involvement as in the abdomen, bone marrow and SNC. Primary hepatic lymphoma is extremely rare. Despite its aggressiveness if suitably treated is potentially curable. The role of hepatitis C virus (HCV) infection in the development of the non haematological disease and its association with about 10-15 % of indolent lymphomas is well known. We describe a rare case of a 25 year- old man with HCV infection in association with primary hepatic BL. The patient as a child received blood transfusion for a glucoso-6-phosphate dehydrogenase (G6PD) deficiency. In August 2000 there was a chance discovery of an increase levels of ALT and AST, positive HCV infection and presence of multiple hepatic nodules following ultrasonography. A CT abdominal scan and MR confirmed the presence of these lesions. Histopathological and immunohistochemical examinations revealed BL according REAL classification and a modest activity of hepatitis. There was no other site of involvement based on physical or radiological examination. The patient was treated with initial cytoridution phase to minimize the risk to increase enzymes and potential tumour lyses syndrome with a first cycle of CVP chemotherapy after which an improvement was noted in enzymes and less lesions. Subsequently, he continued the treatment following Magrath schedule, resulting in a complete remission and persisting HCV infection. Negativity HCV infection was obtained after a treatment with alpha-interferon 3 MU three/times/week and PEG-Interferon (1.5 micrograms/Kg/week) for 2 years. Our experience suggests the possible key role of HCV in the cause of lymphoma as well as in more aggressive form such Burkitt's lymphoma.

P516

ASYMPTOMATIC HCV PATIENTS WITH B NON-HODGKIN LYMPHOMA SUFFER A MILDER CHRONIC LIVER DISEASE THAN PATIENTS WITHOUT LYMPHOMA: A CLINICAL AND PATHOLOGICAL STUDY

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Patients infected with Hepatitis C virus (HCV) may also develop B-Non-Hodgkin Lymphoma (B-NHL). Pathological and clinical differences between HCV patients with and without B-NHL have been investigated in a group of asymptomatic carriers: anti-HCV positive patients with persistently normal levels of aminotransferase (ALT). 55 asymptomatic carriers (18 with and 37 without B-NHL) entered the study and underwent liver biopsy that was assessed according Ishack' score. During the follow-up ALT levels were determined each 2 months. The increase of ALT above normal levels, a marker of liver disease recurrence, was the end point of the study. Pathological-documented liver damage was significantly milder, both in terms of grading and staging, in B-NHL patients. All patients with B-NHL showed still normal ALT values after 12 years of follow-up. In contrast, 20.8% (Kaplan-Meyer estimate) of the patients without B-NHL showed an abnormal increase of ALT. Thus, HCV positive patients with B-NHL seem to have a less severe and more stable liver disease than patients without B-NHL. This suggests that, despite the tropism of HCV for both hepatocytes and lymphocytes, pathogenetic mechanism of hepatic and haematologic involvement might be different, and that HCV infection might lead either to chronic liver disease or to B-NHL.

P517

GENE TRANSFER OF A MODIFIED IDIOTYPE INTO DENDRITIC CELLS FOR IMMUNOTHERAPY OF NON-HODGKIN LYMPHOMAS

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The majority of B cell non-Hodgkin Lymphomas (NHL) are characterized by the clonal expansion of a single B cell expressing a tumor-specific antigen, the idiotype (Id). Studies in animal models and clinical trials have shown that the therapeutic efficacy of Id-based vaccines correlates with cellular immune responses, although humoral responses have been better characterized. We recently reported a molecular approach to modify and transfer the unique Id of the murine 38C13 lymphoma into dendritic cells (DCs) for simultaneous stimulation of CD8+ and CD4+ tumor reactive T lymphocytes. The strategy relies on chimeric constructs coding for Id fused with the targeting signal of the lysosomal associated membrane protein 1 (LAMP1), able to enhance antigen processing in the context of MHC class II. Recombinant vaccinia viruses (rVV) were con-

structed coding for Id fused with the targeting signal of the lysosomal-associated membrane protein1 (Id-LAMP1) to promote antigen presentation in the context of MHC class II. Mature DCs infected with rVV/Id-LAMP1 elicited both CD4+ and CD8+ Id-specific T cells, and protected animals from tumor challenge. Id-specific CD8+ cells were required to mediate the effector phase of a therapeutic response, and CD4+ cells were beneficial in the induction phase of the response (Muraro *et al.,* Blood 2005). To facilitate translation to the clinical setting, we tested transfection into DCs of idiotype-RNA. Despite a lower gene transfer efficiency, the ability of RNA-transfected DC to elicit Id-specific CD8+ and CD4+ responses was higher than what observed with rVV. 90% of the animals vaccinated with DCs transfected with RNA coding for Id-LAMP1 survived tumor free for more than sixty days. To evaluate the possibility of tumor escape in the few animals that developed tumors despite vaccination, the tumors-Id were sequenced. Results showed that VL is more susceptible to mutations than VH, and most importantly, that Id-based vaccines are limited by tumor escape in a higher proportion than Id-LAMP1-based vaccines. These results indicate that DC transfected with Id-LAMP1 induce efficient CD8+ and CD4+ cellular responses to Id, and may be useful therapeutically.

P518

DENDRITIC CELLS EXPRESSING LANGERIN AND CD1A IN EARLY MYCOSIS FUNGOIDES

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Background. In Mycosis fungoides (MF) dendritic cells (DCs) are increased when compared to healthy skin. DCs are immune accessory cells involved in the uptake, processing and presentation of antigens to reactive T cells. The activation of the so called TILs (tumor infiltrating lymphocytes) is more efficient when DCs have completed their maturational process. Therefore, blockage of DCs maturation by MF neoplastic cells is retained a mechanism of tumoral escape.

Aims. We aimed to analyze the distribution and the density of dendritic cells expressing CD207/langerin molecule and CD1a - the immature stage of their differentiation - in MF skin lesions, and to investigate the prognostic impact of langerin+ DCs density in terms of patients responsiveness to treatment and disease free survival (DFS).

Material and Methods. Thirty-three MF patients (20 F, 13 M; mean age, 55.7 yrs, range 28-76) were included in the present study. Eleven patients were in stage IA, 17 in stage IB, 4 in stage IIA and one in stage IIB. After treatment with PUVA and interferon alpha, 30 obtained a clinical complete remission, while 3 did not respond. After a mean follow-up of 29.3 months (range, 2-79), 8 patients relapsed, whereas 22 are still in complete remission. Immunostaining for langerin and CD1a was performed on skin formalin-fixed paraffin-embedded tissue sections obtained before the beginning of treatment and langerin and CD1a cell density was evaluated at light microscopy. Statistical analysis was performed using a SPPS statistical package.

Results. Density of CD1a+ and langerin+ epidermal DCs was, respectively, low in 6 and 11 cases, and high in 27 and 22. Density of CD1a+ and langerin+ dermal DCs was, respectively, low in 6 and 16 cases, and high in 27 and 17. Density of dermal langerin+ DCs was more frequently high in cases with a band-like/nodular infiltrate (78.6%) than in cases with a not confluent infiltrate (31.6%; Fisher's exact test: p=0.02). Density of langerin+ epidermal DCs was low in all 3 cases in which therapy with PUVA and interferon did not achieve a complete remission (Fisher's exact test: p=0.03). A shorter DFS was observed in patients with a high density of both langerin+ dermal DCs (log rank test, p=0.005) and CD1a+ dermal DCs (log rank test, p=0.06): at 45 months all patients with a high density of langerin+ dermal DCs had the probability to relapse of 35% compared to 25% of those with a low density of langerin+ dermal DCs.

Discussion. A low number of epidermal langerin+ DCs appears to be an unfavourable parameter in order to obtaining a complete clinical remission in early MF treated with a combination of PUVA plus interferon alpha: it is conceivable that these agents may activate epidermal DCs maturation and promote their maturation, thus facilitating the clearance of neoplastic cells by anti-tumour specific lymphocytes. Moreover, we can hypothesize that a high number of immature langerin+ dermal DCs is related to an increased risk of disease relapse, and denote a less favourable disease behaviour, since MF neoplastic cells, by blocking dermal DCs maturation, may reduce TILs recruitment, thus impairing the anti-tumour immune reponse.

P519

SEQUENTIAL BRIEF CHEMO-IMMUNOTHERAPY FND + RITUXIMAB AS FIRST LINE TREATMENT FOR ELDERLY PATIENTS WITH FOLLICULAR LYMPHOMA IS ABLE TO INDUCE CLINICAL AND MOLECULAR REMISSION WITH PROLONGED FAILURE-FREE SURVIVAL

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Introduction. Elderly patients (pts) with FL do worse than younger ones and the treatment is usually palliation rather than cure. In order to reduce the toxicity due to a prolonged chemotherapy in elderly pts, we investigated the efficacy and safety of a brief regimen FND plus Rituximab.

Patients and Methods. from March 1999 to March 2003, 71 elderly pts (age >60) with advanced stage FL at diagnosis were enrolled. Treatment plan was: 4 courses of FND (Fludarabine 25 mg/m^2 days 1-3, Mitoxantrone 10 mg/m^2 day 1 and Dexamethasone 20 mg days 1-3) followed by 4 Rituximab infusions at 375 mg/m^2/week; pts in PR received 2

further FND and 2 Rituximab infusions. PCR molecular monitoring for the presence of IgH/Bcl2 and/or Ig heavy chain (IgH) gene rearrangement was performed at the beginning of the treatment, after FND, after Rituximab and during follow-up time on bone marrow (BM) samples.

Results. median age was 66 (range 60-78); 15% had stage II, 13% stage III and 72% stage IV disease; 62% had BM involvement; 41% had bulky disease and 24% were at high risk according to IPI score. At the end of the treatment overall response was achieved in 63 pts (90%) and CR in 58 pts (83%). Five pts (7%) were in PR, 6 pts (9%) did not respond and one patient (1%) died of neutropenic sepsis during FND course. The addition of Rituximab allowed to increase CR rate from 44% (31 pts) after FND to 83% (58 pts); 73% of responding pts did so with a brief treatment program (4 FND + 4 Rituximab). Pts with adverse prognostic features as BM+, high IPI score and bulky disease at diagnosis responded as well as those with more favorable ones with no significant differences in CR rates. The toxicity was mild with grade 3-4 neutropenia reported in 22% of FND courses, but only 3 pts developed grade 3-4 infections. With a median follow-up of 31 months, failure free survival was 50% at 39 months. Thirty-five pts had a molecular marker (Bcl2 or IgH rearrangement) at diagnosis. Twenty-seven of these 35 pts had PCR analysis following completion of treatment: after FND 9/27 pts (33%) did not show anymore BM molecular disease, while PCR negativity was achieved in 23/27 pts (85%) after Rituximab treatment. After FND chemotherapy 4/9 PCR negative pts were in CR while all 23 PCR negative pts at the end of treatment were in CR. 3-yr FFS rate was significantly higher in PCR negative pts: PCR- 71% vs PCR+ 0% ($p<0.01$). Eighteen PCR negative pts in continuous clinical remission had regular molecular monitoring at 6, 12, 24 months off therapy. Fifteen are in continuous clinical and molecular remission; one had a molecular relapse at six months without evidence of disease recurrence and one patient with molecular relapse at 6 months subsequently converted to negativity still present at 36 months of follow-up. *Conclusions.* a brief course of chemo-immunotherapy is able to achieve high clinical and molecular response rates in elderly pts with low toxicity. The sequential use of FND and Rituximab induces a CR rate >80% also in unfavorable subsets of pts. The achievement of PCR negative status correlates with a lower risk of failure.

P520

INCIDENCE OF HEMATOLOGICAL TUMORS IN PATIENTS WITH MYCOSIS FUNGOIDES IN AN ACADEMIC HOSPITAL-BASED SERIES

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The occurrence of hematological neoplasms in association with Mycosis Fungoides (MF) has been the subject of sporadic case-reports. At the best of our knowledge, in the last thirty years at least seventy cases of MF have been reported in the literature in association with several cutaneous and extracutaneous haematological disorders, including lymphomatoid papulosis, anaplastic large cell lymphoma, B-cell chronic lymphocytic leukaemia (CLL), Hodgkin's lymphoma, plasma cell dyscrasia and myeloma, as well as chronic and acute myelogenous leukemias. The aim of our study was to evaluate the incidence of this rare association in our series of patients with MF collected by the Cutaneous Lymphoma Study Group of Marche Region and to describe the clinico-pathological findings of the cases showing such an association.

From January 1994 to December 2004, about 160 patients with a median age of 60 years (range 15-83) were referred to the Department of Haematology, Polytechnic University of Marche Medical School at Ancona, for a clinical and histologically confirmed diagnosis of MF for staging, treatment and follow-up. Out of them 5 (3%) showed an association with another type of haematological tumours. The patients were 3 women and 2 men, ranging in age from 46 to 68 years (median age, 59 years). Two cases showed a B-cell CLL, one case showed an IgG-k+ plasmocytoma, and 2 cases were affected also by acute myelogenous leukemias (M2 and M3 FAB subtypes). Diagnosis of MF preceded the appearance of the other haematological malignancy in the cases with CLL and multiple myeloma (MM). In these patients the second tumour was diagnosed during the follow-up, when the patients were monitored for the treatment of MF, respectively 1, 2 and 4 years later the diagnosis of MF. Patients had been treated for the cutaneous lymphoma by either steroid, or interferon, or PUVA and interferon, when laboratory investigations allowed an early detection of increasing peripheral monoclonal B cell lymphocytosis with the typical CD19+, CD5+ and CD23+ phenotype in the 2 B-CLL cases and an increasing circulating monoclonal IgG-k+ component in the case of multiple myeloma (MM). The bone marrow was partially infiltrated by B lymphocytes in CLL cases and diffusely infiltrated by monotypic plasma cells in MM case. Only the patient with MM stopped the treatment for cutaneous lymphoma, and received thalidomide, whereas the other two B-CLL patients were watched carefully and not treated for the second lymphoproliferative disorder. In cases with acute myeloid leukemias MF appeared subsequently, respectively 6 and 7 years later, with the clinical appearance of large pruriginous patches in the trunk and limbs. Histological examination of skin biopsy specimens showed a lichenoid superficial infiltrate of cerebriform lymphocytes with CD4+ phenotype and monoclonal T cell receptor gamma gene rearrangement by PCR. These findings were considered sufficient for excluding a reactive dermatosis with MF-like appearance, expecially the drug-reactions. In the same period we observed a case of Chronic Myelogenous Leukemia (CML) showing multiple cutaneous lesions suggestive for MF during the treatment with Gleevec: histology in this case revealed the presence of a T cell infiltrate with a mixed CD4+ and CD8+ phenotype and polyclonal by PCR: in this case skin lesions recovered completely after drug discontinuation. In conclusion, our series showed that about 3% of patients with MF may have a subsequent or a previous malignant lymphoma or myelogenous leukaemia. When MF appears first, a careful monitoring of the disease can allow an early diagnosis of the second tumour. When MF appears subsequently, dif-

ferential diagnosis with MF simulants needs to be entertained and sustained by an accurate histological, immunophenothypical and molecular analysis. We can speculate that MF, CLL and MM are relatively frequent diseases occurring in elderly and therefore they can occur simultaneously by chance; a fortuitous association, more than an increased risk for other haematological malignancies, might exist. However, we cannot exclude that either a genetic predisposition, or an underlying viral infection, or therapy-related carcinogenesis or an alteration in common progenitor cells, might account for the coexistence of different haematological tumours in the same patients.

P521

PRIMARY EFFUSION LYMPHOMA: AN IELSG INTERNATIONAL SURVEY

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International Extranodal Lymphoma Study Group (IELSG)

The International Extranodal Lymphoma Study Group (IELSG) coordinated a retrospective survey aimed to describe the clinico-pathological features and patterns of outcome of PEL, a rare B-cell neoplasm characterized by a preferential involvement of fluid-filled body spaces, consistent infection of the tumour clone by human herpesvirus type-8 (HHV-8) and a close relationship with underlying immunodeficiency status of the host. The study involved 15 international institutions. Forty-three patients (38 males and 5 females) were registered. Median age at diagnosis was 59 years (range 27-102). In 23 (53%) patients an associated human immunodeficiency virus (HIV) infection was reported, in one case the diagnosis of PEL was made after a solid organ transplantation, in two patients other immunodeficiency conditions were present. The tumour HHV-8 infection was demonstrated in 35 out of the 39 tested cases, Epstein-Barr virus infection in 13 of 30 cases. CD4 count was lower than 200/μL in 18 of the 25 cases in whom the data was available. An ECOG performance status score ≥ 2 was observed in 29 patients and the presence of B-symptoms in 20 patients. Serum LDH was elevated in 21 of the 39 tested patients. In 4 patients nodal involvement at diagnosis was reported, in 4 cases at least one extranodal site of localization other than serous cavities was present. A low/low-intermediate risk score according to International Prognostic Index was reported in 10 cases, an intermediate-high/high risk score in 29 cases. Heterogeneous treatment approaches were reported. Nineteen patients received systemic chemotherapy, in 16 cases an anthracycline-based regimen. Intrapleural cidofovir was administered in 3 patients. Eleven HIV+ patients received highly active anti-retroviral therapy (HAART), three of them as single therapy. Adequate follow-up data were available in 39 patients: among them median overall survival was 6.7 months, median cause-specific survival was 13.6 months and median progression-free survival was 8.3 months. Interestingly, cases of tumour complete regression after implementation of the sole HAART, after intrapleural administration of cidofovir or without any treatment were reported. Our data confirm the poor prognosis of PEL but suggest a possible biological and clinical heterogeneity. A review of the pathological, phenotypical and virological features is ongoing to validate these observations.

P522

INTERIM RESULTS OF A RANDOMISED STUDY PERFORMED BY THE INTERGRUPPO ITALIANO LINFOMI (IIL) COMPARING R-CHOP AND R-MINI-CEOP IN A POPULATION OF ELDERLY PATIENTS (OVER 65) WITH DIFFUSE LARGE B CELL LYMPHOMA (DLCL)

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Introduction and Objectives. Chop plus Rituximab (R-CHOP) is considered the standard treatment for elderly patients with diffuse large B cell lymphoma (DLCL). However the rate of elderly patients who could tolerate a full dose regimen has never been exensively investigated. The IIL designed a phase III study to compare R-CHOP with a regimen especially devised for elderly patients (R-mini-CEOP) in terms of Event Free Survival (EFS). Secondary objectives were: 2) to compare the two regimens in terms of response, toxicity and overall survival; 2)to verify the feasibility of a multidimensional score for excluding frail patients from the study.

Patients and methods. Prior to randomization elderly patients (>65 yrs) with DLCL, were administered a 4 multi-item scales questionnaire including activities of daily living scale (ADL), instrumental activities of daily living scale (IADL), comorbidity scale and geriatric scale. Fragile patients were not eligible for the study and treated according to phisicyans' decision. The others were randomised between R-CHOP (Day 1:Rituximab 375 mg/m^2, Cyclophosphamide 750 mg/m^2, Doxorubicin 50 mg/m^2; Vincristine 1,4 mg/m^2;Days 1-4: Prednisone 60 mg/m^2)and R-mini-CEOP (Day 1: Day 1:Rituximab 375 mg/m^2, Cyclophosphamide 750 mg/m^2, Epidoxorubicin 50 mg/m^2; Vinblastine 5 mg/m^2; Days 1-4 Prednisone 60 mg/m^2). Both regimens recycled every 21 days for a total of 6 courses; G-CSF was scheduled from day 2 to 7. From March 2003 until December 2004, 114 patients were registered from 21 Hematology/Oncology centers, 17 of whom were classified "frail". The remaining 97 patients were randomized between R-CHOP (48) and R-miniCEOP (49). Most of these patients had a severe clinical presentation. Median age was 73 years, LDH upper than normal value was found in 59%, 71% had stage III-IV, 20% presented a bulky disease. 53 patients (26 R-CHOP and 27 R-mini-CEOP) completed the chemotherapy and were assessed for the response. CR rate was 64% and ORR 73%. Despite the use of G-CSF the more frequent side effect was a grade 3/4 leucopenia, which occurred in 41% of patients. Toxic deaths were 2%; events which suggest to stop the ran-

domisation have not occurred. Frail patients had similar clinical characteristics to the randomized ones except for age (median age 78 vs 73); the overall survival estimated at 12 months was 82% vs 32% in frail group (p=0.0001). *Conclusions.* treatment of elderly patients with a full-dose chemoimmunotherapy regimen is feasible and encouraging. The use of a multidimensional score system seems a good tool to identify the patients who are able to tolerate a full dose regimen.

P523

RITUXIMAB PLUS CHOP- 14 FOR THE TREATMENT OF PATIENTS WITH AGGRESSIVE NON HODGKIN'S LYMPHOMA

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Introduction. Standard first line therapy for aggressive Non Hodgkin's Lymphoma (NHL) consists of CHOP chemotherapy. The addition of Rituximab to CHOP has produced a survival benefit. Moreover the dose-dense CHOP has recently shown an improvement in response and survival rate of patients with aggressive NHL.

Methods. In order to evaluate the efficacy and feasibility of dose-dense CHOP plus Rituximab we planned a schedule with Rituximab 375 mg/m^2 and CHOP therapy on day +1 given every 2 weeks, with G-CSF starting from day +7 to +11 for 6 cycles. We administered prophylaxis with sulfamethoxazole and trimethoprim and itraconazole. From January 2002, we have enrolled 44 patients with aggressive NHL (FL grade III = 13, DLBCL = 31). The mean age was 62 years (range 29-76). Two patients were stage I with bulky disease, 8 stage II, 7 stage III and 27 stage IV. Eighteen/44 (41%) patients presented B symptoms, 18 (41%) bone marrow involvement, 28 (64%) increased LDH values and 31 (71%) had at least one extranodal station involvement. According to the IPI score, 24 patients were Low Risk (IPI = 0-1) and 24 High Risk (IPI =2-3). *Results.* All patients completed the planned 6 cycles and were evaluable for response. Thirteen (29%) patients delayed treatment (anemia=1, neutropenia=11, thrombocytopenia=1). No important extrahematological toxicities were observed, particularly with regard to mucositis and cardiotoxicity. Thirty-six patients (82%) obtained a CR and 8 (18%) a PR (the overall response rate was 100%). After a median follow-up of 20 months (range 3 - 36), 6 patients (14%) have relapsed. The 2-year OS and DFS were 89% and 78% respectively.

Conclusions. R-CHOP-14 is a feasible and active therapeutic protocol devoid of severe toxicity for the management of patients with aggressive NHL.

P524

COMPREHENSIVE GERIATRIC ASSESSMENT AND TAILORED THERAPY IN ELDERLY B-DIFFUSE LARGE CELL LYMPHOMA PATIENTS

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Comprehensive Geriatric Assessment (CGA) is a multidimensional, interdisciplinary process that identifies medical, functional, and psychosocial problems and helps to develop a comprehensive management of older cancer patients. In march 2003 we began a multicentric study for all newly diagnosed elderly patients (≥ 65 years) affected by B-Diffuse Large Cell Lymphoma (B-DLCL) CD20+; all patients underwent a tailored treatment based on CGA: Activity of Daily Living (ADL) score, Cumulative Illness Rating Scale Geriatric (CIRS-G), Geriatric Syndromes (GS), Mini Mental State Examination (MMSE), Geriatric Depression Scale (GDS). "Frail" patients (Group 3), defined by ADL score < 6 or ≥ 3 comorbidities or ≥ 1 geriatric syndromes, received 4-6 cycles of a mini-CHOP regimen (cyclophosphamide 350 mg/sm day 1, doxorubicin 25 mg/sm day 1, vincristine 1 mg/sm day 1, prednisone 40 mg/sm days 1-5). Patients with ADL score = 6 and without specific comorbidities or GS (Group 1) received 4-6 cycles of standard CHOP + Rituximab (RTX) regimen (Coiffier, NEJM ' 02). Patients with ADL score= 6, ≤ 2 comorbidities and without GS (Group 2) received 4-6 cycles of CCHOP + RTX regimen including pegylated liposomal doxorubicin (Caelyx) 30 mg/sm in place of standard doxorubicin 50 mg/sm and RTX on day 1 of each cycle. This choice was based on our preliminary results with CCHOP-RTX in 15 refractory or relapsed B-DLCL patients aged > 65 years or with several comorbidities, who obtained an Overall Response (OR) of 57 % and a Continue Complete Remission (CCR) rate of 43 %.

At February 2005 all 75 patients referred at our 9 institutions have been enrolled in the study: median age is 74 years (range: 65-92), 32 patients were in stage I-II and 43 in stage III-IV. Forty-two (56 %) patients (median age: 70 years) were assigned to group 1 (CHOP + Rituximab), 20 (26.6 %) patients (median age: 77 years) to group 2 (CCHOP + Rituximab) and 13 (17.4 %) "frail"patients (median age: 80 years) to group 3 (mini-CHOP), the overall response rate to tailored therapy and follow-up of each group of patients are under evaluation and definitive data will be showed. Our preliminary results confirme that this patient-tailored approach based on CGA is feasible and that about 82% of elderly patients can receive a potentially curative treatment. Moreover this approach allows to identify a relevant percentage of patients (26 %) in whom a tailored therapy can be offered instead of a potentially life-threatening conventional antracycline-based chemotherapy or instead a palliative therapy without antracyclines.

P525

THE ROLE OF ANTIVIRAL TREATMENT IN HCV-RELATED NON-HODGKIN LYMPHOMA: AN UPDATE OF A MULTICENTER STUDY

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Hepatitis C Virus (HCV) is largely, although not homogenously, diffuse in Northwestern Europe and U.S.A. It has been shown to play a role both in hepatocellular carcinoma and in B-cell non-Hodgkin lymphoma (B-NHL). Up to now the exact biological mechanisms that could explain the lymphomagenic role of the virus are unknown, although several hypothesises are under investigation. We have previously published a series of 13 pateints, affected by low grade B-cell NHL and characterized by an indolent course (i.e. doubling time less than 1 year, no bulky disease), who underwent antiviral treatment only with peghilated interferon and ribavirin (peghilated interferon 50-70 microgram once a week, ribavirin 1000-1200 mg daily). Now we report an update of this study. Up to now we were able to evaluate 15 patients with a mean follow up of 18,1±7,6 months (range 2-28 months). Eight patients experienced complete or good partial haematological response that has lasted up to now with a mean follow up of 16,1months. Two other patients achived a long lasting partial response. The only one relapse occurred about one year after the end of treatment, hematological relapse happened toghether with viral relapse, the lymphoma reappeared as highy chemoresistant high grade lymphoma, and two months later the patient died. Interestingly complete and good partial responses were more likely to be seen in viral genotype 2 (p=0.035) and were strictly related to the decrease of viral load under treatment (p=<0.001). Toxicity causes the stop of the treatment in 3 patients, however one of them was able to achieve complete response. Time to achieve hematological response was quite long (mean 9±2,5 months).

This kind of experience strongly provides a role for antiviral treatment in patients affected by HCV related low grade B-cell NHL. Especially viral genotype 2 infection may be considered a good prognostic marker for hematological response as well as decrease of viral load under treatment. Toxicity in our hands was however significant and further experiences are warrented in order to better modulate antiviral therapy doses.

P526

HAIRY CELL LEUKEMIA: TREATMENT RESULTS AND PROGNOSTIC FACTOR ANALISIS IN A MONOCENTRIC EXPERIENCE OF 151 PATIENTS

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Background. Factors predicting treatment response and survival in Hairy Cell Leukemia (HCL) have not been extensively studied. The aim of this study was to analyze clinical and laboratory features at presentation in correlation to treatment response and overall survival, and the role of different treatment approaches in disease free survival (DFS), progression free survival (PFS) and overall survival (OS).

Metods. The data of 151 consecutive HCL patients observed between 1982 and 2004 were retrospectively analyzed.

Results. Median age was 53 (range 30 to 80) years, with 126 males, and 25 females. The following data at presentation were analyzed and compared with response, DFS,PFS and OS: Hb<10g/dL (observed in 27% of patients); Plt<100.000/mL (72%); WBC>10.000/mL (15 %); splenomegaly (75%); >70% bone marrow infiltration (27%). At univariate analisis, only WBC>10.000/mL resulted significantly correlated to a reduced PFS, while none of the other factors considered affected DFS, PFS nor OS . 88 patients received as first line treatment alpha2-interferon (IFN) alone, 49 purine analogues (PA) alone or in combination with IFN, 5 were treated with splenectomy. Among IFN treated patients, CR, PR and SD were obtained in 21.6%, 73.8% and 4.5% of the patients respectively; while among PA-treated patients in: 26.5%, 71.4% and 2.0%, respectively. Nevertheless, DFS was significantly prolonged in patients treated with PA with respect to IFN. No significant difference in OS was observed. The median PFS is 27.6 months, the median OS is projected at 238 months after a median follow-up of 131 months.

Conclusions. Among the routine clinical and hematochemical baseline features, only the presence of WBC count >10000/mL was significantly correlated to a lower PFS. First line treatment with purine analogues was associated with prolonged PFS and DFS with respect to IFN; nevertheless, no difference was observed in OS.

Non-Hodgkin's Lymphoma IV

P527

IEV PLUS RITUXIMAB AS SALVAGE TREATMENT FOR RELAPSED/ REFRACTORY NON HODGKIN LYMPHOMA

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Autologous stem cell transplantation (ASCT) is commonly used as part of the treatment for patients with relapsed or refractory non Hodgkin lymphoma (NHL). Thus the salvage chemotherapy for such patients has a dual aim: to induce a therapeutic response and to permit to mobilize a sufficient number of peripheral blood stem cells (PBSC). Previous reports have shown favourable results, in terms of either response rate or mobilization, with the combination of ifosfamide, epirubicin, and etoposide (IEV). Rituximab is a chimeric anti-CD20 monoclonal antibody highly effective in NHL as first line treatment in combination with CHOP; promising results have been also reported for refractory or relapsed patients in combination with ICE (R-ICE). In order to evaluate feasibility and efficacy in terms of disease response, tocixity and PBSC mobilization of IEV with the addition of rituximab, we treated a series of 30 patients with refractory/relapsed aggressive NHL. Four different Hematology institutions from Campania were involved in this study. Schedule of the regimen, called R-IEV, was as follows: rituximab 375mg/sqm on days 0 and 7; epirubicin 100mg/sqm on day 1; ifosfamide 2500mg/sqm on days 1 to 3; etoposide 150mg/sqm on days 1 to 3; G-CSF was added from day 6 at 5 microg/kg after the first course and at 10 microg/kg after the second course for PBSC mobilization. ASCT was planned after administration of two courses. Median age of patients was 48 years (17-75). Twelve patients had relapsed and 18 were refractory. At the time of writing, 21/30 patients are evaluable. Overall response (OR) rate was 70% (15/21 patients); CR and partial remission rates were 47% (10/21) and 23% (5/21), respectively. Of the remaining patients, one maintaned stable disease and was autografted as planned, 4 patients were refractory to salvage and died from disease progression, and the last patient died from septic shock during severe neutropenia. Hematologic toxicity consisted of WHO grade IV neutropenia in 11 cases (52%), with a median to ANC>500/microL of 13 days, severe anemia in 5 patients (23%), and WHO grade IV thrombocytopenia in 5 cases (23%). FUO occurred in 3 cases, all treated with empirical antibiotics, while no other documented infections occurred. Mobilization was successful in 17/21 patients (80%), with a median $CD34^+$ PBSC harvest of $14,7x10^6$/kg (range 2-61), with a median of 2 apheresis. In 16 cases (94% of mobilizers, 76% of evaluable cases) ASCT was actually performed. In 5 HCV+ patients no viral reactivation was observed. In conclusion, R-IEV is an effective rescue therapy for NHL with relatively low toxicity. The efficacy of IEV seems to be improved by the addition of rituximab [47% CR in this series vs 38% in a published series of 28 patients treated with IEV alone (Pocali et al, Leuk&Lymphoma 2004)], expecially if we consider that more than 50% of the former patients had primary refractory disease. A longer follow up on a larger series in a randomized study is clearly needed for a definitive statement of the real superiority of R-IEV as compared to IEV. Mobilization rate is satisfying.

P528

RITUXIMAB THERAPY MAY IMPAIR STEM CELL COLLECTION IN PATIENTS WITH NON-HODGKIN'S LYMPHOMAS

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Introduction. Rituximab (RTX) as single agent or added to various combination chemotherapy regimens (CHOP, DHAP, MACOP-B, etc) has demonstrated significant increases in response rates, overall and event-free survival in patients with indolent and aggressive B-cell lymphomas, without significant increases in hematological toxicity. Inability to collect adequate numbers of peripheral blood stem cells remains an obstacle to perform adequate autologous transplantation in many patients. However, the effect of RTX administration for *in vivo* purging on mobilization and stem cell harvest remains unclear.

Methods. We retrospectively studied 36 patients (pts) with B-cell non-Hodgkin's lymphomas (NHL) who underwent to stem cell mobilization from July 1998 to December 2004.

RTX-group. Fifteen pts (9 males and 6 females) received 4 doses of RTX (range 2-8) at 375 mg/m^2 at any time. Median age was 51 (range 27-69) years. Histology comprised 9 indolent and 6 aggressive NHL. The disease stage was I in 1 pt, II in 3 and IV in 11 (bone marrow involvement). They received 2 (range 1-4) regimens of chemotherapy. At the time of mobilization 3 pts were relapsed, 6 in partial remission (PR), 6 in complete remission (CR). Mobilization was induced by DHAP + G-CSF in 6 pts, CHOP + G-CSF in 1, Endoxan 7 g/m^2 + RTX (days +3 and + 11) in 3 and G-CSF-alone in 5.

NO-RTX group. Twenty-one pts (16 males and 5 females) receveid 2 lines of treatment (range 1-5). Median age was 52 (range 13-69). Low grade were 12 and high grade 9. Nine pts were stage II, 2 stage III and 10 stage IV (bone marrow involvement). Seven pts were relapsed, 2 refractory, 6 in PR, 6 in CR at mobilization. Peripheral stem cells were mobilised with DHAP + G-CSF in 17 pts, MACOP-B + G-CSF in 3, G-CSF-alone in 1.

Results. The median number of apheresis was similar for the two groups: 1 (range 1-3) for RTX group vs 1 (range 1-2) (p=0.15). All 21 pts in no-RTX group mobilized a target

cell dose of 2 x 10(6) CD34+ cells/Kg and 1 pt had 2 subsequent collections. In the RTX group 2 pts (13%) were poor mobilizers and 2 pts required a 2nd attempt after mobilization failure. The median day of apheresis was 11 in no-RTX group (range 11-15) vs 9 (range 4-13) in RTX group (p=0.38). The median number of CD34+ collected/kg was lower in RTX group: 4.2 (0.13-27.10) vs 8.14 (range 2.3-54.6) (p=0.0083). The median CD34+/mcl count also was lower in RTX group [34.9 (range 17.9-207.9) vs 44.6 (range 20.8-590) (p=0.0001)].

Conclusions. Rituximab used for *in vivo* purging of lymphoma cells prior or concurrent to the mobilizing regimen may impair the ability to collect adequate number of stem cells in B-cell NHL patients eligible for autologous stem cell transplantation.

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P529

HYPER-CVAD AND RITUXIMAB AS *IN VIVO* PURGING FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANT AND RITUXIMAB MAINTENANCE IN NEWLY DIAGNOSED MANTLE CELL LYMPHOMA

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Previously untreated mantle cell lymphoma (MCL) are consistently associated with poor prognosis when treated with CHOP-like regimens. Typically the CR rate is 20-30%, median FFS = 10-16 months and median OS = 3 years. In the attempt to improve outcome we used a high dose intensity regimen such as Hyper-CVAD (HCVAD) with autologous stem cell transplant (ASCT). Seventeen patients patients entered the study but only 7 are valuable. Patients were apheresed after 2nd course of HCVAD and if apheresis (LPH) were PCR positive (Bcl1+/JH+) a second set of LPH were performed after completion of 4th cycle. To perform an *in vivo* purging Rituximab 375 mg/m^2 was added at day +1 and +9 after last dose of ARA-C; GCSF 10µg/kg was commenced on day +5 until LPH was ultimated. Rituximab maintenance (375 mg/m^2 once weekly for 4 consecutive weeks) started 2 month post-ASCT and was repeated every 6 months. Seven patient have finished the program and all 7 had bone marrow PCR positive (Bcl1+/JH+) at diagnosis. In 4/7 (57%) in-vivo-purging allowed to collect PCR negative (PCR neg.) products. Median age at diagnosis was 60 years (range 41-69). All patients receiving ASCT were conditioned with BEAM. Five patients out of 7 received 3 courses of HCVAD and 5/7 completed 4 courses. Status pre-ASCT was: 43% CR, 43% PR, 14% PD and 71.5% bone marrow PCR negativity. Status-post ASCT was: 71.5% CR, 14% PR and 14% SD, and 71.5% bone marrow PCR neg. Status post Rituximab maintenance was 90% CR, 10% PR and 90% BM-PCR neg. With a median follow up of 34 months 6/7 are alive and 5/6 are disease free. *Conclusions.* purging *in vivo* allowed to collect tumor free graft in 57% of patients. HCVAD plus Rituxan allowed to obtain 86% overall response rate with 43% CR and 43% PR . ASCT allowed to increase CR rate from 46% to 71.5% and allowed to increase bone marrow PCR negativity to 71.5%. One patient was transplanted with PCR positive products and reached PCR negativity post-ASCT which might suggest that this result was conditioning-dependet. Rituximab maintenance post-ASCT did increase CR rate and PCR negativity. This high-dose-intensity program associated with Rituximab allowed to collect tumor free grafts and to obtain an encouraging rate of CR and of bone marrow PCR negativity. A longer follow up and and larger randomized trials with larger accrual are needed to better define the role of this therapy for MCL.

P530

TREATMENT OF PRIMARY CNS LYMPHOMA IN IMMUNOCOMPETENT PATIENTS WITH A COMBINED TREATMENT MODALITY: EXPERIENCE OF A SINGLE CENTRE

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Primary CNS lymphoma (PCNSL) is a highly aggressive lymphoma limited to the brain with a poor prognosis. As PCNSL is a rare form of extra-nodal lymphoma in immunocompetent patients, current therapeutic knowledge results from studies that often include small numbers of patients. Here, we report a single-centre experience on 41 consecutive patients with HIV-negative PCNSL diagnosed between march 1995 and may 2004 and treated at our institution. Patients had a median age of 59 years (range, 31-78 years), 20 patients were male and 21 female. Fifteen patients presented a single lesion and 26 patients multiple lesions. The treatment plan for all patients included an induction phase of chemotherapy based on high-dose methotrexate (MTX) (3.5 g/m^2) followed by a consolidation with whole brain-radiotherapy (40 Gy + 20 Gy boost to sites of original lesions). In 19 patients 2 cycles of MTX were planned. In 22 patients addition of 2 cycles of high-dose Cytosine Arabinoside (AraC) (2 g/m^2 q12h x 4) was planned, either in a sequential schedule (13 patients) or in combination with high-dose MTX (9 patients). In the MTX alone group, 15 of 19 patients (79%) received the treatment as scheduled, in the MTX/AraC sequential arm 9/13 (69%) patients completed the chemotherapy phase as programmed, while in the combined MTX/AraC arm only 4 of 9 patients (44%) received the treatment as planned. Causes for therapy modifications were: renal toxicity in 4 pts, hepatic toxicity in 2 pts, infections in 6 patients receiving AraC, and progressive disease in 1pt. Radiotherapy was performed in 36 patients. At the end treatment, 24 pts (59%) were in complete remission, while 10 pts (24%) had persistent disease and 7 pts were dead due to toxicity (17%). While CR rates were 63 and 62 % for patients treated with MTX and sequential MTX/AraC, respectively, the CR rate was only 44% for patients in the combined MTX/AraC arm

due to a high toxic death rate (33%). Predicted overall survival at 5 years was 34%. There was a trend to confirm the prognostic value of the IELSG scoring system (Ferreri *et al.*; J Clin Oncol 2003; 21:266). Twenty-five patients so far died: 11 pts for progressive disease, 10 pts due to toxicity (infections, 3pts; thrombo-embolic events 3 pts; late neurologic complications, 4 pts) and 4 for other causes (other neoplasia, 2pts; myocardial infarction, 1 pt; accident, 1 pt). Predicted disease-free survival (DFS) for patients who achieved a complete remission after chemo- and radiotherapy was 65% at 5 years. There was no statistically significant difference in DFS between pts treated with MTX alone (80% at 5 yrs) and MTX/AraC (50 % at 5 yrs) (p=0.09).

In conclusion, treatment with high-dose MTX followed by radiotherapy appears to result in at least the same probability of disease control in PCNSL as combinations of MTX with AraC followed by radiotherapy. The addition of AraC, in particular in the combined treatment schedule, is associated with significant toxicity. However, results of ongoing randomised studies are needed to definitely evaluate the role of addition of AraC in the treatment of PCNSL. More targeted and less toxic therapies are needed to improve outcome in this lymphoma type with poor prognosis.

P531

DELETION OF GLUTATHIONE S-TRANSFERASE M1 IS A RISK FACTOR AND PROGNOSTIC MARKER IN FOLLICULAR LYMPHOMA

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Glutathione S-transferases (GSTs) are enzymes involved in the detoxification of several environmental mutagens, carcinogens, and anticancer drugs. GST polymorphisms resulting in decreased enzymatic activity have been associated with the risk to develop several types of solid tumours. We studied the frequency and the prognostic significance of the deletion of 2 GST subfamily genes, M1and T1 and of the GSTP1 Ile105Val polymorphism in patients with Follicular Lymphoma (FL). The study population included 67 patients with FL (31 males, 36 females) with a median age of 54 years (range 19-80 years). Allele frequencies were compared with a case-matched cohort of 134 normal blood donors(62 males, 72 females) with a median age of 56 years (range 27-71 years). GST gene polymorphisms for GSTT1 and M1 were studied using a multiplex PCR method, including the BCL2 gene as an internal control. The Ile105Val polymorphism of the GSTP1 gene was analyzed using the PCR-RFLP technique. Deletion of the GSTM1 gene was present in 41% (55/134) of normal donors, while in patients with FL the prevalence was 61% (41/67) (p=0.007), resulting in an increased risk for FL in individuals with the GSTM1-null genotype (odds ratio 2.31; 95% confidence interval 1.3-4.2). The GSTM1-null genotype particularly increased the FL risk in males (odds ratio 5.67, 95% confidence interval 2.1-15.2, p<0.001). The risk of FL was not increased in individuals with GSTT1-null and the GSTP1Val105 allele. The GST polymorphisms were studied for associations with patient characteristics, response to therapy, and survival. No association between GSTT1-null and GSTP1 allelic variants and patients' characteristics or prognosis were found. We found an association between the GSTM1-null genotype and an advanced stage of disease (I/II versus II-IV, 41% versus 70 %, p=0.075), high grade of malignancy (grade 1/2 versus 3, 49% versus 81%, p=0.027) and poor ECOG performance status (p=0.035). First-line treatment consisted of radiotherapy in 5% of patients, chemotherapy containing Fludarabine in 17%, and CHOP or CHOP-like chemotherapy in 78%. In 57% of patients, rituximab was included in the first-line therapy. The rate of complete remissions was 66% and the progression-free survival at 3 years was 53%. Patients with GSTM1 deletions had a significant worse progression-free survival when compared with those with undeleted GSTM1 (p=0.0047). In our patient series, a FLIPI score of >3 (Solal-Celigny et al, Blood 2004; 104:1258) was associated with a poor prognosis (p=0.0015). The multivariate analysis showed that FLIPI score and GSTM1 genotype were independent prognostic factors for progression-free survival. GSTM1 was of particular prognostic value in the patients with FLIPI score <2 (p=0.0028). In conclusion the GSTM1-null genotype may increase the risk for FL and is associated with unfavourable prognosis.

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PRIMARY GASTRIC LYMPHOMA PRESENTATION: AN ITALIAN MULTICENTER STUDY

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Background. The stomach is the most frequent site of intestinal lymphomas. However, few data are available on the clinical-endoscopic presentation of gastric lymphoma as well as on possible differences in clinical pattern and endoscopic features between low-grade (LG) and high-grade (HG) lymphomas. In this study, we evaluated such aspects on consecutive primary gastric lymphoma patients observed in the last 10 years in four Hospitals (1 North, 2 Centre, and 1 South of Italy).

Methods. Clinical, histological, and endocospic records of consecutive patients diagnosed with LG or HG gastric lymphoma were retrieved and accurately evaluated. Symptoms were categorized as *alarm* (anaemia/melaena/ heamorrage, persistent vomiting, weight loss) or *no alarm* (epigastric/abdominal pain, heartburn, dyspepsia/ bloating). The endoscopic findings were classified as *normal* (no macroscopic lesions) or *abnormal* (ulcer, erosions, nodular pattern, hypertrophic folds, polypoid mass). Statistical analysis was carried out by using the Chi squared test.

Results. During the study period, 91 patients with gastric lymphoma were detected. Overall, 40 patients were

observed in the first 5 years and 51 in the last 5 years (p=0.09). The other results of the study are summarized in the following Table.

Table.

	Overall (91patients)	LG lymphoma (52 patients)	HG lymphoma (39 patients)	P value
Age (mean ± SD) years	67 ± 15	66 ± 16	68 ± 14	0.4
Sex (M/F)	56/35	34/18	22/17	0.6
Alarm symptoms	39 (43%)	16 (31%)	23 (59%)	0.005
Normal endoscopy	10 (11%)	10 (19%)	0 (0%)	0.01
H. pylori infection	66 (73%)	47 (90%)	19 (49%)	<0.0001
Stage (IA / >IA)	59/32	45/7	14/25	<0.0001

Conclusions. The incidence of primary gastric lymphoma seems to be increasing. The overall prevalence of alarm symptoms is quite low, and they may be absent in near 70% of LG lymphoma patients. Moreover, contrarily to HG, LG lymphoma may present as a normal endoscopic finding and it is more frequently associated with H. pylori infection. At diagnosis, HG lymphoma is more frequently detected in an advanced stage as compared to LG lymphoma.

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LOW GRADE GASTRIC LYMPHOMA HELICOBACTER PYLORI RELATED: LONG TERM FOLLOW-UP

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Background. There are few data on the long term outcome of low grade gastric lymphoma HP related.

Aims. to evaluate prospectively the 5-years survival and 5-years disease free survival of low grade gastric lymphoma HP related.

Patients and Methods. 39 consecutive patients (24 men and 15 women), mean age 61 years (24 – 82) with low grade gastric lymphoma Stage 1-2 (Lugano Classification) were diagnosed from 1993 to 2004 in 3 Hematological and Gastrontestinal Departments (Palermo,Roma ,MIlano). All the patients were treated with the same eradicating therapy (ET): PPI + Amoxicillin + Claritromicyn for 2 weeks. Patients were considered in remission if after 6 months endoscopy showed a complete regression of the istological lesions. Patients were followed up every 6 months with endoscopic controls. If after 6 months no remission was observed the patients were treated by chemotherapy. The main outcome was the survival and secondary outcome was the relapse rate. Kaplan-Maier was used to evaluate survival.

Results. after a median follow up period of 36 months (4 - 119) only 3 patients died (one for progression of lymphoma and two for cause different from lymphoma). The 5 and 10 yrs cumulative survival was 97% and 92% respectively . The helicobacter pylori infection was cured in 30 (70%) patients after ET. 26 (67%) patients went on histological remission after eradication, in particular 24 (80%) out of the 30 eradicated pts and 2 (22%) out of the 9 not eradicated (p<0.005). 5 out of 26 in remission relapsed and were treated 3 by immuno-chemotherapy, 1 by surgery and 1 by ET ; 4 out of 5 responded to treatment and one died for progression of lymphoma. 1 out of 21 patients in long term remission died for cause not related to lymphoma. 13 out of 39 (33%) did not respond to eradicating therapy and 12 were treated by chemotherapy and/or immunotherapy and one died for cause not related to lymphoma. 7 out of 12 achieved CR after treatment. 3 were resistant to chemotherapy (one treated by Radioterapy, one by chemotherapy and one by surgery) and the other 2 are under evaluation.

Conclusions. The prognosis of low grade gastric lymphoma is benign with a 5 years survival of 97% and with a 5 years disease free survival of 81% .

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ROLE OF 18 F-FLUORODEOXYGLUCOSE-POSITRON EMISSION TOMOGRAPHY IN THE STAGING OF HODGKIN DISEASE AND AGGRESSIVE NON HODGKIN LYMPHOMA: COMPARISON OF PET-SCAN AND CONVENTIONAL STAGING

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Introduction. The accuracy in staging of HD and aggressive NHL patients is an important feature to plan treatment. The aim of the study was to evaluate the clinical significance of FDG-PET compared with computed tomography (TC) plus BM biopsy in these patients.

Materials and methods. From January 2004 to December 2004, 30 consecutive patients were analysed: there were 19 males, 11 females with a median age of 48 years (17-83), 18 HD and 12 aggressive NHL patients (11 DLCL and 1 mantle cell), 23 pts at the diagnosis and 7 in relapse respectively. All pts underwent conventional staging and FDG-PET scan. According to standard staging results there were 1 patient in stage I, 10 pts in stage II, 8 pts in stage III and 11 in stage IV. FDG-PET results were compared with results of chest and abdomen CT and bone marrow biopsy. When a discordant result was detected, magnetic resonance imaging (MRI) and/or ultrasonography were performed. At the end of treatment all patients underwent PET/TC scan and BM biopsy, if previously involved.

Results. FDG-PET changed the disease stage in 7/30 pts (23%): 5/30 were upstaged and 2/30 were downstaged respectively. In the 2 pts downstaged a minimal BM involvement (< 20%) was detected only by BM biopsy but not by FDG-PET scan. In the 5 pts upstaged by PET-scan the following new lesions were detected: 2 vertebral bone lesions, 2 sub-diaphragmatic involvement (TC scan showed adenopathies < 15 mm) and 1 hepatic involvement respectively.

Conclusions. FDG-PET is a non-invasive and efficient imaging modality to stage patients affected by HD and aggressive NHL and it must be considered complementary to CT scan. Large prospective studies are needed to demonstrate the real impact of FDG-PET on management in HD and aggressive NHL patients.

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PERIPHERAL T CELL LYMPHOMAS TREATED WITH PROMACE-CYTABOM: SINGLE CENTER ANALYSIS OF PROGNOSTIC FACTORS AND SURVIVAL WITH A LONG TERM FOLLOW UP

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Introduction. PTCLs represent 10% to 15% of all Lymphomas in western countries. They arise from post-thymic lymphocytes and express mature T immunophenotype. They are aggressive, with high incidence of relapse and low chemo-sensitivity. We performed a retrospective analysis of a subset of PTCLs reviewed according to the REAL/WHO criteria and homogeneously treated by a third generation chemotherapy regimen "ProMACE-CytaBOM (PC)", evaluating the impact of prognostic factors and of treatment on overall survival (OS).

Methods. Among 197 aggressive non Hodgkin Lymphomas (nHL) observed between Jan 1991 to Jan 2000, we analysed 36 patients (pts) (18%) with PTCLs: 16 PTCLs unspecified, 18 large T cell nHL, 2 NK/T cell nHL.

Pts' characteristics were: median age (49yrs), performance status >2 in 7/36 (18%) pts, stage III-IV in 25/36 (68%) pts, constitutional symptoms in 15/36 (40%) pts, Bulky disease in 16/36 (45%) pts, high serum level of LDH in 7/36 (18%) pts, and of beta2microglobulin in 8/36 (23%) pts, Bone Marrow involvement in 13/36 (36%) pts, extranodal involvement in 21/36 (59%) pts, IPI 3-4 in 7/36 (18%). Treatment regimen was PC (Cyclophosphamide 650 mg/sqm, Doxorubicin 25 mg/sqm, Etoposide 120 mg/sqm. on day 1, and Cytarabine 300 mg/smq, Bleomycin 5 UI/sqm, Vincristine 1,4 mg/sqm, Methotrexate 120 mg/sqm, on day 8, and Prednisone 60mg/sqm for 14 days). Median cycles administered were 6.

Results. 202 cycles of chemotherapy were administered to 36 pts. The schedule was well tolerated; the most important toxicity was myelosuppression, with incidence of grade III-IV neutropenia in 28% of cycles. One treatment related death occurred for septic shock. Complete Remissions (CR) were 18/36 (50%), Partial Remissions (PR) 10/36 (28%); Relapses 10/18 (55%). Median Event Free Survival was 26 months (7-88m), 5 years overall survival rate was 45% with a median follow up of 32 months (4-169). Univariate analysis of preognostic factors at diagnosis showed that response inversely correlated with: stage III-IV, cutaneous involvement and PS>2.

Conclusions. PC is an effective and well tolerated schedule in PTCLS, but CR rate and OS rate are worse than those observed in a subset of pts with B-Diffuse Large Cell Lymphomas treated in the same period with the same schedule in our institution. Therefore, to improve CR rate and OS rate, new therapeutic strategies should be investigated in multicentric clinical trials.

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PRE-AUTOLOGOUS TRANSPLANTATION TREATMENT WITH RITUXIMAB DOES NOT IMPAIR STEM CELL HARVEST AND ENGRAFTMENT IN YOUNG PATIENTS WITH DE-NOVO DIFFUSE LARGE B-CELL LYMPHOMA AT POOR PROGNOSIS

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Introduction. Rituximab is a monoclonal antibody commonly used in salvage regimens and also as first line in combination with chemotherapy. However its effects on peripheral stem cell (PBPC) harvest, engraftment and toxicity are actually poorly understood.

Patients and methods. We analyze the impact of Rituximab on stem cell mobilization, engraftment (median time to absolute neutrophil count >500/mm^3 for three consecutive days and platelets >50.000) and toxicity after ASCT procedure in two groups of patients affected by diffuse large B-cell lymphoma (B-DLCL) at diagnosis enrolled into two consecutive trials. Group A: an intensified chemoimmunotherapy regimen R-MegaCEOP (Rituximab 375 mg/m^2 day 1, CTX 1200 mg/m^2 + EPI 110 mg/m^2 + VCR 1.4 mg/m^2 day 3 and PDN 40 mg/m^2 days 3 to 7) every 14 days with G-CSF support for 4 courses ± 2 DHAP; patients in CR or PR received two courses of intensified chemotherapy R-MAD (Mitoxantrone 8 mg/m^2 + ARA-C 2000 mg/m^2/12h + Dexamethasone 4 mg/m^2/12h days 1 to 3 and Rituximab 375 mg/m^2 day 4 and before peripheral blood stem cell harvest). Group B: MACOP-B 8 weeks ± 2 DHAP + 2 courses of MAD (in patients in CR or PR) without Rituximab. Both groups were given ASCT with BEAM as conditioning regimen. All patients were <60 years with B-DLCL at age-adjusted IPI intermediate-high (IH) or high (H) risk and/or with bone marrow (BM) involvement. Clinical characteristics at diagnosis were well balanced between the two groups.

Results. 68 pts are evaluable: 35 pts in group A (with Rituximab) and 33 pts in group B (without Rituximab). All patients of the two groups collected >2 x 10^6 CD34$^+$ cell/kg. Median CD34$^+$ cell/kg in group A was 13 x 10^6 compared with 41 x 10^6 in group B with no statistically significant difference. Median time to neutrophils engraftment after ASCT was similar in the two groups: 9 days in group A and 10 days in group B. No delay in the platelet recovery was observed in patients treated with chemoimmunotherapy: 15 days in group A vs 16 days in group B. No differences were observed regarding severe early (<30 days after ASCT) toxicities (WHO grade 3-4) between group A vs B: severe mucositis in 11 pts vs 23 pts, gastrointestinal in 6 pts vs 4 pts. Severe infections occurred: group A 6 patients (2 Gram+ sepsis, 1 Gram- sepsis, 1 FUO, 2 bacterial pneumonia); group B 4 patients (2 FUO, 1 bacterial and 1 viral pneumonia). One patient in group A died of bacterial pneumonia after ASCT.

Conclusions. The results of this study suggest that Rituximab may be safely included in a pre-ASCT high dose chemotherapy regimen with no evidence of reduced stem cell harvest, nor delayed engraftment and nor increased early toxicity after ASCT.

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EFFECT OF ADDING RITUXIMAB TO INTENSIFIED AND HIGH DOSE CHEMOTHERAPY WITH ASCT AS FIRST LINE TREATMENT IN STAGE III-IV AT INTERMEDIATE-HIGH AND HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction. The addition of Rituximab to standard chemotherapy CHOP improves the outcome of advanced stage B-DLCL, however patients at high risk continue to do worse. The feasibility and efficacy of adding Rituximab to HDC is still poorly studied so far. We tested the effect of adding Rituximab to HDC comparing the results with a group of B-DLCL patients treated in a previous GIMURELL trial (J Clin Oncol 1997; 15:491-498) with the same eligibility criteria.

Patients and methods. From January 2001 to December 2004, 73 previously untreated patients <61 years affected by B-DLCL, stage III-IV at age-adjusted IPI IH or H risk were enrolled (R-HDC group). They were treated with four courses of R-MegaCEOP (R 375 mg/m^2 day 1, CTX 1200 mg/m^2 + EPI 110 mg/m^2 + VCR 1.4 mg/m^2 day 3 and PDN 40 mg/m^2 days 3 to 7) every 14 days with G-CSF support for 4 courses; then two courses of intensified chemoimmunotherapy R-MAD (Mitoxantrone 8 mg/m^2 + ARA-C 2000 mg/m^2/ 12h + Dexamethasone 4 mg/m^2/12h days 1 to 3 and R 375 mg/m^2 day 4 and before peripheral blood stem cell harvest as *in vivo* purging) followed by ASCT with BEAM as conditioning regimen ± IF RT. All patients were given antibacterial and antifungal prophylaxis throughout the whole treatment.

Results. Median age was 45 years (19-60); 53% at IH and 47% at H risk according to IPI; 32% had bone marrow (BM) involvement and 80% LDH level >normal. CR/CRu at the end of the treatment was achieved in 56 pts (77%), PR in 2 (3%), NR in 11 (15%) and 4 (5%) died of toxicity. With a median follow-up of 2 years, the failure-free survival (FFS) and overall survival (OS) measured at 2 years was: 70% and 76%. These results were compared to those achieved in 41 B-DLCL patients enrolled with the same eligibility criteria in the previous trial without Rituximab. These previously reported patients have a median follow-up of 6 years and were treated with: MACOPB x 8 weeks + MAD x 2 + BEAM and ASCT (HDC group). CR/CRu in this group of patients was 71%, NR 22% and toxic deaths 7%. FFS and OS at 2 years was 49% and 56% respectively. In both IPI groups (IH and H risk), patients treated with R-HDC had a better outcome than those treated with HDC: R-HDC vs HDC (IH risk 2-yr OS 85% vs 65%; H risk 2-yr 67% vs 50%). Short-term toxicity appeared similar. A Cox's model was performed to adjust the effect of treatment for risk factors (age, IPI, BM involvement, number of extranodal sites). In this multivariate analysis the risk of failure and death was significantly reduced by the addition of Rituximab to HDC: adjusted hazard ratio for FFS (R-HDC vs HDC)=0.49 (95% CI=0.27-0.33, p=.02), adjusted hazard ratio for OS (R-HDC vs HDC)=0.47 (95% CI=0.23-0.54, p=0.03). Also age >45 years and H risk IPI adversely affected the outcome in multivariate analysis. Germinal center and non germinal center subtype analysis is ongoing in both treatment groups.

Conclusions. These results suggest that the addition of Rituximab to intensified and high dose chemotherapy with ASCT support is feasible and effective in B-DLCL at poor prognosis and compare favorably to our historical experience.

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99MTC-MIBI UPTAKE IN BCL-2 OVEREXPRESSING LYMPHOMA CELLS AND NON-HODGKIN'S LYMPHOMA PATIENTS DURING DRUG INDUCED APOPTOSIS

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Our previous studies showed that Bcl-2 overexpression in transfected cultured breast cancer cell lines prevents 99mTc-MIBI uptake. Treatment with staurosporine, a potent inducer of apoptosis, causes an early transitory recover of tracer uptake in Bcl-2 overexpressing clones. Similar findings have been obtained by inducing apoptosis with several anti-cancer drugs. These observations raised the possibility that 99mTcMIBI uptake, assessed in basal conditions and in the early phases of drug-induced apoptosis, may be used as *in vivo* fuctional test for the detection of Bcl-2 overexpression in tumors such as follicular and large B-cell non-Hodgkin's lyphoma in which translocation between chromosome 18 and 14 often occurs resulting in the transcriptional up-regulation of Bcl-2 gene expression in a high percentage of cases. Aim: To test this hypotesis, we first studied the effect of Doxorubcine and Fludarabine, used in the therapeutic regimens of large and follicular B cell lymphoma respectively, on 99mTc-MIBI uptake in Human B cell precursor leukemia cells and Human B cell lymphoma cells expressing low and high levels of Bcl-2 respectively. Then we studied the effect of standard chemotherapeutic regimens on 99mTc-MIBI uptake in patients with follicular and large B-cell non-Hodgkin's lyphoma.

Methods. Human B cell precursor leukemia cells NALM-6 and Human B cell lymphoma cells DHL-4 were treated with Doxorubcine and Fludarabine. 99mTc-MIBI uptake was assessed after 5 and 24 hours of incubation in both cell lines and expressed as percentage of untreated corresponding clone. The study *in vivo* included twelve patients that were evaluated by 99mTc-MIBI scan prior to any therapy. They were e.v. injected with 740 BMq of 99mTc-MIBI and underwent whole-body scan 10 min post-injection. Seven days after the basal 99mTc-MIBI scan, patients received the first adnministration of the planned standard chemotherapeutic regimen and after 5 hours they underwent a second 99mTc-MIBI scan. Basal and post-treatment 99mTc-MIBI scan were then compared. Visualization of new lesions was recored for each patient and tumor-to-heart ratios were obtained from both basal and post-treatment 99mTc-MIBI scan.

Results. After 5 hrs of drug exposure, Bcl-2 overexpressing cells showed an increase of 99mTc-MIBI uptake, conversely, cells with low levels of Bcl-2 showed a decrease of 99mTc-MIBI uptake. Six out of the twelve patients studied showed the appareance of new lesions in the post-treatment 99mTc-MIBI scan. An increase of tumor-to-heart ratios varying between 10% and 60% was observed in the post-treatment 99mTc-MIBI scan in seven patients. *Conclusion.* Our findings provide a rational basis for the development of an *in vivo* functional test to assess Bcl-2 overexpression in cancer. In fact, an increase of 99mTc-MIBI uptake in lymphoma lesions early after chemotherapy may reveal overexpression of Bcl-2 anti-apoptotic protein. Since Bcl-2 antagonists are undergoing clinical evaluation, changes of early 99mTc-MIBI uptake may also be used to assess the efficacy of such inhibitors.

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CHEMOIMMUNOTHERAPY WITH FLUDARABINE + MITOXANTRONE + DEXAMETHASONE (FND) FOLLOWED BY ANTI-CD20 (RITUXIMAB) IN INDOLENT NON FOLLICULAR LYMPHOMA

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Introduction. Indolent lymphoma usually have a high clinical response rate with conventional treatment, but relapses are frequently and molecular remissions are rare. The combination of Fludarabine containing regimens and Rituximab induce good clinical response but few data are available regarding its feasibility, safety and possible immunosuppressive effect. Moreover no data are yet available regarding molecular remissions.

Patients and methods. From February 2000 to March 2005, 22 untreated patients with advanced stage indolent non follicular lymphoma were treated with 4 courses of FND (Fludarabine 25 mg/m^2 days 1,2,3 + Mitoxantrone 10 mg/m^2 day 1 + Dexamethasone 20 mg days 1,2,3) followed by 4 Rituximab infusions at standard dose of 375 mg/m^2/week. Patients in partial remission after this treatment further received 2 FND courses and 2 Rituximab infusions. PCR molecular monitoring for the presence of IgH rearrangement was performed on bone marrow (BM) at the beginning of the treatment and on BM after FND and after Rituximab.

Results. Median age was 56 years (range 37-74); 9 males and 13 females; 6 lymphocitic NHL, 8 marginal NHL, 8 immunocytoma; 21 had stage IV disease, 21 BM involvement, 8 bulky disease and 5 had >1 extranodal sites: skin, kidney, parotid, liver. Two patients were HCV positive. 18/21 patients completed the therapeutic plan; 4 patients did not receive Rituximab due to progression of disease after FND. Treatment has been entirely performed in an outpatient setting. No toxic deaths were observed. Severe toxicity (WHO Grade 3-4) was: neutrophenya 2 cases after FND and one after Rituximab; one bacterial infection after FND; one acute myocardial infarction after FND and immunotherapy. Clinical response to treatment was evaluated after FND and after Rituximab. Clinical response to 4 FND was as follows: Complete Remission (CR) 4 patients, Complete Remission Unconfirmed (CRu) 4, Partial Remission (PR) 10, Non Response (NR) 5. Clinical response to Rituximab, at the end of the whole treatment program, was as follows: CR 11, Cru 1, PR 5, NR 5. With addition of Rituximab, complete response (CR + Cru) increased from 36% to 59%. A PCR-negative status was achieved in 2/18 patients after FND chemotherapy, while molecular remission was observed in 9/18 patients after Rituximab treatment. All these 11 patients were in clinical CR.

Conclusions. FND chemotherapy followed by Rituximab has been showed feasible in an outpatient setting with low toxicity. Rituximab improves clinical and molecular response achievable after chemotherapy in indolent non follicular lymphomas.

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SEQUENTIAL USE OF RITUXIMAB AFTER CHOP AS FIRST-LINE THERAPY IN FOLLICULAR LYMPHOMA

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Rituximab is a chimeric murine/human monoclonal antibody that reacts specifically with the B-cell antigen CD20. The lack of myelotoxicity indicated that Rituximab would be well suited for use in combination with chemotherapy. The obtimal schedule for administering rituximab and chemotherapy has not yet been determined. We have used Rituximab after chemotherapy with the aim to purge *in vivo* minimal residual disease in patients with diagnosis of follicular lymphoma grade I or II according REAL classification. Starting from march 1999 untill march 2004, thirty-eight patients after the obtainment of complete remission (CR) or very good partial remission (VGPR) were treated with four infusions of Rituximab once a week. The characteristics of patients were the following: 9 female and 29 male; median age was 52 years (range 30 – 69); 4 patients were stage 1, 8 were in stage II, 14 stage III and 12 stage IV; according to IPI index 26 were low-risk, 11 were intermediate-low risk and 1 was intemediate-high risk. Eleven patients (29%) had histological positive bone marrow; bulky disease was present in seven patients; pathologic LDH value in eight patients. All patients were treated with antracyclin containing regimens (CHOP or CHOP-like) for six cycles. All patients which obtained at least a very good partial remission were treated with Rituximab at the dosage of 375 mg/m^2 weekly for four weeks. No severe hematological or extrahematological toxicity were observed either with chemotherapy or with immunotherapy. All patients were treated as outpatient. After a median follow-up of 43 months (range 11 – 72) two patients died and overall survival was 92%. No clinical characteristics were associated with overall survival. After a median period of 36 months (range 3 – 65 months) eight patients experienced a relapse (24%) and the disease-free survival was 59%. Two out 8 relapsed patients were treated with chemotherapy and obtained a new complete remission one is alive with disease and five are at the moment in treatment. This study confirm the safety and feasibility of this

procedure. In comparison with literature we report a bettere result in term of disease free survival in fact after three years we do not reach the median time to relapse. We can conclude that the use of antibody anti-CD20 could increase the relapse free survival even if will be necessary to raise the follow-up period to confirm this data.

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IMPORTANCE OF COMBINED-MODALITY THERAPY FOR PRIMARY BONE LYMPHOMA

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Primary bone lymphoma (PBL) is a rare entity and comprises about 5% of all extranodal non-Hodgkin's lymphomas (NHL) and 5-7% of all primary bone tumors. To date there is no consensus about the optimal treatment for PBL. We report the experience of the Hematology Unit of Federico II University of Naples.

Eight patients with newly diagnosed PBL were treated from august 1998 to December 2004. Four of them were male and four were female; the median age was 47 years (range 15-78 years). Pain at the local site and soft tissue swelling were the commonest symptoms. Involvement of the bones in the lower half of the body was more frequent than the bones in the upper half. All patients had B-cell high-grade histology. The median follow-up was 50 months. Four patients had a solitary bone lesion; and in four patients, the tumor was spread to many bone sides. All patients were treated with combined-modality therapy (chemotherapy and radiotherapy) and seven patients received 6-12 courses of pamidronate or zolendronate. No patient had any fracture during clinical course.

All patients are in complete remission. Primary bone lymphoma is a malignancy that is highly curable with a combination of chemotherapy and radiotherapy.

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SAFETY OF A 14-DAYS SCHEDULE WITH ALTERNATION OF EPIRUBICIN AND MITOXANTRONE IN A CHOP-LIKE REGIMEN IN ELDERLY PATIENTS WITH AGGRESSIVE NON HODGKIN LYMPHOMAS

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Although CHOP is considered a standard treatment for Large Cell Non Hodgkin Lymphomas (NHL), in patients over 65 years of age there are several modification of this regimen in order to reduce cardiotoxicity. We have already demonstrated that a treatment with the alternation of epirubicin and mitoxantrone in a CHOP-like regimen retains efficacy and reduces overall toxicity in elderly patients with intermediate and high grade NHL. Furthermore, recent studies have demonstrated that an intensified schedule (CHOP-14) has higher event-free and overall survival rates than standard CHOP-21. In this perspective, we have adapted our standard CEOP/CNOP to a 14 days schedule while maintaining the same doses of antineoplastic drugs (Epirubicin 50 mg/m^2, and Mitoxantrone 10 mg/m^2). Twenty elderly patients with Diffuse Large Cell Lymphoma (18 B-cell and 2 T-cell) and one patient with Marginal Zone Lymphoma were treated with alternating CEOP/CNOP at 14 days of interval. Median age was 72 years (range 62-8), 15 patients had stage III or IV while the age adjusted IPI score was higher than 2 in 5 of them. Furthermore, 10 out of 21 patients treated showed extranodal involvement at diagnosis (6 G.I tract., 2 skin, 2 eye). Treatment was well tolerated in all patients but one who needed hospitalization for bronchopneumonia. Supportive care with G-CSF was given to 18 and with EPO to 11 patients. Four patients developed fever successfully treated with broad spectrum antibiotics. Mild mucositis occurred in 2 patients and delayed administration of chemotherapy was required in only 3 patients. No treatment related deaths were registered. Finally, in 5 patients anti-CD20 immunotherapy was combined to CEOP/CNOP treatment while only in three patients radiotherapy was used. So far, all 21 patients are evaluable for response: 13 (62%) patients reached complete remission, 6 (28%) patients showed partial remission and only two patients were resistant to treatment. We conclude that in elderly patients a 14 days schedule with the alternating CEOP/CNOP scheme is feasible such as the use of G-CSF might be avoided in some patients. The efficacy of this treatment should be evaluated in a higher number of patients and with a longer follow up.

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SUCCESSFUL TREATMENT OF TWO PRIMARY EFFUSION LYMPHOMA IN HIV NEGATIVE PATIENTS WITH ANTI CD20 MONOCLONAL ANTIBODY (RITUXIMAB®)

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Primary effusion lymphoma (PEL) exhibits exclusive or dominant involvement of serous body cavities without a detectable tumor mass. The most common form arises in human immunodeficiency virus (HIV) positive subjects; the neoplastic cells usually display a null phenotype and carry both Epstein Barr virus and Human Herpes Virus 8 (HHV8). A few cases of PEL without signs of HHV8 infection and showing a B-cell phenotype in HIV negative subjects have been reported. Herein we present two elderly patients with HHV8 negative PEL successfully treated with the anti CD20 monoclonal antibody Mabthera (Rituximab®). A 91 year old man (case A) and a 62 year old woman (case B) were admitted in our ward because of dyspnea, fever, night sweats and bilateral pleural effusion. The cytologic and cytofluorimetric examination of pleural fluid revealed highly atypical and pleomorphic cells with a B phenotype (CD19/CD20/CD22). Human herpes-virus type 8/Kaposi sarcoma herpes virus (HHV-8/KSHV) genomic sequence was not present in the lymphoma cells and there was no serologic evidence of HIV infection. A chest-abdomen CT scan showed bilateral pleural effusion without lymphoadenomegaly and hepatosplenomegaly. Both patients were diagnosed as PEL and received front line therapy with Rituximab 375 mg/m^2 weekly for six weeks. After the fourth week of therapy the CT scans were negative and both patients were symptoms free. At time of this writing both patients are still in CR (Case A 8 months; case B + 6 months). In conclusion our experience confirms that PEL in HIV negative patients shows B-cell associated antigens. The prompt and sustained responses we have obtained suggest that Mabthera could be a suitable option for the treatment of HIV negative PEL patients.

P544

SINGLE AGENT RITUXIMAB THERAPY FOR SPLENIC MANTLE CELL LYMPHOMA: CLINICAL AND BIOLOGIC IMPLICATIONS

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Mantle cell lymphoma (MCL) is an aggressive form of non-Hodgkin (NHL) lymphoma, typically occurring in older males and usually associated with the BCL1/JH gene rearrangement. It is characterized by lymphadenopathy, bone marrow (BM) and gastrointestinal tract involvement at diagnosis. Due to a reduced effectiveness of anthracycline-based CHOP-like chemotherapy, the prognosis of MCL is usually poor with a median survival of 3-4 years. About 80% of MCLs derive from pregerminal center (GC) B cells with an unmutated status of V(H) genes. A smaller subset (20% of cases) originates from more mature B-cells undergone somatic hypermutation in response to T cell-dependent antigens. Inclusion of Rituximab (R) in chemotherapy strategies for MCL appear to provide a significant clinical advantage, since it has been shown that activity of R-Hyper-CVAD/MTX-AraC is comparable, in terms of overall response rate, relapse free and overall survival, to Hyper-CVAD/MTX-AraC plus stem cell transplantation. We present the case of a 64 year old male who, based on a BM biopsy, in the absence of lymphadenopathy, was generically diagnosed, at two different institutions, as affected by 'B-cell lymphoproliferative disease'. After three years of watch and wait strategy, the patient came to our observation because of spleen enlargement and progressive thrombocytopenia. A new BM biopsy showed a diffuse infiltration of CD20+, CD79+, CD5-, Cyclin D1+, CD3-, BCL6-, CD23-, CD10- small lymphocytes, with a proliferation index (Ki67) <10%. A BCL-1 rearrangement due to the t(11;14) translocation, was also detected in BM neoplastic cells. The mutational analysis of V(H) genes showed an unmutated molecular pattern and a diagnosis of splenic MCL was then made. PET examination displayed several FDG-avid sites in the spleen. Due to the usually indolent clinical course of splenic MCL, the patient underwent splenectomy for therapeutical purposes. MCL diagnosis was confirmed on histologic, phenotypic and molecular grounds on spleen tissues. BCL-1 rearrangement was detected also in peripheral blood (PB) lymphocytes. In order to manage residual disease and prevent disease progression, the patient was given 8 courses of weekly Rituximab with a strict molecular monitoring of PB and BM samples. Disappearance of the BCL-1 rearrangement was obtained on PB starting from the 4th course of therapy while a persistent 'nested' PCR positivity for BCL-1 rearrangement was observed in the BM one month after the end of Rituximab treatment. At a current follow up of 12 months the patient remains clinically progression-free but still displaying a 'nested' BCL1 signal in the BM and a negative PB. This case may be of interest under several aspects. First, it confirms the 'indolent' course of the splenic form of MCL; second, it suggest that Rituximab may represent a useful tool to treat residual disease, without significant toxicity, in this rare subset of MCL; third, it emphasizes, that the lack of eradication of PCR-detectable BM disease in splenic MCL does not necessarily predict a rapid disease progression. Finally, our data indicate that, at variance with CD5+ lymphoproliferative disorders, the presence of a unmutated pattern of V(H) genes did not predict a unfavorable clinical course in splenic CD5- MCL. In such rare MCL subset, unmutated and putatively pre-GC tumor B-cells, might have arisen in a non-GC ectopic environment, such as the spleen, where somatic mutation is silent. These tumor cells however could potentially respond to T-cell-independent antigens and retain a indolent biologic behaviour. The molecular and biologic heterogeneity of tumor cells arising from the mantle zones of anatomically distinct lymphoid tissues, may have therefore significant clinical implications.

P545

EFFICACY AND TOLERABILITY OF LIPOSOMIAL CYTOSINE ARABINOSIDE (DEPOCYTE®) FOR THE TREATMENT OF LINFOMATOUS MENINGITIS

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Background. Meningeal blastic infiltration is a severe complication of non-Hodgkin lymphoma (NHL) and acute lymphoblastic leukaemia (ALL). Standard therapy consists in craniospinal radiationintrathecal (IT) or high-dose intravenous administration of cytarabine (ara-c) and methotrexate (MTX), +/-methylprednisolone. DepoCyte® is an unique ara-c liposomial formulation for IT administration, that provides a lower toxicity profile and a more convenient dosing regimen compared with unencapsulated ara-c or MTX. Patients and methods. Ten patients (pts) with meningeal involvement were treated with IT Depocyte® at standard dose of 50 mg every 2 weeks, given for compassionate use from July 2004 until March 2005. Six pts had diagnosis of NHL (5 with primary lymphonodal disease, 1 with primary central nervous system, CNS, disease) and 4 pts of ALL. Median age was 46 years (range 22-70). All pts had been previously treated with standard chemotherapy (CT) regimens for their primary disease. Two pts had received CNS prophylaxis (radiotherapy and IT MTX). Five patients had received MTX-based IT CT within 2 weeks before starting Depocyte®. Three patients had received IT dexamethasone in concomitance with Depocyte®. A total of 28 cycles were administered (range 1-4/pt). No dose reductions were required for toxicity. Results. Six pts (3 NHL and 3 ALL) experienced a complete cytological and neurological response; 1 ALL pt had a cytological and neurological partial response; 1 NHL pt with primary CNS disease remained in stable disease; 1 NHL pt had a neurological progressive disease; 1 NHL pt was lost to follow up. Mild toxicity was recorded: grade 2 aracnoiditis (1/9), headache (1/9), bone pain (1/9), grade 1 nausea and vomiting (2/9).

Conclusions. Depocyte® appears to be a feasible and active therapeutic option for lymphomatous meningitis; phase II-III trials are needed to confirm efficacy and tolerability.

Myeloma and Plasma Cell Dyscrasias III

P546

PERSISTENT PARTIAL RESPONSE IN PATIENT WITH POEMS SYNDROME IN ASSOCIATION WITH CASTLEMAN' S DISEASE TREATED ONLY WITH HIGH DOSE OF DESAMETASONE: DESCRIPTION A CASE AND REVIEW OF LICTERATURE

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The Poems syndrome is an uncommon multisystemic disorder characterized by the association of polyneuropathy, organomegaly, endocrinopathy, M protein , skin changes and various other systemic clinical signs that occur in the setting of a plasma cell dyscrasia. POEMS syndrome is rare: several hundred cases have been described in the literature. However, the incidence may be higher because the presence of the syndrome may go unrecognized. Although many plasma cell disorders have been reported in patients with POEMS syndrome, most patients are seen with osteosclerotic myeloma or monoclonal gammopathy of unknown significance (MGUS). The M proteins most frequently found are the immunoglobulin A (IgA) and immunoglobulin G (IgG) and light chains. The type of plasma cell disorder has not been shown to be correlated with the constellation of symptoms noted in patients with POEMS syndrome. The pathophysiologic link between the constellation of symptoms and the underlying disease is not well understood, but the link may be related to changes in the levels of a cytokine or a growth factor. Circulating levels of proinflammatory cytokines (IL-2 beta, TNF alfa, IL-6) are increased in patients with POEMS and their pleiotropic effects released secondary to a strong activation of the monocyte /macrophage system, take part in the multisystemic expression of the disease. Serum IL-6 levels in POEMS reflected the disease activity (higher in active than in stable disease) but not the severity of accompanying plasma cell dyscrasia. Sites of IL-1 beta production include lymphonode and bone marrow tissues. Increase of production of IL-6 could support the efficacy of corticosteroid therapy, particularly in acute clinical situations. Most recently significant elevations in vascular endothelial growth factor (VEGF) levels have been noted. Increases in VEGF levels have been postulated to lead to enhanced vascular permeability, which allows deposition of plasma cell-derived material. Stimulated vascular proliferation has been postulated to result in some of the skin changes noted in the disease. Serum levels of other growth factors, including epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), are not increased in patients with POEMS syndrome. Because POEMS syndrome is associated with Castleman's disease and angioma formation, a role for human herpes virus 8 (HHV-8) has been postulated; however early studies have not demonstrated an association. Treatments with demonstrated benefit include corticosteroids, low-dose alkylator therapy, and high-dose chemotherapy with peripheral blood stem cell transplant and recently thalidomide. The

author report a case of 63 years old man that seven years ago admitted to his institution with a diagnosis of probable liver disease. A physical examination disclosed polyadenopathies, epatosplenomegaly, skin abnormalities, ginecomastie, sloping's edema, ascites, pleural effusion, and peripheral neuropathy. The lymph node biopsy showed lymph node modification typical of Castleman's disease. At the same time was demonstrated monoclonal gammopathy of the IgA type and bone marrow plasmocytosis (about 10%). A rectal biopsy specimen for deposits of amyloid was negative. The percutaneos liver biopsy not revelead hepatic involvement. The final diagnosis was Castleman's disease with POEMS syndrome. The patient was treated with success with high dose of desametasone (40 mg iv) for 4 days every three weeks for twelve months. The patient got the partial remission of her symptoms and this condition is present still to 8 year-old distance from the initial diagnosis and the patient has not needed anymore of desametasone. I conclude that POEMS syndrome is a hypercytokinemic syndrome in wich bone marrow plasma cells are not of malignant type. The pathogenesis of the POEMS syndrome is therefore far from established.

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LOW DOSE OF GEMCITABINE IN RELAPSED MULTIPLE MYELOMA: A CASE REPORT

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Multiple Myeloma (MM) is a B-cell neoplasia with an unfavorable prognosis because of its inherent resistance to chemotherapy. Therapeutic options are poor in relapsed or refractory patients, in the elderly particularly. Gemcitabine, a cytosine arabinoside analougue, is a pyrimidine nucleoside with antitumor activity against solid tumor and hematological malignancies. We reported the case of 73 year-old male patient with IgG-lambda type of MM, in relapse after three treatment lines of chemotherapy. Previous treatments included Vinorelbine, Cyclophosphamide, Melphalan, Idarubicine, Dexamethasone and Zoledronate. He was treated with anticoagulant therapy for thrombophilic state (DVT and TIA) then he wasn't eligible for thalidomide. At diagnosis (2001, December), the patient showed stage III-A with multiple vertebral fractures. At starting Gemcitabine (2004, May), patient's characteristics were: Hb:8.7 g/dL; IgG:2530 mg/dL; IgM<17mg/dL; IgA 32 mg/dL; marrow plasmacells as 45 %. Gemcitabine 200 mg/m^2 i.v. was administered on days 1 and 8 , every four weeks, for six courses. Dexamethasone 4 mg/i.v. twice in week and Zoledronate 4 mg/i.v. monthly were associated. Hematologic response was evaluated at second and sixth course. The time elapsed between diagnosis and starting of gemcitabine was twenty-eight months. At second course the patient showed stable improvement of Hb level (>11 g/dL) and a decrease of marrow plasmacells (13%). At sixt course the patient showed a further decrease of marrow plasmacells (8%). Patient no required hospitalisation and oral anticoagulant treatment was effective. The only hematological toxicity observed was a grade II neutropenia. No extramedullar or infectious toxicity was observed. After a follow-up of ten months, disease was stable then the patient was submitted to maintenance schedule including Gemcitabine. Our single observation suggest that the combination of low doses of Gemcitabine, Dexamethasone and Zoledronate seems have good activity in relapsed MM, with an accetable toxicity and feasibility profile.

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SYMPTOMS ARE THE LEADING CLINICAL FACTORS IN THE EVOLUTION OF IgM MONOCLONAL GAMMOPATHIES

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Background. The purpose was to verify the reliability of the new criteria for the differential diagnosis of IgM gammopathies recently proposed by an international panel of experts (Athens Conference, 2002).

Methods. A retrospective series of 698 patients with IgM gammopathy was reviewed paying attention to symptoms, serum IgM concentration, bone marrow infiltration, blood cell count and clinical course. Four clinical entities can be identified – IgM monoclonal gammopathy of undetermined significance (IgM-MGUS), asymptomatic and symptomatic Wandenström's macroglobulinemia (A-WM and S-WM, respectively) and IgM-related disorders (IgM-RD), – although this last was excluded from the study because of the scarcity of patients due to probable selection biases. The observed mortality was studied related to that expected in the general population of comparable age and sex and over an equivalent period of follow-up (standardized mortality ratio, SMR).

Results. IgM-MGUS, A-WM and S-WM shared many clinical aspects but, with respect to the general population, patients with IgM-MGUS had a slight, but definite, survival advantage, those with A-WM had a mortality rate equivalent to that of the general population, whereas the SMR of patients with S-WM was 5.4. Within A-WM and S-WM the SMR values did not vary significantly in relation to marrow lymphocyte counts or serum IgM concentrations.

Conclusions. Our findings represent a prognostic validation of the applied diagnostic criteria for three out of the four identifiable clinical entities and, moreover, highlight the importance of symptoms over serum IgM concentration and marrow infiltration.

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THE ADDITION OF BORTEZOMIB (VELCADE) RESTORED SENSITIVITY TO THALIDOMIDE AND DEXAMETHASONE (THAL-DEX) THERAPY IN A CASE OF RELAPSED-REFRACTORY MULTIPLE MYELOMA

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Patients with relapsed or refractory Multiple Myeloma have limited treatment options due to development of chemoresistance. The proteasome inhibitor Bortezomib (VELCADE) has shown promising activity in this group of patients as single agent and its combination with other anti-myeloma agents is currently under evaluation. In february 2003, a 57-year old woman presented with pancytopenia associated with massive bone marrow infiltration by monoclonal plasma cells. No serum or urine monoclonal components were detectable. Cytogenetic analysis evidenced deletion of chromosome 13q. The patient was treated with VAD regimen followed by double high dose chemotherapy (melphalan 200 mg/mq with stem cell support), achieving complete remission (RC). Interferon-alpha was then given subcutaneously as maintenance therapy. Seven months after the second autologous stem cell transplant the patient relapsed showing pancytopenia and a monoclonal bone-marrow plasmacytosis of 98 percent. Pancytopenia required an intensive transfusion regimen of packed red cells and platelets. We started Thalidomide 200mg/day plus Dexametasone 40 mg/day on days 1-4, 9-12, 17-20 (Thal-Dex) as salvage therapy but the disease was refractory to the first two months of treatment. We therefore explored the efficacy and safety of adding Bortezomib to Thal-Dex regimen (1.0 mg/mq administered on day 1,4,8 and 11 followed by one-week break). Starting on the third cycle of the Bortezomib administration, serum calcium and LDH levels decreased rapidly and peripheral blood counts returned to normal. The patient became transfusion-independent. Response was confirmed by demonstration of 40 percent and 70 percent reduction of bone-marrow plasmacytosis after the third and the sixth cycle, respectively. The treatment was administered in a out-patient, ambulatory care setting. It was well tolerated with a manageable side-effect profile characterized by a dose-related decrease in platelet count (obviously evaluable after their normalization) with a nadir at day 11 and a good recovery by the next cycle. Although Bortezomib was administered simultaneously with Thalidomide no neuropathy was documented. Previous in vitro studies evidenced that Bortezomib markedly enhances sensitivity of MM cells to subtoxic concentration of doxorubicin and melphalan. Our case documented that Bortezomib can restore sensitivity to Thal-Dex resistant cells and that it can be safely added with a manageable toxicity profile.

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WASHOUT OF TECHNETIUM SESTAMIBI AND MULTI DRUG RESISTANCE IS THERE A CORRELATION WITH THE RESPONSE TO THALIDOMIDE AND TO CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA?

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Total Body scintigraphy with Sestamibi is considered less effective than NMR and standard X-ray in evaluating bone litic lesions in Multiple Myeloma.

This is due to the molecular characteristics of Technetium Sestamibi which enters the plasmacells and gives informations about their number and metabolism rather than about the bone status. On the other hand, this can be used for other informations, for example the follow up of patients with non secreting Multiple Myeloma. The Sestamibi is expelled from plasmacells with mechanisms linked to P170, so that it could be possible to plan the therapy by evaluating *in vivo* the Multi Drug Resistance. The amount of Sestamibi that is expelled from plasmacells can be calculated by performing a second scan two hours after the first one and comparing the radioactivity levels of the two scans. We have evaluated 18 (8 at diagnosis and 10 treated) patients with Multiple Myeloma and tried to correlate the results obtained before the therapy with talidomide or chemotherapy with the response to the therapy.

We defined a positive washout that corresponding to a minimum 50 % reduction in uptake and, according to this criterion, we observed that the washout was positive in 13 patients and negative in 5. 7 patients treated with thalidomide had a positive washout (from 52 to 100%). 6 of them had a good response and only one was resistant to therapy. 4 patients out of the 10 patients treated with chemotherapy had a negative washout and 6 had a positive washout. 3 of the patients with a negative washout responded to therapy, 1 did not; only one of the patients with a positive wash out responded to therapy, the remaining 5 were resistant to treatment.

Conclusions. 1) Following our observations, we conclude that the evaluation of Sestamibi washout is feasible, the effectiveness of thalidomide is not influenced by mechanisms of cellular excretion but the response to chemotherapy seems to be influenced by these. 2) According to these data the proposed technique could therefore offer useful indications in planning the therapy, by identifying at diagnosis those patients with a greater possibility of response to chemotherapy. Anyway we have to define a more accurate cut off criterion and overall to extend the study to a wider number of patients

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DEBULKING BY PULSE-VAD HIBRID REGIMEN, FOLLOWED BY THALIDOMIDE AND DEXAMETASONE, IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS NOT ELIGIBLE FOR HIGH-DOSE THERAPY

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Autologus stem cell-supported high-dose melphalan has emerged as standard therapy for multiple myeloma younger patients. Intermittent melphalan-prednisone (MP) is still considered the treatment of choice for patients not eligible for high-dose therapy. More rapid cytoreduction with VAD in comparison with MP was generally observed, reflecting the different activity of these treatments against more or less mature myeloma cell subpopolations, respectively. However, the occurrence of serius infection and cardiovascular complications were reported in most studies using VAD regimen. Studies are ongoing to determine the efficacy of thalidomide when combined with other effective agents such as dexamethasone, combination chemotherapy, and the proteosome inhibitor PS 341. Between October 2001 and December 2004 we conducted a pilot study in a total of 20 elderly patients with previously untreated Multiple Myeloma based on 2 cycles Pulse-VAD hybrid (Vincristine 1.2 mg/sqm –maximum 2 mg-day 1; pegylated liposomal Doxorubicin (Caelix) 15 mg/sqm day 1; Dexamethasone 20 mg day 1-4) month x 2 courses, followed by Thalidomide 200 mg/day continuously and Dexamethasone 20 mg day 1-4 every month. The characteristic of the patients were the following: median age = 77 years (72-85), M/F = 12/8, IgG/IgA = 16/4 kappa/lambda = 17/3, Stage II/III = 8/12, Median beta 2 microglobulin 3.8 mg/l (1.9-14), Median C reactive protein 2.66 mg/l (0.14-42), Median lactate dehydrogenase 480 U/l (330-1050), Median BMPC 50% (30-75), Median PLT 120.000 mm3 (40.000-195.000), Median Hb 8,6 g/dL (7.2-11.5). After a minimum of 6 months of treatment, 14 patients showed a significant response (reduction of M-component >50% in 10 cases and >75% in 4); in a restant 6 patients a decrease >25% was observed; 18/20 patients are alive and progression-free (12 months of median follow-up). Adverse effects were moderate (grade </= 2); constipation (all patients), sedation (8/20 patients) and deep venous thrombosis (5/20) was observed. All the patients reduced the assumption of analgesics against bone pain and had an improvement of performance status. No patient had to stop therapy because of cardiac toxicity and no patient had to be hospitalised because of infectious or other complications. These data showed that pulse-VAD hybrid regimen (modified by substituting doxorubicin with pegylated liposomal doxorubicin) followed by thalidomide and dexamethasone is well tolerated in elderly patients and is active as front line therapy for newly diagnosed disease. A longer follow-up is needed to evaluate the duration of the favourable responses and the effect on survival.

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INTRAVENOUS LOW-DOSE MELPHALAN PLUS ORAL PREDNISONE AND THALIDOMIDE (MIVPT) AS SALVAGE THERAPY FOR ADVANCED MYELOMA

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Thalidomide alone or in combination has been tested in patients with advanced and, more recently, untreated multiple myeloma. In untreated patients, we have showed the synergistic effect of oral melphalan prednisone and thalidomide. In advanced myeloma, the combination of thalidomide and dexamethasone has been proved safe and effective. Short courses of intravenous low-dose melphalan showed limited and lower hematologic toxicity in comparison with oral melphalan. This approach might be specifically suitable for advanced myeloma. To address this issue, we evaluated intermittent short courses of low dose intravenous melphalan in association with prednisone and thalidomide (MivPT) in order to asses toxicity and efficacy. Between April 2003 and January 2005, 24 patients that relapsed or were refractory to induction chemotherapy were treated with short courses of intravenous low dose melphalan plus prednisone and thalidomide. Median age was 64 years, 58% were male, and 60% were in III stage. Median time from diagnosis was 47 months. Four patients (17%) showed progressive disease despite thalidomide plus dexamethasone salvage therapy. Melphalan was administered i.v. at the dose of 20 mg/mq every 3 months. Prednisone was administered orally 3 times a week and doses ranged from 12,5 mg to 50 mg. Thalidomide was continuously administered at daily doses ranging from 50 to 100 mg. Twelve patients received 1 course of melphalan, 3 patients 2 courses, 4 patients 3 courses, 5 patients 4 courses. Twenty-three patients were evaluable for response and toxicity: median time to response was 3 months: 4 patients (17%) showed a M-protein reduction >75%, 6 patients (26%) showed response 50-75%, 4 patients (17%) response 25-50% and 5 patients (22%) response <25%. Progressive disease was observed in 4 patients (17%). Reduction in the paraprotein levels was associated with decreased number of bone marrow plasmacells and increased hemoglobin levels. After a median follow up of 12 months, median event-free survival from start of MPT was 8 months. In 33% of patients the duration of remission was longer than the previous one. The treatment was relatively well tolerated, most adverse events were recorded as CTC grade I-II. Constipation was frequent (26%) but well controlled. Tingling and numbness were recorded in 21%, vertigo in 8% as grade I-II. Sedation (17%) was common but usually well tolerated. One patient experienced neuralgia and 2 patients vertigo as grade III. Hematological toxicity was recorded as grade IV in 13% of patients: most of patients showed toxicity grade I-II. Infective toxicity grade III was recorded in 2 patients (8%) who discharged MPT treatment; grade I-II in 13%. Among other adverse events a cardiac failure grade II and a deep-vein thrombosis: both required treatment discontinuation. Thalidomide alone was discontinued in 33% of patients mostly due to tingling and numbness (13%) and less frequently to vertigo, constipation and sedation.

The combination of MivPT is active against advanced myeloma. This combination showed a very limited hematological toxicity. MivPT might be successfully used when the combination talidomide and dexamethasone is ineffective.

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MPTV (MELPHALAN, PREDNISONE, THALIDOMIDE AND VELCADE) COMBINATION THERAPY IN ADVANCED/REFRACTORY MULTIPLE MYELOMA

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Bortezomib (VELCADE™) is a new drug that is effective for the treatment of refractory multiple myeloma (MM). Both in vitro studies and preliminary clinical experiences have shown promising activity of VELCADE in combination with other anti-myeloma agents. VELCADE can restore sensitivity to Melphalan-resistent MM cell lines. In relapsed refractory patients the combination of VELCADE plus Thalidomide induced a 60% of overall response rate (≥25% M protein reduction); the combination VELCADE plus Melphalan induced a 67% of overall response. In newly diagnosed patients, the addition of Thalidomide to the standard oral Melphalan/Prednisone combination significantly increased response rate and event free survival. The aim of this study is evaluate the efficacy and the safety of combination therapy Melphalan/ Prednisone/ Thalidomide/VELCADE (MPTV) as salvage treatment in advanced/ refractory myeloma patients.

Multiple myeloma patients relapsed/refractory after 1 or 2 lines of treatment were eligible for this trial. The MPTV regimen consisted of oral Melphalan 6 mg/sqm administered on days 1-5, oral Prednisone 60 mg/sqm on days 1-5, Thalidomide 100 mg continuously, and VELCADE administrated on days 1, 4, 15, 22 of each course, at 1 mg/sqm in the first 10 patients cohort; 1,3 mg/sqm in the second 10 patients cohort and 1,6 mg/sqm in the third 10 patients cohort. Each course was repeated every 35 days for a total of 6 courses. Sixteen patients have been enrolled in this study, median age 63 years, 75% IgG, 12% IgA, 12% Bence Jones. Four patients were treated with MPTV as second line therapy, 12 patients as third line. Eleven patients received prior autologous transplant, 5 Thalidomide based regimen and 7 conventional chemotherapy.

Ten patients have completed at least 1 course and were available for response and toxicity evaluation. Three patients received 1 entire course of MPTV, 4 patients 2 courses, 1 patient 3 courses, 1 patient 5 courses, 1 patient 6 courses. All this patients received VELCADE at dose of 1 mg/sqm. Responses were evaluated according to the EBMT criteria, and toxicities according to Common Terminology Criteria (CTCAE ver.3.0). In evaluable patients response rate was: 20% near complete response (1 patient after 2 courses, 1 patient after 6 courses), 30% partial response, 20% minimal response, 30% stable disease. Grade 4 adverse events were 1 neutropenia and 1 thrombocytopenia. Grade 3 events were haematological toxicities: neutropenia (6 patients), thrombocytopenia (3 patients), anaemia (1 patients) and infective toxicities: febrile neutropenia (1 patient), sepsis (1 patient), pneumonia (1 patient). The most common grade 2 non hematologic toxicities were: constipation (4 patients), sedation (1 patient), paroxysmal atrial tachycardia (1 patient). Among the 5 patients with baseline peripheral neuropathy, 4 patients remained stable, and 1 patient worsened (grade 2). One patient developed de novo treatment-related neuropathy grade 1. These preliminary data showed that MPTV combination is a promising regimen for relapsed/refractory myeloma. Updated results of the trial will be presented.

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THALIDOMIDE AND DEXAMETHASONE IS AN EFFECTIVE SALVAGE TREATMENT FOR MYELOMA PATIENTS RELAPSING AFTER AUTOLOGOUS TRANSPLANT

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High-dose therapy followed by autologous stem cell transplantation (AT) is the standard treatment for untreated multiple myeloma (MM) patients, aged 65 or less. AT improves survival and quality of life but relapse always occurs and the best treatment option for relapsed patients has not been defined. Between January 1999 and September 2003 we retrospectively analysed 90 MM patients (median age 61) treated in first relapse after AT: 43 patients received Thalidomide and Dexamethasone (TD group), 28 patients repeated AT (AT group), and 19 patients were treated with conventional chemotherapy (CC group). No difference in patients characteristics were found among different group. We evaluated response, PFS and OS from first relapse. TD regimen included Thalidomide 100 mg/day associated with DEX 40 mg on days 1-4 each month. In the AT grounp 96% of patients were conditioned with Melphalan 100 mg/mq and 4% with Melphalan 200 mg/mq. In the CC group 84% of patients received Doxorubicin and/or Cyclophosphamide regimens. The median time from diagnosis was 32 months for TD and CC patients and 29 months for AT group. The median follow-up was 34 months (range 4.5-45) from the start of TD salvage, 18 months (range 3.5-24) from the start of AT and of 21 months (range 2-19.5) from the start of CC. Response rate, defined according to the EBMT/IBMTR criteria, was significantly lower for patients receiving salvage CC in comparison with AT and TD ($p<0.001$). In details, response rate among patients who received TD was: 19% near CR (nCR) (absence of M protein detected by electrophoresis), 28% partial remission (PR) (M-protein reduction 50-99%), 35% stable disease (SD) (M-protein reduction 0-49%) and 19% progressive disease (PD). The response rate after AT was 11% nCR, 71% PR, 11% SD and 7% PD; after CC was: 16% PR, 32% SD and 53% PD. Patients treated with

TD experienced a response rate slightly lower than patients treated with AT but the time to progression was significantly prolonged. Median PFS from relapse for TD group was 20.3 months vs 9 months for AT group and 4.5 months for CC group (p<0.001). Moreover 20% of patients treated with TD showed a longer PFS at relapse than at diagnosis. TD also improved OS from relapse: median OS from first relapse was not reached for patients receiving TD; it was 15 months after AT and 27.5 months after CC (p=0.008). The multivariate analyis shows that TD, age and Beta2microglobulin were independent risk factors associated with improved outcome. In conclusion TD is an effective first salvage approach for MM patients relapsing after AT. It significantly improves PFS and OS from first relapse.

P555

ELEVATED SERUM CONCENTRATION OF ANGIOPOIETIN-2 IN PATIENTS WITH MULTIPLE MYELOMA: CORRELATION WITH MARKERS OF DISEASE ACTIVITY

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The association between the expression of Angiopoietin-2 (Ang-2) and cancer progression and poor outcome in solid tumors has been documented, but its significance in haematologic malignancies has not been evaluated.

Using an Elisa technique (Quantikine human angiopoietin-2 immunoassay; R&D Systems), we measured serum angiopoietin-2 levels in 24 patients with newly diagnosed or relapsed Multiple Myeloma (MM), in 6 patients with monoclonal gammopathies of undetermined significance (MGUS) and in 15 healthy controls. Four MM patients were in stage I; ten in stage II and ten in stage III (Durie-Salmon classification). Bone lesions were scored from 0 to 3, according to X-ray findings. No difference in Ang-2 serum levels could be found between MGUS patients (median 409 pg/mL; range 220-570 pg/mL) and healthy controls (median 563,75 pg/mL; range 110-1025 pg/mL) (p=NS). In MM patients serum levels of Ang-2 were significantly higher in comparison to controls (p<0,001). A significant difference was observed in the Ang-2 concentrations at different disease stages with elevated levels at stage III compared to stage II and I (median 2543,3 pg/mL, 1772,2 pg/mL, 681,25 pg/mL, respectively). There was a positive association between Ang-2 and bone involvement as its levels increased with increasing bone lesion score. A positive correlation was found between serum Ang-2 and b2-microglobulin (r=0,317). In conclusion, a high serum Angiopoietin-2 level detected in MM patients and its correlation with known factors of disease activity show that Ang-2 may be implicated in Multiple Myeloma; however, further additional studies are required.

P556

THALIDOMIDE-INDUCED PERIPHERAL NEUROPATHY IN NEWLY DIAGNOSED AND PRE-TREATED MULTIPLE MYELOMA PATIENTS

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Thalidomide has shown remarkable activity in advanced, relapsed or refractory multiple myeloma (MM), so that its use has been recently proposed either in newly diagnosed patients or as maintenance treatment after conventional or high-dose therapy. This latter therapeutic approach has risen the concern of peripheral neuropathy, known to occur after long-term therapy with this drug. In order to investigate this issue we analyzed the outcome of 74 patients who have been treated with thalidomide +/- dexamethasone for longer than 8 months at our Institution. Thirty-four patients (18M, 16F, median age = 55 years) had newly diagnosed symptomatic MM and were treated with four monthly courses of thalidomide 200mg/day + dexamethasone 40 mg on days 1 to 4 (even cycles), or on days 1 to 4, 9 to 12 and 17 to 20 (odd cycles), followed by cyclophosphamide 7g/m^2 + G-CSF, peripheral blood stem cell (PBSC) collection, and double autologous PBSC transplant. Thalidomide + dexamethasone was administered throughout the whole treatment program. Forty patients (27M, 13F, median age = 61 years) were treated with thalidomide 200mg/day $^{+/-}$ dexamehasone 40mg on days 1-4 every four weeks as salvage therapy for relapsed (n = 14) or progressive (n=26) MM. Neurotoxicity was the most troublesome and frequent toxic effect that was observed after > 8 months treatment, the incidence averaging 74% in newly diagnosed patients and 75% in pretreated ones. Symptoms included paresthesias, tremor and dizziness; serial electrophysiological studies revealed a symmetrical, mainly sensory peripheral neuropathy, with minor motor involvement. The severity of neuropathy, graded according the NCI-CTC 2.0 scoring system, varied greatly in the two groups of patients, as pretreated patients showed grade 2 and 3 toxicity in 32.5% and 27.5% of the cases, respectively, while the majority of newly diagnosed patients complained about grade 1 toxicity (57%), and none of them experienced grade 3 toxicity. In both groups thalidomide neurotoxicity was not related to sex, M protein isotype, and thalidomide daily dose. In pretreated patients, a significant correlation between grade 2 + 3 neurotoxicty and longer disease duration was found, thus suggesting that preexisting (sub)clinical neurotoxicity related both to MM and/or to prior therapies could favour thalidomide-induced toxic effects. These results suggest that long-term thalidomide therapy in MM may be hampered by the remarkable neurotoxicity of the drug, and that a neurological evaluation should be mandatory prior to thalidomide treatment, in order to identify patients at risk of developing a peripheral neuropathy.

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BONE RESORPTION MARKERS ARE INCREASED IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS WITH VERTEBRAL LESIONS AT SPINAL MAGNETIC RESONANCE IMAGING

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Magnetic resonance imaging (MRI) has been demonstrated to be more sensitive than plain x rays in detecting spinal involvement in multiple myeloma (MM) patients, as both vertebral lesions and pattern of bone marrow infiltration can be depicted. In the present study we aimed at evaluating whether bone involvement of the spine, as analyzed by MRI, can be related to the extent of bone remodeling in symptomatic newly diagnosed MM patients. For this purpose, whole spine MRI was performed in 48 newly diagnosed MM patients (31M, 17F, median age = 54 yrs) and, at the same time, markers of bone resorption (urinary NTX, PYR and DPYR, and serum crosslaps) and bone formation (bone alkaline phosphatase-BAP and osteocalcin) were measured. MRI pattern of bone involvement was normal in 7 patients (15%), diffuse in 20 (41%) and focal in 21 (44%). These three groups of patients did not differ significantly in terms of median age, distribution of M protein isotype and light chain, beta-2 microglobulin, bone marrow plasma cell infiltration and disease stage. No difference in bone resorption and bone formation markers was observed in patients showing a diffuse, focal or negative MRI pattern. In 22 patients (48%), MRI revealed also either a vertebral compression fracture (n = 16) or a vertebral mass (n = 6); in these patients a focal (68%) rather than a diffuse (32%) pattern of MM involvement was more frequently detected. Urinary NTX (70.8 nmol/mmol crea ±10.45SE vs 42.6 nmol/mmol crea ±7.57SE, p= 0.04) and serum crosslaps (7556 pmol/L±1292SE vs 4585pmol/L±625SE p=0.04) were significantly increased in patients with vertebral compression fractures or vertebral masses compared to individuals with abnormal MRI but without vertebral lesions. Our data indicate that NTX and serum crosslaps correlate with the extent of spinal damage in MM patients, as detected by MRI.

P558

CLINICAL AND PROGNOSTIC FEATURES OF IGM-RELATED DISORDERS

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Introduction. IgM-related disorders (RDs) are variably-sized IgM monoclonal components (MCs) presenting cryoglobulinemic activity or mediating autoimmune phenomena, in the absence of clear evidence of lymphoma. Although distinctive clinical features and need of immunosuppression in view of the MC-associated symptoms suggest IgM-RDs to have a different natural history from that of asymptomatic IgM-monoclonal gammopathies (MGs), studies on their prognosis are lacking.

Patients and Methods. One-hundred eighteen IgM-RDs [101 cryoglobulinemias, 11 peripheral neuropathies (PNs), 3 idiopathic thrombocytopenic purpuras, and 3 cold agglutinine hemolytic anemias] diagnosed between 1984 and 2004 were studied in order to identify predictors of transformation into malignant lymphoproliferative disease (MLD). Survival probability was calculated by means of the Kaplan-Meyer estimator. Survival curves were compared using the log-rank test.

Nineteen patients had type I cryoglobulinemia [11 idiopatic, 6 hepatitis C virus (HCV)-related, and 2 hepatitis B virus (HBV)-related], and 82 had type II essential mixed cryoglobulinemia (EMC)(15 idiopatic, 66 HCV-related, 1 HBV-related). Twenty-one cryoglobulinemias presented a mild to moderate hepatomegaly with/without splenomegaly, mostly HCV-related. Of EMCs, 30 had arthralgias and/or vascular purpura (25 receiving corticosteroids), and 17 presented PN. Sixteen out of 28 PNs (57%), either with or without EMC, were treated with steroids, cyclophosphamide or polychemotherapy with/without plasma-exchange. One further anti-myelin-associated antigen-related PN achieved symptoms remission after anti-CD20 antibody treatment. The cumulative probability of evolution to MLD at 5 and 10 years was 7% (95%CI, 2-12%) and 12% (95%CI, 3-21%), respectively. At a median follow-up of 55 months (7-195), 8 IgM-RDs (7%) presented transformation into overt Waldenström's Macroglobulinemia (n=6), non-Hodgkin's lymphoma (n=1) and B-chronic lymphocytic leukemia (n=1). At univariate analysis, age (p=0.8), type of light chain (p=0.6), beta2-microglobulin levels (p=0.8), degree of bone marrow lymphoplasmacytic infiltration (p=0.6), hemoglobin level (p=0.7), neutrophil counts (p=0.3), platelet counts (p=0.8), presence (p=0.9) and type (p=0.4) of cryoglobulinemia, and HCV-positivity (p=0.7) did not significantly correlate with transformation. On the contrary, male sex (p=0.01), IgM MC level 3 g/dL or more (p<0.0001), detectable Bence Jones proteinuria (p=0.005), absolute lymphocyte counts > 4 x 10^9/L (p=0.02), and erythrocyte sedimentation rate > 40 mm/h (p=0.003) significantly correlated with the evolution risk to malignant lymphoproliferative disease.

Conclusions. Although IgM-RDs represent a distinct clinical entity frequently requiring treatment in view of the IgM-related symptoms, their evolution probability is similar to that of IgM-MGs of undetermined significance. Prognostic factors for malignant transformation seem to widely overlap those observed in asymptomatic IgM-MGs.

P559

THE EVOLUTION OF MULTIPLE MYELOMA DURING THE LAST THIRTY YEARS: A SINGLE INSTITUTE EXPERIENCE

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Background. MM is an incurable plasma cell neoplasm characterized by a great variability of clinical presentation. Its natural history is changed after the introduction of new therapies for MM as high dose chemotherapy or supportive treatments (bisphosphonates) obtaining a better control of symptoms, a higher response rate and a prolonged overall survival compared to the conventional treatments.

Aims and study population. The aims of this work were: 1. to evaluate within a population of 772 MM patients, diagnosed from 1973 to 2003 at our department, the possible differences in clinical presentation between two groups of patients diagnosed before (group I) or after 1994 (group II); 2. to compare in the same groups, patients aged as or less than 65 years treated at onset with high dose therapy (HDT) or with conventional therapy (CT) in order to assess response rate, progression free and overall survival (PFS, OS), post progression survival (PPS) and type of relapse; 3. to compare patients aged more than 65 years treated with CT before or after 1994 in order to value the impact of supportive care and new recent salvage therapies on response and survival.

Results. Epidemiology: The two groups statistically differed for the reduced incidence of bone pain (p=0.019), and increased incidence of lytic lesions (p=0.017) in group II. After 1994 were diagnosed more MM evolving from MGUS (p=0.000) and a reduced incidence of early deaths (within four months from diagnosis) was registered (p=0.000). Regarding the other clinical parameters no statistically differences were observed. Follow up: Younger patients divided in the two groups (HDT: 96 patients, CT: 295 patients), presenting comparable characteristics at onset, showed a better quality of response when treated with HDT (p= 0.000): 94.4% of responsive patients (CR and VGPR and PR) versus 42.9% in CT group. The median OS was 50 months in CT group, while in the in HDT group median value was not reached (p=0.000) and the incidence rate of death was halved in HDT patients (from 0.012 to 0.0057). Also PFS was longer in HDT group (37 versus 23 months, p=0.000), while PPS was not statistically different (22 18 months, p=0.41). The modality of relapse was similar after HDT or CT (0.052) even if a trend in a decreasing skeletal relapse was observed after HDT. The analysis of valuable older population (116 before and 89 after 1994) revealed better significant results for patients diagnosed after 1994: responsive patients 50% versus 29.7% in CT group (p=0.019), median OS 46 versus 21 months (p=0.000).

Conclusions. Our data did not show strong differences in clinical presentation during the last thirty years; in particular, the incidence of asymptomatic or early stage patients is not increased. On the contrary, the observed higher incidence of bone involvement may be related to a better and systematic bone evaluation in all patients. The response and survival data confirm the advantage of high dose therapy with respect to conventional chemotherapy without a difference in the outcome after progression. The better results obtained in the older population diagnosed and treated more recently suggest the efficacy of the new supportive therapies.

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HIGH-DOSE MELPHALAN WITH STEM CELL SUPPORT IN MYELOMA PATIENTS OLDER THAN 65 YEARS

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Background. High dose chemotherapy with peripheral stem cell support is the best treatment for patients with newly diagnosed multiple myeloma (MM). Compared to conventional chemotherapy, it increases the complete remission rate up to 50% and the overall survival (OS) up to about a median of 5 years. The risk of serious complications limits this approach to patients younger than 65 years and in good performance status. At our institution we did not exclude from this potentially effective approach patients aged 66-71 years, who had at least a partial response to VAD regimen.

Aim. In this study we evaluated the toxicity and efficacy of high dose chemotherapy with stem cell support in patients aged of 66-71 years (group A), compared with a similar group of patients 60-65 years old (group B).

Patients and methods. From January 1995 until December 2002, 301 new cases of multiple myeloma were diagnosed at our Institution: among them 66 (22%) were aged 66-71 years, 52 (17%) 60-65 years,183 (61%) less than 60 or more than 72 years. Twenty of 66 (30%) consecutive patients in group A and 30/52 (58%) in group B (p=0.004) actually received high dose chemotherapy with stem cell support: 4 VAD, CTX 4 g/mq and G-CSF used to mobilize peripheral blood stem cells (PBSC), followed by single or tandem L-PAM 100/200 mg/mq with PBSC support.

There were no significant differences in the characteristics of two group, which listed in Table 1.

Table 1.

All patients of group A received CTX 4 g/mq and successfully mobilised PBSC, median $CD34^+$ $5x10^6$/ kg (2.4-17.2 $x10^6$/Kg). Thirteen patients received one course of Mel 200 mg/mq, 2 cases tandem Mel 200 mg/mq, 4 pts tandem Mel 100 mg/mq, one FTBI/Mel 140 mg/mq. In the group B (age 60-65 years) 7 patients received CTX 4 g/mq, 23 CTX 7 g/mq and all patients mobilised PBSC, median

CD34+ 5x10^6/ kg (1.8- 12.2 x10^6/Kg). Only one patients received 3 tandem Mel 100 mg/mq, 2 cases tandem Mel 200 mg/mq, the others one course of Mel 200 mg/mq .

Results. All patients in both groups completed the program and no toxic death was observed. There was no significant difference in neutrophil (N>500/mmc) and platelet (>20000/mmc) recovery: median 9 days (range 5-12) and 10 days (range 6-14) respectively in both groups.

No significant difference in the incidence of treatment complications was observed in both group: deep venous trombosis (n 2 in group A, n1 in group B), sepsis(n 2 in each group), pneumonia (n 2 in group A, n 3 in group B), mucositis grade 3/4 (n1 in each group). Response to treatment was evaluated accordig to Bladè criteria (Br. J. Haematol 1198; 102:1115). CR and VGPR in group A and B were obtain in 9 (45%) and 25 (83%) patients respectively (p=0.004), PR in 11 (55%) and 5(17%), relapse/progression occurred in 15 (75%) and 18 (60%)(p=0.227), death for disease in 4 (20%) and 9(30%) patients. Median EFS was 27 months in group A and 37 months in B (p=0.001), median OS has not yet been reached in both group. Projected OS at 57 months is 65% in group A and 73% in group B (p=0.71)(See Table 2).

Table 2.

Response	Group B (40-65yrs)	Group A(66-71yrs)	
CR+VGPR	25 (83%)	9 (45%)	P=0.004
PR	5 (17%)	11 (55%)	
Relapse/Progression	18 (60%)	15 (75%)	P=0.227
Death	9 (30%)	4 (20%)	
EFS (median; months)	37 m.	27 m.	P=0.001
OS at 57 months	73%	65%	P=0.71

Conclusion. High dose chemotherapy with stem cells support is feasible with a limited toxicity in elderly myeloma patients. Nevertheless on an intention to treat basis significantly less patients aged 66-71 could receive high dose therapy with stem cell support compared to patients 60-65 years old and the response rate and EFS were better in the younger group.

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NK AND CD8+ CELLS EXPANSION IN BONE MARROW AFTER LOW DOSE THALIDOMIDE IN RELAPSED MULTIPLE MYELOMA PATIENT, CONTROL OF MYELOMA DISEASE: A CASE REPORT

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The antiangiogeneic activity of thalidomide (Thal) in Multiple Myeloma (MM) is the rational for the use of thal in MM relapsed patients.

We report a case of relapsed MM patient after double autologous stem cells transplantation (aSCT) and vaccine-therapy has been treated with thal with very good response of disease and with expansion in bone marrow of cytotoxic cells. Male 57 years-old have received diagnosis of MM, IgG lambda stage IIIA, in 1997 in other institution. Treated with VAD regimen and double aSCT has obtained a complete remission (CR). Relapsed in 2001 the patient has effected vaccine-therapy with a partial remission. In August 2002 has been recovery in our Division for relapse of myeloma disease, monoclonal component (MC): 4000 mg/dL and bone marrow aspiration (BMA): 90% of plasma-cells. Treated with thal at dose of 200 mg/day, no steroid has been used. At month +6: MC: 303 mg/dL and BMA: poor cellularity and nodular infiltration of plasma-cells. The patient has reduced thal at dose of 50 mg/day. At month + 20: MC: 345 mg/dL and BMA: poor cellularity, lymphocytes: 50% and plasma-cells: 5%. The patient has suspended thal therapy.

At month +26: MC: 668 mg/dL and BMA: poor cellularity, lymphocytes: 40% and plasma-cells: 10%. The immunophenotipe has shown increased levels of CD8+ (28%) and NK (25%) cells in the bone marrow.

At month +30: MC: 1008 mg/dL and BMA: good cellularity and lymphocytes: 20% and plasma-cells: 20%. The immunophenotype has shown a reduction of the populations CD8 (10%) and NK (7%) cells. Initial relapse of MM disease and the patient has started therapy with thal 100 mg/day. At month + 31: MC: 387 mg/dL, the patient continuous thal therapy. The low dose thal therapy in this patient has shown an expansion of CD8+ and NK cells in the marrow, this expansion probably has controlled the disease because to the suspension of the thal a decrement of the value of CD8+ and NK cells is observed with initial relapse of disease. Probably in this patient also the vaccine-therapy has a role and the synergy among thal therapy and vaccine-therapy is the real motive of expansion cytotoxic cells and control minimal residual disease

P562

TECNETIUM-99M SESTAMIBI SCINTIGRAPHY: POTENTIAL ROLE IN STAGING AND FOLLOW-UP OF PATIENTS WITH MYELOMA AND MGUS: A MULTICENTRIC STUDY

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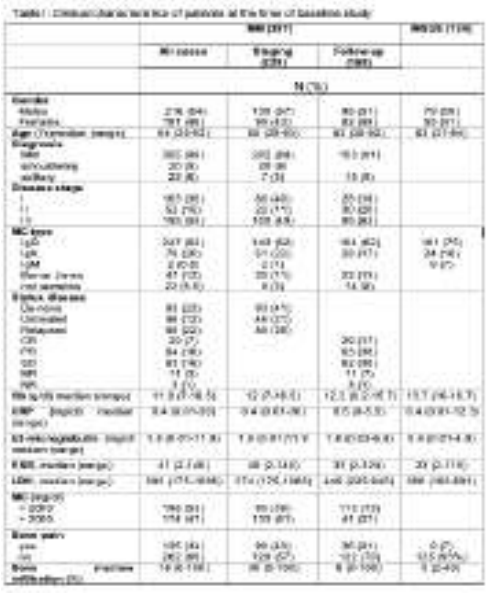
Introduction. Appropriate imaging is essential for diagnosis/staging and follow-up of monoclonal gammopathies. Standard skeletal X-ray is the important baseline but is not sensitive and may be a counfonding tool in the staging and follow-up, respectively. In this multicentric study, we have therefore evaluated the additional benefit of Tc 99m sestamibi scanning in patients with myeloma and MGUS, focusing on 1) sensitivity and specificity in myeloma diagnosis/staging and MGUS, respectively; 2) comparison to Xray in staging myeloma; 3) its clinical usefulness in the follow-up of myeloma patients; 4) relationship with clinical status and biological parameters.

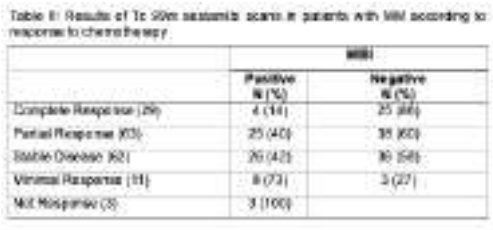
Design and Methods. Between February 2001 and January 2005, 531 scans (397 myeloma and 134 MGUS) were studied by whole body scans obtained 20 minutes after administration of 740 MBq of Tc99m sestamibi. The clinical char-

acteristics of patients are summarized in Table 1.

Results. On 134 MGUS scans, 34 (25%) were positive. Xray was false positive in 11 cases (9%). The specificity of Tc99m sestamibi in this setting was 75%. Among the 397 myeloma scans, 229 (58%) were patients at diagnosis/ staging (smouldering myeloma (SM), de-novo/untreated multiple myeloma (MM) or solitary myeloma and relapsed MM). Tc99m sestamibi and Xray were positive in 146 (64%) and 97(45%) cases, respectively. On 119 cases with negative Xray, Tc99m sestamibi was positive in 50% of cases although it was false negative in the 23%. The sensitivity of Tc99m sestamibi was 77% whereas Xray sensitivity was lower (45%). At univariate analysis, the Tc99m sestamibi correlated with diagnosis (MM vs SM vs solitary; p=0.01) and disease activity as determined by b2 microglobulin (p=0.06), bone marrow infiltration (p=0.004), CRP (p= 0.0002), hemoglobin levels (p< 0.01), status of disease (relapsed vs untreated; p 0.005), disease stage (I vs II-III; p= 0.0001), bone pain (p=0.00001) and gender (p=0.004). We found no correlation between positive Tc99m sestamibi and age, LDH, ESR, immunoglobulin value. At multivariate analysis, a positive pattern correlated with higher CRP (0.0005), higher BMI (p=0.02) and bone pain (0.002).Among 168 scans (42%) with myeloma in follow-up post chemotherapy, Tc99m sestamibi and Xray were positive in 39% and 55% of cases, respectively. Bear in mind that Xray osteolytic lesions do not disappeared with chemotherapy, Table 2 showes the results of Tc99m sestamibi in myeloma patients according to response to treatment.



We found a statistical significant correlation between positive Tc99m sestamibi and high BMI (p=0.01), high immunoglobulin value (p=0.03), disease status(CR vs evolutive disease; p=0.002) and response to therapy (CR vs PR, SD, MR, NR; p=0.001), gender (p=0.02) and number of previous chemotherapy (p=0.09). At multivariate analysis, a positive pattern correlated with high BMI (p=0.002), disease status other than CR (p=0.03). In patients with solitary myeloma, Tc99m sestamibi was positive on 5 out 6 cases at diangosis/staging and showed more active lesions than Xray. In solitary myeloma post-chemotherapy (16 scans), Tc99m sestamibi correlated with response and disease status (negative pattern in CR and PR patients).



Conclusions. Tc99m sestamibi and myeloma at diagnosis/staging. This study provides evidence that Tc99m sestamibi scanning have higher sensitivity (77%) compared to Xray (45%). Moreover, a positive pattern is associated with biological parameters related to tumor burden (p=0.02) and disease activity (p=0.0005). Tc99m sestamibi and myeloma in the post-chemotherapy follow-up. Tc99m sestamibi is a useful adjunct, with a significant relationship to BMI (p=0.002) and response to the therapy (p=0.03). Solitary myeloma. Tc99m sestamibi correlates with response and status disease and may have a role in the "staging", modifing treatment approaches. Tc99m sestamibi and MGUS. The Tc99m sestamibi scintigraphy is positive in 25% of patients: could be interesting to monitor the patients during long term follow-up.

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MAINTENANCE WITH LOW DOSE THALIDOMIDE AFTER AUTO-SCT IN MULTIPLE MYELOMA

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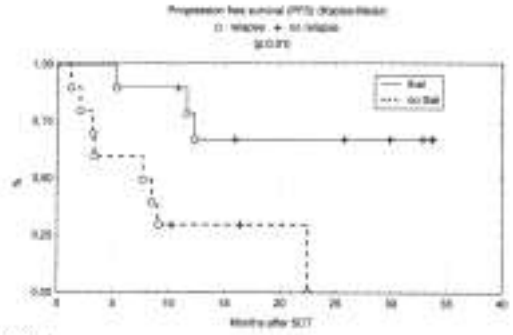
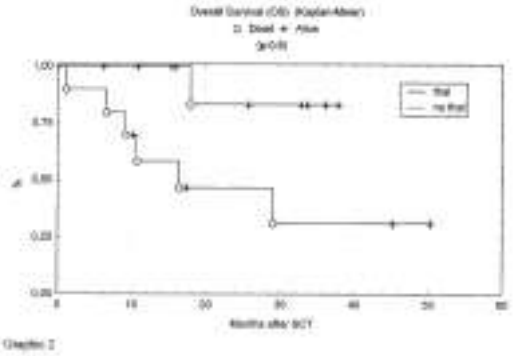
High dose therapy with single or double transplantation (auto-SCT) has improved prognosis of multiple myeloma (MM). New drugs are promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is been investigated as first line therapy. It could be useful in the control of minimal residual disease. We used thal as maintenance after autologous transplantation (single or double) and compare the outcome with other maintenance or none. From January 2001 to January 2005 20 patients (10 males and 10 females) with MM have been treated in our institution. Median age was 58 years (range 51-72). 9 were IgG, 6 IgA, 2 light chains and 2 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 10/20 performed double auto-SCT. 3 months after SCT, 10 patients (5 single and 5 double SCT) began thal 50 mg/die as maintenance therapy. 10 patients (5 single and 5 double SCT) received IFN-g (3/10), dexa (3/10) or no therapy (4/10). The 2 groups were regarding the type of myeloma: 5 IgG, 2 IgA, 2 light chains and 1 plasma-cell leukaemia

in the thal group; 4 IgG, 4 IgA, 1 IgD and 1 plasmacell leukaemia in the other. Response to SCT: 2 CR and 8 PR in the thal group; 5 CR, 4 PR and 1 NR in the other. In the thal group 3/10 patient relapsed. Median follow up from the beginning of maintenance therapy was 21 months (range 7-35) with 7/10 patients in CR or stable disease, with a progression free survival (PFS) and overall survival (OS) projected at 35 months of 70% and 83% respectively. In the other group, 8/10 patients relapsed. Median follow up was 30 months (range 5-50) with a median PFS of 8 months and OS projected at 50 months of 30%. The difference between the 2 groups is statistically significant for PFS (p: 0,01), and not significant for OS (p: 0,6) even if difference (80% vs. 30%) appears clear. (Graph. 1-2). Thal was administered for a median period of 6 months, being neurological toxicity the main reason of suspension (3/10 patients). Neurological toxicity grade I-III was present in 65% of patients, while haematological toxicity grade I occur in 55% of patients. In conclusion, in a small number of patients low dose thal as maintenance after auto-SCT resulted in an improved PFS and OS when compared with other or none maintenance, with acceptable toxicity. Further studies in larger cohorts and randomized trials are needed to confirm this experience.





Myeloma and Plasma Cell Dyscrasias IV

P564

MULTIPLE MYELOMA WITH RENAL INVOLVEMENT AT DIAGNOSIS. A RETROSPECTIVE REVIEW OF 34 CASES

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Background. Renal involvement at diagnosis is a poor prognostic factor in multiple myeloma (MM). In recent years high dose chemotherapy followed by autologous stem cell transplantation has improved the prognosis of these high risk patients.

Aim of the work. We have retrospectively reviewed patients affected by MM, whose diagnosis was made in our two divisions from 1980 to 2003; patients whose creatinine level was over 3 mg/dL at diagnosis were considered.

Results. Thirty-four patients were found. 22 were males and 12 females. Their mean age was 64.8 (range 39-85). Mean creatinine level was 5.46 mg/dL (range 3-13.2). Mean haemoglobin level was 9.1 g/dL (range 6.1-13.5). Thirteen patients had hemoglobin level < 8.5 g/dL, 12 patients had hemoglobin between 8.5 and 10 g/dL, while 9 had hemoglobin > 10 g/dL. Mean serum calcium level was 11.6 mg/dL (range 6.6-18.6); twelve patients had calcium level over 12 mg/dL. Mean bone marrow plasmacell infiltration was 50.6 % (range 20-95). Fourteen patients had a Bence-Jones MM, nine patients a IgA, one patient a IgD and ten a IgG myeloma. Twenty-eight patients had a stage III myeloma according to Salmon, while six patients had a stage II myeloma. Eleven patients are alive with a mean follow-up of 33.6 months (range 15-109); twenty-three patients died after a median of 16 months (range 1-107). Up to ten years ago, patients were treated with melphalan and prednisone or with alkylating-containing regimens. Three patients died before any chemotherapy could be begun. In recent years, VAD-like regimens have become standard therapy. Six patients had received an autologous stem cells transplantation; four of them are alive after a median of thirty-eight months.

P565

INEFFICACY OF NOVEL BIOLOGICAL AGENTS IN EXTRAMEDULLARY RELAPSES OF MULTIPLE MYELOMA

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Background. Despite the recent experiences about the efficacy of novel biological agents for treatment of extramedullary relapses of Multiple Myeloma (MM), we report the case of a 57-year-old male patient affected by

refractory stage IIIA light chain MM, who developed extramedullary disease progression during treatment both with thalidomide and bortezomib, though a good haematological response and significant decrease of the original urinary monoclonal paraprotein.

Case report. In May 2001 our patient received standard chemotherapy according to VAD-like protocol and radiotherapy to the right mandible for extramedullary disease; thereafter, because of stable disease, he was treated with cyclophosphamide (1 gr/m^2, days 1 and 3 i.v.) and dexamethasone (40 mg, days 1-3 i.v.). In November 2001 the patient, in haematological remission, underwent local radiotherapy on the pelvis for 1 masse involving the right wing of the ilium. In April and August 2002 the patient received double autologous PBSC transplantation, after conditioning regimen with 200 mg/m^2 Melphalan. In November 2003 the patient relapsed with extensive skeletal disease and increase of M-protein but without any evidence of extramedullary disease; bone marrow biopsy revealed 50% plasma cell involvement; therapy with thalidomide and dexamethasone was started. In April 2004 Magnetic Resonance Imaging (MRI) of the thorax showed a mass 10x10 cm arising from 5th ring, despite haematological remission; local radiotherapy was performed after stopping thalidomide and dexamethasone treatment. In October 2004 because of a new haematological relapse (bone marrow malignant plasma cell rate = 40%) treatment with bortezomib at the dose of 1.3 mg/m^2 according to conventional scheduled treatment was started. In January 2005, after the 4th cycle of treatment, the patient suddenly presented convulsion and loss of consciousness. Cerebral MRI showed abnormal enhancement of the meninges of the left cerebral hemisphere and into left frontoparietal site. The cytological examination of the cerebrospinal fluid revealed atypical plasma cells; the immunophenotypic analysis showed expression of CD38 and CD138 on cells obtained from cerebrospinal fluid. Aggressive systemic and intrathecal treatments together with craniospinal irradiation did not modify any neurological sign and the patient died in March 2005.

Discussions. Our clinical experience extends the very small number of published cases on extramedullary disease progression of MM during treatment with bortezomib and suggests that treatment with novel biological agents does not prevent and does not cure extramedullary relapses. According to Juliusson the differences in microvascular supply of marrow and the extramedullary tumor might explain the different effects of thalidomide. Regarding bortezomib, experimental studies, conducted in non-human primates by i.v. administration of radiolabeled [14C]PS341, report a reduced distribution of bortezomib in some organs and tissues (skeletal muscle and skin) as well as its difficulty to penetrate the brain, spinal cord, testes and eye. Kropff has recently described extramedullary disease progression during bortezomib treatment in 2 patients with normal bone marrow, hypothesizing "a bone marrow restricted activity of bortezomib"; contrarily, Patriarca reports the efficacy of bortezomib for extramedullary relapses of MM including CNS involvements after autologous and allogeneic transplantation.

Conclusions. Our data do not support the use of novel biological agents alone for the treatment of extramedullary disease progression. Further experiences, from other groups with larger series of patients, examining the role of novel biological drugs specifically on extramedullary relapses including CNS involvements are needed.

P566

MAXILLARY OSTEONECROSIS AND BISPHOSPHONATES

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The widespread use of chronic bisphosphonate (bp) treatment in metastatic disease to the bone and in the treatment of osteoporosis has been recently associated to increased risk of oral osteonecrosis. The largest number of patients (99) was described so far in two reports (Ruggiero, J Oral Maxillofac Surg 62: 527-534, 2004; Marx, J Oral Maxillofac Surg 61: 1115-1118, 2003), which mainly included myeloma patients (46%), receiving or having received chemotherapy (without local radiotherapy) and prolonged bp treatment, mostly pamidronate or zoledronate. In the majority of cases (83%), local surgical procedures preceded the onset of refractory osteomyelitis of the jaw, not healed by medical conservative treatments, and often requiring major surgical procedures. Histologic examination of the lesions ruled out metastatic diseases and showed necrotic bone with signs of avascular necrosis and bacterial infection. We report 7 cases (all females) of this heretofore rarely reported adverse event, occurred in the last three years in myeloma patients followed in four Departments of Hematology in the Campania region. Patients' mean age at diagnosis was 69 yrs (r 58-76). Six patients were in chemotherapy (MP, VMCP); in one case bp was the only drug administered. Mean number of bp administrations was 25 (r 14-37). Local procedures were traceable in 4/7 cases (dental extraction). Bone damage was in the maxilla (2/7) or in the mandibula (6/7). Histology excluded plasma cell localization in all cases. Zoledronic acid was administered in all cases but one, who was initially treated by pamidronate (13 doses) and then shifted to zoledronate. Bp were stopped soon after the onset of the adverse event; antibiotics and debridement of oral cavity were the therapeutic procedures adopted. Several hypotheses have been raised to link the use of bp to the onset of this peculiar complication: i) osteoclast blockage and consequent deficient bone renewal; ii)osteoblast inhibition; iii)antiangiogenic activity. From an epidemiological point of view, two factors appear significantly associated to increased risk of oral osteonecrosis in these patients: prolonged bp administration and concomitant local events. Hence, while waiting for a better definition of risk criteria for this severe complication, it seems reasonable to interrupt bp administration before surgical oral procedures and to avoid bp administration in patients with concomitant jaw osteopathy. Further attention should also be paid to continuous prolonged bp administration.

P567

OUR EXPERIENCE IN THE TREATMENT OF LIGHT CHAIN DISEASE

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Light chain disease (LCD), first described by Randall in 1976, is characterized by tissue deposits of monoclonal LC . Kappa chain deposition occurs in 85% of cases, lambda in 15%, but mixed deposits may also occur. LCD is usually diagnosed in association with plasma cell proliferation or other lymphoproliferative diseases, but one third of cases occur in absence of overt hematologic disorder. Kidney is the main target of LCD, as pivotal site of LC clearance. However, other localizations may involve liver, spleen, nodes or heart, and greatly affect disease evolution. Renal damage is common and may cause massive proteinuria (30%) or progressive renal failure (70%).

Diagnosis of LCD may be difficult and can be suggested by renal abnormalities of unknown origin in course of myeloma or monoclonal gammopathy. Formal diagnosis is based on the demonstration of LC deposits in renal biopsy by immunofluorescence, as histologic features by standard stains may overlap with diabetic glomerulosclerosis or membrane- proliferative glomerulonefritis. Differential diagnosis may benefit by electron microscopy, which shows linear electrondense granular deposits along basal glomerular and tubular membranes, as well as in arterial vessel walls. Natural history of LCD is characterized by rapid progression, leading to dialysis in case of renal disease and death when multiple organs are involved.

LCD therapy is not yet addressed by common guidelines, and treatment is based on single institution experience. Treatments often include chemotherapy and may control disease progression or even achieve clinical improvement. In the last three years we have followed four patients affected by LCD with renal involvement: the table shows main clinical features and outcome.

We treated by chemotherapy only patients in whom a clinical improvement could be expected. Hence, dialytic patients without further visceral complications do not usually receive chemotherapy. Cases with minor kidney damage are treated by courses of melphalan and prednisone, if older than 65-70 yrs, or courses of high dose dexamethasone +/- adriamycin if high dose chemotherapy plus stem cell support is programmed in case of systemic progression.

P568

TISSUE DOPPLER ULTRASONOGRAPY FOR CARDIAC AMYLOIDOSIS

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Tissue Doppler (TD) is an ultrasound tool of growing clinical application, since it allows quantification of Doppler shift within the range of myocardial tissue motion. It can provide quantitative information about myocardial diastolic and systolic function. Few information is available about TD parameters in patients with primary amyloidosis (AL). Aim of the present study was to characterize TD patterns as expression of ventricular longitudinal function in patients with AL, in relation to a healthy control group. The study population included 10 patients with AL at diagnosis and 10 normal subjects. Diagnosis of AL was confirmed by histological evidence in 8 patients, while in two patients the echocardiographic evidences were highly evocative of this disease. All the participants underwent standard Doppler echocardiography and pulsed TD, by placing sample volume at the level of posterior septum, mitral lateral annulus and tricuspid lateral annulus (apical 4-chamber section). Myocardial systolic and diastolic velocities (Myocardial early diastolic velocity -Em-, myocardial atrial velocity -Am- and their ratio) and time intervals (relaxation time [RTm], pre-contraction and contraction time) were measured. The ratio between Doppler transmitral E velocity and TD-derived Em of mitral lateral annulus (E/Em) was calculated as an index of left ventricular (LV) end-diastolic pressure. Age (59±6.8 years in AL vs. 58.1± 5.7 years in controls), blood pressure (125/75.2± 18.7/10.9 mmHg vs. 120/73 ± 10.5/6.7 mmHg) and heart rate (78±10.8 bpm vs. 67.5±17.3 bpm), were comparable between the 2 groups. Also LV ejection fraction (55±22% vs. 63±7.4%), E/A ratio (1.6±0.9 vs. 0.9± 0.29) and E velocity deceleration time (191.8±46.7 vs. 203.6±33 msec) did not differ. LV mass was higher in AL than in controls (267.6±62.0 g vs. 161.4±44.2 g, $p<0.001$). Among TD measurements, Em and Sm peak velocity were lower in AL patients, at septum level (respectively 0.05±0.2 vs. 0.05±0.01 m/sec; 0.08±0.1 m/sec vs. 0.10±0 3 m/sec: both $p<0.02$) and at mitral annular level (0.05±0.01 vs. 0.07±0.01 msec $p=0.04$; 0.05±0.01 vs. 0.07±0.01 m/sec $p=0.03$). The differences were not significant at the tricuspid annulus. Myocardial time intervals were not significantly different between the two groups at any assessed level. LV E/Em ratio was 16±6.3 in AL and 7.6 ± 1.9 in controls ($p<0.01$).

In conclusion, a reduced Em peak velocity at septal and mitral annular levels allowed to detect a sub-clinical myocardial diastolic dysfunction in AL patients, while standard Doppler indexes were still within normal range. LV E/Em ratio was higher than in controls, indicating an early increase of LV end-diastolic pressure and proposing itself as a reliable index to evaluate the progression of LV involvement. Right ventricle did not appear to be involved at diagnosis.

P569

B-FGF, IL-6, TNF-ALPHA AND VEGF SERUM LEVELS IN NEWLY DIAGNOSIS MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE, DEXAMETHASONE AND ZOLEDRONATE

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The observation that increased bone marrow angiogenesis correlates with advanced phases of multiple myeloma (MM), along with the well documented in vitro anti-angiogenic activity of thalidomide has provided the rationale of the investigational use of this agent in patients with advanced refractory MM and, more recently, with newly diagnosed disease. The aims of the present study were to investigate the relationship between serum concentrations of angiogenic cytokines and both baseline characteristics and response to therapy in a series of 96 patients with symptomatic MM who were enrolled in the "Bologna 2002" clinical study. All patients were previously untreated and received four months of therapy with thalidomide (200 mg/d), dexamethasone (40 md/ for 4 days, repeated monthly) and zoledronic acid (4 mg/month) in preparation for subsequent autologous transplantation. Serum levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) were measured at baseline and therapy. For this purpose, laboratory assays were performed using quantitative sandwich ELISA (manufacturer Pierce Endogen and R & D Systems). At baseline, serum levels of bFGF and IL-6 were significantly higher in MM patients than in healthy controls (p<0.0001 and p= 0.04, respectively). We found that serum levels of IL-6 were closely related with both TNF-alpha and VEGF levels (p<0,0001 and p=0,0142); serum levels of VEGF were significantly correlated with platelet count (p=0,0015). On an intent-to-treat basis and using stringent criteria (EBMT criteria), the overall response rate (> or = partial response) to thalidomide-dexamethasone was 85%; 31 patients (32%) showed at least a very good partial remission (VGPR). Among responders, there was no significant change in pre-treatment and post-treatment plasma levels of angiogenic cytokines. In the group of patients who attained at least a VGPR, post-treatment bFGF levels showed a trend towards a reduction in comparison with baseline, but the difference was not statistically significant (p=0,08). Additionally, after therapy we observed a significant increase in the levels of VEGF (pre-treatment mean levels 247,66 pg/mL versus post-treatment mean levels 403,10 pg/mL, p<0,0001), without any difference between responders and non-responders. The limited number of patients who failed to respond to thalidomide prevented any formal comparison between responders and non-responders with respect to baseline plasma levels of angiogenic cytokines. Further research in understanding the role of angiogenesis and angiogenic cytokines in newly diagnosed MM, and more properly clarifying their relationship with primary thalidomide therapy is needed.

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P570

PERCUTANEOUS VERTEBRAL BODY PLASTY IN PATIENTS WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) is characterized by osteolytic lesions causing skeletal destruction with bone pain and pathological fractures mainly with vertebral involvement. Medical treatments including chemotherapy, bisphosphonates analgesic drugs, and radiotherapy are often not effective in pain control. Vertebroplasty and kyphoplasty represent the main techniques performed for treatment of sever pain in these cases. The first one is employed to reinforce and stabilise vertebral fractures, whereas the second has the advantage of reexpanding the collapsed vertebra and restoring the anterior and midvertebral height. The most frequent complications of these procedures are cement leakage in the epidural or fora area and pneumonia or venous thromboembolism.

We report the outcome of 15 procedures (8 kypho and 7 vertebroplasty) in 13 patients affected by MM with vertebral body fractures documented by MRI or spiral CT scan and pain refractory to conventional medical therapy referred to our Unit from September 2003 to December 2004. Patients characteristics were: median age 55 years (range 49-72), 9 male/4 female, 12/13 patients were in stage IIIA and 1 in stage IIA, 10 IgG (7 kappa and 3 lambda) and 3 Ig A (2 kappa and 1 lambda). 4 patients were at diagnosis, 6 in chemotherapy treatment, 1 after autologous transplantation, 2 relapsed after allogeneic stem cell transplantation. The median pre-treatment Karnofsky performances status and VAS-score (Visual Analog Scale Pain Scores) were 50 (range 30-70) and 9 (range 8-10) (0 no pain - 10 maximal pain), respectively. The levels involved ranged from T6 to S1; the maximum number of levels treated in one patient was three. The procedures were performed via a percutaneous extrapedicular or transpedicular approach using local anaesthesia and light sedation. At 12 hours from the procedure all patients were controlled with spiral CT scan, and were discharged from hospital the day after the surgical treatment. All patients reported marked pain relief as soon as 24 h after the procedure; Karnofsky grade and VAS score were 70 (range 60-90) and 3 (range 2-5), respectively, at 24 hours and at 1 month after treatment. No major complication was observed. With a median follow–up of 8 months (range 1-16), all 13 patients tolerated well the procedure with an effective pain control.

P571

IN MULTIPLE MYELOMA RESPONSE TO PRIMARY THERAPY WITH THALIDOMIDE AND DEXAMETHASONE IS NOT ADVERSELY AFFECTED BY T(4; 14) AND/OR CHROMOSOME 13 ABNORMALITIES

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Multiple Myeloma (MM) is a plasma cell (PC) malignancy characterized by a profound genomic instability. Recently, several recurrent genomic abnormalities have been identified; these abnormalities define specific subgroups of patients with MM and are supposed to be involved in the pathogenesis of the disease. In this context, it has been reported that one of these subgroups, involving at least one-half of patients, carries at diagnosis translocations involving the immunoglobulin heavy-chain (IgH) locus and/or deletion of chromosome 13 (del(13)). Among IgH translocations, t(4;14)(p16;q32) is one of the most commonly reported. Both del(13) and t(4;14) are associated with a poor prognosis. Translocation t(4;14) results in the production of a chimeric fusion transcript between MMSET and IgH, and, in about 70% of t(4;14) positive cases, it leads to the activation of the fibroblast growth factor receptor 3 (FGFR3). In the present study we investigated the frequency of t(4;14) and del(13) in a series of 52 previously untreated patients with symptomatic MM who were enrolled in the "Bologna 2002" clinical study and received first-line therapy with thalidomide and dexamethasone in preparation for subsequent autologous transplantation. The relationship between these chromosomal abnormalities and response to treatment was also analyzed. For this purpose we isolated the CD138+ plasma cell fraction from the bone marrow taken at diagnosis from patients who entered the study. We analyzed 1) the presence of t(4;14) by RT-PCR of the hybrid transcript IgH/MMSET, 2) the over-expression of FGFR3 by Real-time RT-PCR, and 3) the presence of del(13) by FISH analysis. The relationship between these two chromosomal abnormalities and response to thalidomide-dexamethasone was also investigated. Translocation t(4;14) was detected in 15/52 patients (29%). Among these patients, 10/15 (67%) displayed both IgH/MMSET hybrid transcripts and FGFR3 over-expression, thus supporting the discrepancy between IgH/MMSET positivity and FGFR3 over-expression. Deletion del(13) was detected in 19/47 patients who could be evaluated (40%). Patients with translocation t(4;14) were more likely to carry also del(13) than t(4;14) negative patients (64% vs.31%, respectively; p=0.05). Importantly, patients with translocation t(4;14) and/or del(13) had the same probability to respond to thalidomide-dexamethasone (76%) than patients who lacked these unfavourable abnormalities (70%). In conclusion, using a RT-nested PCR assay designed to detect IgH/MMSET hybrid transcripts as an indicator of translocation t(4;14) we found that this karyotipically cryptic chromosomal abnormality could be demonstrated at the onset of the disease in 29% of cases. This frequency was higher than that previously reported by others using either FISH or RT-PCR, probably as a result of the high sensitivity of our RT-nested PCR assay At the opposite, the presence of del(13) (40%) was in accordance with previous data from the literature and, consistently with other reports, was closely associated with translocation t(4;14) (63% of cases). Remarkably, neither translocation t(4;14) nor del(13) had an adverse influence on response to first-line therapy with thalidomide-dexamethasone. This effect is worthy of note, because del(13) was previously shown to be a negative prognostic factor for response to thalidomide in patients with advanced refractory/relapsed MM and a prior analysis of our group translocation t(4;14) had an adverse influence on response to high-dose therapy and subsequent autologous transplantation. Combined thalidomide-dexamethasone can thus be considered as a valid treatment option in preparation for autologous transplantation even for patients with poor prognosis chromosomal abnormalities.

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P572

MELPHALAN AND DEXAMETHASONE AS FIRST LINE TREATMENT OF ELDERLY MULTIPLE MYELOMA PATIENTS

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Multiple myeloma (MM) is an incurable disease that since 1960 has been treated with a combination of alkylating agents and prednisone. Despite the new drugs that we are testing, this combination is still a gold standard for the management of MM patients older than 65 years or for those patients who cannot undergo high-dose chemotherapy followed by allogeneic or autologous stem cell transplantation. In order to improve the results obtained using melphalan and prednisone and considering the good results obtained, by other investigators, using dexamethasone alone, we treated MM patients not eligible for high dose chemotherapy programs or older than 65 years with melphalan (10 mg/m^2/die x 4 days) associated to dexamethasone (20 mg/die: 1-4 days and 15-18 days) for six cycles every 28 days. Between October 1991 and August 2004, 101 consecutive patients were treated with this protocol at the Hematology of the University "La Sapienza" of Rome. The diagnosis of MM was made using the criteria and staging of Durie and Salmon. Forty-nine patients were men and 52 women, median age 67 years (range 42-78), 66 patients had IgG, 24 IgA, 6 light chains and 5 non secretive MM. At diagnosis according to Durie-Salmon classification 10 patients were in Stage IA (9 plasmocytoma and 1 amyloidosis), 1 IB, 38 IIA, 2 IIB, 43 in IIIA, and 7 in IIIB. The response defined as reduction > 25% of the initial monoclonal component value associated with disappearance of the clinical symptoms was obtained in 79 patients: 27 (34%) had a reduction of the initial monoclonal component > 75%, 31 (39%) > 50% but < 75% and 21 (27%) > 25% but < 50%. At present 39 patients are alive and 21 are

progression free. The overall median response duration was 33 months, the event free survival was 25.5 months and the median overall survival was 56 months. No severe (Grade 3-4 of the WHO) haematological as well as non haematological toxicities were observed. The lack of severe toxicity allowed us to administer the drugs on an outpatient basis. In conclusion these encouraging results suggest the need of a randomised prospective trial to evaluate the efficacy of melphalan plus dexamethasone in MM patients.

P573

A CASE OF SEVERE SYSTEMIC AMYLOIDOSIS ASSOCIATED WITH MICROMOLECULAR MYELOMA

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Patients with unexplained heart failure, hepatomegaly, nephrotic syndrome, or peripheral neuropathy should be evaluated for primary systemic amyloidosis (amyloid light-chain, or AL) by seeking evidence of a clonal plasma cell disorder with serum and urine immunofixation studies. Although the presence of amyloidosis may be suggested by the anamnesis, symptoms and noninvasive tests (such as serum amyloid P component scintigraphy), histological diagnosis is established by biopsy of an affected organ or by abdominal fat biopsy. It has been reported that abdominal fat biopsy is often the diagnostic procedure of choice in the early evaluation of suspected amyloidosis, since causes minimal patient discomfort and is accompanied by virtually no risk of complications. A positive result has a high predictive value of amyloid disease (100%) in patients of unknown clinical status, but the procedure has a low sensitivity (57%).

We describe a case of an 80 years old woman with a typical clinical presentation but negative fat pad biopsy. Ten months before admission she had a syncope due to a supraventricular arrhythmia of unexplained origin and a diastolic dysfunction was found on ultrasonography examination. Subsequently she was admitted to another hospital for asthenia, weight loss and peripheral edema. Blood chemistry revealed mild cholestasis and reduced hepatic function, electrophoresis showed hypogammaglobulinemia, and bence-jones kappa light chains positivity with mild proteinuria on urine analysis. There was no history of alchool abuse, markers of viral infection, ANA, AMA, ASMA, anti-LKM were negative. Ultrasonography showed hepatomegaly with disomogeneous echogenicity without focal lesions, cholangio-RMN revealed no dilatation of the biliar tract, and X-ray ruled out bone lytic lesions. Echocardiography revealed severe systolic disfunction, with symmetric left ventricular wall thickening, small ventricular chambers and bilateral atrial dilatation. Abdominal fat biopsy was performed and stained negative with Congo Red. During two months of hospitalization her clinical condition progressively worsened, a bone marrow biopsy was then carried out and revealed 30% of monoclonal K light chains plasma cells, allowing the diagnosis of micromolecular myeloma. At the admission to our Department she was dyspnoic, with generalized edema and jaundice (blirubin=6,3 mg/dL), and despite of a course of high dose dexamethasone (20 mg for 4 days), the general conditions rapidly deteriorated and she died for cardio-respiratory failure. Autopsy revealed massive amyloid infiltration of heart, liver, spleen, kidney and adrenal glands. This case confirms that the clinical grounds are very important for a correct diagnosis and that unexplained signs of heart failure together with epatomegaly and progressive hepatic failure are important signs to suspect systemic amyloidosis. Moreover, abdominal fat pad biopsy has very low sensitivity and early biopsies of suspected involved organs are strongly recomanded in order to estabilish soon specific therapies such as those including high dose chemotherapy with autologous hematopoietic stem cell transplantation, oral melphalan and prednisone or 4'-iodo-4'-deoxydoxorubicin.

P574

THE ROLE OF [18F] FDG PET AND [99MTC] MIBI SCINTIGRAPHY IN PREDICTING THE CLINICAL COURSE OF PATIENTS WITH MULTIPLE MYELOMA

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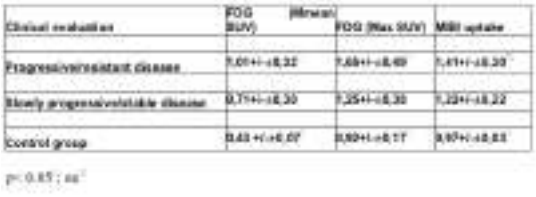
Methods. Between March 2002 and May 2003, 24 patients (pts) with MM, 18 males and 6 females, were enrolled. All the pts were evaluated before therapy. All the 24 pts underwent PET examination and MIBI scanning. PET images were acquired 45 minutes after injection of a mean activity of 370 MBq. Whole body planar scintigraphy was performed within a week from the PET study and 30 minutes after the injection of 740 MBq of MIBI. The mean and maximum Standardized Uptake Value (SUV) and MIBI target-background ratio were calculated by means of Region of Interest (ROIs) positioned on the femura and thighs, in order to assess the involvement of bone marrow. The uptake of both tracers was calculated also in 19 control pts: 13 pts with PET and 6 with MIBI myocardium scintigraphy.



Results. After a mean follow-up of 21 months, 14 pts had conventional chemotherapy (CT) and 6 received high dose CT with autologous peripheral blood stem cell (PBSC) infusion; among these latter, two had an allogeneic PBSC transplantation with low dose conditioning regimen. Four pts haven't been treated with any CT. Six pts died, five because of MM and one for unrelated reasons. We observed that FDG and MIBI uptake was significantly greater in our pts in respect to control subjects. In this connection, the uptake of FDG was significantly greater in the bone marrow of pts with subsequent rapid clinical pro-

gression, or resistant-to-therapy disease, than in pts with slowly progressive/stable disease (see Table). A greater MIBI uptake was also noted in the former group of patients in whom six deaths were observed. In conclusion, PET and MIBI scintigraphy seem to be useful in predicting clinical evolution of MM pts.

P575

PREDICTIVE VALUE OF MINIMAL RESIDUAL DISEASE QUANTITATIVE DETECTION IN MULTIPLE MYELOMA PATIENTS IN COMPLETE REMISSION AFTER STEM CELL TRANSPLANTATION

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Molecular evaluation of minimal residual disease (MRD) following high-dose therapy and stem cell transplantation (SCT) for Multiple Myeloma (MM) can be performed by means of patient-specific PCR for IgH rearrangement. Attainment of sustained molecular complete remission (MCR) was previously reported to offer an advantage in terms of prolonged event-free survival and reduced risk of relapse in comparison with persistence of molecularly detectable MRD. Within this latter group the use of quantitative methods of molecular analysis could be of value in identifying patients with different residual tumor burden and, consequently, different risk of relapse.

In the present study we developed a simple and reliable assay to quantify MRD in bone marrow samples obtained from patients who received either autologous or allogeneic SCT for symptomatic MM.

By using Real-time PCR, we used SYBR Green chromophore as "universal probe" and we adopted the comparative Ct method to determine the amount of MRD. More specifically, the number of IgH copies detected in the diagnostic sample was set to 100% and the value of the post-transplant samples was given as a percentage of the sample taken at baseline (IgH ratio).

We performed a retrospective quantitative analysis of 71 BM samples from 12 patients; all of whom were persistently PCR positive by qualitative monitoring of MRD. With a median follow-up of 24 months, 4 patients out of 12 showed signs of relapse after 20 to 52 months (1 after an allogeneic SCT and 3 following an autologous transplantation), while the remaining 8 patients were still in CR (1 after an allogeneic SCT and 7 following an autologous transplantation) after 35 to 69 (median 57 months). Results of our analysis showed a statistically significant difference in IgH ratio values between patients who relapsed and those who remained stable (mean ln IgH value = -0.81 vs. 0.86, respectively; p = 0.018). In order to obtain a cut-off value that could predict for relapse, we subsequently evaluated our results by applying a Bayesian approach. The objective of this analysis was to obtain a Positive Predictive Value (PPV), which is the likelihood of relapse for an individual patient with a positive test. A cut-off value of 2.1 was identified. The sensitivity of our test was 100%; in fact all relapsed patients overcame the cut-off IgH value. Nevertheless, the specificity of the test was 75%, as two patients in CR overcame at least once the cut-off value (resulting false positive): therefore, the PPV was 0.67, which actually means that the probability of relapse will be 67%, when the IgH cut-off value has been overcome during follow-up. These results need to be validated by the analysis of a larger series of patients. If confirmed, IgH ratio could provide a simple and reliable method for predicting early relapse after SCT for MM.

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P576

CUMULATIVE INCIDENCE OF PROGRESSION TO LYMPHOPROLIFERATIVE DISEASE IN 198 IGM MONOCLONAL GAMMOPATHIES OF UNDETERMINED SIGNIFICANCE WITH A LONG FOLLOW-UP

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Approximately 1% of person aged >50 years as well as 3% of those > 70 years may have a monoclonal gammopathy of undetermined significance (MGUS) . Of these, the majority are of IgG or IgA type while MGUS of IgM are less frequently observed. The aim of this study was to analyse the the risk of progression to lymphoproliferative disorders of IgM MGUS . From July 1973 to June 2004, 323 monoclonal gammopathies of IgM type were observed at the haematology of the University "La Sapienza" of Rome. At diagnosis, 198 (61%) were MGUS, 112 (34%) were WM, 8 (2,4%) were non Hodgkin Lymphoma (NHL) 4 (1,5%) were IgM Myeloma and only 1 had Chronic Lymphatic leukaemia. Of the 198 MGUS, 125 (63%) were men and 73 (37%) were women (ratio 1.71); median age 65.7 (range, 33-89 years) . At diagnosis, all patients had an IgM M protein concentration less than 2 gr/dL with a median concentration of 1.7 g/dL. Median haemoglobin, serum creatinine and albumin levels were 13.2 gr/dL(range 6,9-17.9), 1.04 mg/dL (range 0.6-2.5) and 3,9 gr/dL (range 3.4-5) respectively. Hepatomegaly and splenomegaly were present in 24 % and 14% of patients, respectively. These 198 patients were monitored for 8741 person-months; during the follow-up, a lymphoproliferative disorder developed in 17 (8.5%) patients. In particular, 11 patients (5.5%) evolved in WM and 6 (3%) in NHL. Progression incidence to lymphoproliferative disorder was 0.001 months/person; cumulative incidence of progression to lymphoproliferative disease was 6.8% at 5 years, 23,2 % at 10 years and 45.5 % at 15 years. The results, at 5 and 10 years, are more similar to those reported by Morra et al (Leukemia 2004;18:1512-1517) with a cumulative probability of evolution to lymphoid malignancy at 5 and 10 years of 8% and 29% in asymptomatics IgM monoclonal gammopathies than to those obtained by Kyle et al (Blood 2003;102:3759-3764) who observed a cumulative incidence of progression of 10% at 5 years, 18% at 10 years and 24% at 15 years . These differences, as for the cumulative incidence of pro-

gression to lymphoproliferative disease among Italian and USA IgM asymptomatic monoclonal gammopathies, may be related to geographical unknown factors.

P577

INTERMITTENT LOW DOSES OF THALIDOMIDE IN THE MAINTENANCE TREATMENT OF MULTIPLE MYELOMA AND PERIPHERAL NEUROPATHY

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Background. The role of thalidomide in previously treated, refractory or relapsed myeloma patients is well known, as reported by other studies. However, sometimes peripheral neurophaty limits its clinical use.

Casistics. Thirty patients with refractory/relapsed MM (22 female, 8 male), aged from 53 to 81 yrs, were treated with conventional escalated doses of thalidomide. Of the 30 patients, 19 obtained a clinical response (4 CR, 15 PR); among responders, 8 patients (2 CR, 6 PR) had clinical signs and electrophysiological evidence of peripheral neuropathy, varied from mild to moderate, such as we introduced a reduced schedule of thalidomide:100 mg daily 10 days a month. In 2 out of 8 patients (25 %) the diagnosis of neuropathy was performed before starting thalidomide standard therapy with a progression of the electrophysiological data after 5 months. In the other 6 patients the neuropathy began from 7 to 14 months after treatment. The first clinical symptoms were painful paresthesias and numbness in all patients. At neurological examination ipopallestesia and ipo-areflexia at lower limbs were the only clinical signs; none of the patients showed weakness at the neurological examination. In 5 out of 8 patients an axonal sensory-motor neuropathy was found; in 3 patients a pure sensitive neuropathy, more evident at lower limbs, was detected.

Conclusion and discussion. In the subsequent 6-31 months follow-up evaluation, none of the patients treated with intermittent low doses of thalidomide developed progression of electrophysiological findings. Two of them, however, had to discontinue thalidomide therapy for severe painful paresthesias. Three out of 8 patients in PR, had to stop therapy because of progression MM (1,5,10 months). Low doses of thalidomide may be applicable in the maintenance treatment of myeloma patients, as its clinical response and stable neuropathy.

P578

CHROMOSOME 13 ABNORMALITIES IN MULTIPLE MYELOMA PATIENTS: IMPACT ON PROGNOSIS AND SURVIVAL

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Chromosome 13 abnormalities (del 13q or monosomy) have been associated with poor prognosis in patients affected by Multiple Myeloma (MM). Indipendently from the method of detection (karyotype versus FISH) or the type of treatment (standard versus high-dose chemotherapy), these abnormalities are associated with lower response rate and shorter survival. Moreover, they have been found also in monoclonal gammopathy of uncertain significance (MGUS), suggesting that they are an early genetic event, even thought it is not clear if they have a role in disease progression or represent only an initiation event. In our Institution, 18 MM patients (9 at diagnosis, 6 at relapse and 3 during follow-up post CHT) were studied by conventional cytogenetics and interphase FISH analysis. The mean age was 62,5(range 43-77), with male prevalence.The class of the monoclonal component was IgG in 12 and IgA in 3 patients; 3 patients had light chain disease. At the time of analysis, the stages were: I/II (n=7), IIIA (n=5)and IIIB (n=6). At the time of cytogenetic analysis, mean of bone marrow plasma cells percentage was 59,4% (range 6-95%). Cytogenetic and FISH analysis were performed simultaneously. Among the 18 patients, an abnormal karyotype was detected in 5/8 successful cultures: 2 patients showed chromosome 13 deletion, one patient monosomy 13 and two showed hypodiploid karyotype. FISH analysis was informative in all 18 patients : all five patients with abnormal karyotype had del(13q); in addition, five patients with normal or non informative karyotype showed del(13q) by FISH analysis. The mean number of cells carrying del(13q) was 34% (range 15-80%), probably reflecting tumor heterogeneity; the highest values were found in patients who had hypodiploid karyotype or del(13q) or chromosome 13 monosomy by conventional cytogenetic analysis. Due to the small number of patients, the impact of chromosome 13 deletion on overall survival and time to progression was analyzed only considering the most sensitive technique (FISH) used. The cohort of patients carrying del(13q) had a shorter survival, even though the difference did not reach statistical significance. However, even with our small numbers, time to progression was significantly shorter in del(13q) patients (p=0.0398). Thus, we confirm the worse impact of chromosome 13 deletions on the outcome of MM patients. The usefulness of a sensitive technique for the assessment of the chromosome 13 status such as the FISH analysis has to be stressed in the context of clinical trials specifically designed for MM patients with adverse prognostic features.

P579

BISPHOSPHONATES AND JAW OSTEONECROSIS IN MYELOMA PATIENTS

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Introduction. Bisphosphonates are widely used in the treatment of cancer patients with hypercalcemia of malignancy and osteolytic bone lesions of multiple myeloma. Recently osteonecrosis of the jaw has been described in patients treated with intravenous bisphosphonates, pamidronate and zoledronate. We describe 3 cases of jaw osteonecrosis associated with intravenous bisphosphonates therapy for bone osteolytic lesions of multiple myeloma.

Case reports. Case 1. A 54- year- old woman who was diagnosed with multiple myeloma five years ago, was admitted to our Hospital because of pain in the left region of the mandible. In the previous years she had received chemotherapy with vincristine, melphalan, cyclofosfamide and prednisone (VMCP). In the last 16 months she only received intravenous zoledronate (4 mg iv, every 28 days). It was diagnosed osteonecrosis of mandibular body, left side 34, after a previous dental extraction. She was treated with sequestrectomy successfully. Case 2. A 71 year – old woman with a previous history of MGUS advanced then in multiple myeloma, had received chemotheraphy with melphalan plus prednisone (MP) (12 cycles) and pamidronate (90 mg iv, every 28 days) and then zoledronate (4 mg iv, every 28 days). During the treatment with zoledronate, she showed an osteonecrosis of the mandibular body, right side 47, after bone biopsy. The conservative treatment was a failure. Case 3. A 67 year – old woman with multiple myeloma, treated with chemotherapy with melphalan and prednisone MP (12 cycles), showed an osteonecrosis of mandibular body, left side 37, after dental extraction. At the time of the osteonecrosis she has been treating with pamidronate (90 mg iv, every 28 days) for two years. The sequestrectomy was not resolutive.

Conclusions. Most cases of jaw osteonecrosis reported in the literature concern cancer patients who are receiving intravenous bisphosphonates. These lesions occur especially after dental extractions. The jaw is particularly exposed to exterior environment through frequent gum lesions. Moreover the special jaw vascularization (especially terminal arteries) associated with antiangiogenic effect of bisphosphonates, may explain the avascular bone necrosis described in this bone, often after invasive procedures.

P580

CASE REPORT OF A PLASMACYTOMA ATIPICAL CLINICAL BEHAVIOR

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We describe an unusual case of bone plasmacitoma treated by conventional chemotherapy and double autologous peripheral blood stem cells transplantation who later relapsed with hepatic lesions. On may 2002 a 39 years old man presented bone pain not responsive to common analgesics. A CT scan revealed a large osteolitic lesion on bone pelvis.A bone biopsy documented the diagnosis of Plasmacytoma (CD79a weak positiviy,CD138,IgG and k chains positivity). M proteins was not detectable .Not bone marrow localizations neither other bone litic lesions were detectable. Patient received six courses of DAV conventional regimen followed by peripheral blood stem cells mobilization with Cyclophosphamide (7 grams /m^2) and double autologous peripheral blood stem cells transplantation. Radiotherapy on bulky lesion was added. On June 2004 a complete remission was obtained (documentated by CT ,MRI,PET). From June to October 2004 he was in good clinical conditions .In October 2004 a PET scan showed multiple bone lesions and ten intrahepatic lesions (from 1.2 to 5.3 centimeters of diameter). Histological examination by CT guided hepatic biopsy revealed the same immunophenotype of the previous disease . No other lesions neither bone marrow involvement was demonstrated. We decided to treat with two courses of DT-PACE and allogenic bone marrow transplantation from identical sibling donor. This case demonstrate an atipical course of Plasmacytoma resembling to the behavior of solid cancer with hepatic repetitive lesions without bone marrow involvement .

P581

EXTRA-MEDULLARY PROGRESSION IN A MULTIPLE MYELOMA PATIENT: DISCORDANT RESULTS OF PET-FDG AND TECHNETIUM-99M-MIBI IMAGING

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Clinical presentation of Multiple myeloma (MM) is higly variable spanning from asymptomatic disease to diffuse bone disease, renal failure, anemia and hypercalcemia. Median survival is about 3-5 years with a range varying from few weeks and more than ten years. Disease progression may also occurr at extramedullary sites, being often associated with a poorer clinical outcome. It is has been speculated that soft tissue involvement may take place more frequently in thalidomide refractory/relapsed patients. While different reports have underscored the role of either PET-FDG and technetium-99m-MIBI in evidencing soft tissues infiltration by neoplastic plasma cells, the differential sensitivity and specificity of each imaging technique are yet to be fully defined in the setting of extramedullary MM. We report the case of a MM patient whose disease progressed in soft tissues, without any evidence of MIBI uptake but with a very impressive PET-FDG positivity. A 75 year-old woman was diagnosed IgG Kappa stage IIIA MM in 1999. A good partial response, maintained for two years, was obtained after a standard Melphalan/Prednisone (MP) therapy. On December 2001, the patient showed disease relapse with the appearance of new bone lytic lesions and serum MC increase. Thalidomide and dexamethasone salvage at the standard dose of 200 mg daily of Thalidomide and 20 mg daily (d 1-4) of dexamethasone was then started. Disease stabilization was obtained after two months of therapy but treatment was

discontinued due to the onset of unacceptable toxicities including peripheral neurologic symptoms, constipation, nausea, tremor, dizziness and somnolence. The patient was therefore switched to 4 courses of vinorelbine (25 mg/m^2) plus dexamethasone 20 mg daily (d 1-4) plus monthly bisphosphonates. On September 2004, after 29 months of disease stabilization, the patient devoloped a solid mass on the left side of anterior chest wall involving a thoracic rib and spreading to contiguous soft tissues, as confirmed by CT. The technetium-99m-MIBI scan was totally unremarkable, while PET evidenced a strong FDG uptake limited to the left chest wall mass, all other sites being negative. A fine needle aspiration of the solid mass revealed a diffuse infiltration of CD138+ neoplastic plasmacells. RT-PCR studies disclosed MDR-1 and MDR-3 overexpression by soft tissue plasmacells. Both MIBI and PET scannings are helpful tools for detecting occult lesions both within bone and in extramedullary sites, in MM patients. It is usually believed that PET-FDG is more sensitive in evidencing lower spine and pelvic disease due to the unpredictable gastro-intestinal uptake of MIBI. Our data suggest that PET-FDG may also turn of great relevance for detection of extramedullary disease progression in specific situations. Since technetium-99m-MIBI is a transporter substrate of P-glycoprotein (Pgp), the overexpression of Pgp in soft tissue plasma cells could explain, in our patient, the negativity of the thoracic mass to MIBI scanning, probably related to an intra-cellular wash out of the radionuclide. A prospective comparison of these two imaging techniques for evaluation of extramedullary disease in MM patients appears of relevant interest.

Quality of Life and Support Therapy

P582

DON'T CALL US SURVIVORS! RESULTS OF A SELF-HELP GROUP EXPERIENCE FOR YOUNG PATIENTS WITH HODGKIN'S AND NON HODGKIN'S LYMPHOMA

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Background. Given the great improvement of clinical results in most fields of oncohematology, it is likely that new psychosocial end-points and human needs might come out from those patients who expect to be long survivors or even cured.

Aims. To understand what catching cancer in a significant period of life for professional and family planning has meant for a cohort of young patients with lymphoma and what emotional and cognitive changes they have played to cope with such an experience.

Methods. Preliminarly, patients with HD and NHL <45 years old who were treated at our department were invited to take part in a focus group guided by a psychologist and an hematologist in order to agree on main psychosocial items on which they focused their interest. In a second phase, the items were discussed with them in depth during four consecutive meetings. Every meeting was recorded, transcribed and analyzed using an human science approach with the purpose of tracing specific patterns of understanding. Contents were then categorized in five areas: self, health, family, social aspects and coping strategies. Only those conclusions with full agreement by all partecipants are reported.

Results. Six of ten patients (2 female and 4 male, median age 35,5 yrs; 4HD and 2 NHL; 1 still under treatment and 5 off therapy with a median follow-up of 27,6 months accepted our invitation and went through all meetings. Concerning self, the strongest feeling was an addiction to react against disease constraints in order to maintain an autonomous life without changing their targets. Greater sensitivity toward other people suffering was also repeatedly mentioned. Patients'; main concern about health was fear of recurrence of disease, of pain and personal suffering as well as of thoughts of death; the feeling of being dissected and catalogued as well as the presence of physical long-term consequences such as early menopause and loss of fertility were also reported. Concerning their relation within the family a significant difficulty in supporting their parents and their children was underlined. However, while a feeling of guilt with respect to their parents for making then worried is specially bothersome, the presence of children is viewed as a great resource. Concerning social aspects, patients underlined that despite maintaining their occupation was difficult during treatment, it was still viewed as an important help for coping with the disease. Further problems were finding a new job once one was lost, obtaining a life insurance.Coping strategies included deleting of negative thoughts, constrains to maintaining of normal life style, the continuous search of family and social support, the last including of a continuous and frequent contact with their doctor.

Conclusions. When a young patient has the potential of becoming a long survivor from an oncologic disease, participation to open groups in which to confront with other patients, their family and doctors under the supervision of a psychologist is most advisable in order to focus on relevant psychosocial needs that might be overlooked otherwise. The family session was particularly important to open the family communication about the illness.. All patients have agreed to participate in further groups in order to introduce new patients

P583

EVALUATION PATIENT OUTCOMES IN HAEMATHOLOGICAL HOME CARE: SUPPORT TEAM ASSESSMENT SCHEDULE: PRELIMINARY RESULTS

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Quality assurance is a necessary component of contemporary health care. In the past, palliative care has not generally been subject to systems of audit and quality management. From the last years the policy of palliative care organizations was the most significant issue for mantaining the quality of care. The Support Team Assessment Schedule (STAS) is a psychometric evaluation of clinical audit tool. The STAS comprises nine items pertaining to the patient and his family, and seven items concerning the service provided. From March 2004 STAS is done on patients entered in Home Care (HC) at Haemathology Department at Florence which are affected by acute leukemia and lymphoproliferative disorders. This study aims to evaluate avaibility of this autoclinical audit tool as evaluation of effectiveness of palliative support team. Staff assessment is part of routine data collection at first point of contact.The patient is assessed at each contact:the timing of successive assessment is dependent on the nature of the service providing care (range 2-10 days). From March 2004 to February 2005 35 patients are recruited and 216 evaluations are completed; staff completed 168 assessments on 25 patients, actually 10 patients are assessed. Preliminary results demonstrate that the support team has given the relief not only of the symptoms (pain,fatigue) but also of emotional,social and psychological problems in the most patients in HC. Good comunication is the real support for the family.

P584

ACUTE LEUKEMIA IN THE ELDERLY: HOME CARE EXPERIENCE

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The majority of patients wih Acute Myeloid Leukemia (AML) are 60 years of age or older.Therapeutic results are limited in outcome among old patients.Age from a clinical perspective is heterogeneous and poorly reflected by chronological age. The Comprehensive Geriatric Assessment is a multidimensional diagnostic process; it provides an stimate of life expectancy, i.e. a critical step in the process of therapeutic decisions in elderly patients, choosing between aggressive or palliative treatment.Palliative care is the combination of active an compasionate therapies to support the "frail" patient. From June 1992 to February 2005 352 patients (pts) entered the Home Care (HC) at Haemathology Department of Florence; 156pts (44%) were affected by AMLand 81% were 60 years older.

Clinical status of 127 old pts at the moment of inclusion in HC:

1) Ending in death:39 pts 2) Resistant AML: 58 pts 3) AML at diagnosis: 30 pts

Results.

39 ending in death PTS: AGE 62-96 PS3 17 PS4 22,Median assistance period 15 days (range 3-45),Medical examinations 310,Blood transfusions 113,Hours nurse assistance 540(daily assistance),70%pts died at home

58 resistant AML: age 63-88 PS2 25 PS3 33,Median assistance period 129 days (36-720),Medical examinations 2046,Blood transfusions 698,Hours nurse assistance 4050,80% pts died at home

30 AML at diagnosis: AGE 61-88 PS1 8 PS2 18 PS3 4,Median assistance period 348 days (39-975),Medical examinations 2708,Blood transfusions 892,Hours nurse assistance 4590; all pts were treated by ARA-C low doses (62 cycles ,range 1-7 cycle/patient),3 pts achieved complete remission; 82% pts died at home.

These data confirm the utility of HC not only for ending in death patients ,but also for frail patients treated by not aggressive chemotherapy such as ARA-C low doses.

P585

ECONOMIC ANALYSIS OF A DOMICILIARY PROGRAM OF SUPPORTIVE AND PALLIATIVE CARE FOR PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background. The implementation of home care programs for hematological patients requires the assessment of the specific costs of the domiciliary setting.

Aims. To analyze the use of resources and the global costs of the services provided at home of the patients according their phase of disease.

Methods. During 2 yrs, 185 patients were assisted at home to provide:

- supportive care, for pts. early discharged (ED) with infections after chemotherapy;
- palliative care, for pts. with different life expectancy: < 3 months (terminal, TE); >3 <6 months (advanced, AD); > 6 months (chronic, CH).

The cost drivers evaluated concerned: 1) health care providers and coordination/support team; 2) materials and medicines; 3) laboratory; 4) transfusional support. In 40 cases indirect costs (for the patient and the family) have been also evaluated. Mean monthly costs (MMC) are expressed in Euro (?) and have been compared with national Diagnostic Related Groups (DRGs) scale charges for hematological diseases and with district fares for palliative

care for patients with oncological diseases.

Results. Intensity of care: among the 4 groups of pts., ED and TE pts. required the higher mean monthly No. of home visits (24.1 and 23.2, respectively) and of transfusions (6.8 and 6.22); they were assisted for a median No. of 19 days and 22.8 days, respectively. Economic analysis: the MMC was affected by the following variables: disease status, transfusional needs, type of hematological disease. MMC for TE pts (€ 4.232,82) and ED pts. (€ 3.986,57) resulted higher than for AD pts. (€ 2.304,04) and CH pts (€ 1488,43). With the exclusion of CH groups, these costs exceed the district fares for home care of oncological pts. The highest MMC, for pts. with acute leukemia and > 4 transfusion/mos. (€ 11.080), resulted lower as compared to the highest corresponding DRG. The MMC sustained by the families was € 2.027,72.

Conclusions. From the purchaser/provider perspective of the health care system , costs of home care for some categories of hematological pts. are lower than those of hospitalization, although superior to current national fares for home care programs. From the perspective of the health care as a whole, the economic burden charged to the families must be taken into account when evaluating the costs of home care.

P586

OSTEONECROSIS OF THE JAW ASSOCIATED WITH CHRONIC BISPHOSPHONATES THERAPY IN MULTIPLE MYELOMA LONG SURVIVORS: A NEW CLINICAL ENTITY?

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Background. Bisphoshonates (BS) are generally used to treat patients with advanced multiple myeloma (MM) and other neoplasms that infiltrate bone. Therapy is given by diagnosis of bone lyses over an extended period of time and it is well tolerated by patients. Recently, severe osteonecrosis (ON) of the jaw have been reported as an adverse effect. In biopsy Actinomyces spp were recovered from culture. The aetiology is not understood, although it has been postulated to be secondary to the antiangiogenic effect of bisphoshonates. An increase risk is described in patients undergoing major dental procedure.

Method and Population Characteristics. We performed a retrospective review of patients who presented to our Haematology Department between January 2004 and December 2004 with the diagnosis of MM and an history of chronic monthly intravenous BS therapy (Pamidronato 90 mg and than zoledronate 4 mg). 65 patients (pzs) were observed: M=36; F=29; stadium III= 60, stadium II=5, median age 64 years (range 34-80). All received two or more anti-myeloma therapy and 37 were taking Thalidomide when ON arose.

Results. Eight patients (12.3%) presented ON of the maxilla (4/8 pzs) and mandible (4/8 pzs). 7/8 pzs had a history of dental extraction in the site of ON. The median duration of BS therapy was 5 months for pamindronate and 20 months for zolendronate. The median dose of BS therapy was: 360 mg (range 0-6480 mg) for Pamidronate, 80 mg (range 0-152 mg) for zoledronate. The biopsy revealed osteomyelitis in 4 pzs, osteonecrosis in 2, and gingival inflammation in one. Actinomyces spp were recovered in only one patient. The therapy was variable: osteotomia (n=3), prolonged antibiotic therapy (n=2), gingival curettage (n=2) and hyperbaric oxygen (n=1). Only one patient presented a complete resolution. Two patients presented an important exposed jawbone.

Statistical Analysis. Data analysis showed that the administration of more than 360 mg of Pamidronate and of more than 80 mg of zoledronate is significantly associated (p=0.039) to ON lesions with an Odds Ratio of 3.68 (C.I.95% 1.065-12.714)

Conclusion. ON may be a new complication in MM patients long survivors. Our data, even if preliminaries, induced these considerations: 1.The basis for new recommendations for bisphosphonate use needs to be written. 2. Major debridement surgeries are to be avoided if at all possible. 3.Oncologists should pay attention to this new problem.

P587

INTERMITTENT NON-INVASIVE VENTILATION IN IMMUNOCOMPROMISED PATIENTS WITH ACUTE RESPIRATORY FAILURE

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Avoiding intubation is a major goal in the management of acute respiratory failure in immunocompromised patients, affected by haematological malignancies. The transferring to the intensive care unit, endotracheal intubation and mechanical ventilation are associated with a significant risk of death. The early use of intermittent non-invasive ventilation (NIV) during acute respiratory failure can help to avoid the need for endotracheal intubation and improve the outcomes of patients. We conducted a feasibility study of intermittent NIV, in 10 haematological pts at an early stage of hypoxemic acute respiratory failure: in these patients immunosuppression was caused by neutropenia after intensive chemotherapy (grade IV, 7/10 pts), bone marrow transplantation (3/10 autologous, 4/10 allogeneic) or it was a result of intensive corticosteroid therapy (4/10 pts). The enrolled patients showed: pulmonary infiltrates; fever; severe dyspnea at rest; a respiratory rate of more then 30 breaths per minute; a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO2:FiO2) of less than 200 while the patient was breathing oxygen through a Venturi mask.

Non-invasive ventilation was delivered to the patients through a helmet. The helmet was adjusted and connected to a "Venturimeter" with a pressure support of oxygen. Positive end-expiratory pressure (PEEP) was repeatedly increased by 2cm of water (up to a level 10 cm of water) through a valve The end point of each procedure was to maintain the arterial oxygen saturation above 90 percent. Period of non-invasive ventilation lasted at least 60 minutes and alternated every 2 - 5 - 10 hours, according the arterial oxygen saturation. The mean duration of NIV was 8 days

(range 6-14). During the first 4 days, the NIV was administrated for a mean of 5-6-9 and 6 hours respectively; subsequently the mean duration of NIV was 5 hours per days. Pts stopped NIV when the PaO2:FiO2 Ratio was more than 200 while the patient was breathing oxygen through a Venturi mask. Fourteen patients overcame the respiratory failure: nine of them were discharged; two patients died for cardiac failure and three for progressive disease. Four patients died for respiratory failure after intubation. We think that the beneficial effects of PEEP on alveolar recruitment and in treating atelectasia at an early stage of respiratory infection help to maintain adequate alveolar ventilation during the acute respiratory failure. In our study the intermittent use of NIV was possible without transferring the patients in intensive care unit and it permitted to avoid endotracheal intubation, with encouraging results.

P588

THE ASSESSMENT AND THE IMPLEMENTATION OF A QUALITY MANAGEMENT SYSTEM OF AN HEMATOLOGY UNIT ACCORDING TO UNI EN ISO 9001:2000

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A quality management system (QMS) improves quality in health care units. This report describes the introduction of a QMS according to ISO 9001:2000 in the Hematology department at the Policlinico Universitario Tor Vergata of Rome. First, a throughout analysis of all processes was performed. Two main QMS processes have been selected: treatment of hematological malignant disease and clinical research project management. These processes have been defined and integrated with the hospital top management policy. The sequence and the interactions of these processes with the other clinical departments, diagnostic services as well as with administration units were also defined. Thereafter, the organization has determined all the criteria and methods to ensure monitoring, analysis and continual improvement of these processes. We started quality rounds including medical and nursing personnel and we redefined and explained all processes including their responsibilities in the QMS. We evaluated and optimized all necessary resources in terms of purchasing processes and personnel training, evaluating customer satisfaction using patients' surveys. A complete documentation, including quality manual, documented procedures and all documents and records required, has been built up. After a process of 12 months, an independent, accredited organization recommended that our QMS be given certification according to ISO 9001:2000. In conclusion, certification of an hematology department according to ISO 9001:2000: i) may represent the first step towards total quality management in complex health care institution, ii) makes patients highly guaranteed for their health care, iii) should represent the common basis, shared among different centers involved in multicenter clinical studies.

P589

EVALUATION OF COPING AND COMPLIANCE IN ONCO-HEMATOLOGICAL PATIENTS

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On the basis of the Transactional Model [Lazarus and Folkman, 1984], coping is defined as a process induced by a stressful event that changes over time and in different contexts. Psycho-oncology has recently embraced this definition as it represents how people cope with the stressful event of "cancer": the two principal coping strategies are known as the "problem-focused" (aimed at eliminating the event causing stress) and "emotion-focused" strategy, which is intended to control the emotional response. In 1987, Watson and Greer describd four main coping styles: anxious preoccupation, fighting spirit, fatalism and denial.

Some trials have been conducted with the aim of relating coping styles to the outcome of illness and, although the data are still equivocal, a relationship between fighting spirit and a positive outcome has been identified in some cases, and hopelessness and depression seem to be related to a negative outcome [Petticrew *et al.*, 2002]. Analyses of the multiple variables involved have indicated the role of compliance to treatment, which Sackett and Haynes (1979) defined as "how much a patient cooperates with medical staff, agrees with clinical choices, and participates actively in their accomplishment". Compliance to treatment also plays an important role in treatment outcome, and patients adopting non-adaptive coping strategies have been found to be less compliant [Ayres and Hoon, 1994].

We conducted a 9-month longitudinal study of 41 patients suffering from onco-hematological pathologies in order to investigate the relationship between coping strategies and compliance to therapeutic regimens. The assessed strategies were classified as adaptive or non-adaptive using the Mental Adjustment to Cancer Scale [Watson and Greer, 1987], and compliance was evaluated using a specific questionnaire designed with the aim of analysing all of the behavioural variables identified by means of a systematic review of the pertinent literature. Statistical analysis revealed a relationship between adaptive coping strategies and high levels of treatment adherence ($p<0.05$), whereas poorly compliant patients adopted less adaptive coping responses. We also assessed psychological distress (Symptom Checklist 90), performance status (Karnofsky Performance Index) and pain perception (VAS), but the results were not included in this variable analysis as they will be used for further studies.

P590

ECONOMICAL ANALYSIS OF A ONE-YEAR ACTIVITY OF THE HEMATOLOGICAL HOME CARE PROGRAM

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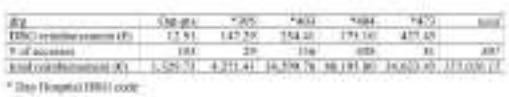
Background and Obyectives. During the year 2004, the Hematological Home Care Program (HHCP) of Niguarda Ca' Granda Hospital (Milan) has assisted 57 outpatients (chronic patients, previously hospitalised, terminal patients needing palliative care, and acute patients discharged after chemotherapy or after treatment of infections) Aim of the study is the economical evaluation of the HHCP during the year 2004, comparing the overall costs of this service with the Lombardy Diagnosis Related Group (DRG) reimbursement due for hospitalised pts.

Design and Methods. At this stage of the investigation, the most suitable way to evaluate the cost of the HHCP system was the simple addition of all costs (labor, overheads, transportation, consultants, etc.) generated during 2004, rather than performing an analytical evaluation of each single-patient cost. The total cost amount was therefore divided by single access, after stratifying by pathology for further, more detailed analysis.The total cost was then compared with the DRG reimbursement during the same period. Pts diagnosis were: HLC/CLL (10 cases), ALL/AML (7), HL/NHL (6), MDS (5), MM (19), MPS (7) and other haematological diseases (3). A total of 807 HHCP therapeutic contacts for 57 outpatients (22 male, 35 female, mean age 76.7 y) were provided during the year 2004.

Results. Given the disease distribution of the patients, the total cost (labor, overheads, etc.) of the HHCP system was 105,661.55 € (130.9 € per access; 1853.7 € per patient), whereas the total DRG reimbursement for these outpatients would have been 155,020.15 € (see table below).

Thus, HHCP has allowed a total savings of 49,358.60 € (31.8% less), i.e., about 865 € per outpatient. The result is even better if we consider that 103 of the 807 accesses (12.8%) would have been reimbursed as outpatient examination at very low (and not realistic) DRG price (12.91 €). Moreover, this figure does not include the social expense of working-day loss by patient parents which, if taken into considerayion, would further reduce the impact of medical care for this subgroup of pts.

Interpretation and Conclusions. The HHC Program ongoing at the Niguarda Ca' Granda Hospital is still at an early stage, but it has already provided savings for some 850 ? per patient per year. The increase in number of outpatients treated by means of HHC Program will probably increase total savings in a sensitive way.



P591

PSYCHO-HAEMATOLOGIST: EXPERIENCE OF MODENA

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The Italian Leukaemia Association (AIL), Modena branch, in November 2001 set themselves a target to provide complimentary support in addition to the haematologist and the domestic day-care nursing for patients, with the provision of a psycho-oncologist. Initially this was only provided at the patients home, where a relationship would develop between the patient, the family, and the psychologist. Over time this support was also provided at the hospital to set up some continuity in the process, in a more structured way.

Actually, in the case of haematologist patients the psycho-oncologist would need to be less generic and more specialised in the field of haematology and subsequently a specialist Psycho-haematologist is preferred, because the specific management needs for that illness and the fact that the patient needs to be admitted in the hospital for longer periods of time. Our psycho-haematologist's experience, both at home and in the hospital, must be considered in a transversal and circular way.

A transversal assistance because the patient needs to be followed from the beginning of his treatment in all the different phases: induction and consolidation in the haematologist ward; the maintenance of the therapy in Day Hospital and, if necessary, in Marrow Unit Transplant (UTM). The scope is not simply in or out of the hospital, but wherever the patient invites you and wherever the doctors allow the entrance. A circular assistance because it is a team that is all working together with the patient, with the chance to share feedback on the patient's health and on his feelings in the different phases. In fact information that could have previously been missed is now more able to be retrieved and recorded by the various professionals who look after him. In this way the hospital is not a place which keeps the patient at a distance, with the psycho-haematologist now acting as an important bridge between it and the home care assistance.

P592

TRANSFUSION OF INACTIVATED PLATELETS WITH AMOTOSALEN HCL IN HEMATOLOGICAL PATIENTS

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Introduction. In the last years, an increase of platelet (PLT) transfusion associated to the larger use of high dose chemotherapy was observed and up to now, PLTs still represent a major risk of transfusion-transmitted-disease for possible bacterial contamination. Aim of this study is to evaluate the *in vitro* characteristics of inactivated PLT products and their efficacy in haematological patients.

Methods. PLT inactivation was performed with a photo-

chemical treatment process (amotosalen HCl and an ultraviolet A light irradiation) by using "Intercept Blood System" (Baxter). PLT concentrates were processed within 24 hours of collection (pre-inactivation median time: 21,4±1,2 h). The PLTs were mixed with Amotosalen HCl and exposed to UVA light at the dose of 3J/cm^2 for 4±0,04 minute and then agitated for a minimum of 4 hours with CAD absorbing device in order to reduce the residual free Amotosalen HCl . At the end of the procedure, platelets were collected in a storage bag and kept in agitation until the time of transfusion. PLT samples were collected before, after inactivation process and at time of transfusion in order to evalutate the following parameters: pH, pO2 and pCO2, glucose and lactate. The clinical efficacy was evaluated by measuring the corrected count increment (CCI).

Results. PLTs collected from 39 healthy donors with AMICUS Baxter blood cell separator were stored in a solution with 35% of autologous plasma and 65% of Intersol. The PLT yield was a median of 4.28x10^{11} (2.88-5.68x10^{11}) in a final volume of 320 ml (232-407 mL). After inactivation the PLT yield was 4.02 (2.84-5.62) x10^{11} with a PLT loss of 6,1% ± 2,2. The *in vitro* data are summarized in table 1. The inactivated PLT products were transfused in 39 hematological patients (24 LMA, 9 LnH, 6 LAL) without any adverse transfusion reaction. The mean CCI at 18 – 24 hours was 6087 ± 4915 with a value > 4500 in 64% of the transfusions, a value similar to our historical data.

Conclusion. The inactivated PLTs maintain a good biological property and satisfactory clinical efficacy up to 5 day storage, therefore, the cost/benefit of this procedure has to be defined.

P593

THE EFFICACY OF PLATELET TRANSFUSION IN ACUTE LEUKEMIA: THE ROLE OF ABSOLUTE INCREMENT

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Introduction. The efficacy of platelet (plt) transfusion is usually measured by means of the corrected count increment (CCI) value at 18-24 hours post-trasfusion. Aim of this study was to evaluate whether the post-transfusion absolute increment (AI), of easier and more immediate utilization, could represent a valid alternative to the CCI; we also investigated factors related to a satisfactory AI.

Methods. From September 2001 to August 2004, we evaluated 5100 plt transfusion episodes in 264 patients with acute Leukemia (195 AML, 69 ALL). Plt concentrates were obtained from single-donor apheresis (N=3374) or from whole blood donors using the Buffy-Coat method (N=1726). We used a CCI >4500x10^9/L to define a satisfactory response. The value of the cut-off of AI that better predicted a CCI>4500 x 10^9/L was identified using the ROC curve. The multiple logistic regression analysis was used to investigate factors correlated to a satisfactory response. Results were expressed as Odds Ratios (ORs) with their 95% confidence intervals (95% CI).

Results. The mean amount (±DS) of plt infused was of 3.80±1.2 x 10(e)11. Pre and post-transfusion mean plt values were 12784±9087x10^9/L and 27594±19142 x 10^9/L, respectively. We obtained a mean CCI of 6202±6922 x 10^9/L and a mean AI of 14282±16513x10^9/L. The cut-off of the AI that better predicted a CCI>4.500x10^9/L resulted to be equal to 10.500x10^9/L, with a sensitivity of 92%, a specificity of 92.1%, a positive predictive value of 92.8%, and a negative predictive value of 91.3%. At multivariate analysis, the likelihood of an AI>10500 was 60% higher when the dose of plt transfused was between 4 and 6 x 10(e)11 (OR=1.60; 1.39-1.84) and almost double when the dose exceeded 6 x 10(e)11 (OR=1.94; 1.40-2.70). The transfusion of AB0 identical plt also increased the likelihood of a satisfactory response (OR=1.35; 1.13-1.62). The probability of a satisfactory response decreased by 13% for each day of plt storage (OR=0.87; 0.82-0.92) and was significantly lower in the presence of plt antibodies (OR=0.30; 0.25-0.36). In the latter case, the transfusion of cross-match negative plt increased by 50% the likelihood of a satisfactory response (OR=1.54; 1.09-2.18).

Conclusions. In our experience, an AI>10500x10^9/L represents a valid and easy to use indicator of plt transfusion efficacy in patients with acute leukaemia. The attainment of a satisfactory response is strongly related to the dose of plt transfused, the storage duration, the presence of alloimmunization and AB0 compatibility.

P594

TOTAL PAIN AND ISOLATION: THE CENTRAL ROLE FOR THE NURSE IN THE PROCESS OF THE LEUKEMIC PATIENT'S CARE

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Does the total pain, as defined by the World Health Organization, affect the hematologic patient? From the very beginning of the diagnosis of acute leukaemia, the hematologic patient enters a multistep therapeutic process, in which isolation is the rule, for obvious reasons related to the immunosuppression state following chemotherapy. Simultaneously, an early and profound changing either in the mood or in the behavioural and psychological attitudes does occur in the patient, who is compelled to recognize the severity of the illness and to accept, almost immediately, the chemotherapeutic program, proposed by the physician. The total pain stems from the need to face this unexpected condition and the nurse, with whom the patient has the first and long lasting contact, should play a crucial role in the patient management to provide a global care. Thus, the aim of our study is to better define and characterize the total pain perceived and reported by the leukemic patient, by identifying possibly specific and dis-

tinctive physical, emotional and psycho-social features, related to various factors including environmental isolation, the dose intensity and the time duration of the chemotherapeutic regimens, the frequency and the severity of the complications, related either to the disease itself or to the therapy. We plan to apply the following methodology: 1) the Visive Analogic Scale (VAS) to quantify and monitor the total pain; 2) a 10-item self-report questionnaire, the Hospital Anxiety and Depression Scale (HADS), and the Schedule for Evaluation of Individual Quality of Life (SEIQOL); 3) weekly audits involving all the professional figures surrounding the hematologic patient, namely the nurse, the psycologist and the physician to promote discussion of clinical cases. Our study is oriented to improve the quality of life of the leukemic patients, by the promotion, the collection and the proper use of information and emotional burden of each professional figure taking care of the patient.

P596

QUALITY OF LIFE IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Therapeutic approach for acute myeloid leukemia (AML) in elderly patients is generally tailored on the basis of age, performance status, concomitant diseases and patient consent. Toxicity and low-response rates are major constraints and the therapeutic options are most likely conditioned by the clinicians' opinion about the patients' health status and preferences. The patient's own perception of health (health-related quality of life – HRQOL) may be found very useful in such situations. We designed a prospective multicenter study to evaluate the predictive potentials of HRQOL measures on prognosis in elderly AML patients aged over 60 years.

We present preliminary baseline data in 41 AML patients of median age 72 (range 69 - 90) yrs. HRQOL measures were obtained by applying the QOL-E and the EORTC QLQ-C30 questionnaires at diagnosis. Demographic and disease-related factors were also evaluated. Both questionnaires showed good internal consistencies in the AML population, except for the sexual, cognitive and nausea/vomiting domains (Table 1).

Table 1

QOL-E domains	Standardized Cronbach alpha coefficients	EORTC QLQ-C30 domains	Standardized Cronbach alpha coefficients
Physical	0.89	Physical	0.85
Functional	0.83	Role function	0.89
Social	0.71	Social	0.82
Sexual (n=24)	0.53	Emotional	0.80
Fatigue	0.85	Fatigue	0.84
Disease-Specific	0.76	Cognitive	0.72
Total	0.93	Global Health Status	0.85
		Nausea/Vomiting	0.68
		Pain	0.87

QOL-E scores were particularly low (reflecting poor HRQOL) in the fatigue and disease-specific domains (Table 2).

Table 2

QOL-E domains	Median (interquartile range)	EORTC QLQ-C30	Median (interquartile range)
Physical	68 (58-90)	Physical	68 (63-89)
Functional	69 (46-82)	Role function	67 (33-83)
Social	56 (28-78)	Social	83 (66-100)
Sexual	67 (67-100)	Emotional	75 (67-92)
Fatigue	45 (28-45)	Fatigue	44 (33-67)
Disease-specific	33 (29-54)	Cognitive	83 (67-100)
Total	86 (79-91)	Global Health Status	58 (46-67)

The preliminary analyses of the associations in our data suggested that age might be a major determinant of QOL: in fact it was inversely correlated with cognitive function (r=-0.267, p=0.026), global health status (r=-0.293, p=0.011), physical function (EORTC QLQ-C30 r=-0.392, p<0.0001; QOL-E r = 0.-0.241, p=0.04), role function (r=-0.0369, p=0.002) and fatigue (EORTC QLQ C30 r= 0.343, p=0.003; QOL-E r= 0.306, p==.008). The lack of domestic assistance (reflecting no previous need for help at home) was associated with better physical (QOL-E p=0.02; EORTC QLQ-C30 p=0.012), functional (p<0.0001), role function (p=0.003), fatigue (QOL-E p=0.017; EORTC p=0.015), general (p=0.002), QOL-E specific (p=0.03), dyspnea (p=0.003) and total (QOL-E p=0.009; EORTC p=0.003) scores. Fatigue was also associated with a longer duration of fever (r=0.882, p=0.04). The presence of concomitant diseases was associated with poorer physical well-being (p=0.006), role function (p=0.02), emotional well-being (p=0.05), and cognitive function (p=0.05). The presence of hemorrhages at diagnosis was associated with poorer emotional well-being (p=0.016). Interestingly, QOL was not correlated with baseline Hb levels. Noteworthy, objective well-being measured by ECOG PS grade was associated with fever (p=0.012), but did not correlate with other dimensions of well-being perception. Twenty-eight patients were alive after 1 month for re-evaluation. Changes in QOL scores were reported: a tendency for worsening of the EORTC QLQ-C30 perception of financial problems (p=0.13) and a decrease in social function (p=0.006); and a decrease in QOL-E physical (p=0.02), specific (p=0.04), total (p=0.008) and treatment outcome index (p=0.013) scores. In conclusion, at diagnosis HRQOL is poor, especially with increasing age and in patients with concomitant diseases and it deteriorates soon after the diagnosis, during the initial treatment. The future prospective data, in an adequate number of patients, may provide more detailed and robust results on the role of patient-tailored therapy in this particular category of AML patients.

P597

LONG TERM TREATMENT OF TRANSFUSIONAL IRON OVERLOAD WITH SUBCUTANEOUS DEFEROXAMINE IN ADULT PATIENTS AFFECTED BY ONCO-HEMATOLOGIC DISEASES

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Background. transfusional iron overload may occur in several chronic onco-hematologic diseases, chiefly in Myelodysplastic Syndromes (MDS) and in idiopathic myelofibrosis (IMF). As iron overload may cause symptoms when the amount of total body iron reaches 100-200 mg/kg, iron chelating therapy is worth-while if the life-expectancy is sufficiently long to predict that signs and symptoms due to iron overload will appear.

Aims. In consideration of the discomfort and the costs of a long term chelation therapy with subcutaneous Deferoxamine (DFO), the only chelating drug at present licenced in Italy for non-thalassemic patients, and of the reduced compliance due to the old age of MDS and IMF patients, a retrospective analysis of the feasibility, effectiveness and toxicity of DFO treatment is justified.

Methods. From June 1997, 18 pts (11 males, median age: 73, range 57-85 yrs) with low-or-intermediate risk MDS (12 patients) or with IMF (6 patients), and with a significant transfusional iron overload were treated with subcutaneous DFO, either by 8-10 hours pump-driven subcutaneous continuous infusion (5 pts) or by once-or-twice daily subcutaneous bolus injection (13 pts), for 5 days/week.

Results. The median time-lapse from diagnosis to the start of DFO was of 16.5 (range 1-132) months, and the median duration of transfusion-dependance before the start of chelating treatment was of 12.5 (range 1-48) months, with a pre-treatment median transfusion burden of 34.5 (range 4-126) packed red cell units. The median pre-treatment serum ferritin level was of 1834 (range 730-4531) ng/mL. The median duration of DFO treatment was of 21 (range 2-72) months, and the median ferritin value at the last medical examination was of 2280 (range 418-6777) ng/mL. 2 pts discontinued DFO, after 2 and 8 months respectively, because of important side effects.

Conclusions. DFO, either by pump-driven subcutaneous continuous infusion or by subcutaneous bolus injection, is a feasible treatment even in elderly MDS or IMF pts, and could delay the progression of transfusional iron overload.

Published Abstracts

L001

ROLE OF IMAGE-GUIDED FINE-NEEDLE ASPIRATION BIOPSY IN THE MANAGEMENT OF PATIENTS WITH SPLENIC METASTASIS

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Objectives. Splenic metastases are very rare and are mostly diagnosed at the terminal phase of the disease or at the time of autopsy. The cytohistologic diagnosis, when done, is made prevalently by splenectomy. Reports on splenic percutaneous biopsies in the diagnosis of splenic metastasis are fragmentary and very poor.

The aims of this study are to analyse retrospectively the accuracy, safety and the clinical impact of ultrasound (US)-guided fine-needle aspiration biopsy (UG-FNAB) in patients with suspected splenic metastasis.

Methods. A retrospective analysis of 1800 percutaneous abdominal biopsies performed at our institute during the period from 1993 to 2003 was done and 160 patients that underwent splenic biopsy were found. Among these 160 patients, 12 cases with the final diagnosis of solitary splenic metastases were encountered and they form the basis of this report. The biopsies were performed under US guidance using a 22-gauge Chiba needle. Results. There were 5 women and 7 men, median age 65 years (range 48-80). Height patients had a known primary cancer at the time of the diagnosis of splenic metastasis: 3 had breast adenocarcinoma, 2 colon adenocarcinoma, 2 melanoma and 1 lung adenocarcinoma. Four patients were undiagnosed at the time of the appearance of splenic metastasis and subsequent investigations showed adenocarcinoma of the lung in 2 patients and colon adenocarcinoma in the remaining 2. The splenic biopsies allowed a cytological diagnosis of splenic metastasis in all the 12 patients and a specific therapeutic approach in all the patients. There were no complications related to the biopsies.

Conclusions. These results indicate that UG-FNAB is a successful technique for diagnosis of splenic metastasis allowing an adequate treatment of the affected patients.

L002

IN THE STEM CELL ERA WHAT DO DOCTORS KNOW ABOUT VITAMIN B12 DEFICIENCY?

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Background. Vitamin B12 (cobalamin) and acid folic deficiency can lead to a wide spectrum of potentially serious disorders, mainly haematologic and neuropsychiatric, that can be often reversed by early diagnosis and prompt treatment. The clinical presentation of these deficiencies varies considerably and the severity of cobalamin deficiency is unrelated to vitamin B12 concentrations. The diagnosis of vitamin B12 deficiency has traditionally been based on low serum vitamin B12 levels, usually less than 200 pg/mL or 150 pmo/L. Cobalamin deficiency is often unrecognised or not investigated because the clinical manifestations are subtle (1).

Aim of the study. The recent observation of a young woman (case 7, table) with a megaloblastic anemia (MA) lasting for three years prompt us to review our experience in this field.

Methods and Results. The table shows the main clinical findings of selected case of MA we have observed since January 2002. We have excluded cases with more obvious causes of MA (liver disease, drugs, gastrointestinal disease, etc.), seen in this period at our Department, which serves a population of about 60,000 inhabitants. The clinical presentation was variable from a stomatitis with elevated MCV without anemia (case 6, Table) to a life-threatining MA anemia (case 1, Table). In all the six cases (2-7, table) treated with parenteral cobalamin the clinical signs and the hematologic/neuropsychiatric manifestations promptly disappeared with the therapy. We think it could be useful to describe briefly the case of a 44-old year woman that we consider as a paradigmatic one of the clinical presentation of MA in our era. The patient (case 7, Table) was seen in January 2005 because of parestesias and numbness in the fingertips of the arms and legs associated with megaloblastic anemias. She had a personal history of iron deficiency and hypothyroidism secondary to autoimmune thyroiditis (AT). Her mother and grand-mother both suffered from pernicious anemia. In February 2002 she presented macrocytosis with normal RBC and Hct. In April 2003 she was seen at a referral Department of Hematology. At that time peripheral blood cells counts (PBC) were: WBC 6.3 x 10^9/L, RBC 3.08x10^{12}/L, Hb 132 g/L, Hct 38%, MCV 123 fL, PLTS 456 x 10^9/L. Serum vitamin B12 level was 228 pg/mL (range 160-970) with normal serum folate level. A bone marrow aspiration showed dyserythropoiesis and a generic diagnosis of macrocytic anemia was done. No therapy was prescribed. Because of progressive anemia, in the next two years she was seen several times at her GP&'s office and, in October 2004, also at a referral Department of General Medicine of another hospital. At that time her PBC were WBC 7.2x10^9/L, RBC 2.97x10^{12}/L, Hb 119 g/L, Hct 36%, MCV 121 fL, PLTS 320x10^9/L. She was treated with 2 gr of endovenous iron without benefit on her anemia. Three months later she presented to our attention with a clear picture of MA and neuropsichiatric signs. The table (case 7) reports the main laboratory abnormalities. She was put on cobalamin therapy with a prompt improvement of her symptoms and anemia.

Conclusions. We think that the cases we have briefly described here are paradigmatic of the variable clinical pre-

sentation of cobalamin deficiency. Also in our experience MA is a frequent diagnosis, particularly in elderly people. If a bone marrow biopsy is performed, a pathological diagnosis of myelodysplasia is frequently done. Serum cobalamin levels in the normal-low range are also frequent and, when associated with macrocytosis, they can precede also for years the appearance of a clinical picture clearly compatible with a diagnosis of MA. During this time MA could be unrecognised and an appropriated treatment denied to patients, particularly if the use of low serum cobalamin level as the sole means of diagnosis is considered. In absence of more sophisticated diagnostic tools, a trial of cobalamin therapy could reverse the clinical picture and the laboratory abnormalities as seen in our patients.

Table 1. Main clinical data at presentation of selected cases of MA seen since 2002 at our Department.

Patient	Sex/y	Clinical presentation	RBC x10^{12}/L	HCt %	MCV	VitB12 pg/mL	Bone marrow biopsy	Other
1	M/73	Pancytopenia	0.94	10,5	111	104	Myelodysplasia	Cardiomegaly, exitus
2	F/66	MA, Aftous stomatitis	1.96	23.9	122.5	67	AREB	AG
3	M/63*	MA	1.49	21.4	117	147	IM	AT, AG
4	M/91	Pancytopenia	1.87	22.3	129	136	ND	GIE ND
5	F/49	Leucopenia, MA	3.92	38.8	101.7	296	ND	GIE refused
6	M/61	Aftous stomatitis	4.11	43.8	106	223	ND	AG, intestinal metaplasia
7	F/44	MA, tinnitus, ataxia	2.80	36	128.6	117	ND	GIE refused, AT,

MA=Megaloblastic anemia; AG= autoimmune thyroiditis; GIE=Gastrointestinal endoscopy; AG= Autoimmune gastritis

L003

EPSTEIN-BARR VIRUS REACTIVATION IN A PATIENT TRATED WITH ATG FOR A SEVERE APLASTIC ANEMIA. A CASE REPORT

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Epstein-Barr virus (EBV) disease has been increasingly observed in immune deficient patients, particularly after allogeneic hematopoietic stem cell transplantation (HSCT). Acquired severe aplastic anemia (SAA) is a rare disease, in which bone marrow failure is thought to result from an immune mediated mechanism. Immunosuppression is the treatment of choice in patients without a suitable donor for HSCT and anti-thymocyte globulin (ATG) is the single most effective drug in SAA. Several studies have shown that the use of ATG in allogeneic HSCT reduces the incidence and severity of acute and chronic graft-versus-host disease (GVHD) but increases the risk of EBV infections and EBV-related lymphoproliferative disorders. To our knowledge, however, this risk is not documented in patients treated with ATG for SAA. Here we report a case of infectious mononucleosis in a patient treated with rabbit ATG and horse ATG for SAA.A 38-year-old man was diagnosed as having SAA in October 2003. Blood count was: Hb 104 g/L, platelets 5000/mmc, WBC 4200/mmc. The bone marrow cellularity was 10%. The patient was treated with methylprednisolone (1 mg/kg/day day 1-30), cyclosporine A (5 mg/kg/die) and rabbit ATG (3.5 mg/kg/day 1-5), without response at one and three months. In February 2004 he received therapy with horse ATG (3.5 mg/kg/die). On day +19 he developed fever with enlarged and painful cervical lymph nodes. WBC count was 6500/mmc with 67% atypical lymphocytes. Flow cytometry of circulating lymphocytes revealed 85% CD3+, 28% CD4+, 58% CD8+ cells. Most CD8+ cells expressed HLA-DR, CD45RO and CD28. By real time PCR, on day +24 the EBV copy number resulted 30,000/150,000 cells. A diagnosis of infectious mononucleosis was made; cyclosporine was stopped and, considering the risk of developing an EBV-associated lymphoproliferative disease, the patient received two doses of rituximab on days +27 and +36. A decrease of viral load was observed early after the first infusion of anti-CD20 (EBV copy number: 300/150,000 cells on day +29; 30/150,000 cells on day +31; 1/150,000 cells on day +34). Concomitantly there was a progressive resolution of the clinical picture, despite persistency of SAA (Hb 105 g/L, platelets 30000/mmc, bone marrow cellularity 5%). During the following months the plasmatic level of EBV-DNA was closely monitored, always resulting negative. The patient refused an allogeneic MUD transplant, and was treated with androgens achieving a partial hematologic response. To the best of our knowledge, this is the first report of an EBV reactivation in a SAA patient treated with ATG. Of note, despite the in vivo purging of T cells due to ATG, EBV reactivation induced the clinical picture of an infectious mononucleosis. Because of bone marrow failure caused by SAA and the two lines of immunosuppressive therapy, our patient was considered at high risk of developing EBV-related lymphoma. Moreover, the viral load is a significant predictor of BLPD in the allogeneic transplant setting. Immediately after the first rituximab infusion, EBV DNA copies were markedly reduced and the clinical conditions improved greatly. To date, 12 months after viral reactivation, the patient remains negative for EBV DNA in plasma. Our observation suggests that ATG treatment may represent a risk for EBV reactivation also in the SAA patients and we confirm the efficacy of rituximab in preventing EBV-related lymphoproliferative disease in the immunocompromised hosts.

L004

SEVERE APLASTIC ANEMIA TREATED WITH SUCCESS WITH MYCOFENOLATE MOFETIL AFTER TREATMENT FAILURE WITH SERUM ANTI LINFOCITARIO, STEROIDS AND CICLOSPORINE IN A PATIENT WITHOUT FAMILY DONOR IDENTICAL HLA: CLINICAL CASE

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Severe aplastic anaemia (SAA) has to poor prognosis in the absence of treatment. Current accepted therapeutic strategies include immune-suppression (with ATG; PDN and CSA with G. - CSF) and allogeneic stem-cell trans-

plantation. In last years new immune-suppressor drugs have been produced as the mycofenolate mofetil (MM) and alemtuzumab (anti-CD52).

We reported a case of SAA not responsive to the classical immunosuppressive therapy, no HLA sibling donor in family, and successfully treated whit MM. Female, 38 years-old has been recovery in our division in December 2003 for pancytopenia (WBC: 400 mm^3; Hgb: 9 gr/dL and Plts: 20000 mm^3) and fever. Diagnosis of SAA has received with normal cariotype, and immunosuppressive treatment with ATG, PDN, CSA and G-CSF to start. At day +30 WBC: 4500 mm^3; Hgb: 8.9 gr/dL and Plts: 46000 mm3, the patient is in treatment with CSA, steroids and G-CSF, the support with blood bags and platelets was necessary. At day +150 WBC: 2150 mm^3; Hgb: 10 gr/dL; Plts: 15000 mm^3, the necessity of support continues, for partial response the patient suspend CSA and MM therapy to start (2000 mg/day). At month +6 WBC: 13100 mm^3; Hgb: 10.1 gr/dL; Plts: 48000 mm3, only blood bags is necessary with low frequency. At month +12 WBC: 10000 mm^3; Hgb: 12 gr/dL; Plts: 125000 mm3, the patient continues therapy with MM 4 cps / day and G-CSF weekly. The patient at month +15 is in very good condition and blood value is normal. MM an effective alternative therapy is shown for the treatment of the SAA, for such motive and for the low toxicity she could be considered an alternative drug to the cyclosporine in the immunosuppressive treatment of the SAA.

L005

CYCLIC THROMBOCYTOPENIAS: A PROPOS TWO CASES OF DIFFERENT ETIOLOGY

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Cyclic thrombocytopenia (CTP) is a rare disorder in which the platelet count falls periodically to very low levels, such as $10x10^9$/L, and then rises to very high levels (up to $1500x10^9$/L). This condition often presents associated with a clonal T-cell disorder or an autoimmune disease. The etiology is not well established so far: the antiplatelet antibodies are seldom detected, and thrombopoietin (TPO) levels can be normal or even elevated to an extent incompatible with thrombocytopenia.

We describe 2 cases of CTP observed in our institution, associated the first with celiac disease, and the other with a pure white cell aplasia related to a clonal T-cell lymphoprolipheration.

Case 1: V.B., female, 16 y.o., was admitted in 2004, March, because of fever and weakness: hemoglobin level was 4.2 g/dL, MCV 56, RDW 29.4, WBC count was $4.1x10^9$/L (neutrophils 58%), platelets (Plts) $650x10^9$/L. The patient received red blood cell concentrates, and intravenous iron therapy. A diagnosis of celiac disease was made by the detection of anti-transglutaminases, anti-gliadine, and anti-endomisius antibodies and by the histological examination of intestinal mucosa. Three days after the admission the platelet count began a rapid decrease until 23 $x10^9$/L. Bone marrow aspiration showed a reduction in megakariocytes count, an erythroid ipoplasia, and a slight eosinophilia. Gluten-free diet was prescribed and high-dosage immunoglobulins were administered with rapid increase in platelet count, which reached to $1040x10^9$/L within 6 days; then a second fall supervened and 2 weeks later the platelet count was $100x10^9$/L. In the following 2 weeks the platelets reached again normal levels, and so it is nowadays, with a normalization of celiac disease specific antibodies and a full recovery from anemia.

Case 2: P.L, female, 59 y.o., who presented on 2002, November, with a history of recurrent cutaneous infections, was diagnosed as affected by a peripheral T-cell lymphoma, AILD-like, based on the histological examination of an axillary lymph-node, the detection of a circulating clonal T-cell population and the presence of a mediastinal mass, revealed by a CT scan. This clinical picture was associated to a severe central neutropenia (neutrophil count < 100/mmc): a bone marrow biopsy indeed showed a severe myeloid hypoplasia and a deep infiltration by a CD3+ T-lymphocytic population. The megacariocytes were normal and so the erythroid series. Four weeks after the diagnosis the patient became thrombocytopenic (Plts $10x10^9$/L): a bone marrow aspiration showed the absence of megacariocytes. In the following 2 months, Plts count showed a cyclic oscillation with a 25 days period: the first cycle ranged from $5x10^9$/L to $960x10^9$/L, and the second from $6x10^9$/L and $1550x10^9$/L. Finally the bone marrow became definitively aplastic, and the patient underwent recurrent polymicrobic systemic sepsis, until death 4 months from diagnosis of lymphoma.

Conclusions. The association of celiac disease and immune thrombocytopenia is a well recognized clinical occurrence. T-cell mediated disorders can underlie autoimmune disease as well as B-cell lymphoprolipherations do, and it is well established that T-cell large granular leukemia (LGL) is often associated to central and/or peripheral cyclic granulocytopenia. In the first case we described, the hematological and non-hematological remissions ran parallel after the introduction of gluten-free diet, suggesting a common immune mechanism for the 2 different pathological conditions. Case 2 was characterized by two central-origin complications related to a T-cell lymphoprolipherative disease: continuous neutropenia and cyclic thrombocytopenia. Reports on this topic are becoming common, as the knowledge about immune-mediated diseases increases: recent findings in active ITP patients suggest an etiological role for an apoptotic resistance of CD3+ cells, resulting in a defective clearance of autoreactive clones.A similar role can be hypothesized for neoplastic T-cell clones.

L006

CD40 AND CD40L PLATELET EXPRESSION IN DIABETIC PATIENTS CORRELATE WITH THE SEVERITY OF METABOLIC SYNDROME

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CD40 and its ligand CD40L are involved in thrombogenesis and atheromatous plaque formation. An increase in their expression on platelets membrane is associated with

an increase of thrombotic events and atheroscleosis progression, while the experimental use of their inhibitors results in a risk reduction for atherosclerosis, and similar findings will be observed in defective animal models. All the clinical forms of diabetes mellitus are associated with an enhanced risk of atherosclerosis and thrombosis, and in the sera of patients the levels of CD40 and CD40L are higher than in normal people. As expected on the diabetic's platelets membrane these molecules resulted overexpressed. In the hypothesis that in diabetes the CD40 and CD40L expression on the platelets, could correlate with the severity of the metabolic impairment, we have recorded the percentage of platelets bearing these molecules in a series of patients with type 2 diabetes, needing different burden of therapy to maintain a metabolic balance. We detected the positive platelets by an indirect fluorescence method, using an anti CD61 fuorescinated mouse serum and anti CD40 or anti CD40L ficoeritrinated mouse sera, and estimated the percentage of positive platelets from the ratio of positive CD40 or CD40L counts over positive CD61 counts. The overall median percentages were: CD40 16.86 (0.02–94.4); CD40L 3.1 (0.09–19.6). In the group of patients who needed only diet to be in balance the percentages were: CD40 20.39 (3.77–94.4), CD40L 1.46 (0.3–9.1); for the patients treated with oral drugs were: CD40 17,07 (5,7–69,4), CD40L 2,85 (0,3–9,1) and in the group of patients needing insulin the percentages were: CD40 6,48 (0,02–53,7), CD40L 5,52 (0,09– 19,7). Though the series is small and the variance is great with evident overlapping values, the percentage of platelets expressing CD40 or CD40L correlate with the treatment needed, with a linear trend statistically significant ($p<0.05$), possibly mirroring a parallel increase in the risk of trombotic events. The inverse correlation between the CD40 and CD40L deserves further studies.

L007

ANTIFUNGAL TREATMENT WITH CASPOFUNGIN: A SINGLE CENTRE EXPERIENCE

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Infections are the main complication of the patients with hematologic diseases during severe neutropenia and among them fungal infections are the most diffucult to treat and the major cause of mortality for these patients. Because now we have a new antifungal class, Echinocandins, we have wanted to verify the tolerability and efficacy of Caspofungin.

From January 2004 until now we have treated 12 consecutive oncohemopatic and neutropenic patients. The schedule was: in case of persistent fever (at least 4 days) during broad spectrum antibiotic therapy a high-resolution CT scan of the lungs, an abdomen US scan, swabs from pharynx, nose and rectum and blood cultures were performed. In case of positivity of one or more of these findings Caspofungin was administered i.v. at the dosage of 70 mg on the first day and then 50 mg from the second day; the infusion time was 1 hour. The patients were 8 males and 4 females, the mean age was 47 yrs (range 30-60 yrs). The diagnoses were: acute myeloid leukemia 7, acute lymphoblastic leukemia 2, lymphoma 3; the disease's phases were: onset 2, first CR 2, CR>I 2, PR 4, Relapse 1, Resistant 1. Two patients were subjected to an allogeneic BMT, 1 to an autologous BMT, the other patients to an induction or consolidation or rescue chemotherapy course. In four cases Caspofungin was administered as secondary prophylaxis of a previous probable or proven fungal infection (in 2 of these patients the infection was proven and was from Aspergillus spp), for the other patients Caspofungin was administered for persistent fever and at least one lesion of the lungs with no evidence of bacterial or viral infection. The mean time of treatment was 18 days (range 7-21 days); the treatment was not discontinued for anyone of them because of adverse events; the dosage of Caspofungin was not changed for anyone. For the 2 allogeneic transplants cyclosporine A administration was not changed and we did not found any renal or liver alterations. No adverse events during the infusion of Caspofungin were seen and it was not necessary to administer any drug before the infusion. We did not seen breakthrough fungal infections. In only one case a proven fungal infection (Aspergillus fumigatus) was demonstrated so the other cases were probable or possible infections. No progression of the infection was seen. All the infections were completely cured. Four patients died: 3 of them for leukaemia and one for bacterial infection (Pseudomonas aeruginosa) after the fungal infection. These cases may show that now we have a new treatment option for fungal infections in neutropenic patients and this option is safe for the patients, does not preclude any other treatment (such as CsA), is well tolerated and the resolution rate of the infections is very high, probably because of the new mechanism of action of the drug. Our study should have been verified in a larger cohort of patients especially about its efficacy.

L008

EFFICACY OF SEQUENTIAL-COMBINED THERAPY WITH NEW ANTIFUNGAL DRUGS IN ADULT ACUTE NON LYMPHOID LEUKEMIA WITH INVASIVE ASPERGILLOSIS

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In adult ANLL induction fungal infection represent the main adverse event since can induce either high death rate as well as treatment delay, that may have a significant impact on disease outcome. The role of prolonged antifungal treatment for invasive aspergillosis was tested in 5 adult ANLL enrolled in intensive therapeutic protocol including stem cell traspantation.Of these 5 cases - 3 F,2 M, median age 31 (min 21- max 48 yrs) - 4 were de novo ANLL - FAB subtype M2 2,M5 2 - and 1 Acute Promyelocitic Leukemia (APL)in molecular relapse.As induction schedule ,all patients receveid standard or high dose citosyne-arabinoside + anthracyclines +/- etoposide;infection prophilaxis included itraconazole oral solution and chinolones.During induction, median length of severe neutropenia (PMN<500/mmc) was 23d (min18 - max 29). Febrile episodes have been empirically treated with broad

spectrum antibiotics (piperacilline/tazobactam plus aminoglicoside with or without a glycopeptide). Invasive fungal infection was diagnosed according to the International Consensus:in 4 cases aspergillosis was defined as probable since lung CT scan showed several spread parenchimal lesion but sputum and blood cultures were negative;in 1 case proven ,at nose level, by istology. First approach with liposomal-amphotericin B - total dose 3 gr/pt - failed; then 4 pts received voriconazole (median total dose 84 gr/pt) for a median of 210 d (min 120 – max 240) and 1 voriconazole (total dose 120 gr) for 300 d plus caspofungin (total dose 5.8 gr) for 170 d ,respectively.All patients were discharged after complete remission (CR)achievment,continuing antifungal treatment on outpatient basis.Complete infection recovery,documented by CT scan and histology ,was obtained in all patients after a median time of 3 months (min 2 - max 9).Hematological treatment plan,if delayed ,was continued.3 pts received consolidation with intermediate dose citosyne-arabynoside + doxorubicin,followed by stem dell trasplantation (STC): 2 allogenic and 1 autologous.The patient with proven aspergillosis uderwent soon ,after infection recovery,to allogenic STC.The APL pt had hematological relapse,was treated with arsenic trioxide,obtained 2nd CR and then uderwent allo-STC,while continuing voriconazole therapy.As antifungal related toxicity,we recordered peripheral neuropaty due to cyanocobalamin deficiency,promptly improved with B12 replacement. To date,all patients are alive at +13,+15,+18,+22 and +29 months;3 are in first CR at +17,+19 and +27;1 in 2nd CR at +10,while 1 pt relapsed 5 months after allo-trasplant.

In conclusion, these data ,if reported in a small serie,shows that prolonged treatment with these new azoles and echinicandines, represents an efficace and safe approach to cure severe aspergillosis in adult ANLL,thus to continue intensive antineoplastic plan.

L009

ANTIFUNGAL PROPHYLAXIS IN ELDERLY AFFECTED BY ACUTE LEUKEMIA

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Invasive fungal infection is an increasing source of morbidity and mortality in patients (pts) with acute leukemia, particulary in patients with prolonged severe neutropenia, preexisting myelodisplasia and advanced age. Early diagnosis of invasive fungal infection is difficult, suggesting that antifungal prophylaxis could be the best approach for these pts. In this work we retrospectively evaluated the incidence of the invasive fungal infection and of the employment of empiric antifungal treatment in elderly pts (>60 years old) affected by acute leukemia. All the pts have been treated with standard induction therapy and with different regimens of primary antifungal prophylaxis. Between Jan 2002 and Dec 2004 we treated 20 pts (mean age 69 years, range 60-74 years) with acute leukemia: 12 pts with acute myeloid leukemia (AML), 4 pts with acute promyelocitic leukemia (APL) and 4 pts with acute lymphoblastic leukemia (ALL). Antifungal prophylaxis regimens were: fluconazole 200mg day PO, itraconazol oral solution 200mg bid/day PO, nystatin 10 mLx3/day PO. Three pts was treated with fluconazole(1 ALL, 1 APL, 1 AML); 4 pts with itraconazol(1 APL, 3 AML) and 13 pts with nystatin(3 ALL, 2 APL, 8 AML).

During induction therapy the mean duration of absolute white blood cell count < 500 was 12 days for ALL, 11 days for APL and 18 days for AML. Empirical antifungal treatment with liposomial amphotericin B for fever unresponsive to broad spectrum antibiotic therapy was emploied in 3 pts (15%), with AML; fungal infection prophylaxis was nystatin for 2 pts and fluconazole for 1 pt. Only in 1 (5%) of these 3 pts a diagnosis of invasive fungal infection was confirmed by pulmonary biopsy ; this pt had received prophylaxis with fluconazole.

In conclusion in our elderly affected by acute leukemia the incidence (5%) of invasive fungal infection was within the range of incidence reported in literature in younger patients. Elderly with AML were at greater risk of invasive fungal infection in comparison with pts affected by ALL or APL. Our study group was too small in order to compar the efficacy of different antifungal prophylaxis regimen. However the results seem to indicate a greater efficacy of itraconazol (no patients treated with this regimen needed empiric antifungal therapy). More extensive studies are needed in order to identify the optimal antifungal prophylaxis for pts with AML.

L010

ANTIFUNGAL PROPHYLAXIS IN HIGH-RISK NEUTROPENIC PATIENTS WITH HAEMATOLOGICAL NEOPLASMS. A PRACTICAL EXPERIENCE IN A SINGLE INSTITUTION

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Background. The antifungal prophylaxis in neutropenic patients undergoing intensive chemotherapy for haematological neoplasm is still undefined and not uniformly applied in various experiences. Instead agreements and guide lines are underway concerning empiric and pre-emptive therapy or when a documented infection exists. In an attempt to emphasise the role of antifungal prophylaxis we present a retrospective experience on progressively modified approach with high risk patients with haematological neoplasms.

Patients and methods. Since Jenuary 95 to December 2004 we crossed three different antifungal prophylactic periods in severy neutropenic patients following chemotherapy: the first from 95 to December 97 with the use of Fluconazole and Nistatine per os, the second period from 98 to December 2002 with the use of low dose Deoxicolate Amphotericine B (20 mg/day) and a third period since January 2003 to present time with the use of low dose liphosomal Amphotericine B (1 mg/Kg x day). 328 patients entered the first period, 843 patients in the second period and 980 patients the third period. The evaluation consisted on the incidence of invasive fungal infections in the three periods under study.

Results. 845 blood culture were done during fever in the first period and 9 patients (3.2%) resulted positive for fungi with a positivity of 4% of cultures ; 8 patients for Candida Parapsilosis and one Candida Tropicalis. 2320 blood culture were done in the second period and 2% resulted positive for fungi; 2 patients with Candida Parapsilosis and one patient with Candida Albicans. 2700 blood cultures were done in the third period and 1% resulted positive for fungi; 3 patients showed a positivity for Candida Parapsilosis, 3 Candida Albicans, one Candita Guillermondi and one Geotricum Capitatum. A further evaluation consisted on the invasive infections involving other organs than blood; the first period included infections of Aspergillus Flavus (3 pts), Calingumella Bertholletine (1 pt) Rizopus Oryzoe (1 pt) and the last period Clostridium Neoformans (1 pt), Aspergillus Niger (1 Pt) and Aspergillus Fumigatus (1 pt).

Conclusive remarks. Our study demonstrated that the modification of fungal infection prophylaxis over the time, despite the increase of patient's risk, produced a general reduction of the incidence of documented infections. This observation leads to conclude that low dose Amphotericine B, given intravenously, Deoxicolate or liphosomal formulation, is effective in preventing invasive fungal infections in most of patients at high risk with malignant haematological diseases.

L011

AGROBACTERIUM RADIOBACTER, A RARE HUMAN PATHOGEN: REPORT OF A SEPSIS IN A LEUKAEMIA PATIENT

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The genus Agrobacterium is composed by small aerobic, gram-negative bacilli, generally involved in the pathogenesis of plant tumors. Among them, only the species Agrobacterium radiobacter has been occasionally reported to have a role in human infections. It has been generally isolated in immuno-compromised patients and is, therefore, considered an opportunistic pathogen.

We have observed a patient, female aged 78, with acute myeloid leukaemia onset after myelodisplastic syndrome, who during chemotherapeutic treatment developed a sepsis by Agrobacterium radiobacter, whose pathogenic role was confirmed by repeated isolations. The patient presented her 1st febrile episode under prophylaxis with laevofloxacin. The antimicrobial susceptibility testing, successively performed on the isolates showed that they were susceptible to this drug. An empiric antimicrobial treatment with piperacillin/tazobactam was started but the fever did not stop. The strain was still isolated in a further set of blood cultures after 4 days of treatment. Only the addition of Amikacin determined a persistent apyrexia confirmed by negative repeated blood cultures.

Our case, together with the few other cases reported in literature (about 40) confirm the potential pathogenic role of this bacterium in particular in hematologic patients.

L012

ACUTE RENAL FAILURE FOLLOWED BY DYSARTHRIA AFTER A SECOND COURSE OF INTERMEDIATE-DOSE CYTARABINE

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We report the case of a 53 year-old gentleman with 2 years story of chronic lymphocytic leukemia recently transformed in B-large-cell lymphoma, stage IVB for multiple lymphnodes, vertebral, liver, and bone-marrow localizations. He had been treated with fludarabin and cyclofosfamide for his low-grade disease, and with DHAP chemotherapy (cysplatin 100 mg/m^2 d 1, cytarabine 2 g/m^2 d 2, dexamethasone 40 mg d 1-4) after transformation. He achieved a partial response after the first DHAP, with complete regression of systemic symptoms, and a 80% reduction of measurable disease. Twenty-eight days later, one day after a second DHAP, the patient developed acute renal failure with anuria and severe hypocalcemia, followed by acute dysarthria. Creatinine levels arised to a maximum of 5 mg/dl, and after two dyalitic treatments levels started to lower and spontaneous emunction re-appeared. Calcium was delivered ev until full recovery. Two consecutive computerized tomographies (CT) scans of the brain were negative, and a magnetic resonance imaging (MRI) did not reveal any alteration of the signal, both in T1 and in T2. The patient died three days later because of a Clostridium Difficile sepsis. Others have reported acute cerebellar syndrome with nistagmus, ataxia and lack of coordination as a consequence of DHAP chemotherapy. To our knowledge this is the first case of acute isolated dysarthria as a consequence of cytarabine neurotoxicity, and with routinary images that could not explain the symptom.

However, acute renal failure is described as one of the major risk factors for cytarabine neurotoxicity, together with age over 50, high dose administered, male gender, and past history of neurologic dysfunction. Renal function should be carefully monitored when treating high-grade high-kinetic lymphomas, also when a good cytoreduction has been achieved by a previous cycle.

L013

COMBINED LAMIVUDINE AND ADEFOVIR DIPIVOXIL TREATMENT ALLOWS SAFE AND LONG-TERM CAMPATH-1H THERAPY IN REFRACTORY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA WITH HBV REACTIVATION

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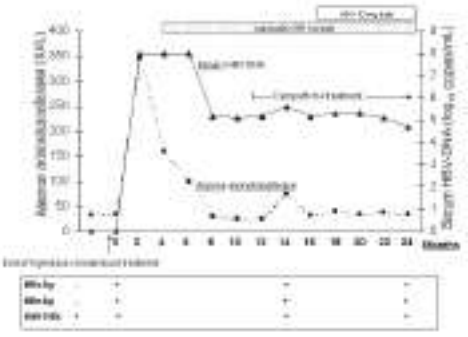
Hepatitis B virus reactivation in HBsAg-positive or HBsAg-negative/HBcAb-positive patients is a well known complication of chemotherapy. Binet stage A, B-cell chronic lymphocytic leukemia (B-CLL) was diagnosed in 1989 in a 49-year-old man, who tested HBsAg-negative/HBcAb-

positive. One year later, because of disease progression, low-dose chlorambucil therapy (5mg/day) was started and a partial remission was obtained. Subsequently the disease was maintained under clinical control by repeated cycles of low-dose chlorambucil during a period of 12 years.

In March 2002, because of a new relapse, the patient underwent 6 cycles of high-dose chlorambucil (0.5 mg/Kg/day for 5 days monthly) and obtained a new partial remission. Even at start of this treatment the only marker of HBV infection was anti-HBc positivity. Soon after the end of alkylators, liver function tests levels for the first time showed an abnormal increase; concomitantly HBsAg positivity, HBeAg positivity and HBV DNA levels of 8 log10 copies/mL (by PCR assay, LLQ <3.3 log10 copies/mL) were detected. Ultrasound examination of the abdomen was otherwise unremarkable. The temporal relationship between hepatitis onset and chlorambucil withdrawal, and the exclusion of any other known factor for HBV infection strongly indicate the reverse seroconversion of a silent HBV infection as the only responsible for the acute hepatic flare observed. In April 2003, due to the persistence of HBsAg and high levels of serum HBV-DNA, and in order to prevent hepatic flares, lamivudine (200mg/day) was started with normalization of ALT levels in the first 4 months; serum viral load decreased to 5.2 log10 copies/mL. In November 2003, due to a further leukemic B-CLL relapse, low-dose alemtuzumab (Campath-1H) (10 mg three times a week subcutaneously) was initiated. In March 2004, because there was not further reduction of serum virus load and because of the need of increasing the dose of Campath to 30 mg three times a week, adefovir dipivoxil 10 mg/day was added to ongoing lamivudine. This combined antiviral therapy enabled the MoAb therapy to be successfully continued for 10 months; in the meantime, ALT levels remained within the normal range and serum HBV-DNA progressively declined to 4.7 log10 copies/mL. No adverse events were recorded. To the best of our knowledge, this is the first report that the association of lamivudine with adefovir dipivoxil could allow a safe and long-term Campath-1H treatment in patients with advanced B-CLL and chronic hepatitis B.



L014

DEVELOPMENT OF MYCOSIS FUNGOIDES IN A PATIENT AFFECTED BY B-CELL CHRONIC LYMPHOCYTE LEUKEMIA PREVIUOSLY TREATED WITH FLUDARABINE PLUS CYCLOPHOSPHAMIDE

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In B-CLL an evolution to an aggressive B cell lymphoma, known as Richter's syndrome, can occur. The appearance of other secondary lymphomas is uncommon. Here we report a case of mycosis fungoides in a pre-treated patient with B-CLL.

On September 2002 a 52 year old woman was diagnosed as B Cell Chronic Lymphocyte Leukemia according to NCI–WG guidelines, by morphologic and immunophenotypic analyses of peripheral blood and bone marrow. At diagnosis the disease was stage 0 according to Rai classification after evaluation by ultrasonografy and TC scan. On November 2003 she had disease progression for increasing lymphocytosis and appearance of relevant lymphadenopathy, as showed by ultrasonografy and TC scan. A neck lymph nodal biopsy was performed and the diagnosis was consistent with small lymphocytic lymphoma or B-CLL according to WHO classification. She was treated with FC protocol (fludarabine / cyclophosphamide) for 4 cycles every 28 days. On may 2004 the TC scan didn't show any lymphadenopathy, and the cytofluorimetric assay of bone marrow revealed a pathologic cell infiltration equal to 2,8 %. On June 2004 she started suffering from pruriginous papules and nodules on her skin. A skin biopsy diagnosed Mycosis Fungoides CD 79-, CD 3+, CD4+, CD 8-, Ki 67 15%, TIA 1-, Gran Zyme B-. Therefore she has being treated with interferon plus PUVA therapy since then. At the moment there are no pruritus and cutaneous manifestations.

Conclusions. In this case the development of mycosis fungoides can depend on the immunological deregulation caused by both the first disease and its related treatment. Fludarabine induces prolonged immunosuppression which increases potentially the risk for secondary malignancies from 2 months forward after the start of therapy. At the moment in literature even if a slight increase of secondary cancers in patient treated with fludarabine compared with the general population is reported, the relative risk seems to be similar to that which has been described before the use of purine analogs (Cheson JCO, Vol. 17, 1999). In our case the secondary lymphoma doesn't show a worse prognosis compared with previous disease. Cytogenetic and molecular studies could explain the pathogenetic mechanism of secondary lymphoproliferative disorders.

L015

A SINGULAR CASE OF POLYCLONAL LYMPHOCYTOSIS

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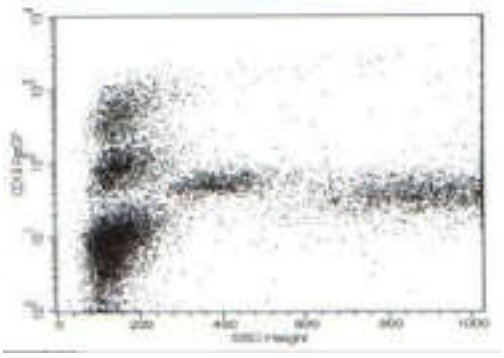
In 2004 a woman 55 years old had come to our observation, in apparent good healt, with peripheral lymphocytosis (WBC = 9.72 k/microliters; LYM = 5.03 k/microliters; %LYM = 51.2%). The patient is a smoker. Nothing to report in remote anamnesis. The clinical examination was usual. The QSPE showed hypogammaglobulinemia; the routine tests were usual; the hepatosplenic echography was negative. Therefore we executed the immunophenotyping in peripheral blood. With a cytometric plot, using the monoclonal antibody CD19 – PerCP versus SSC, two different CD19+ cell subsets have been pointed out, that differ for density of CD19 expression (see the next figure).



The clonal restriction has been investigated in these two cell subsets: taken together, these showed polyclonality; analyzed individually, it has been showed that the lower CD19 expression density cell subset had a kappa – clonal restriction, whereas the higher CD19 expression density cell subset had a lambda – clonal restriction. At this point some molecular biology tests have been executed in peripheral blood to evaluate the presence of the Ig genes clonal restriction, but these tests resulted negative for the Ig genes remanage. The cytometric analysis of the CD19+ cell subset in its entirety underlined polyclonality, confirmed by the molecular biology investigations, too. But the peculiarity of the clinical case consists in the fact that in the cytometric analysis within the CD19+ elements we distinguish two cell subsets with different CD19 exspression density and every one with a definite clonal restriction. Why two CD19+ cell subsets with different CD19 exspession density? Only the prolonged observation of the patient can give an answer to this question.

L016

PULMONARY LEUKOSTASIS SYNDROME SECONDARY TO B CELL CHRONIC LYMPHOCYTIC LEUKAEMIA. A CASE REPORT

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B-cell chronic lymphocytic leukemia (B-CLL) is frequently diagnosed with marked lymphocytosis or, during the observation, WBC count may be more than 100.000/mmc. The occurrence of very high lymphocytosis is rare and , even if present , in contrast to myelocytic leukemia , leukostasis is a rare complication in patients with B-CLL. We report a case of B-CLL presenting with marked lymphocytosis and leukostasis syndrome. A 61-year-old woman was admitted to our department with high circulating lymphocyte count and dyspnea. The day before she had had a right thoracic trauma. The physical exam showed diffuse lymphadenopathies, marked hepatosplenomegaly and right pleural effusion .

Haematological parameters revealed the leukocyte count to be 744.0x10^9/L with > 99% small lymphocytes, hemoglobin level 8,7 g/dl, and platelets count 269.0 x 109/L . Flow cytometry of peripheral blood lymphocytes showed a typical B-CLL phenotype (CD19+, CD20+, CD5+, CD23+, CD10-,CD38-) and cytogenetic study trisomy 12. Abdominal echography showed sub diaphragmatic lymphadenopaties. Cardiac echocardiography was evocative of pulmonary hypertension. She was severely hypoxemic and slightly hypercapnic with pO2 35 mm Hg, pCO2 52 mm Hg, sat.O2 74,5 %. Perfusional pulmonary scintigraphy excluded pulmonary embolism and chest high risolution CT scan didn't show any alteration. Laboratory parameters showed light increase of LDH of 662 mU/; D-dimers was 0,39 mg/L (nv < 0.2) ; all the other parameters were in the normale range. A pulmonary leukostasis syndrome secondary to B-CLL was diagnosed and treatment was started with vigorous idratation with saline solution, low molecular height heparin, methyl-prednisone 30 mg/mq, leukapheresis for 5 consecutive days and chemotherapy with the association of Fludarabine 25 mg/mq/day for three days, Cyclophosfamide 250 mg/mq/day for three days and a modified schedule of Rituximab (65 mg/mq days 2,3,4 and 130 day 5) in the hope to avoid tumor lysis syndrome. The WBC count decreased rapidly to 350.0x10^9/L on the 5th day and to 10.0x10^9/L on the 15th day from the start of chemotherapy. Contemporary to WBC decrease, the hypoxemia and hypercapnia improved to pO2 64 mmHg and pCO2 37.9 mmHg , the echocardiographic signs of pulmonary hypertension regressed and the patient was dismissed. The patient was further treated with other five cycles of Fludarabine, Cyclophosphamide, and Rituximab and achieved complete remission lasting 15 months. At this moment she is in a second complete remission achieved with Mabcampath. This case demonstrates that leukostasis syndrome may be present in B-CLL and that intensive treatment with leukapheresis and chemotherapy is indicated.

L017

CONCOMITANT CHRONIC LYMPHOCYTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA: EVIDENCE OF SIMULTANEOUS EXPANSION OF TWO INDEPENDENT CLONES

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B-CLL is known to increase the risk of developing subsequent solid neoplasms while the coexistence with other hematologic malignancies, and with AML in particular, has been described very rarely in previously untreated patients. We recently admitted to our unit a 69-year old male with an unremarkable past medical history presenting anemia (Hb 7.3 gr/dL, MCV 100 fl) and leukocytosis (WBC: 27520/mmc, 38% myeloid blasts, 44% mature lymphocytes, 18% neutrophils). Trephine biopsy revealed complete filling of intertrabecular space by MPO+/CD34+ blast cells and a nodular pattern of accumulation of CD5+/CD23+/CD20+ lymphocytes. Immunophenotyping of peripheral blood cells confirmed the coexistence of a blast cell population CD34+/CD13+/CD33+/HLA-DR+/CD7+ of myeloid lineage and a B-cell population CD19+/CD5+/CD23+ characterized by low surface immunoglobulin density with lambda light chain restriction. No chromosomal abnormalities were detected in both myeloid and lymphoid cells. Consistently, no molecular evidence of chromosomal translocations were observed by investigating in RT-PCR a number of fusion transcripts, including BCR-ABL (M-BCR and m-BCR), CBFb-SMMHC, AML1-ETO, TEL-AML1, DEK-CAN, MLL-AF4, E2a-PBX1. Based on these findings, the patient was diagnosed as being simultaneously affected by acute myeloid leukaemia and B-cell chronic lymphocytic leukaemia. The occurrence of these two malignancies raises the question about the possibility of a common origin as the result of a single transforming event. To check this hypothesis, we investigated the IgJH rearrangement pattern in DNA extracted from purified CD34+/CD19- and CD34-/CD19+ cell fractions, as obtained by immunomagnetic cell-sorting with CD34- and CD19-conjugated immunomagnetic beads. IgJH gene rearrangement, however, was detected only in the CD19+/CD34- fraction but not in the CD34+/CD19- one. These results provide evidence that the rare concomitant association of CLL and AML likely arise from simultaneous expansion of two independent clones.

L018

FLOW CYTOMETRY AND CONCURRENT DIAGNOSIS OF HAIRY CELL LEUKEMA AND CHRONIC LYMPHOCYTIC LEUKEMIA

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We report the case of a 60 year old man who came to our observation because of asthenia, mild cytopenia and splenomegaly. Examination of the bone marrow aspirate by standard microscopic morphology and immunocytochemistry showed the characteristic picture of hairy cell leukemia (HCL) infiltrate whereas flow cytometry (FC) was able to identify two distinct B lymphoid populations (CD19+) by the analysis of forward and side scatter. Interestingly, FC revealed a larger subpopulation of lymphoid cells (10 % of total leucocytes) expressing strong lambda light restriction and immunophenotype CD5-, CD11c+, CD19+,CD20+, CD23-, CD103+, FMC7+ consistent with HCL and some (3%) of the small B-cell without surface membrane immunoglobulins and associated immunophenotype CD5+, CD11c-, CD19+,CD20+, CD23+, CD103-, FMC7- typical of chronic lymphoid leukaemia (B-CLL). Additional FC procedures on the small B lymphocytes CD5+ revealed cytoplasm kappa light chains. The expression of different light chains immunoglobulin on HCL (lambda) and on CLL (kappa) cells suggested two coexistent and independent malignant clones. The patient was treated for HCL with IFN 3MU x 3/ week for 12 months. After this period, the revaluation of the patient by histologic analysis of the bone marrow aspirates showed, despite the therapy, a residual (10 %) presence of the HCL population but did not reveal typical cells of the B-CLL. The FC instead was able to detect both the hairy cell clone and an increased (12 % of total leukocytes) number of the lymphocytes CD19+CD5+CD23+.

The morphologic criteria failed to distinguish small clonal B-cells from normal lymphocytes because staining of neoplastic cells for kappa and light chain restriction was unsuccessful. FC was more efficient to detect the coexistence of HCL and CLL for its characteristic capability to identify also small amount of these subsets by the simultaneous and accurate assessment of cell size and antigen expression.

This case suggests how FC, being more sensitive than histology in detecting low levels of malignant cells, can play an important role in the study of B-cell expansions. Therefore the flow cytometry can be an invaluable tool in the analysis of bone marrow because the histological examination alone could be inadeguate for revealing small amount of neoplastic infiltrations.

L019

DE NOVO PH+ (P210) ACUTE MYELOID LEUKEMIA. A BLASTIC CRISIS WITHOUT CHRONIC PHASE? A CASE REPORT

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We describe a case of *de novo* acute myeloid leukemia (AML) who presented Philadelphia (Ph) chromosome with p210 hybrid transcript without a precedent chronic phase.

Case report. a 69 years old man was referred to our observation with typical features of AML, M1 subtype according to FAB classification. One months ago blood cell count showed a normal peripheral blood picture. We defined the AML as *de novo* onset. Anamnesis of patient revealed three previous neoplastic diseases: gastric and kidney adenocarcinoma surgically treated and cured, and one year ago a bowel adenocarcinoma subjected to surgical resection, which relapsed with liver metastasises and successively treated with palliative chemotherapy. For this reason periodic analyses , included monthly blood stain, were performed, and only the last one showed marked leucocytosis (63 x 10^9/L) with peripheral blasts 95%, and thrombocytopenia (47 x 10^9/L). Haemoglobin (14 g/dL), red blood cells count (5,19 x 10^9/L) and medium globular volume (86 microliter) were normal. Except a bad performance status, physical examination did not reveal particular features: lymph-nodes, liver and spleen enlargement were not present. Bone marrow aspirate confirmed the diagnosis of AML, FAB M1, with a massive blast infiltration (80%). Unexpectedly cytogenetic analysis revealed the presence of Ph chromosome associated to trisomy 8. This datum was confirmed with RT-PCR analysis who revealed t(9;22)(q34;q11) with major breakpoint, compatible with p210 hybrid transcript. So we considered this Ph+ AML similar to a blastic crisis without a precedent clinically evident chronic phase. After the initial induction treatment with Idarubicin-Cytarabine schedule, the patient continued with Imatinib 400 mg/die without significant adverse events. The period of observation is now shortest (30 days) to determine the cytogenetic and molecular response to Imatinib.

Discussion. Ph+ AMLs are rarely described, and are characterized by elevated aggressiveness and poor prognosis. The majority of the authors retains that the origin of this disease is at stem cell level, and the pathophysiology is similar to blastic phase of chronic myeloid leukaemia, but differently from this one no previous chronic phase is clinically evident. We retain interest two aspects of our case: the first is the previous normality of blood features one month before the onset who supports the "de novo" origin of this leukaemia, and the second is the anamnesis of the patient who revealed an elevated susceptibility to neoplastic disease, so it is hypotisable any kind of genetic instability who leads to rapid evolution of a transforming lesion such as Ph chromosome. Gene profile studies would be required for a better evaluation of this aspect.

L020

ASSOCIATION OF ARA-C, MITOXANTRONE AND ETOPOSIDE IN ELDERLY AFFECTED BY ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a frequent disease among elderly people (>60 years). Only few elderly pts can be treated with standard chemotherapy because of an high incidence of complications and deaths related to treatment. Moreover complete remission (CR) is achieved in less than 60% of elderly with AML and the mean of overall survival is 8 months. In order to reduce the toxicity and the number of toxic death we developed a specific regimen for elderly affected by AML: ARA-C 100mg/m^2 bid day IV on days 1-7, if pts > 65 years ARA-C 100mg/m^2 bid day IV on days 1-5, etoposide 100mg/m^2/day IV on days 1-3, mitoxantrone 10mg/m^2/day IV on days 1-3. If pts achieved CR, they received four courses of postremission therapy with ARA-C 100mg/m^2 day on days 1-5. From Jan 2002 to Dec 2004 we treated 9 elderly pts (6 males, 3 females) mean age 70 years old (range 60-74) affected by AML. A known or presumed myelodisplastic syndrome was present in 4 (45%) cases. Karyotype analisis showed: normal karyotype in 4 pts; del (20q) in 2 pts; del(12q) in 1 pt; t(8-21) in 1 pt, complex karyotype in 1 pt. During induction therapy the median duration of absolute white blood cells < 500 was 18 days (range 15-27). All the patients experienced fever > 38.3°C for a mean time of 9 days (range 5-19) and 2 pts (22%) needed empirical antifungal therapy with liposomial amphotericin. No toxic death was observed. Complete remission was obteined in 7 (77%) pts and 6 pts (66%) received at least 3 courses of postremission therapy. Four pts (57%) relapsed at 2,8,9 and 12 months and died within 3 months from relapse. The median duration of follow-up was 7 months and the exstimated 12 months overall survival was 34%. In our experience the emploiment of an association of ARA-C, mitoxantrone and etoposide is feaseble and seems to induce a good rate of CR (77%) in elderly with AML without any toxic death. Despite the favourable response to induction therapy, 57% of the pts relapsed within 12 months. These results suggest the need to develop novel therapeutic strategies for elderly with AML.

L021

USE OF MYLOTARG: EXPERIENCE OF A SINGLE UNIT

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Mylotarg is brand name of a monoclonal antibody (Gentuzumab ozogamicin – GO)-anti-CD33, conjugated with an anti-cancer protein (Calicheomicin), produced by Wyeth-Ayerst and approved by FDA on May - 17 – 2000 for refractory Acute Myeloid Leukemias (AML) therapy, initially only for elderly (aged > 60), now for young people

too. It is the first conjugated monoclonal antibody used for targeted therapy. It is active as single agent against poor risk AML (Hamann, Bioconjug. Chem.- 2000) and against relapsed AML (Larson, Leukemia – 2000) with a C. R.-rate of 30 %, but its effect is increased by adding conventional chemotherapy, particulary Aracytin, as reported by up to date multinational study (2004), that followed up foregoing experiences by German Groups (GO + Ara - C + Dauno), French (MRC: 4 studies of Phase II , with 4 different protocols), American (MFAC: GO + Fludara + Ara–C + Cyclosporine), Italian – EORTC – LG -GIMEMA (Phase II : patients aged 61 – 75) and provides for the administration of GO– 9 mg/sqm on 1 th and 15 th day, followed up a MICE-cycle (C.R. = 50 %). The above-mentioned study concerned patients aged > 60 (Baccarani, Durrant, Linkesh – Blood,2002 ; 100 : 341 a. Abstract 1322) with GO – 9 mg/sqm on 1 th and 8 th day + Ara-C 100 mg/sqm c. i. 1 th - 7 th day and re-proposed by another Italian multiple experience (Piccaluga, Baccarani, Visani – Bologna + Pesaro - Leukemia Research – 2004) with the use of GO – 6 mg/sqm intravenous 1 th day and 4 mg/sqm intravenous 8 th day + Ara-C at the same dose (C.R. = 56 %). Our Group treated from Genuary to Dicember 2004 10 patients suffering from AML, 6 relapsed and 4 refractory patients. The mean age was 54 years (40 - 73). 4 relapsed patients receaved mean-low doses of GO (3 mg/sqm) day 1 and day 15 with intermediate administration of MICE, 2 relapsed patients received GO + Ara–C (as Bologna + Pesaro), 2 refractory patients receaved low doses of GO (2 mg/sqm) repeated day 1, 3, 5; two refractory patients receaved low doses of GO (2 mg/sqm) weekly for 4 weeks and after montly. Relapsed patients deceased all. Of the refractory patients two deceased and two are still alive and fairly good clinic conditions. Our experience, even if of restricted number and even if we consider that deceased patients had all relapsed and, therefore, were in a very bad prognosis, seems to show that therapy with GO is more right for refractory patients, especially aged, better if with reduced leukemic burden.

This conclusion is in accordance with all that already reported by other experiences and receivesfurther confirmation by an up-to-date report (Van der Velden, Leukemia – 2004) that a high charge of antigens CD33 into the peripheral blood is an unfavourable prognostic factor, because the peripheral captation carried out by CD 33 towards GO sensibly reduces the saturation of CD33 into the bone marrow (40 – 60 %) and therefore is the determining factor of smaller effect of killingagainst blastic cells.

The immediate conclusion we get is that GO has to be administered using higher doses, but the acquired experience about this problem reports a fairly good effect of Veno-occlusive disease (VOD). Therefore, we have to resort to splitting up of the doses, repeated in short time.

But still more we can obtain with the association GO + conventional chemotherapy. The most way seems to be, therefore, not the infusion of chemotherapy within two administrations of GO, but the preliminary reduction of the leukemic burden before Mylotarg.

L022

GASTROINTESTINAL COMPLICATIONS IN ACUTE MYELOID LEUKEMIA: THE TROUBLE OF DIFFERENTIAL DIAGNOSIS. A CASE REPORT

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Gastrointestinal complications during induction chemotherapy for AML are usually due to chemotherapy toxicity or infection. Neutropenic enterocolitis and leukemia localizations in the bowel wall are rarer, but potentially fatal.

The case of a 44 year old woman is reported. AML M4, according to the FAB classification, with hyperleukocytosis (116,000 WBC/mmc, blasts 68%), dermal and mucosal involvement, was diagnosed in April 2004. On day 5 of induction chemotherapy (ICE: idarubicin, cytarabine and etoposide) the patient developed neutropenic fever (38° C), diffuse abdominal pain and subsequently diarrhea. All microbiological findings were negative. The abdominal X-ray performed on +9 showed a distended and fluid-filled colic tract demonstrating an occlusive ileus, and the sonographic scan documented an abnormal thickening of the bowel wall (14 mm), consistent with the diagnosis of neutropenic enterocolitis. Due to the worsening of the clinical findings and the risk of perforation (increasing abdominal pain, absence of bowel motility, vomiting and hyperbilirubinemia) an emergency laparotomy with resection of 1.5 m of jejunum and a derivative jejunum-ileostomy was performed on day 11 in presence of severe neutropenia and thrombocytopenia. The histology demonstrated a massive infiltration of the bowel by leukemia cells with diffuse necrosis. The patient required intensive care but the dehiscence of the anastomosis caused a peritonitis sustained by a Klebsiella Pneumoniae sepsis and complicated by multiorgan failure. Further surgical interventions were necessary during the hospitalization in the ICU in order to treat an acute cholelithiasis, for the conversion of the external stomy in a jejunum-ileum anastomosis and the recanalization after the dehiscence. During this period, which lasted 3 months, the patient achieved complete remission. She was then treated with 4 consolidation cycles (IC, HidAC 8g/sqm and subsequent PBSC collection, 3 cycles of HidAC 20g/sqm + Idarubicin 40mg/mq each with reinfusion of PBSC). At present the patient is still in complete remission after 10 months from diagnosis and 1 months off therapy, with a completely restored enteric function.

Comment. The clininal and US findings consistent with a diagnosis of neutropenic enterocolitis during the pancytopenia subsequent to the induction chemotherapy could overlap or mask a necrotizing colitis due to massive infiltration of the bowel by leukemia cells. For this reason we recommend to consider this complication in case of diagnosis of AML M4.

L023

FLAG-IDA-MYLOTARG IN THE THERAPY OF RELAPSED AND REFRACTORY ACUTE MYELOID LEUKEMIA. PRELIMINARY RESULTS

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Patients with relapsed and refractory AML have a bad prognosis. In this setting is often difficult to obtain a complete remission. In the last years fludarabine and high dose Citarabine with or without Idarubicine and G-CSF have been frequently used (FLAG and FLAG-IDA schedules). The immunotoxin gemtuzumab ozogamicin (Mylotarg) (GO) Wyeth is a humanized IgG4 monoclonal antibody directed against the CD33 epitope, which is chemically linked to calicheamicin, a highly potent antitumor antibiotic. As a single agent it has been shown to be an effective agent in the treatment of relapsed AML with a tolerable toxicity profile. Recently the United Kingdom Medical Research Council (MRC) published the preliminary results of AML15 trial , which was designed to evaluate the effect of adding GO to each course of intensive induction or consolidation chemotherapy in patients younger than 60 years as first-line treatment. These results are encouraging.

We are using this association in the treatment of relapsed and refractory adult AML. Until now 7 patients have been enrolled in this trial and we show our preliminary results.

Patients and methods. 2 patients were resistant to AML12 EORTC-GIMEMA induction ; 1 patient was in the first relapse and 1 in the third after conventional chemotherapy, 1 was relapsed after autologous transplant and 2 were relapsed after allogeneic transplantation. The demography and characteristics are shown in the Table.

pts	age yrs	disease status	treat. response	further therapy	outcome
pt 1	46	resistant	CR	BMT	CR +6M
pt 2	52	resistant	resistant	--	died
pt 3	69	1 th relapse	CR	--	CR +4M
pt 4	65	3th relapse	died		
pt 5	57	relapse after auBMT	died		
pt 6	58	relapse after BMT	resistant	--	died
pt 7	28	relapse after BMT	CR	2th BMT	CR +3M

All were treated with the same schedule. Fludarabine 30 mg/sqm dd 1-5, Citarabine 2,0 gr/sqm dd 1-5, Mylotarg 3 mg/sqm d -1, G-CSF 375 mg dd 1-6.

Results. Three patients obtained Complete Remission , 2 died after therapy and 2 were resistant to this schedule.The three patients in CR are in continuous complete remission at 3, 4 and 6 months. Two of these underwent allogeneic BMT with no significant toxicity. In our experience the toxicity of FLAG-IDA-GO schedule is acceptable and the results are promising in refractory and relapsed AML.

L024

BIPHENOTIPIC ACUTE LEUKEMIA WITH COEXPRESSION OF CD79A AND MARKERS OF MYELOID LINEAGE: A CASE REPORT

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Biphenotipic acute leukemia blasts expresses markers of two different lineages, most commonly myeloid and either B or T-lymphoid. This entity has been defined by a scoring system from the European Group for the Immunological Characterization of Acute Leukemias (EGIL): CD79a expression in association with blast antigens is considered to be indicative of B lineage ALL.

We report an unusual case of biphenotipic acute leukaemia, according to EGIL criteria, with CD79a expression along with other lymphoid and myeloid antigens and its modified expression during induction treatment when a myeloid population appears.

A 51-years-old woman presented with dyspnoea on exertion, joint pain and cytopenia. Clinical examination revealed splenomegaly, cervical lymphadenopathy and oral candidiasis. At admission blood cell count was: Hb 7.5 grams/deciliter, WBC $4.7x10^9$/Liter, N $0.45x10^9$/Liter, PLT $49x10^9$/Liter. A blast population representing 90% of bone marrow nucleated hematopoietic cells and 24% of the peripheral blood cells was identified. Blasts have a variable appearance, with a few elements with a small number of cytoplasmic granules. Myeloperoxidase stain (BM) was weak positive (4%) while nonspecific esterase stain was negative. Cytogenetics showed no abnormalities. Flow cytometric immunophenotipic analysis (BM) showed coexpression of myeloid and B-lymphoid antigens, with a medium-strong positivity for CD79a. The same analysis was performed in the protocol centralization centre and diagnosis of acute lymphoblastic leukemia (ALL) with a not well defined myeloid component (reactive? biphenotipic?) was done. Standard induction treatment for ALL was given until g+14 when, subsequently to G-CSF administration, leukocytosis was observed.

Morfologic and flow cytometric immunophenotipic analysis revealed a single myeloid blast population. Therapy was consequently modified by adding high-dose cytosine arabinoside. Complete remission was obtained and consolidation chemotherapy was given; peripheral blood stem cells were harvested. No sibling donor was available so patient underwent autologous peripheral stem cell transplantation (PBSCT). At complete recovery from PBSCT, prophylactic intrathecal therapy was started. Patient is actually at > 200 day from PBSCT, in continuous complete remission and good clinical conditions.

Biphenotipic leukemias are uncommon and frequently demonstrate an aggressive disease course with survival rates inferior to those of leukemias derived from a single-cell lineage. Cytogenetics abnormalities and age under 15 years are strongly associated with a poor prognosis. Patients with biphenotipic leukemia should have risk stratification with treatment tailored to their prognostic factors, in absence of which a myeloid-based treatment seems to be a reasonable option.

L025

ACUTE MYELOID LEUKEMIA IN ELDERLY PATIENT: A TWO-CENTER STUDY ON TREATMENT OUTCOME AND THERAPY-RELATED PHARMACOECONOMIC ANALYSIS

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Which is the best treatment for AML in elderly patients is still debated. Actually there are three main options: supportive treatment (ST), conventional chemotherapy (CC) and low dose chemotherapy (LDC).

Aim of this study is to define the best therapeutic and pharmacoeconomic approach in elderly AML. This is a retrospective nonrandomized study. A cost analysis on 17 patients hospitalization was performed. The monthly cost of hospitalisation or specific care was calculated dividing the global expense of hospitalisation or specific care in each group of treatment, for the sum of survival months of all patients in that group. We present a two center study. 21 patients (12F/9M), median age 72 years (R 65-80), were treated as follows: 9 with CC, 9 with LDC, 3 with ST. 14 patients presented comorbidity, 13 had PS 0-1, 8 had secondary leukaemia and M2-M4 were the most represented FAB subtypes. The most frequent comorbidities were diabetes (7pts), second neoplasms (5pts) and ischemic cardiopathy (4pts). Global median survival for all patients, without regard for the treatment received, was 3 months (R1-10). Median survival was 5 months for patients treated with CC, 5.5 for LDC and 1 for ST. Median hospitalisation was 1 month for ST (R0.5-1), 2 months for CC (R1-8) and 1 month for LDC (R0.2-3). Monthly cost of hospitalisation was €5100 for ST, €3700 for CC and ?1000 for LDC.

The antibiotic expense was higher in ST (€2900/month vs €1100/month in CC and €300/month in LDC), but transfusion expense was higher in CC (€1200/month vs €900/month in ST vs €500/month in LDC).

Chemotherapy expense was €500/month in CC vs €6/month in LDC. New drugs (Mylotarg and Glivec) increased significatively chemotherapy costs (€900/month vs €40/month) and hospitalisation expense (€4100/month vs €2300/month).

Erythropoietin use didn't reduce transfusional expense (€1200/month vs €700/month in patients without erythropoietin).

G-CSF administration wasn't effective in antibiotic expense reduction (€1800/month vs €1100/month in patients without G-CSF).

Supportive measures were higer in CC and ST (€1200/month) than in LDC (€200/month). Albumin, parenteral nutrition and growth factors use are the main cause of increased expense in supportive measures.

In conclusion LDC seems to be an economic and effective therapeutic option especially if performed in outpatient setting.

A more appropriate use of albumin and growth factors would be desirable to reduce an inappropriate and eccessive sanitary expense.

Use of new drugs in elderly AML is still to well define under therapeutic and pharmacoeconomic aspect.

Nevertheless these data need further confirmation on a larger patient cohort.

L026

FLA(G) OR IDA-FLA(G) REGIMENS IN THE TREATMENT OF REFRACTORY OR RELAPSED ACUTE MYELOID LEUKAEMIA

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The prognosis of patients with primary refractory or relapsed acute myeloid leukaemia is poor. Regimens containing Fludarabine and Cytarabine have been proved effective in this setting. We retrospectively evaluated the results obtained with FLA(G) or Idarubicine-FLA(G) in 9 refractory and 13 relapsed acute leukaemia patients treated between 2001-2004.

Patients characteristic and results of therapy. Primary refractory AML (Table 1): the median age is 36 years (range 9 - 70); Pts 1,2,3,5,6,8 were at high risk for clinico-biological markers at diagnosis; all were refractory to induction chemotherapy with standard doses Cytarabine. All were treated with one or two courses of FLA(G) or Ida-FLA(G) therapy and 7/9 obtained a Complete Remission (CR). The patient 7 and 9 (age 63 and 70yr) received, after consolidation therapy with FLAG, two further courses of high doses ARA-C; one is in continuous 1^CR after 13 months, the second relapsed after 10 months. The others 5 patients, after consolidation therapy, relapsed at a median time of 3 months (range 3-4). 2 patients were again treated with Ida-FLAG and obtained a 2^ CR : the first received an unrelated SCT and is living in CR at 43 months; the other received haploidentical SCT and died for infective complications. The others 3 patients were transplanted in 1^ early relapse: two with unrelated donor and died of progressive leukaemia, the third patient from a matched related donor (MRD) and he is currently alive in 2^ CR at 13 months. Patients relapsed after autologous BMT (table 2, pts 1-6): median age is 39 years (range 19-53yrs); they received FLA(G) or Ida-FLA(G): 5/6 patients obtained a 2^ CR. 3 of them, after consolidation therapy (FLA(G) or HDARA-C), underwent SCT (pt 1 haploidentical and pts 3 ad 6 MUD) They are in CR, respectively at 24, 31, 14 months. Pt 4 refused further therapy and is actually in CR at 29 months. Pt 5 didn't find any donor, relapsed after 14 months and died. Patients relapsed after conventional chemotherapy (table 2 pts 7-13): median age is 62 years (range 51-67 yrs); the previous induction regimens were with standard doses Citarabine for pts 1-6 and with high doses citarabine for pt 7. Pt 1 was in 2^ relapse, the others were in 1^ relapse. All were treated with FLA(G) or Ida-FLA(G) and Pts 1,2,3,4,5 obtained a new CR. Pt 1 underwent autologous SCT in 3^CR and is in CR at 26 months. The others patients relapsed: three didn't received any fur-

ther therapy, Pt 2 was transplanted with a non myeloablative conditioning regimen from a MRD in early relapse and is alive at 35 months in 3^CR.

Conclusion. FLA(G) and Ida-FLA(G) regimens are effective to overcome standard doses Cytarabine resistence also when poor prognostic biological markers are present; they are well tolerated in elderly patients and have a low extra-hematological toxicity allowing further heavy transplant procedures. The CR is short in refractory patients, while long disease free survival may be obtained in relapsed patients. It is notable that most patients were previously treated with standard doses Cytarabine.

Table 1. Patients primary refractory to Standard Doses Ara-C induction

Pts	Age (yrs)	risk	2^line chemo	Response	Further treat.	Outcome
1	9	46,XY,11q-	FLAG	resist.	Haplo SCT	died
2	16	45,XX,der(6),-9	FLAG	1^ CR(4m)	MUD SCT	died
3	17	46,XY,t(1;7),t(10;11)	FLAG	1^ CR(3m)	MUD SCT *	2^CR (+43m)
4	26	46,XY	IdaFLAG	1^ CR(3m)	MRD SCT**	2^CR (+13M)
5	36	WBC >100.000	IdaFLAG	1^ CR(3m)	Haplo SCT*	died
6	38	FLT3,ITD WBC >100.000	IdaFLAG	1^ CR(3m)	MUD SCT**	died
7	63	FLT3,ITD	IdaFLAG	1^ CR(10m)	HD-ARA-C	relapse
8	70	46,XX	FLA	resist.	no	died
9	70	46,XY	FLAG	1^ CR(+13m)	HD-Ara-C	1^CR (+13)

**BMT in 2^ CR **BMT in early relapse*

Table 2.Patients relapsed after autologous transplant (pts 1-6) or conventional therapy (pts 7-13)

Pts	Age (yrs)	Cytogenetic	1^CR m	Chemio	Response	Further treat.	Outcome
1	19	47,XY,+8,INV16	20	FLA	2^CR	FLA + HAPLO SCT	CR +24 m
2	55	46,XX	4	IdaFLAG	resist		died
3	21	46,XX, t(8;21)	31	FLA	2^CR	FLA + MUD SCT	CR +31 m
4	53	45,X,-X, t(8;21)	12	FLA	2^CR	FLA	CR +29m
5	39	46,XY, t(8;21)	14	FLAG	2^CR	HD ARA-C X2	died for AML
6	39	46,XY, t(8;21)	57	IdaFLAG	2^CR	IdaFLAG+MUD SCT	CR +14m
7	51	45,X-Y, t(10;11)	96 (2^CR)	FLAG	3^CR	FLA + Auto	CR+26 m
8	64	46, XY	10	FLAG	2^CR	FLAG + HD Ara-C+ alloBMT*	CR+36 m
9	67	45, X,-Y, t(8;21)	10	FLA	2^CR	FLAG	relapse 11 m
10	62	failed	13	FLAG	2^CR	FLAG	relapse 12 m
11	58	46, XY	18	IdaFLAG	2^CR	FLAG	relapse 6 m
12	67	46, XY	9	FLAG	resist		died for AML
13	60	45,XX,-5,+8,-15,t(3;12)	3	FLAG	resist		died for AML

**BMT in 2^ early relapse*

L027

ACUTE MYELOID LEUKEMIA (FAB M4) FOLLOWING LONG TERM TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Acute myelogenous leukemia (AML) is estremely rare in chronic lymphocytic leukemia (CLL) despite extensive use of alkylant agents. We present the case of a patients with CLL treated for long time with clorambucil who developed acute myeloid leukemia. This 67 year-old caucasian man was affected by chronic lymphocytic leukemia and monoclonal IgG kappa gammopathy since 1997. At the beginning, the peripheral blood and bone marrow smears revealed a proliferation of mature and small lymphocytes with typical immunophenotype (CD5+, CD19+, CD23+, CD20+ , kappa+) and the atypical presence of CD11c. At the disease progression (lymphocytosis, spleen enlargement), the patient started treatment with clorambucil. Over the period of 5 year (January 1999 - December 2004) the total dose was 4800 mg. During an outpatient routine control in December 2004 he appeared in partial remission for CLL, but he developed high grade fever and severe pancytopenia (leucocytes < 1000, platelets < 20000 and anaemia < 8 gr/dL). The peripheral blood and bone marrow smears revealed a partial infiltration by lymphocytes with small and mature phenotype and an unexpected proliferation of blast cells with this immunophenotype: CD15+, CD64+, CD33+, CD34+, HLADR + and CD11c and MPO+ inside cells. The bone marrow trephine biopsy revealed diffuse reticular fibrosis, lymphocytes population with nodular arrangement and middle size blast cells (30% of total cells) with the same characteristics. This histological picture was consistent with AML (FAB M4). The patient's general clinical condition did not allow intensive treatment and he died after 2 months because of hemorrhagic complications. The occurrence of AML treated with alkylant agents is extremely rare but we can speculate that the presence of a marker such CD11c which was present since the beginning of the lymphoid disease and in both lymphoid and myeloid proliferation, during the end stage disease could have a role in this patient with severe prognosis heavily treated with alkylant agents.

L028

THE ETOPOSIDE IN THE TREATMENT OF ACUTE MYELOMONOCYTIC AND MONOCYTIC MYELOID LEUKEMIA IN THE ELDERLY: OUR EXPERIENCE COMPARING SCHEMES WITH AND WITHOUT VP-16

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The treatment of the acute myeloid leukemia (AML) in the elderly is still debated. Different cycles have been used in literature (FLAI; I.C. FLAG; MICE etc.) with similar results in terms of DFS and OS. A very poor prognosis is correlated with the subtypes FAB M4 and M5. The aim of our study is valuate the difference among cycles with or without etoposide. The etoposide is a inibitor of topoisomerase II, this drug produces a damage to the Dna of the cells and interferes with the replication and the transcript, the etoposide is commonly used in the treatment of the AML, especially it results active in acute myelomonocytic and monocytic leukaemia. From June 2001 to March 2005 we have treated in our division 20 AML (FAB M4/M5) 10 male and 10 female with median age of 70 old years (range 61-84 years). 11 patients (4 male and 7 female with median age of 72 years) have been treated with the I.C. FLAG scheme (Fludarabine 20 mg/m^2 in continuous infusion for

three days and cytarabine 1450 mg/m^2 in continuous infusion for 4 days) while 9 patients (6 male and 3 female with median age of 68 years) have been treated with the MICE scheme (Mitoxantrone 6 mg/m^2 days 1-3-5; etoposide 80 mg/m^2 days 1-5 and cytarabine 100 mg/m^2 in continuous infusion for 7 days). In IC FLAG group 4 patients (36%) have obtained a complete remission, 6 patients (66.5%) in the MICE group, with significant difference. The median DFS in the IC FLAG and MICE groups has been of 6, 67 and 6, 53 months respectively while the median OS has been of two months in the IC FLAG group and 6, 67 months in the MICE group. The rate of TRM has been of 18% and 10% in the groups IC FLAG and MICE respectively, no statistical difference. These results have shown a best rate of CR and overall survival in patients treated with etoposide, this difference is not statistically significant for the group MICE. Not significant the DFS among the 2 groups. In conclusion the etoposide is a good drug in the treatment of these patients but one more large cohort is necessary to appraise the real difference among the 2 treatments. For the poor prognosis new therapeutic strategies are necessary. Mini allogeneic transplantation is to possibility of cure but it appears feasible only in patients <65 years and in very good condition. Autotransplant is a therapeutic option but impotant is to value a regime of based conditioning on the high dose etoposide. Another important drug is the gemtuzumab ozogamicin in induction, consolidation or maintenance therapy in AML (FAB M4 and M5) patients.

L029

ACUTE LEUKEMIA AFTER FLUDARABINE AND CYCLOPHOSPHAMIDE T HERAPY IN PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA: MUTAGENIC OR IMMUNOSUPPRESSIVE EFFECT? A CASE REPORT

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The development of acute myeloid leukemia secondary to treatment for chronic lymphocytic leukemia is a rare event. Only few reports describe this evenience in a very limited number of patients. We report a case of acute erythroblastic leukemia developed after fludarabine therapy for chronic lymphocytic leukemia. A 62 years old man underwent to our observation because of severe trilinear pancytopenia casually detected at a routine control. Sixteen months before, in another institution, chronic lymphocytic leukemia stage C of Binet was diagnosed. Subsequently patient received six courses of fludarabine phosphate (25 mg/sqm day 1-3) and cyclophosphamide (300 mg/sqm day 1), with normalization of CBC. A bone marrow biopsy was performed at the moment of our first observation and showed a diffuse infiltrate rappresented by blasts CD34-, MPO-, CD68/PGM1-, glicophorin C+. Then diagnosis of acute myeloid erythroblastic leukemia M6 FAB was posed. Karyotype analysis showed a complex feature. Treatment with "3 + 7" schedule was started. Twenty five days after this therapy, a bone marrow aspiration showed that patient achieved morphological complete remission with persistent alteration of karyotype. A new course of "3 + 7"schedule was performed but without significant benefit and the progression of leukemia. Three months later patient died.

The existence of secondary leukemia post myelodysplasia, alchylating agents, topoisomerase inhibitors, antracyclins and radiotherapy administration is well known. Reports regarding acute leukemia onset after fludarabine are scarcely signaled. Nowadays is still unclear if fludarabine has a direct mutagenic activity. On the other hand, fludarabine administration and chronic lymphocytic leukemia are either immunosuppressant conditions. In this setting acute leukemia development might be a condition equal to a second neoplasia onset (event not infrequent in the two conditions previously mentionned). Our patient presented also a cytogenetic alteration involving 5q, that tipically is found in acute myeloid leukemia secondary to alchilating agents. If cyclophosphamide has had a mutagenic role in this patient, could have been the leukemogenic effect enhanced by the concurrent immunosoppression of fludarabine and chronic lymphocytic leukemia ? In any case we have to consider that our patient received only low dose of alchilating agent and the interval of time between the two neoplasie has been too much short.

This report underlines the increased necessity of a strict, global monitoring in patient affected by lymphoproliferative disorders and treated with fludarabine, with the aim to detect precociously secondary neoplasia onset.

L030

MAJOR CYTOGENETIC RESPONSE AFTER PROLONGED MYELOSUPPRESSION DURING A SHORT TIME IMATINIB THERAPY IN LATE CHRONIC PHASE OF CHRONIC MYELOID LEUKAEMIA

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Myelosuppression after imatinib therapy in chronic myeloid leukaemia occurs frequently in late chronic phase and is more common in patients with advanced disease. Patients in late chronic phase after early imatinib therapy, that degree myelosuppression ,interrupt treatment and have a high probably not achieve complete cytogenetic response (CCR) than those who had no interruptions. We present a case of chronic myeloid leukaemia ,female 64 years old , in late chronic phase refractoriness to alfa IFN , who obtained major cytogenetic response (MCR) after prolonged myelosuppression then starting low-standard dose imatinib therapy. A female patient 65 years old received diagnosis LMC t(9;22) in 1997, intermediate Sokal score. She was refractoriness to three years discontinous alfa IFN therapy and obtaining a transitory minor cytogenetic response, failured at she has interrupted in 2001. Then her treatment was hydroxiurea (HU) only until February 2002. Imatinib therapy started in march 2002 , dose standard 400 mg daily in chronic phase and 100% Ph positive. Myelosuppression grade III-IV occurs after 20 days through out six months far from starting , no infectious complications occurred. Prednisone (40-80 mg daily first month , then 25 mg/daily all over myelosuppression) and blood transfusions were required. In September 2002 she came back in chronic phase disease – Ph 100% positive- and during five next months, no therapy was in need because

of haematologic response. Resumption of disease occurs and an unusual schedule with Imatinib was approached (dose: 100 mg/daily for 5 days/month) starting in February 2003 until November 2003, obtaining haematologic response only. In January 2004 , for facing leucocytes increasing , a new rise of Imatinib dose was made for continuous therapy (300 mg/daily) and this time myelosuppression does not occur. In May 2004 she obtained haematologic and major cytogenetic response, still mainteined. Myelosuppression is an Imatinib therapy side effect that occurs in patients in late chronic phase, accelerated and blast crisis of chronic myeloid leukaemia. In patients who failed alfa IFN therapy there is an high incidence of grade III-IV myelosuppression and it usually begins within the first weeks of starting therapy. An increased percentage of bone marrow blasts , a low hemoglobin level , a long time diagnosis, a previous therapy with alfa IFN , can be associated with myelosuppression. Grade III-IV neutropenia and trombocytopenia can induce stop Imatinib therapy or reduction of daily dose (not less than 300 mg, in current literature). Another experience shows there is a difference for progression free survival and achievement of CCR between patients who have cytopenias and interrupt treatment than those whose treatment not had to be stopped. This case report shows that continuous Imatinib therapy could be more useful, also in prolonged myelosuppression, to achieve MCR or CCR, rather than stop therapy: we need more trials in more patients to validate this, perhaps using growth factors to reduce myelosuppression lasting.

L031

18FDG-PET, 67GALLIUM SCAN AND COMPUTED TOMOGRAPHY IN THE BASELINE STAGING, THERAPY RESPONSE EVALUATION AND FOLLOW UP OF PATIENTS WITH HODGKIN DISEASE: A CASE REPORT

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In patients with Hodgkin disease (HD) an accurate staging at diagnosis is mandatory for planning the optimum therapeutic course and schedule. Different imaging techniques are now available and they are important not only for diagnosis but also in the course of therapeutic treatment and follow up because the individuation of residual masses or relapsing disease could allow the beginning of a second line therapy. We report on a case of a 65-year-old woman affected by rheumatoid arthritis treated with steroids and methotrexate, and previously treated for a papillary carcinoma of the thyroid with total thyroidectomy and therapeutic doses of 131I. The patient came to our attention in June 2003 because of a large right axillary lymph node which was removed; the histopathological examination was consistent with the diagnosis of HD, nodular sclerosis subtype. The patient underwent a standard baseline staging procedure with total body CT scan and bone marrow trephine biopsy; the latter resulted negative and the CT did show no suspected masses nor lymphoadenopathy. A whole body 67Gallium scan detected a positive signal in the right axillary region (where the node had been taken away) and another one in the upper stomach. In order to investigate this result the patient underwent esophagogastroduodenoscopy with endoscopic biopsy, showing only the presence of a chronic gastritis without Helicobacter pylori infection.

The disease seemed therefore to be a limited stage I Hodgkin Disease, according to the Ann Arbor staging system. Instead of beginning therapy we decided to perform a 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) where foci of hyperactivity outside areas of known physiologic uptake are considered positive for HD. Despite the negative results of CT scan and Gallium scan, the FDG-PET revealed sites of hyperactivity in the spleen and in mediastinal and retroperitoneal lymph nodes. After these result CT scan was repeated but, again, no significant lymphoadenopathies were detected.

The patient started chemoterapy (ABVD regimen) in August 2003. At the end of 4 courses of chemotherapy whole body CT scan and FDG-PET resulted both negative. At the last follow up, 21 months after the diagnosis, the patient is in good condition; FDG-PET repeated 14 months after the end of therapy is still negative. This case highlights the fact that FDG-PET allowed a better diagnostic definition compared to CT scan and Gallium scan, thus resulting in both stage modification (from a stage I HD to a stage IIIs HD according to Ann Arbor staging) and treatment modification. Even though our experience is limited this case, according to recent literature data, pointed out the role of whole body FDG-PET in staging, management and follow up of Hodgkin disease.

L032

PRIMARY LUNG LYMPHOMA (BALT) PRESENTING AS SECOND LYMPHOMA

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Primary lung lymphoma of the bronchial associated mucosae (BALT) is a rare event, accounting for about 0,3-0,4% of primitive non Hodgkin's lymphoma. Lung tissue could be interested as extranodal localization in about 10% of lymphomas, especially high grade diffuse lymphomas, low grade lymphocityc lymphoma and T-lymphoma.

The onset of a second lymphoma after chemotherapy localized in the lung and histologically definable as BALT, to our knowledge, has not been reported.

Case Report: a male, 56 years old, presented to our observation in January 1997 because of monoclonal gammopathy (IgM lambda) of minimal entity. It was present spleen enlargement (18 cm) but no lymph node enlargement in superficial sites. Blood peripheral counts were normal (wbc: 6700/mmc, hb: 13,5 gr/dl, plt: 140000). TC scan was negative and so was bone marrow cytological examination. Only bone marrow biopsy revealed a nodular infiltrate of little lymphocytes with a quota of large lymphocytes, defined by pathologist as follicular lymphoma. Strategy was wait and see. Good clinical conditions were present till December 1999 when a later cervical lymph node appears. Biopsy was then performed. Histological examination showed: diffuse infiltration by small cleaved lymphocytes and about 10-15 centroblast for each field. Immunohistological phenotype was: CD20+, CD79a+, CD10+/-, BCL2+. A large presence of proliferative figures and posi-

tivity for Ki-67 were present. Diagnostic conclusion was then transformation of follicular lymphoma into grade III. First line therapy chosen was MACOP-B. Treatment was well tolerated till the seventh week when it has to be stopped because severe toxicity like mucositis grade IV, mild renal failure, hepatic toxicity grade III and life-threatening infection which needed hospitalization, broad spectrum antibiotics and empiric antifungine treatment. At the resolution of the infection patient received 4 doses of rituximab. In April 2000 complete remission of the disease was documented by negativity of TC scan, PET-scan, cytological and histological examination of bone marrow. Patient was in complete continuous remission till October 2002, when cough and dyspnea presented. Chest RX showed parenchimal infiltrate, measured by TC scan in 2,5 cm and localized in right lung. It was the only detectable localization. Neither superficial lymphnode nor spleen enlargement was present. Peripheral blood counts were normal and so bone marrow cytological and histological examination. So was negative PET scan, except for the lung. A FNAB TC scan guided was not conclusive, so lung biopsy was performed. Histological examination of lung lesion revealed low grade lymphoma with a certain quota of plasmocytoid/plasmocytic cells. Phenotype was CD20+, CD79a+. All these data were compatible with the definition of BALT. Treatment planned was combination of fludarabine and cyclophosphamide (flu-cy) for three days plus rituximab for a total of three cycles. Treatment was well tolerated, and complete remission could be documented by TC and PET scan, being all the other parameters negative. Patient is nowadays in complete continuous remission. The occurrence of a second lymphoma is not a so rare event, accounting about 13% in a large serie recently published, called multiple histology lymphoma (MHL). In our case there is a first transformation in a higher grade, and 30 months after remission the onset of a second variety of lymphoma of lower grade. It is known the possibility to develop a second lymphoma of lower grade than first, but the lung localization as not yet been reported.

L033

FOLLICULAR LYMPHOMA WITH CUTANEOUS LOCALIZATION AND SEVERE RENAL DAMAGE

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Background. Low-grade Follicular Lymphoma (FCCL) are indolent proliferative disorders characterized by frequent bone marrow localization, long term survival, high incidence in elderly population and abnormal Bcl-2 expression. Extranodal sites as well as absence of bone marrow involvement are infrequent.

Findings. We report a case of a 65-year-old man with a secondary cutaneous manifestation of FCCL and contemporary renal damage. The case dates back to July, 2000 with a frontal cutaneous erythemato-papular lesion. The histologic biopsy, at that time, was not conclusive.

A second frontal punch biopsy performed on July, 2003 found Follicular Lymphoma (predominantly small-cleaved cell type CD20+, BCL-2+). Meanwhile the patient also displaied symptoms of severe nephrosis characterized by hypertension refractory to ACE-INH and sartans, severe declivous edemas with proteinuria >18 gr/24 h. Then he underwent a renal biopsy showing a Membranous Glomerulonephritis in absence of chryoglobulinemia. CT scans showed multiple adenopathy exclusively on mesenteric region. The biopsy excluded the bone marrow involvement and Bcl-2 absence. We concluded for a III stage, high ILI score FCCL which we treated with a chemotherapy of association FND followed by Rituximab

Conclusion. The clinical response was characterized by the early disappearance of the skin lesion after the first course of chemotherapy. Negative CT scans and complete remission of Nephrosis Syndrome persist after one year since the end of chemotherapy .

L034

OVERALL SURVIVAL OF PATIENTS WITH FOLLICULAR LYMPHOMA IN RELATIONSHIP CD56, CD8/CD11A PERIPHERAL BLOOD LYMPHOCYTES SUBSETS DURING A TWO YEAR FOLLOW UP

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Introduction. Follicular Lymphomas (FL) constitute the most important subgroup of low-grade non-Hodgkin's lymphoma (NHL). Biological molecules as LDH, Haemoglobin, Beta 2 microglobulin are well-known prognostic markers of this neoplasm . Probably also immune cells can give some information about disease progression. Therefore we propose the study of two subsets of lymphocytes, CD56 and CD8/ features, during FL, are only sporadically explored. In order to verify this hypothesis we have CD11a normally represented in peripheral blood, comprising cells with antitumour cytotoxic activity whose followed up a group of patients, for two years, at our Centre for Blood Disease.

Patients and Methods. In 27 subjects, (median age 61 years, range 39-87) affected by FL referring to WHO criteria, has been analysed in peripheral blood, using flow cytometry, at the time of the diagnosis, CD56 and the expression of CD11a molecule on T CD8 cells. The over-expression of this molecule seems to be correlated with cytotoxic activity of CD8. After a two year follow-up we have considered the survival rate of the patients according to the lymphocytic subsets results independently from other biological and clinical parameters, including the type of therapy.

Results. 21 subjects (78%) had values of CD56 within the normal range while the remaining 6 (22%) showed increased values and after two years 13 patients (62%) of the first group were still alive while only 2 (33%) in the second did. CD11a molecule on T-CD8 was normally expressed in 10 cases (37%) while it was over-expressed in 17 (63%) and when we assessed the overall survival rate after a two year follow up, we observed that in the group with normal values the surviving subjects were 8 (80%) while only 7 (41%) were alive when CD11a was over-expressed.

Conclusions. Expression of the CD11a molecule on T CD8 within normal values seems to be correlated with longer

survival in patients with FL while it results shorter with increase of CD56 lymphocytes. To confirm these indications a larger number of patients should be studied.

L035

LIPOSOMAL DOXORUBICIN IN THE TREATMENT OF ELDERLY, CARDIOPATIC OR PRETREATED PATIENTS WITH LYMPHOMA

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Myocet (liposomal doxorubicin) has an improved pharmacokinetic profile with less myelosuppression and GI toxicity and has a reduced risk of cardiotoxicity at dose level equivalent to standard formulations of doxorubicin.

We replaced the conventional doxorubicin with Myocet for the treatment of elderly patients (pts), or pts with cardiac disfunction or pts with previous treatment with doxorubicin. From June 2003, we have treated with COMP 13 pts with aggressive NHL and with MBVD 2 pts with Hodgkin's lymphoma, replacing doxorubicin with Myocet (50 mg/m2 in COMP and 25 mg/m^2 in MBVD).

The median age was 70 years (range 54-76). Two pts were stage I, 5 stage II, 2 stage III and 6 stage IV. According to IPI score, for NHL only, 3 pts were low risk, 5 low-intermediate, 4 intermediate-high and 1 high risk. Three pts were pretreated with anthracyclines, 7 pts showed cardiomiopathy (1 ischemic, 4 hypertensive and 2 hypokinetic). The median left ventricular ejection fraction (LVEF) at diagnosis was 59% (range 42%-75%). All pts but one had no change in LVEF, one patient presented a myocardial disfunction resolved with medical therapy and he his alive with disease. At the moment 13 out 15 patients are evaluable for response: 9 pts obtained a complete remission (69%) one a partial remission with an overall response of 77%, one patient stopped therapy due to myocardial disfunction and two patients died one for a stroke and the other for gastrointestinal bleeding. After 64 cycles we have observed one toxic event and two concomitant complications. No significant hematological toxicity was recorded. One pts died of disease and after a median observation period of 10 months (range 2-21) the overall survival was 77%.

Conclusions. We conclude that liposomal doxorubicin allows to treat patients with concomitant diseases which could limit the use of conventional anthracycline. Myocet is feasible and effective in a subset of patients with very negative characteristics at diagnosis. It reduces cardiotoxicity risk without reducing chemotherapeutic efficacy.

L036

CASE REPORT: RELAPSE OF LYMPHOMA AFTER ESTROGENIC THERAPY

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Lymphocytes are very sensible to corticosteroids; in fact this is the most important therapy in the treatment of lymphoproliferative disorders. Sex hormones'structure is very similar to corticosteroids'one. So we asked ourselves if it could be possible that a lymphoproliferative disorder could be sensible to estrogens. Herein we want describe the case of a patient who had a recurrence of lymphoma after estrogenic therapy. A.S., a Caucasian woman 67 years old, came to our attention in April 2002, with a documented relapse of Lymphoma. In January 2001, in another Hospital, a Follicular B cell Lymphoma in Stage IV was diagnosed. From March to June 2001 she had been treated with standard regimen CHOP, including Cyclophosphamide 750 mg/m^2, Adriamycin 50 mg/m^2, Vincristine 1,4 mg/m2 all E.V. day 1st and corticosteroids 100 mg P.O. from day 1st to 5th. Schedule repeated every 21 days for 6 times. In July 2001 she was submitted to Radiotherapy on the involving fields, obtaining complete remission. At the end of the treatment she developed a state of fatigue and difficulty in the movements, probably due to the chemotherapy. At that moment she was in menopause from the age of 50 years old, she had never had particular problems and had never used estrogenic support. Her Ginecologist diagnosed menopausal disorders so, from October 2001, she started a therapy with Estradiol Valerato 2 mgs/die P.O. for 21 consecutive days of a month. With this treatment she obtained the return of the mestrual cycle after 17 years. Concording to the estrogenic therapy, a lot of subcutaneous nodules started to appear all over her body. The patient was submitted to the biopsy of a nodule and the histological examination releaved the recurrence of the same Follicular B cell Lymphoma. When she came to our attention, in April 2002, she was in estrogenic support for 5 months. Clinical and radiologic evaluation showed a diffuse disease in Stage IV (skin and subcutaneous). As the first thing we stopped the estrogenic support; the patient returned to a menopausal state. We started with a corticosteroid treatment with Prednisone P.O. 1 mg/kg/die for 10 days, obtaining a first reduction of the number and the volume of the nodules. In a second moment she started a regimen of chemotherapy with the MINE schedule, including Mitoxantrone 8 mg/m^2/die E.V. day 1st + Ifosfamide 1,3 mg/m^2/die E.V. day 1st-2nd-3rd and Etoposide 200 mg P.O. day 1st-2nd-3rd, schedule repeated every 21 days for 6 times. Using growth factors'support we could respect doses and times of therapy. In September 2002, at the end of the chemotherapy, the clinical and radiologic evaluation releaved a state of complete remission. To consolidate the result, we started a therapy with Rituximab. In December 2002, the patient had a subitaneous relapse with CNS and retroorbitary envolvement, and died in few weeks. The research of estroprogestinic receptors in the lymphoid tissue resulted negative. Our hypothesis is that corticosteroid receptors in some neoplastic tissues, could be sensible to high dose of estroprogestinic therapies. This idea because of the very similar corticosteroid and estroprogestinic hormones'structure.

L037

PRIMARY GASTRIC LYMPHOMA: TREATMENT OUTCOMES FOR 99 PATIENTS OVER A 20-YEAR PERIOD AT A SINGLE INSTITUTION

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Background. Stomach is one of the most common extranodal involvement site of non-Hodgkin lymphoma. The optimal management of primary gastric lymphoma (PGL) remains controversial. No controlled clinical trials have designed widely accepted guidelines. A twenty years experience of a single institution was retrospectively evaluated.

Patients and Methods. A total of 99 patients were evaluated from January 1980 to December 2004. Staging was based on the Ann Harbor system modified by Musshoff and histology on the Working Formulation classification. Survival dates were entered using the Kaplan Meyer curves. The sample represents patients with a mean age of 63 years old (range 16-90) and a slight predominance of male patients (52,5%); stage IE 44,5%, IIE1 24,2%, IIE2 31,3%. Low grade LNH was present in 40 patients, intermediate grade in 28, high grade in 31. Fifty-five (55,6%) underwent to primary gastrectomy, 27 were also treated with multimodality approach receiving chemotherapy or radiotherapy. Forty-four patients had gastric preservation: 14 received oral anti-Helicobacter Pylori regimen and 30 chemotherapy (CHOP or CHOP like).

Results. The median survival of the 55 patients who received surgery-based management (with or without CT/RT) was 232,7 months versus a median survival of 65,1 months of the patients treated with medical approch: the difference was significative (p<0,001). We did not observed a significative difference of median survival according to histological grade and stages. We analysed the median survival of the patients who received CT alone, surgery alone and surgery plus CT: this was 46, 163 and not yet reached at median follow-up of 144 months, respectively (p<0,001).

Conclusions. In this study the role of surgery treatment of PGL is re-evaluated: it seems to be curative in stages I/II and it could be useful also for a correct staging purposes. Surgery may be an indipendent prognostic factor for early stages of PGL patients.

L038

PRIMARY INTESTINAL BLASTIC NK-CELL NON-HODGKIN'S LYMPHOMA: A CASE REPORT

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Blastic NK-cell lymphoma is a very uncommon entity, defined only recently and included in the WHO classification. It is characterized by a cutaneous, nodal and bone marrow involvement due to cells bearing authentic NK markers (CD4+/CD56+/CD3-). It affects mostly the elderly and shows a rapidly fatal course, even if heavily treated. We describe the case of a 43-year-old woman who came to our attention in the year 2002 with diagnosis of primary intestinal, highly aggressive, centroblastic B-cell NHL, with extensive abdominal involvement, in lack of bone marrow infiltration. Patient underwent a VACOP-B regimen for 10 weeks and, during the complete remission phase, stem cells (SC) collection was performed in order to realize an autologous transplantation. Few days before SC transplant, the appearance of a new abdominal tumefaction analyzed by fine needle aspiration cytometry revealed the presence of CD56+ cells: on the basis of this result, a diagnosis of blastic NK-cell lymphoma was performed. A Dexa-BEAM salvage regimen was promptly administered; the following aplastic phase was complicated by intestinal perforation, readily treated with surgery. During the new complete remission phase, patient underwent autologous stem cell transplant, obtaining a 1 year remission. After 18 months from diagnosis, disease relapsed again with intestinal perforation and ovarian involvement. Thus, patient underwent compatible familial donor allogeneic stem cell transplantation with non-myeloablative conditioning regimen. Post-transplant phase was complicated by relapsing disease, with CNS involvement (meninx, spinal cord, facial and acoustic nerves), treated with radiotherapy, medicated rachiocentesis and donor lymphocyte infusion (DLI). During the following months, disease spread widely, with mammalian, pleural and pericardial infiltration by blastic NK elements. Patient's death occurred 30 months after diagnosis, due to cardiac tamponage and ventricular fibrillation. Our experience confirms that blastic NK-cell lymphoma is a highly aggressive disease, unresponsive even to intensive regimen treatments; it is evident also the pleiotropic nature of the illness.

L039

CLINICAL AND MOLECULAR REMISSION OF RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA AFTER R-IEV IMMUNOCHEMOTHERAPY

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Mantle Cell Lymphoma is a mild aggressive lymphoid malignancy (sec. ILGS classification) with poor outcome to therapy. It is characterized, in 70% of patients, by presence of t(11;14)(8q13; q32) and by molecular findings positive for Bcl1 presence and over expession of D1 Cyclin . The immunological pattern is the following : SIg +; CD5+; CD23-; CD20+; CD19+; CD43+; CD11c+; CD10-.

In 2002 november, a 70-year old man was sent to our Hematology Division because of lymphoadenomegalies were present in all superficial sites. Total body CT scan confirmed the increase of spleen size and limphoadenomegalies in many deep sites. The hepatitis C antibodies were negative; it was present a severe coronary disease. The histological examination of one limph node showed a mantle cell lymphoma. The cytological examination of the bone marrow showed a small lymphoid infiltration (23%);

the immunological pattern was: CD5+; CD23-; CD20-; CD43-; CD19+; CD10-. Cariotype was 45X0, t(11;14)(q13;q32); the molecular findings were positive for Bcl1 presence and IgVH rearrangement. Therefore, the patient was subjected to the following treatment schedule: R-CEOP chemotherapy regimen q 28 days for six cycles and this therapy was well tolerated. One month after the last cycle, the patient was subjected to a disease re-staging that showed the partial remission of lymphoadenomegalies with persistence of molecular findings . The patient was subjected to for weekly Rituximab 375mg/sqm induction . The re-staging showed the persistance of Bcl1 with absence of IgVH rearrangement and a stable clinical condition. At 2004 august, the patient returned to our Division because of presence of lymphoadenomegalies in many sites; the bone marrow biopsy showed lymphoid infiltration. The patient was subjected to a treatment with the combination of Ifosfamide, Epirubicin , Etoposide and Rituximab (R-IEV). Four total treatment cycles were performed. One month and three months after the last cycle, the re-staging showed, at the physical examination, the absence of signs of disease; at the bone marrow biopsy absence of lymphoid infiltration, and the molecular findings showed both the absence of IgVH rearrangement and Bcl1. Total body CT scan confirmed the normal spleen size with lymphoadenomegalies absence. Primary end points of this case are to confirm the feasibility and the efficacy of the combination of Ifosfamide, Etoposide , Epirubicina and Rituximab in relapsed/refractory mantle cell lymphomas.

L040

T-CELL RICH B-CELL LYMPHOMA IN A PATIENT WITH COOMBS POSITIVE HEMOLYTIC ANEMIA

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A young 35 year woman came at our observation with a marked anemia (Hb 7g/dL), mild asthenia and nocturnal sweat. At objective examination an inguinal lymphoadenopathy and a mild hepatomegaly was found. The serum examinations for the anemic status revealed a hemolytic anemia positive for Coombs reaction. Therefore, an excissional lymphoadenectomy of an inguinal node was performed and the related histological examination and immunohistochemical analysis showed a Non-Hodgkin Lymphoma type T cell rich B cell Lymphoma. The patient completed the staging with a bone marrow biopsy and a total body CT-scan that demonstrated the absence of other disease sites (stage IB). The patient was subjected to the R-CHOP immuno-chemotherapy regimen every 21 days for a 6 total cycles. After the first cycle Coombs assay became negative and at the end of the therapy also hemoglobin levels became normal. The complete re-staging (CT-scan, bone marrow biopsy and serum examination) performed one month and 6 months after the end of the therapy confirmed the complete remission.

The association between hemolytic anemia positive for Coombs reaction and lymphoproliferative diseases is a common event, but, at our knowledge, until to-day it has been not still described the association with NHL T-cell rich Bcell Lymphoma. The rare reports on the association between Hodgkin Lymphoma and haemolytic anemia might be revised on the basis of the recent advances in the molecular diagnosis of T cell rich B cell lymphoma.

L041

A CASE REPORT OF A CENTRO-FOLLICULAR NON HODGKIN LYMPHOMA WITH A CHROMOSOME 20 DELETION

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A 34 year old man developed a large splenomegaly without superficial lymphoadenomegaly and with normal blood cell counts. Serum examination showed an increase of LDH (500 I.U./L) and erythrosedimentation rate (1st hour: 30 mm). Total body CT-scan showed a large splenomegaly with a dishomogeneous pattern and splenic hilus lymphoadenomegaly. The patient was subsequently subjected to a diagnostic splenectomy and ablation of an infiltrated bowel segment adjacent to the spleen. The preparation showed a CD20+ CD10+ B cell NHL compatible with a grade III centro-follicular lymphoma. The bone marrow (BM) biopsy and the subsequent pathological examination showed a minimal infiltration by small size cells compatible with centro-follicular lymphoma cells. The molecular examination of these cells showed a clonal rearrangement of immunoglobulins and the expression of bcl-2. The cytogenetic examination demonstrated a partial deletion of the chromosome 20 long arm (20q-) as a sole clonal chromosomal aberration. The patient has been therefore subjected to the R-CHOP chemotherapy regimen q 21 days for 6 cycles. The clinical re-staging after 30 days from the last cycle has recorded a complete remission (CT-scan and BM biopsy negative). Molecular examinations were also negative, but the cytogenetic assay still showed the 20q- anomaly in about 3% of the mitoses. The patient was re-evaluated after additional 6 months with similar results and is off-therapy from 8 months. The 20q- chromosomal anomaly has not been described until now, at our knowledge, in centro-follicular NHL. However, it is a rare anomaly in non myeloid haematological neoplasms. A few cases have been described in Chronic Lymphocytic Leukaemia; more recently it has been reported in some splenic NHL of the marginal zone (Hernandez JM et al., Am. J. Pathol., 2001, 158(5), 1843-1850). It is questionable if the 20q- chromosomal anomaly reported in our case could be correlated to the splenic origin of the NHL. Moreover, it has to be investigated if chemotherapy and surgical therapeutic strategies must be followed by bone marrow transplantation regimens in aggressive NHL.

L042

PERSISTENCE OF CHROMOSOME Y ABSENCE IN A CASE OF BCL-2+ NON HODGKIN LYMPHOMA WITH MOLECULAR REMISSION.

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Haematological malignancies are often characterized by chromosomal abnormalities and additional anomalies can succeed, stating subsequent steps in neoplastic evolution both in haematologic and in solid tumours. Some of these abnormalities have a diagnostic or prognostic significance, some are not specific, but can somewhen get a significance for the patient's clinical management. We have recently observed a 65 year old man with a bcl-2+ stage IV NHL. The patient was subjected to 6 cycles of CEOP chemotherapy regimen achieving a partial remission . The bone marrow specimen demonstrated the persistence of NHL expressing bcl-2, as evaluated by molecular techniques, and showed, in addition, the clonal absence of chromosome Y. After a severe abdominal disease progression, the patient was subjected to R-IEV second-line chemotherapy and after 4 cycles achieved a complete haematological and molecular remission. The response was confirmed after 1 month by a bone marrow examen. However, both bone marrow samples demonstrated the clonal chromosome Y absence. The persistence of this chromosomal anomaly in bone marrow but not in peripheral bood is intriguing and suggests that the cromosomal abnormality is acquired, and the remission is not really complete. This hypothesis warrants a very careful monitoring of this patient from both the cytogenetic and the molecular points of view.

L043

ACTIVITY OF RITUXIMAB ALONE ADMINISTERED AS THREE-WEEKLY SCHEDULE IN NON-HODGKIN'S LYMPHOMA ELDERLY PATIENTS

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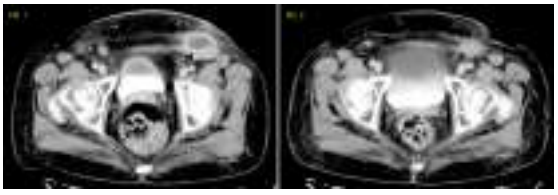
Rituximab is a chimeric monoclonal antibody that binds the CD20 B-cell antigen, a transmembrane phosphoprotein overexpressed in 95% of low-grade follicular non-Hodgkin's lymphoma (NHL), as well as in 35% of diffuse large B-cell lymphomas.[1] Proposed mechanisms of action include complement-dependent and antibody-dependent cellular cytotoxicity, inhibition of tumour growth, and direct induction of apoptosis.[2] The drug exercises proven efficacy in treating chemoresistant low-grade CD20-overexpressing follicular NHLs. It is also used for patients with CD20-positive diffuse large B-cell NHL in combination with standard CHOP chemotherapy.[3] Conventional administration of rituximab as front-line single agent consists in a once a week dose of 375 mg/m2 for four infusions, leading to 60-75% of objective responses. However, several pharmacokinetic studies demonstrated that the optimal therapeutic dose and maintenance schedule may vary among different patient populations.[4,5] Long availability of effective drug serum levels probably depends on lymphoma tumour burden, rates of tumour proliferation, expression of CD20 antigen, and rituximab clearance.[6] On this basis, we are conducting a study for testing activity and safety of single agent rituximab administered as standard dose of 375 mg/m^2 every three weeks in NHL elderly patients. Comprehensively, rituximab is given every 21 days for four cycles as induction treatment followed by an administration every two months for four overall infusions as maintenance schedule. At the moment, 5 patients entered the study since July 2004. Median age was 77 years (range 66-81), median ECOG PS=1. Four patients had untreated follicular NHL, while the oldest one had large B-cell NHL pre-treated with CNOP chemotherapy regimen in which mitoxantrone replaced doxorubicin. Lymphoma stadiation was stage I in two patients and stage II in the remainings. All the patients completed the induction treatment. Four of them started the maintenance phase and are assessable for response. According to NCI response criteria,[7] objective responses were observed in all the patients, with 3/4 complete responses (75%) and 1/4 partial response (25%) [arrows in Figure 1 and Figure 2]. Rituximab as single agent was very well tolerated, without appearance of any relevant side effect. Moreover, the three-weekly administration of the monoclonal antibody yielded complete compliance to treatment and was favourably accepted by the patients.



Considering the preservation of drug activity and the biological characteristics of many indolent NHLs, we propose the above lengthened administration of rituximab alone as effective and feasible treatment approach for elderly patients. A wider analysis is necessary to confirm the early results. The recruitment is ongoing.

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L045

EFFICACY OF MONOCLONAL ANTIBODY ANTI CD20 IN EXTRANODAL MINIMAL RESIDUAL DISEASE ERADICATION

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The role of anti CD20 monoclonal antibody (Rituximab) in B cell lymphoma treatment is well known. Its use in extranodal minimal residual disease is still little investigated. We present a case report in which Rituximab use was important in extranodal residual disease control. A 52 years-old woman underwent to our observation because affected by Follicular non Hodgkin Lymphoma CS III EA. At disease onset axillary and inguinal lymphnodes were involved by disease. On right breast an infiltrating nodule was present. An excissional biopsy on breast nodule was performed and diagnosis of brest involvment by follicular lymphoma was posed. A gallium scintigraphy performed one month after biopsy showed persistent localization of disease in right breast. The patient was submitted to 6 chemotherapy courses with R-CIOP schedule (Rituximab, Cyclophopsphamide, Idarubicine, Vincristine and Prednisone). After chemotherapy patient showed a total regression of lymphoadenopathy. But breast MRI performed because of disease restaging showed a signal alteration localized on surgical cicatrix, suggestive for breast residual disease. The remaining restaging procedures were normal. A maintenance therapy with Rituximab (375 mg/mq) every two months for a total of 6 courses was performed. After maintaining therapy completion restaging tests were performed and breast MRI showed the total disappearance of breast localization of disease. These data were confirmed also by Total Body PET. At a follow up of 19 months the patient is still in complete remission. In our report the use of Rituximab was very useful in minimal residual disease eradication also in an anatomical district apparently bad targeted by monoclonal antibodies. This experience might induce to use Rituximab also in tissutal minimal residual disease.

L046

UNUSUAL CLINICAL AND HISTOLOGICAL TRANSFORMATION OF A FOLLICULAR LYMPHOMA

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Here we report a case of a 46-yr old woman who referred to the ER of our hospital due to leukocytosis (40100/mmc WBC, with 20% of immature lymphocytes), thrombocytopenia (15000/mmc) anemia (8.5g/dL), coupled with rapidly-growing edema of left lower limb. She had had a diagnosis of follicular Non-Hodgkin's Lymphoma (NHL), stage IV, in 1991, received CHOPx8 and abdominal radiotherapy, achieving a Complete Remission (CR). In 1996 she relapsed, and underwent autologous stem cell transplant, achieving a 2nd CR. When admitted to Hospital, she had fever, sweats and arthralgias, physical examination showed bilateral inguinal nodes. Echo scan revealed left hydroureteronephrosis and a 8-cm lymph node mass close to the pelvic wall. LDH was 10.843 U/L, acid uric 22 mg/dl, serum creatinine 2.4 mg/dl. Immunophenotype of lymphoid cells was: CD19+, CD20+,HLA-DR+, CD71+, CD3-, CD5-, TdT-, MPO-.Trephine bone marrow biopsy showed a diffuse substitution by blastic/blastoid cells, frequently with very irregular nuclei, which expressed CD20, CD79a, CD10, BCL-2, and Ki-67 in 80% of them. Marrow cytogenetic analysis showed a complex karyotype in 22 metaphases out of 30, with 51,XX,t(14;18),+X,del(1), t(1;11),add(2),del(6),-13,-16,+21,+4mar,1 ring chromosome. t(14;18) was confirmed by FISH. CSF cytospin was positive for lymphoid cell localization. The clinical, morphological, immunophenotypic and cytogenetic picture of this patient led us to make a diagnosis of follicular lymphoma transformed towards a blastoid variant, which represents an unusual and very aggressive progression of follicular lymphoma, mimicking other poor-prognosis lymphoproliferative diseases, like lymphoblastic lymphoma/leukemia, Burkitt's lymphoma, blastoid variant of mantle-cell lymphoma. Only an integrated diagnostic approach, including morphology, immunophenotyping and cytogenetics, can lead to the correct diagnosis. The patient was treated by HyperCVAD regimen, achieved a 3rd CR, and is now going to receive an allogeneic stem cell transplant from a matched unrelated donor.

L047

PRIMARY LYMPHOMA OF THE CONJUNCTIVA: A CASE REPORT

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Primary lymphoma of the conjunctiva are extremely infrequent and usually belong to extranodal marginal zone B-cell lymphoma of MALT or MALT lymphoma. Radiotherapy is one of the most employed therapeutic options,[1] but complications are frequent and relapses may occur.

Other treatment modalities including resection, cryotherapy, injection of interferon alpha or systemic chemotherapy have been used with varying success.[2] A 43-year-old man visited our clinic for the teatment of a conjunctival tumor in the right eye. Excisional biopsy was performed and revealed that the lesion was a "low grade extranodal marginal zone B-cell lymphoma (lymphoma of MALT)"; the lesion was CD20 and bcl-2 positive imunoistochemically.[3] A clinical staging was preformed including thoracic and abdominal computerised tomoscans, magnetic resonance tomography of the orbita and bone marrow biopsy. No other foci of the lymphoma were found;[4] nevertheless a scintigrafic tomoscan revealed a pathologic positiveness in the controlateral conjunctive.

We treated the patient with the anti-CD20 monoclonal antibody rituximab and chemotherapy with a CVP-like schedule for four courses. As a maintenance, the patient underwent a therapy with rituximab only, once every two months, for four courses. Treatment was well tolerated and resulted in a completed remission. With a follow up of six months further recurrences have not been observed.[2]

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L048

MYCOSIS FUNGOIDES: REPORT OF A THIRD STAGE WITH IMPRESSIVE CUTANEOUS INVOLVEMENT

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L.A. a 64 old man was admitted to our institution on July 2004 for massive cutaneous involvement by neoplastic disease subsequently diagnosed as MF. The patient had begun to suffer from the disease in 2002 when he noted some infiltrative lesions in the abdominal skin. He refused every treatment believing it was a "dirty", incurable disease. He went from his country , Romania , in 2004 after some dermatologists told him he should have died briefly . When we saw the patient for the first time his trunk, abdomen and limbs showed bulky masses (till over 10 cm in diameter) covered with eroded, bloody skin secreting serum and wetting his dresses (Figure1). The first problem to solve was the psychological condition of the patient who did't believe we could succeed in an attempt of curing the disease. The second was a social one , for the illegal position of immigrant from an east Europe country. The third was the idea of beginning a chemotherapy. TC scan didn't showed any lymphonode involvement; on bone biopsy bone marrow was not infiltrated. Finally we could start on September 2004 a CHOP/F-MACHOP alternative regimen that the patient tolerate fairly well. Skin lesions gradually went down and resolved leaving a plain pigmented skin . At the end of 8 cycles of treatment he was in complete clinical remission (Figure 2) . This case is relevant for the extent and the size of cutaneous lesions more than the good therapeutical result . These are findings not usually seen among our people but more easily could be found , as in this case, among immigrant and foreign people from underdeveloped countries.

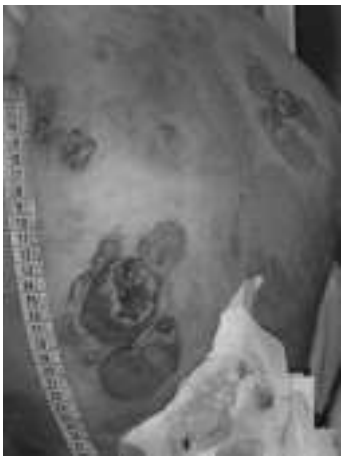

Figure 1.

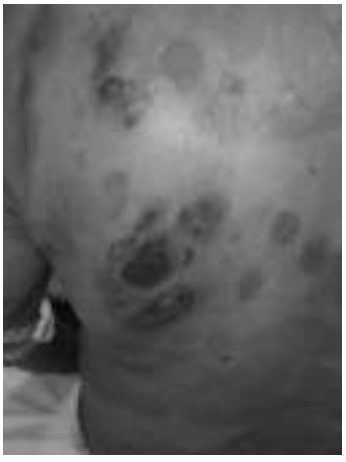

Figure 2.

L049

MANTLE CELL LYMPHOMA IN A PATIENT AFFECTED BY MYELOFIBROSIS

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Myelofibrosis (MF) is a possible evolution of essential thrombocythemia (ET). In a patient with ET evolved into MF, a fast worsening clinical picture, with cachexia and severe transfusion-dependent anaemia, was due to the unexpected occurrence of a mantle cell lymphoma. A 75-year-old male had ET diagnosed in November 1993, successfully treated by hydroxyurea for 10 years . In April 1999 he was treated by intravescical Epirubicin for bladder cancer. In May 2002 his ET evolved into MF. Bone marrow cytogenetics showed 45, X,-Y. In March 2003, the patient became transfusion-dependent, requiring PRBC transfusion once a month. In July the need for transfusion increased to 2 units/week. The spleen size was unchanged. In September 2003 his conditions rapidly deteriorated and a surprising picture of abnormal lymphoid cells, in peripheral blood was noticed. Peripheral blood flow cytometry showed CD22+, CD5+, CD23-, CD10-, SmIg-lambda+. The marrow appeared totally infiltrated by lymphoma cells, and cytogenetics showed the translocation t(11; 14).The patient died in October 2003. In this patient the clinical course went through a number of progressive steps, with two critical moments, in March and July 2003, respectively. The appearance of abnormal lymphoid cells in peripheral blood led us to study the marrow while bone marrow aspirate is usually useless in advanced phase of myelofibrosis. It is likely that the lymphoid infiltration of the marrow coupled with the massive spleen enlargement caused the increased transfusional need. To the best of our knowledge, no cases of mantle cell lymphoma following MF have been elsewhere reported so far. Often this type of lymphoma has splenic and marrow involvement, with splenomegaly and peripheral blood pan-cytopenia, thus overlapping the features of MF. This case report stresses the need for additional investigations in chronically ill patients when the clinical picture tends to modify rapidly.

L050

RAPID DIAGNOSIS OF RELAPSED MANTLE CELL LYMPHOMA BY FLOW CYTOMETRY

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Mantle cell lymphoma represents one of the lymphoma subtypes with the poorest prognosis with a median survival of 3 years. This is due to the frequent advanced state at diagnosis, an aggressive clinical course, absence of a standard treatment and high frequency of relapse. Extranodal involvement is frequent (90% of cases) in this subtype of lymphoma either at diagnosis or at relapse, contributing to make difficult the diagnosis and poor the prognosis. We report two cases of mantle cell lymphoma in which flow cytometry immunophenotyping by fine - needle aspiration of extranodal lesions allowed a rapid diagnosis of relapse. A 53 y-old man was diagnosed in 2000 as low grade follicular lymphoma (stage IV-A) after lymphnode biopsy and was treated with six courses of CHOP; for an early relapse he received fludarabine, achieving a second complete remission (CR). During fludarabine treatment, the lymphnode biopsy was reviewed and a final diagnosis of mantle cell lymphoma was delivered on the basis of a clonal lymphocytic proliferation positive for CD5, CD79a, bcl-2 and cyclin D1. Four months after the end of therapy the patient showed palpebral swelling; a PET scan detected multiple sites of pathological uptake (laterocervical lymphnodes, lung). Flow cytometry immunophenotyping of a fine - needle aspiration (FNAC) of the palpebral swelling documented a clonal lymphocyte population CD5, CD19, CD20, CD22, lambda chain positive and CD23 negative leading to the diagnosis of mantle cell lymphoma relapse. The patient received two courses of DHAP plus rituximab followed by autologous stem cell transplantation and has achieved a prolonged third CR. The second case is a 50 y-old woman, who had received a first diagnosis of low grade follicular lymphoma (stage IV - A), treated with six courses of fludarabine and mitoxantrone and achieving complete remission. Two months after stop therapy, extranodal breast relapse appeared, associated with palpebral swelling. Flow cytometry analysis of the FNAC from extranodal lesions showed a clonal lymphocyte population CD5, CD19, CD20, CD22, kappa chain positive and CD23 negative. Morphological and immunoistochemically analysis of the breast biopsy were consistent with a diagnosis of mantle cell lymphoma (CD20 +, CD79a +, bcl-2 + and cyclin D1 +). After six courses of CHOP - like induction, the patient has been addressed to IEV consolidation followed by autologous stem cell transplantation. In conclusion, flow cytometryc analysis of fine-needle biopsies of both nodal and extranodal lesions may represent a fast, reliable and effective method for diagnosis of mantle-cell lymphoma relapse.

L051

IgA MESANGIAL GLOMERULONEPHRITIS IN MANTLE CELL LYMPHOMA: A CASE REPORT

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Here we described a 52-year-old white man that was referred in February 2005 to our Division for the presence of axillary and inguinal lymph node enlargements. Full blood count was normal and no symptoms were referred. Histological evaluation of an axillary lymph node revealed the presence of a Mantle Cell Non-Hodgkin Lymphoma, with the presence of typical CD5/CD19 positive, CD23 negative cells, also positive for Cyclina 1 expression. Moreover, molecular analysis of peripheral as well of bone marrow specimens revealed the presence of a typical rearrangement in the BCL1-MTC region. Bone marrow trephine biopsy revealed lymphomatous infiltration and CT scan

revealed lymph node enlargement at the retroperitoneal sites. No kidneys alterations were evidenced by ultrasound and CT-scan examination. Nevertheless, since two years before, urine dipstick testing was ever found positive for blood with granular and red cell casts evidenced by microscopy. Serum complement C3 and C4 levels were reduced, and antinuclear antibodies were negative. Creatinine clearance was normal. A percutaneous renal biopsy was performed showing a diffuse mesangial proliferative glomerulonephritis with a positive immunofluorescence for IgA, C3 and lambda light chains. Treatment was started according to CHOP plus Rituximab regimen and sequential high dose chemotherapy with autologous stem cell transplant without nephrotoxic drugs was planned. Glomerulonephritis is a rare complication of lymphoproliferative disorders, most commonly associated to Hodgkin's disease. There are relatively few reports about the presence of glomerulonephritis at the same time in patients with non-Hodgkin lymphomas (NHLs), including both T and B-cell lymphomas. In particular, IgA mesangial glomerular nephropathy is a very rare condition that has been occasionally described in association with NHLs, and in particular in few Mycosis Fungoides or lymphocytic lymphoma cases. At the best of our knowledge, no asociation between IgA nephropaty with mantle cell lymphoma has been reported. Therefore, here we report a very unusual association of an IgA nephropaty with a particular B cell lymphoma, the mantle cell lymphoma, in which aggressive treatments with potentially nephrotoxic agent (i.e. cisplatin) were commonly performed. As consequence, the recognition of this infrequent condition has important implication for therapy. Moreover, interest in this case is also related to the pathogenesis of this association, if it is simply coincidental or due to a lymphoma-related paraneoplastic condition.

L052

NOT PUBLISHED

L053

VERY LOW PLATELET COUNT AND AIR TRAVEL. TO FLY OR NOT TO FLY? AN INTERESTING CLINICAL CASE

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Background. We report the case of a patient referred to us during the last two days of the 2004 for bleeding symptoms. The patient was admitted to our department for frequent epistaxis, gingival hemorrhages and diffuse and large petechiae, spontaneous bruises, gross and frequent hematuria. He was a 65 man suffering of a B-CLL treated with oral Fludarabine. On physical examination we observed a massive splenomegaly and lymphadenopathy. Laboratory evaluation demonstrated a platelet count of 2000/microl; an elevated lymphocyte count of 30000/9/L, and 10,5 gr/L of Hemoglobin. Routine coagulation tests were normal. The patient had an urgent necessity to travel by airplane because of a very heavy personal problem; so we decide to trasfuse filtered and irradiated platelets and to start with Prednisolon therapy and Thranexanic acid to evaluate the day after the platelets recovery.We started a 80 mg/day therapy of Predinisolon i.v. and we trasfused 10 units of platelets. After few hours the bleeding manifestations stopped so we were confident to permit the patient to travel by air the day after. The air jouney sheduled was a 4 hours flight.In the morning of day 31 another blood count was performed, demostrating a very low platelet count(< 1000/microl)and new bleeding symptoms (hemorrhoids bleeding)appeared. The diagnosis of refractory thrombocytopenia due of original disease(B-CLL) progression with massive splenomegaly was made. But Neverless the bleeding symptoms, the very low platelet count, and the very high risks travel the patients was convinced to go back home. At this time we had three possibilities; to not permit the patient jouney informing the airline company; to arrange a private particular flight with a barometric pressure inside the cabin mimic the sea level; to permit the regular flight.After a long discussion the patient forced us to travel alone and in a regular flight.!! He completed his jouney without any major bleeding and other medical problems. At home he started a new combination therapy for his disease progression and now is acheiving a good responce. This case demostrated a lack of concerns about indications and guidelines of air travelling with high risk hematological disease situations. During the meeting we will perform an interview to as many as possible italian haematologist to indagate "how we think and we suggest" in Italy for air travelling patients with haematological disease and critical health situations like thrombocytopenia, anemia, neutropenia, and in the other hand poliglobulia, leukocytosis and thrombocytosis.

L054

ANTICOAGULANT TREATMENT IN A CASE OF THROMBOCYTOPENIC PURPURA REFRACTORY TO PLASMA-EXCHANGE

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Thrombotic Thrombocytopenic Purura (TTP) is a multisystemic disease characterized by thrombocytopenia, hemolytic anemia, renal failure, fever and neurologic abnormalities. Plasma exchange has revolutionized the outcome of this entity from a once fatal disease to a disease that potentially is cured or has prolonged remission. TTP is caused by the persistance of the highly reactive high-molecular-weight multimers of von Willebrand factor (vWF) due to deficiency of the specific vWF cleaving protease ADAMTS-13 resulting in microangiopathic disease. The acquired form is caused by autoantibodies against ADMTS 13, whereas Homozygous or compound heterozygous mutations of ADAMTS-13 are responsible for recessively inherited TTP. We experienced a case of TTP finally relieved after 115 consecutive sessions of plasma exchange (PE). The patient was a 53-year-old female that was examined in emergency because of brown urine and a lowered level of consciousness. As TTP was suspected according to the laboratory findings of abnormally high lactate dehydrogenase and total bilirubin, decreased

platelet counts and numerous fragment erythrocytes, she was admitted to the ICU of our hospital. Immediately after admission, PE was started consecutively using fresh frozen plasma as replacement. Concomitant use of antiplatelet drugs and corticosteroid therapy (Prednisolone 1 mg/kg) was started. After we used cryoprecipitate poor plasma because major allergic reactions and refractoriness compaired. Because complete remission wasn't reached we started vincristine with low molecular weight heparin (LMWH) because hypercoagulation (short PTT, high level of FVIII and clotting in extracorporeal line). When the patient reached CR we reduced and suspended PE and LMWH treatment was replaced with oral sodium warfarin home treatment to keep the international normalized ratio range between 2 and 3. We observed direct correlation between INR (when below the range) and relapse. The patient now is in CR and continue the same therapy. We wonder whether any patient with refractory TTP could gain from anticoagulant treatment.

L055

OPIOIDS IN PAIN MANAGEMENT OF BLOOD-RELATED MALIGNANCIES

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Pain syndromes observed in hematologic malignancies (HM) represent a mixture of pathophysiologic mechanisms, a complex assortment of spontaneous and elicited pain states, and a somewhat unpredictable response to analgesics (Niscola et al, The Hematol J, 2004). Many heterogeneous mechanisms are likely to be involved, such as osteolysis, bone marrow (BM) infiltration, visceral disease localisation, soft tissue involvement, mucosae and cutis lesions, peripheral nerve injuries and space-occupying effects owing to node and organ enlargements. The most cases of bone pain are due to osteolisys, mainly in multiple myeloma patients, and to BM involvement, such as in those with acute leukaemia in which the expansion of the haematopoietic matrix within the rigid-walled space causes intraosseus hypertension, impairment of blood supply and distension of the periosteum, generating severe localized or, more often, migrating pain. Treatment approach to HM-associated pain syndromes should integrate disease-targeted and analgesic measures. The usefulness of causal therapies is based on the rationale that HM may be responsive to these measures even in the advanced phases. Analgesics may be associated to disease treatments or delivered as only measures when the latter are not applicable. The appropriate agent to be administered in this setting must be carefully selected, since many factors, such as concomitant severe neutropenia and thrombocytopenia, are likely to be involved. So, in thrombocytopenic patients or in those presenting coagulopathies or at high risk of renal failure, NSAIDs should be replaced with paracetamol. Moreover, adjuvant drugs, such as gabapentin, should be employed to relieve neuropathic pain. Traditionally, opioids are characterised as weak or strong, depending on their potency and on the presence or not of a ceiling effect. They should be sequentially employed according to the WHO ladder for cancer pain. Initial treatment and titration with opioids should be based on immediate–release (IR) preparations, to be administered at appropriate intervals in order to relieve pain and to reach the individual opioid requirement. Dose titration, using oral IR morphine solution, is recommended with regular oral dosing intervals of four hours followed by repeated doses as needed, until pain relief. Such titration of the dose is generally safe and effective in relieving pain. Once a relatively good pain control has been achieved, a schedule based on slow release (SR) morphine preparation may be administered. While most patients can be adequately managed using the oral opioids, a small percentage require alternative routes, either because they are unable to swallow (i.e. mucositis, end life care and so on), or if a pain relief must be rapidly obtained, intravenous (IV) continuous infusion (CI) or a patient-controlled analgesia (PCA) system are the procedures of choice, employing preselected bolus doses (i.e. 3 mg) at a lockout interval (i.e.15 minutes). No basal rate must be given until the opioid requirement is known. Once adequate analgesia has been attained, the amount of opioid required to control pain in the previous 24 hours should be used to set the patient's CI rate; in patients able to take oral medication, the conversion to a more convenient and less expensive oral regimen should be made as soon as possible. Even though continuous analgesia has been reached, it is imperative to provide for intense episodic (breakthrough) pain. The recommended dose of opioid needed for breakthrough pain, are usually 10% to 15% of the total daily dose. A careful treatment strategy for this difficulty treatable form of pain, based on the availability of rescue doses of a fast onset and short-acting opioid by sublingual or parenteral route in addiction to the continuous analgesic medication, should be determined for each patient. Other available routes of administration for opioids include rectal, transdermal (TD), subcutaneous (SC), epidural, intrathecal and intramuscular (IM). Because of erratic absorption and pain, IM route is generally avoided. SC route is somewhat more convenient. However, caution should be used in patients who become dehydrated because this may hamper systemic absorption and decrease analgesic efficacy. Rectal administration is often uncomfortable for the caregiver and the patient but can be effective, even if the duration of analgesic effect may be shortened with this route; both IR and SR preparations can be used. TD delivering is often the simplest and most convenient for patients who are unable to take oral medications. Thus, the TD fentanyl and buprenorphine patches are best suited for patients with stable pain in whom the 24-hour opioid requirement has already been determined. The invasive neuraxial opioid delivery has a very limited role in patients with HM, given the high risk of infection and bleeding. When opioids are given to a patient, regardless of the route, a bowel regimen should be initiated to prevent constipation, which is almost universal in patients taking opioids. Through a close observation and a careful management, other opioid-related side effects are very rarely observed. In conclusion, the opioids are an essential package of a comprehensive approach to

HM patients and there is no reason for these patients to have ongoing pain with the full range of drugs and strategies currently available.

L056

SYSTEMIC MASTOCYTOSIS WITH ASSOCIATED CLONAL HEMATOLOGICAL NON - MAST CELL LINEAGE DISEASE: A REPORT A CASE WITH MYELODYSPLASTIC SYNDROME AND A REVIEW OF LICTERATURE

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Systemic mastocytoses (SM) are a heterogeneous group of diseases, more frequently diagnosed in adults, which are characterized by accumulation and unusual growth of mast cells (that show on the membrane the markers CD 117, CD 2 and/or CD 25) infiltrating at least two different organs or types of tissue. According to a new WHO classification of 2000, mastocytoses are separated into cutaneous and systemic mastocytoses. Systemic mastocytosis is subdivided into an indolent course with good prognosis and four subgroups with poor prognosis (systemic mastoyctosis with associated clonal hematologic non-mast cell disease, aggressive systemic mastocytosis, mast cell leukaemia, and mast cell sarcoma). Systemic mastocytoses are clonal disorders of mast cells and their progenitor cells , which may show point mutations of the protooncogene c-Kit (the most frequent is in partnership to the codone 816 -Val for Asp - , that results in a spontaneous activation of this tirosinkinasi). This gene codes for the stem cell receptor (CD 117) . The diagnosis is histological with special colorations. Antibody against CD 25 appears today to be a reliable markers for the discriminating neoplastic from normal / reactive mast cells. The clinician can use the dosing of alpha-triptasi that correspondent results to the mass of the mast-cells and therefore in case of an anomalous proliferation of them. Therapy of systemic mastocytosis depend by patient's symptoms. There is no know therapy of the disease. Besides diet and avoidance of skin irritations, symptoms are treated with H(1) – or H(2) – blockers, steroids, leukotriene receptor antagonists, interferon alfa and PUVA therapy. Recently imatinib mesylate is testate in SM. We report a case of a 82 years old patient affected by systemic mastocytosis. In September 2003 it begins dermatological evaluation that brings to a cutaneous biopsy of the region abdominal right: the picture morphological show a marked infiltration of the derma by cellular elements primarily of lengthened form, with clear cytoplasm, with few eosinophils and lympho-hystiocitic. The colorations with the Giemsa made to notice a notable number of mastcells. The anatomopathological picture deposed for a pigmentos urticaria. The laboratory: GB 3100 /mm3 - N. 31% - L 48% - M 18%. EO 3% - Hb 11,1 g/dl - PLT 165.000 / mm^3 - Ves 70 - B2 microglobulin 2558 ng/mL - Ferritin 148 ng/ml – gamma-globulin 21.9 % - LDH 310 UI/L – Bone marrow: generic signs of dyseritropoyesis (SMD). The immunophenotyping was normal. Clinically not nodes neither splenomegaly. Bone marrow biopsy was not accepted. At the Rx of torax and Tomography chest: multiple opacities. Bronchoscopic examination disappeared negative. Bone scan (performed for bony pains): negative except hyperaccumulate on the epiphyses of the humerus right. An examination EGDS results positive only for hiatus gastric hernia and gastritis. He was treated exclusively with topic ant antistaminic therapy during one year. For the progressive anaemia and increase of the number of the white cells that have also reached the 40.000/mm^3 (elements CD 13 +, CD 15+, CD 66 b+, CD14+ and 56+) and discrete reduction of the platelets (values around the 50.000), last summer, he has practised with success therapy with cortisone and epoetin alpha returning to values of Hb of around 11 - 12 g/dl and stabilization of the numbers of the white globules so about 14000/ mm^3. Subsequently for an increase of volume of the right superior member has been submitted to total body thomograpy that has underlined some small and diffused pulmonary opacities and nodes in right armpit and a focal liver lesion. It repeated a bone marrow compatible at with also a dyseritropoiesis . Venous Doppler didn't underline thrombosis. For wish of the patient and the family ones, also seen the progressive one and express decline of his general conditions, he hasn't been possible anymore to proceed to further investigations for which the mutation is not determined D816V of the c-kit and has not been effected the dosing of the seric triptasi and a bone marrow biopsy. Concluding we are able therefore probably to affirm that, despite the impossibility to the individualization of the specific targets, the systemic mastoyctosis with associated clonal hematologic not-mast cell disease from us hypothesized was likely.

L057

A VERY ELDERLY PATIENT WITH SIMULTANEOUS DIAGNOSIS OF ACUTE MYELOID LEUKEMIA AND SEZARY'S SYNDROME EVOLVING FROM REFRACTORY ANEMIA: OUTCOME OF TREATMENT

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We describe the case of a 92 year old woman with myelodysplastic syndrome progressing contemporarily to acute myeloid leukemia and Sezary's syndrome. At 88 years of age, history was negative for any previous and concomitant diseases. She presented with fatigue, dyspnea and pallor. A laboratory examination showed severe macrocytic anemia (Hb 7.7 g/dL, MCV 132 fL) with normal WBC and PLT counts. Bone marrow aspirate and specimen showed refractory anemia with multilinear dysplasia. She was initially treated with supportive care and recombinant human erythropoetin (rHuEpo) obtaining a complete hematological response (Hb 11.0 g/dL). After 23 months of follow-up she developed resistance to rHuEpo and cutaneous and subcutaneous nodules with eczematous lesions. Peripheral blood smear examination showed 5% blasts. Multiple cutaneous biopsies were performed and on histological examination a contemporary diagnosis of Sezary's Syndrome and cutaneous localization of acute monoblastic leukemia (AML) was formulated. The patient

was treated with prednisone and vinblastin (10 mg i.v.) for a total of 3 doses for Sezary's syndrome, obtaining a complete long-lasting remission after 1 month . She also received induction chemotherapy with standard dose Cytarabine and Thioguanine for the AML, obtaining partial remission at 3 months. She maintained a good clinical condition, continuing monthly chemotherapy and supportive care. Patient died after one year from diagnosis of AML at 92 years of age. The decision to treat the patient, regardless of age, improved cutaneous manifestations of malignancies and the clinical condition, thus improving quality of life.

L058

TRANSITION OF POLYCYTEMIA VERA TO CHRONIC MYELOID LEUKEMIA

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Background. Clinical course of Polycythemia Vera (PV) is characterized by a variable risk of disease transformation into myelofibrosis with myeloid metaplasia (MMM). myelodysplastic syndrome (MDS) and less often to acute leukemia (AL). The development of chronic myeloid leukemia (CML) in the course of PV is extremely rare.

Aim. To cytogenetically and molecularly characterize the transition of a case of PV to CML.

Case Report. A 77-years-old Caucasian female was diagnosed as having a PV in September 1996. She showed only a just palpable spleen. Hb 13.3 g/dL, Htc 50, WBC $10.7x10^9$/L with normal differential. Platelets $712x10^9$/L. LAP score 98 (n.v.40-60). Red blood cell mass 55mL/Kg. Arterial oxygen saturation 96.6%. Bone marrow biopsy and aspirate showed marked erythroid and prominent megakaryocytic hyperplasia. Cytogenetic study on bone marrow cells showed a normal female karyotype.RT-PCR analysis of RNA extracted from bone marrow cells showed no rearrangement of bcr gene. Phlebotomies and hydroxyurea (HU) (0.5-1 g/day) were started. One year later, a moderately enlarged liver (3 cm below costal margin) and spleen (0.5 cm) were found. Polycythemia was controlled by phlebotomies and intermittent course of HU for 8 years. On July 2004, the patient suddenly showed the typical features of CML. The physical and ultrasound examination showed an enlarged spleen (3 cm) and liver (3 cm). Hb 10.8 g/dL, Htc 36.8, WBC 160x109/L with neutrophils 51%, eosinophils 1%, basophils 1%, lymphocytes 4%, monocytes 14%, promyelocytes 1%, myelocytes 7%, metamyelocytes 15% and blasts 6%: Platelets $39x10^9$/L. LAP score was 8. Bone marrow aspirate showed an huge granulocytic hyperplasia. Cytogenetics showed the 46,XX,t(9;22) karyotype in the majority of examined bone marrow cells. Rt-PCR revealed the presence of the BCR-ABL chimeric transcript, b2a2 subtype (p210). The patient developed a refractory bilateral pneumonia and died nine days later.

Discussion. In the setting of chronic myeloproliferative disorders , a transition from one subgroup to another is possible, either as natural evolution or owing myelosuppressive treatments. Among the seven reported cases of transition of PV to CML, five have not been investigated for the presence of chromosome or bcr rearrangement during the PV phase. Five of such cases underwent radioactive phosphorus or chlorambucil, but one developed CML in absence of any therapy. Radiations and alkylating agents are known to predispose to leukemogenesis. For the seven reported cases and included our own, and in absence of a marker for the Ph-negative diseases, whether the transition of PV to CML might be a natural or clonal evolution, a treatment dependent progression or a fortuitous occurrence of two distinct disorders, remain to be elucidated.

L059

IMATINIB THERAPY IN SYSTEMIC MASTOCYTOSIS (SM): A CASE REPORT

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Systemic Mastocytosis (SM) is to rare disease, characterized by mast cells proliferation in various organs (bone, skin, liver etc.). Clinical manifestation is related to mast-cells mediators release and the tumoral proliferation involving different organs. The therapeutic strategy includes treatment with symptomatic drugs, idrossiurea and gamma-IFN. Imatimib (STI) has shown to be effective in SM. We report a case of SM treated with STI in our division. Male 55 years-old have received diagnosis of SM in 1973 in other Division. In May 2003 has been recovery in our Division, the patient showed clinical manifestation (urticaria pigmentosa, pruritus, headache, flushing, diarrhea; abdominal and bone pain) organomegaly and leucocytosis. In November 2004 started therapy with STI 100mg/day and after three months the clinical manifestation and abdominal and bone pains are not present, is observed poor reduction of WBC and organomegaly, dramatically resolved of urticaria pigmentosa, no collateral effect is evident for this therapy. In conclusion STI has been shown to be effective in the treatment of SM for clinical manifestation, not good at three months is the haematological response. A longer follow-up is necessary to value the efficacy of such treatment in our patient.

L060

SEQUENTIAL THERAPY RASBURICASE – ALLOPURINOL IN THE TREATMENT OF HYPERURICEMIA IN LEUKEMIAS AND LYMPHOMAS

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Hyperuricemia can be caused by the "Tumor lysis Syndrome" found in patients affected by hyperleukocytosic leukemias or other hematological malignancies with a large tumour burden characterized by a rapid cell turn-over. It is due to the shedding of purinic basis from the tumour following spontaneous or chemotherapy-induced cell tumour lysis. Recent studies have reported that uric acid > 8 mg/dL and white blood cell count > $50x10^9$/L are correlated to a high early death risk. About 25% of patients affected by acute hyperleukocytosic leukemias and by Burkitt Lymphomas in advanced stages develops acute renal failure during chemotherapy, even if these patients are correctly treated for hyperuricemia. The therapeutic weapons that are used until to-day are the hydratant therapy, allopurinol and rasburicase.

Allopurinol acts through the inhibition of Xanthine–Oxydase that catalyzes the formation of uric acid from xanthine inhibiting the synthesis of uric acid, but not eliminating the already accumulated uric acid. Allopurinol has a slow action, is deliverable only per os and can determine a nefropathy with urolithiasis due to high blood concentrations of xanthine. Rasburicase, urate-oxydase from recombinant DNA, an enzyme able to catalyze the oxydation of uric acid in allantoin and to allow its elimination. Rasburicase is i.v. administered and has a prompt action, good tolerability and poor toxicity. The sequential administration of the two drugs could be an effective strategy for the treatment of hyperuricemia and the prevention of uratic nefropathy. Thirteen patients, 8 males and 5 females, mean age 56 years (range: 17-84), affected by hematologic malignancies, were treated with Rasburicase. All the patients have elevated levels of uric acid and two of them have low renal indexes with conserved renal functions. After the beginning of the treatment with Rasburicase all the patients have a constant and gradual decrease of blood uric acid from the day 2 even in presence of chemotherapy administration. Plateau was reached at the third day of therapy with 1 – 2 mg/dL uric acid levels. From day 4 Rasburicase was suspended and 300 mg/die/os allopurinol was begun. Rasburicase administration in one hour 250 ml NaCl i.v. was well tolerated and did not cause important side effects and renal functions were at high limits but normal. Blood uric acid determination was performed maintaining the vial at 4°C to block Rasburicase in vitro activity. The sequential therapy with Rasburicase – Allopurinol has allowed the optimal treatment of hyperuricemia and the maintainance of blood uric acid in a normal range for all the period in which chemotherapy was administered. Moreover, chronic renal failure was solved in the 2 patients who have alteration of renal functions at the presentation. The prevention of uratic nefropathy with this treatment modality has given promising results for the long-term management of these subsets of patients.

L061

INFLIXIMAB TREATMENT OF GRADE IV SKIN ACUTE GRAFT VERSUS HOST DISEASE

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Tumor necrosis factor alpha is one of the principal cytokines involved in the pathogenesis of acute graft-versus-host-disease (aGVHD). Infliximab is a chimeric mouse/human monoclonal antibody that binds to soluble and transmembrane forms of human TNF alpha. Patients and methods: a 39 years old male diagnosed with AML with a mediastinal granulocytic sarcoma and t(10;11) reached 1st CR after induction chemotherapy. The patient relapsed few months from his second consolidation curse and was reinduced with salvage chemotherapy obtaining a PR. Conditioning was with Cy/TBI and received an allogenic PBSC transplant from an HLA identical sibling. He received $3.3x10^6$ CD34/Kg G-CSF mobilized stem cells. GVHD prophylaxis was with ciclosporyne (CSA) and short course MTX. The patient engrafted at day +17 and was in CR by morphology, flow cytometry, cariotype with full donor chimerism. On day +24 the patient had visual disturbances, allucinations and an MRI was consistent with CNS-CSA toxicity. HHV6, HHV8. CMV, EBV were ruled out by PCR on PB and CSF. CSA was changed to FK506 and prednisone was added with at 2 mg/kg with improvement of symptoms 3 days later. On day +33 the patient presented skin aGVHD which progressed by day +35 to grade IV. No liver or GIT GVHD occurred. Infliximab 10 mg/kg was given on day +35 and repeated weekly for total of three doses. Three days out from the first dose skin aGVHD appeared resolving to a "spent phase". As the patient was started on prednisone therapy, fungal prohpylaxis was changed from fluconazole to itraconazole oral solution. On day +80 the patient had a CNS stroke and was admitted with diagnosis (proven by CSF analysis, peripheral blood antigenemia, MRI and pulmonary CT scan) of CNS and pulmonary invasive aspergillosis. The patient received voriconazole associated with caspofungin but expired on day +98. Conclusions: infliximab allowed control of grade IV skin aGVHD added to PDN and FK506 with prompt improvement. Infliximab has shown encouraging activity in intestinal and skin steroid-refractory aGVHD patients, although benefits have been counterbalanced in other reported series by infectious episodes (becaterial, viral and invasive fungal). This patient, despite oral itraconazole prophylaxis, developed pulmonary and CNS aspergillosis. Infliximab was effective to control skin grade IV aGVHD in this patient but a more powerful fungal prhophylaxis might be needed in this setting. ,

L062

IFOSFAMIDE, EPIRUBICIN AND ETOPOSIDE IEV REGIMEN: A SALVAGE AND MOBILIZATION THERAPY FOR PRIMARY REFRACTORY OR RELAPSED HODGKIN&'S AND NON-HODGKIN&'S LYMPHOMA

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The outcome of primary refractory and early or second relapses of Hodgkin's disease (HD) and aggressive Non-Hodgkin's Lymphoma (NHL) is discouraging. For this reason, effective salvage strategies should be able to induce high percentage of good responses as well as to mobilize hematopoietic precursors finalised to transplant procedures as salvage treatment. We report our experience regarding 18 refractory or relapsed patients (12 males and 6 females; median age: 38 years; range 20-59) with HD (12 pts) and NHL (6 pts). Salvage therapeutic approach consisted in 6 cours of a combination of Ifosfamide, Epirubicin and Etoposide (IEV Protocol). We obtained a complete response rate of 75% and 66% in HD and NHL, respectively. In 15 patients (83%) a mobilization of peripheral blood stem cells was performed and a median of CD34+ cells of $4,8x10^6$/Kg, was recovered. All these patients received a consolidation treatment consisting in peripheral stem cell transplantation conditioned by BEAM protocol. Three patients underwent withdrawal of bone marrow ad received autologous bone marrow transplantation. Relate-transplant toxicity was mild, with no occurrence of severe and persisting non-hematologic side-effects. At present (median follow-up: 10 months - range 5-33), after the complete therapeutic program, including IEV and transplant procedures, 17 patients are alive (11 in continuous complete remission - 4 NHL and 7 HD patients). Our results demonstrate that IEV regimen, in our hands, is very effective in relapsed or refractory HD or aggressive NHL, resulting in high percentage of successful stem cell mobilization and feasibility of stem cell transplantation as consolidation therapy.

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